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Handbook of Enzymes

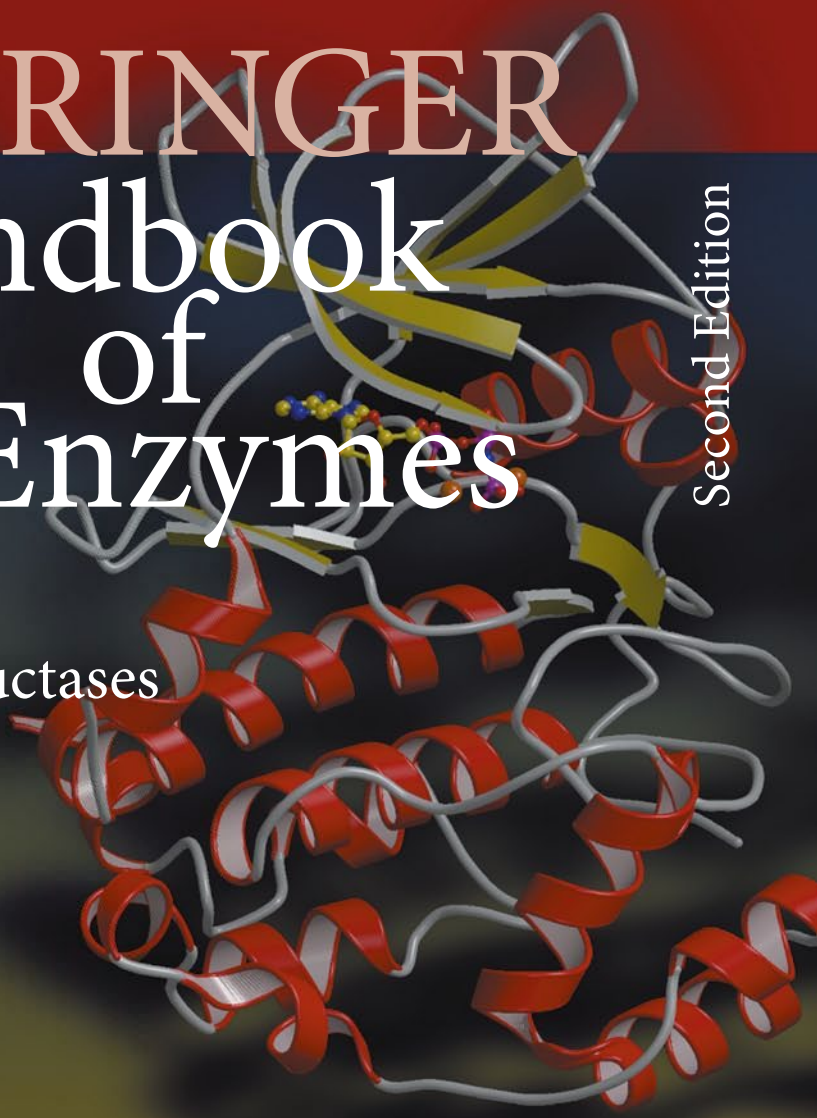
Second Edition

SUPPLEMENT VOLUME S8

CLASS 1

Oxidoreductases

EC 1



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Springer Handbook of Enzymes
Supplement Volume S8

Dietmar Schomburg and
Ida Schomburg (Eds.)

Springer Handbook of Enzymes

Supplement Volume S8
Class 1
Oxidoreductases
EC 1

coedited by Antje Chang

Second Edition

Springer

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Preface

Today, as the full information about the genome is becoming available for a rapidly increasing number of organisms and transcriptome and proteome analyses are beginning to provide us with a much wider image of protein regulation and function, it is obvious that there are limitations to our ability to access functional data for the gene products – the proteins and, in particular, for enzymes. Those data are inherently very difficult to collect, interpret and standardize as they are widely distributed among journals from different fields and are often subject to experimental conditions. Nevertheless a systematic collection is essential for our interpretation of genome information and more so for applications of this knowledge in the fields of medicine, agriculture, etc. Progress on enzyme immobilisation, enzyme production, enzyme inhibition, coenzyme regeneration and enzyme engineering has opened up fascinating new fields for the potential application of enzymes in a wide range of different areas.

The development of the enzyme data information system BRENDA was started in 1987 at the German National Research Centre for Biotechnology in Braunschweig (GBF), continued at the University of Cologne from 1996 to 2007, and then returned to Braunschweig, to the Technical University, Institute of Bioinformatics & Systems Biology. The present book "Springer Handbook of Enzymes" represents the printed version of this data bank. The information system has been developed into a full metabolic database.

The enzymes in this Handbook are arranged according to the Enzyme Commission list of enzymes. Some 5,000 "different" enzymes are covered. Frequently enzymes with very different properties are included under the same EC-number. Although we intend to give a representative overview on the characteristics and variability of each enzyme, the Handbook is not a compendium. The reader will have to go to the primary literature for more detailed information. Naturally it is not possible to cover all the numerous literature references for each enzyme (for some enzymes up to 40,000) if the data representation is to be concise as is intended.

It should be mentioned here that the data have been extracted from the literature and critically evaluated by qualified scientists. On the other hand, the original authors' nomenclature for enzyme forms and subunits is retained. In order to keep the tables concise, redundant information is avoided as far as possible (e.g. if K_m values are measured in the presence of an obvious cosubstrate, only the name of the cosubstrate is given in parentheses as a commentary without reference to its specific role).

The authors are grateful to the following biologists and chemists for invaluable help in the compilation of data: Cornelia Munaretto and Dr. Antje Chang.

Braunschweig
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Dietmar Schomburg, Ida Schomburg

List of Abbreviations

A	adenine
Ac	acetyl
ADP	adenosine 5'-diphosphate
Ala	alanine
All	allose
Alt	altrose
AMP	adenosine 5'-monophosphate
Ara	arabinose
Arg	arginine
Asn	asparagine
Asp	aspartic acid
ATP	adenosine 5'-triphosphate
Bicine	N,N'-bis(2-hydroxyethyl)glycine
C	cytosine
cal	calorie
CDP	cytidine 5'-diphosphate
CDTA	trans-1,2-diaminocyclohexane-N,N,N,N-tetraacetic acid
CMP	cytidine 5'-monophosphate
CoA	coenzyme A
CTP	cytidine 5'-triphosphate
Cys	cysteine
d	deoxy-
D-	(and L-) prefixes indicating configuration
DFP	diisopropyl fluorophosphate
DNA	deoxyribonucleic acid
DPN	diphosphopyridinium nucleotide (now NAD^+)
DTNB	5,5'-dithiobis(2-nitrobenzoate)
DTT	dithiothreitol (i.e. Cleland's reagent)
EC	number of enzyme in Enzyme Commission's system
E. coli	Escherichia coli
EDTA	ethylene diaminetetraacetate
EGTA	ethylene glycol bis(-aminoethyl ether) tetraacetate
ER	endoplasmic reticulum
Et	ethyl
EXAFS	extended X-ray absorption fine structure
FAD	flavin-adenine dinucleotide
FMN	flavin mononucleotide (riboflavin 5'-monophosphate)
Fru	fructose
Fuc	fucose
G	guanine
Gal	galactose

GDP	guanosine 5'-diphosphate
Glc	glucose
GlcN	glucosamine
GlcNAc	N-acetylglucosamine
Gln	glutamine
Glu	glutamic acid
Gly	glycine
GMP	guanosine 5'-monophosphate
GSH	glutathione
GSSG	oxidized glutathione
GTP	guanosine 5'-triphosphate
Gul	gulose
h	hour
H4	tetrahydro
HEPES	4-(2-hydroxyethyl)-1-piperazineethane sulfonic acid
His	histidine
HPLC	high performance liquid chromatography
Hyl	hydroxylysine
Hyp	hydroxyproline
IAA	iodoacetamide
IC 50	50% inhibitory concentration
Ig	immunoglobulin
Ile	isoleucine
Ido	idose
IDP	inosine 5'-diphosphate
IMP	inosine 5'-monophosphate
ITP	inosine 5'-triphosphate
K_m	Michaelis constant
L-	(and D-) prefixes indicating configuration
Leu	leucine
Lys	lysine
Lyx	lyxose
M	mol/l
mM	millimol/l
m-	*meta*-
Man	mannose
MES	2-(N-morpholino)ethane sulfonate
Met	methionine
min	minute
MOPS	3-(N-morpholino)propane sulfonate
Mur	muramic acid
MW	molecular weight
NAD^+	nicotinamide-adenine dinucleotide
NADH	reduced NAD
$NADP^+$	NAD phosphate
NADPH	reduced NADP
NAD(P)H	indicates either NADH or NADPH

NBS	N-bromosuccinimide
NDP	nucleoside 5'-diphosphate
NEM	N-ethylmaleimide
Neu	neuraminic acid
NMN	nicotinamide mononucleotide
NMP	nucleoside 5'-monophosphate
NTP	nucleoside 5'-triphosphate
o-	*ortho*-
Orn	ornithine
p-	*para*-
PBS	phosphate-buffered saline
PCMB	*p*-chloromercuribenzoate
PEP	phosphoenolpyruvate
pH	-log10[H^+]
Ph	phenyl
Phe	phenylalanine
PHMB	*p*-hydroxymercuribenzoate
PIXE	proton-induced X-ray emission
PMSF	phenylmethane-sulfonylfluoride
p-NPP	*p*-nitrophenyl phosphate
Pro	proline
Q_{10}	factor for the change in reaction rate for a 10°C temperature increase
Rha	rhamnose
Rib	ribose
RNA	ribonucleic acid
mRNA	messenger RNA
rRNA	ribosomal RNA
tRNA	transfer RNA
Sar	N-methylglycine (sarcosine)
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
Ser	serine
T	thymine
t_H	time for half-completion of reaction
Tal	talose
TDP	thymidine 5'-diphosphate
TEA	triethanolamine
Thr	threonine
TLCK	N^{α}-*p*-tosyl-L-lysine chloromethyl ketone
T_m	melting temperature
TMP	thymidine 5'-monophosphate
Tos-	tosyl- (*p*-toluenesulfonyl-)
TPN	triphosphopyridinium nucleotide (now $NADP^+$)
Tris	tris(hydroxymethyl)-aminomethane
Trp	tryptophan
TTP	thymidine 5'-triphosphate
Tyr	tyrosine
U	uridine

U/mg	μmol/(mg*min)
UDP	uridine 5'-diphosphate
UMP	uridine 5'-monophosphate
UTP	uridine 5'-triphosphate
Val	valine
Xaa	symbol for an amino acid of unknown constitution in peptide formula
XAS	X-ray absorption spectroscopy
Xyl	xylose

Index of Recommended Enzyme Names

Description of Data Fields

All information except the nomenclature of the enzymes (which is based on the recommendations of the Nomenclature Committee of IUBMB (**I**nternational **U**nion of **B**iochemistry and **M**olecular **B**iology) and IUPAC (**I**nternational **U**nion of **P**ure and **A**pplied **C**hemistry) is extracted from original literature (or reviews for very well characterized enzymes). The quality and reliability of the data depends on the method of determination, and for older literature on the techniques available at that time. This is especially true for the fields *Molecular Weight* and *Subunits*.
The general structure of the fields is: **Information – Organism – Commentary – Literature**
The information can be found in the form of numerical values (temperature, pH, K_m etc.) or as text (cofactors, inhibitors etc.).
Sometimes data are classified as *Additional Information*. Here you may find data that cannot be recalculated to the units required for a field or also general information being valid for all values. For example, for *Inhibitors*, *Additional Information* may contain a list of compounds that are not inhibitory.
The detailed structure and contents of each field is described below. If one of these fields is missing for a particular enzyme, this means that for this field, no data are available.

1 Nomenclature

EC number
The number is as given by the IUBMB, classes of enzymes and subclasses defined according to the reaction catalyzed.

Systematic name
This is the name as given by the IUBMB/IUPAC Nomenclature Committee

Recommended name
This is the name as given by the IUBMB/IUPAC Nomenclature Committee

Synonyms
Synonyms which are found in other databases or in the literature, abbreviations, names of commercially available products. If identical names are frequently used for different enzymes, these will be mentioned here, cross references are given. If another EC number has been included in this entry, it is mentioned here.

CAS registry number
The majority of enzymes have a single chemical abstract (CAS) number. Some have no number at all, some have two or more numbers. Sometimes

two enzymes share a common number. When this occurs, it is mentioned in the commentary.

2 Source Organism

In this data field the organism in which the enzymes has been detected are listed. The systematic names according to the NCBI Taxonomy are preferred. If the scientific name is missing, the synonym or the names from the respective literature references are used. In addition, organism are listed for which a specific protein sequence or nucleotide sequence has been allocated. The accession number and the respective data source, e.g, UNIPROT is given in the commentary.

3 Reaction and Specificity

Catalyzed reaction

The reaction as defined by the IUBMB. The commentary gives information on the mechanism, the stereochemistry, or on thermodynamic data of the reaction.

Reaction type

According to the enzyme class a type can be attributed. These can be oxidation, reduction, elimination, addition, or a name (e.g. Knorr reaction)

Natural substrates and products

These are substrates and products which are metabolized in vivo. A natural substrate is only given if it is mentioned in the literature. The commentary gives information on the pathways for which this enzyme is important. If the enzyme is induced by a specific compound or growth conditions, this will be included in the commentary. In *Additional information* you will find comments on the metabolic role, sometimes only assumptions can be found in the references or the natural substrates are unknown.
In the listings, each natural substrate (indicated by a bold **S**) is followed by its respective product (indicated by a bold **P**). Products are given with organisms and references included only if the respective authors were able to demonstrate the formation of the specific product. If only the disappearance of the substrate was observed, the product is included without organisms of references. In cases with unclear product formation only a **?** as a dummy is given.

Substrates and products

All natural or synthetic substrates are listed (not in stoichiometric quantities). The commentary gives information on the reversibility of the reaction, on isomers accepted as substrates and it compares the efficiency of substrates. If a specific substrate is accepted by only one of several isozymes, this will be stated here.

The field *Additional Information* summarizes compounds that are not accepted as substrates or general comments which are valid for all substrates. In the listings, each substrate (indicated by a bold **S**) is followed by its respective product (indicated by a bold **P**). Products are given with organisms and references included if the respective authors demonstrated the formation of the specific product. If only the disappearance of the substrate was observed, the product will be included without organisms or references. In cases with unclear product formation only a **?** as a dummy is given.

Inhibitors

Compounds found to be inhibitory are listed. The commentary may explain experimental conditions, the concentration yielding a specific degree of inhibition or the inhibition constant. If a substance is activating at a specific concentration but inhibiting at a higher or lower value, the commentary will explain this.

Cofactors, prosthetic groups

This field contains cofactors which participate in the reaction but are not bound to the enzyme, and prosthetic groups being tightly bound. The commentary explains the function or, if known, the stereochemistry, or whether the cofactor can be replaced by a similar compound with higher or lower efficiency.

Activating Compounds

This field lists compounds with a positive effect on the activity. The enzyme may be inactive in the absence of certain compounds or may require activating molecules like sulfhydryl compounds, chelating agents, or lipids. If a substance is activating at a specific concentration but inhibiting at a higher or lower value, the commentary will explain this.

Metals, ions

This field lists all metals or ions that have activating effects. The commentary explains the role each of the cited metal has, being either bound e.g. as Fe-S centers or being required in solution. If an ion plays a dual role, activating at a certain concentration but inhibiting at a higher or lower concentration, this will be given in the commentary.

Turnover number (s^{-1})

The k_{cat} is given in the unit s^{-1}. The commentary lists the names of the substrates, sometimes with information on the reaction conditions or the type of reaction if the enzyme is capable of catalyzing different reactions with a single substrate. For cases where it is impossible to give the turnover number in the defined unit (e.g., substrates without a defined molecular weight, or an undefined amount of protein) this is summarized in *Additional Information*.

Specific activity (U/mg)

The unit is micromol/minute/milligram of protein. The commentary may contain information on specific assay conditions or if another than the natur-

al substrate was used in the assay. Entries in *Additional Information* are included if the units of the activity are missing in the literature or are not calculable to the obligatory unit. Information on literature with a detailed description of the assay method may also be found.

K_m-Value (mM)
The unit is mM. Each value is connected to a substrate name. The commentary gives, if available, information on specific reaction condition, isozymes or presence of activators. The references for values which cannot be expressed in mM (e.g. for macromolecular, not precisely defined substrates) are given in *Additional Information*. In this field we also cite literature with detailed kinetic analyses.

K_i-Value (mM)
The unit of the inhibition constant is mM. Each value is connected to an inhibitor name. The commentary gives, if available, the type of inhibition (e.g. competitive, non-competitive) and the reaction conditions (pH-value and the temperature). Values which cannot be expressed in the requested unit and references for detailed inhibition studies are summerized under *Additional information*.

pH-Optimum
The value is given to one decimal place. The commentary may contain information on specific assay conditions, such as temperature, presence of activators or if this optimum is valid for only one of several isozymes. If the enzyme has a second optimum, this will be mentioned here.

pH-Range
Mostly given as a range e.g. 4.0–7.0 with an added commentary explaining the activity in this range. Sometimes, not a range but a single value indicating the upper or lower limit of enzyme activity is given. In this case, the commentary is obligatory.

pI-Value
The isoelectric point (IEP) of an enzyme is the pH-value at which the protein molecule has no net electric charge, carrying the equal number of positively and negatively ions. In the commentary the method of determination is given, if it is provided by the literature.

Temperature optimum (°C)
Sometimes, if no temperature optimum is found in the literature, the temperature of the assay is given instead. This is always mentioned in the commentary.

Temperature range (°C)
This is the range over which the enzyme is active. The commentary may give the percentage of activity at the outer limits. Also commentaries on specific assay conditions, additives etc.

4 Enzyme Structure

Molecular weight
This field gives the molecular weight of the holoenzyme. For monomeric enzymes it is identical to the value given for subunits. As the accuracy depends on the method of determination this is given in the commentary if provided in the literature. Some enzymes are only active as multienzyme complexes for which the names and/or EC numbers of all participating enzymes are given in the commentary.

Subunits
The tertiary structure of the active species is described. The enzyme can be active as a monomer a dimer, trimer and so on. The stoichiometry of subunit composition is given. Some enzymes can be active in more than one state of complexation with differing effectivities. The analytical method is included.

Posttranslational modifications
The main entries in this field may be proteolytic modification, or side-chain modification, or no modification. The commentary will give details of the modifications e.g.:
- proteolytic modification <1> (<1>, propeptide Name) [1];
- side-chain modification <2> (<2>, N-glycosylated, 12% mannose) [2];
- no modification [3]

5 Isolation / Preparation / Mutation / Application

Source / tissue
For multicellular organisms, the tissue used for isolation of the enzyme or the tissue in which the enzyme is present is given. Cell-lines may also be a source of enzymes.

Localization
The subcellular localization is described. Typical entries are: cytoplasm, nucleus, extracellular, membrane.

Purification
The field consists of an organism and a reference. Only references with a detailed description of the purification procedure are cited.

Renaturation
Commentary on denaturant or renaturation procedure.

Crystallization
The literature is cited which describes the procedure of crystallization, or the X-ray structure.

Cloning
Lists of organisms and references, sometimes a commentary about expression or gene structure.

Engineering
The properties of modified proteins are described.

Application
Actual or possible applications in the fields of pharmacology, medicine, synthesis, analysis, agriculture, nutrition are described.

6 Stability

pH-Stability
This field can either give a range in which the enzyme is stable or a single value. In the latter case the commentary is obligatory and explains the conditions and stability at this value.

Temperature stability
This field can either give a range in which the enzyme is stable or a single value. In the latter case the commentary is obligatory and explains the conditions and stability at this value.

Oxidation stability
Stability in the presence of oxidizing agents, e.g. O_2, H_2O_2, especially important for enzymes which are only active under anaerobic conditions.

Organic solvent stability
The stability in the presence of organic solvents is described.

General stability information
This field summarizes general information on stability, e.g., increased stability of immobilized enzymes, stabilization by SH-reagents, detergents, glycerol or albumins etc.

Storage stability
Storage conditions and reported stability or loss of activity during storage.

References
Authors, Title, Journal, Volume, Pages, Year.

momilactone-A synthase 1.1.1.295

1 Nomenclature

EC number
1.1.1.295

Systematic name
3β-hydroxy-9β-pimara-7,15-diene-19,6β-olide:NAD(P)$^+$ oxidoreductase

Recommended name
momilactone-A synthase

Synonyms
3β-hydroxy-9β-primara-7,15-dien-19,6β-olide dehydrogenase <1> [1]
AK103462 protein <1> [2]
OsMAS <1> [2]

CAS registry number
458569-32-9

2 Source Organism

<1> *Oryza sativa* [1,2]

3 Reaction and Specificity

Catalyzed reaction
3β-hydroxy-9β-pimara-7,15-diene-19,6β-olide + NAD(P)$^+$ = momilactone A + NAD(P)H + H$^+$

Substrates and products
- **S** 3β-hydroxy-9β-pimara-7,15-diene-19,6β-olide + NAD$^+$ <1> (Reversibility: ?) [1]
- **P** momilactone A + NADH
- **S** 3β-hydroxy-9β-pimara-7,15-diene-19,6β-olide + NADP$^+$ <1> (<1> reaction with NADP$^+$ is 70% of the activity with NAD$^+$ [1]) (Reversibility: ?) [1]
- **P** momilactone A + NADPH

Cofactors/prosthetic groups
NAD$^+$ <1> [1]
NADP$^+$ <1> (<1> 70% of the activity with NAD$^+$ [1]) [1]

D. Schomburg and I. Schomburg (eds.), *Class 1 Oxidoreductases: EC 1*,
Springer Handbook of Enzymes S8, DOI 10.1007/978-3-642-36265-1_1,

K_m-Value (mM)
0.036 <1> (3β-hydroxy-9β-pimara-7,15-diene-19,6β-olide, <1> cofactor: NAD^+ [1]) [1]

pH-Optimum
8 <1> [1]

pH-Range
6-10 <1> (<1> pH 6.0: about 50% of maximal activity, pH 10.0: about 60% of maximal activity [1]) [1]

Temperature optimum (°C)
48 <1> [1]

Temperature range (°C)
36-72 <1> (<1> 36°C: about 60% of maximal activity, 72°C: about 60% of maximal activity [1]) [1]

5 Isolation/Preparation/Mutation/Application

Source/tissue
cell suspension culture <1> [2]
husk <1> [1]

Localization
soluble <1> [1]

Cloning
<1> (expression in Escherichia coli) [2]

References

[1] Atawong, A.; Hasegawa, M.; Kodama, O.: Biosynthesis of rice phytoalexin: enzymatic conversion of 3β-hydroxy-9β-pimara-7,15-dien-19,6β-olide to momilactone A. Biosci. Biotechnol. Biochem., **66**, 566-570 (2002)
[2] Shimura, K.; Okada, A.; Okada, K.; Jikumaru, Y.; Ko, K.W.; Toyomasu, T.; Sassa, T.; Hasegawa, M.; Kodama, O.; Shibuya, N.; Koga, J.; Nojiri, H.; Yamane, H.: Identification of a biosynthetic gene cluster in rice for momilactones. J. Biol. Chem., **282**, 34013-34018 (2007)

dihydrocarveol dehydrogenase 1.1.1.296

1 Nomenclature

EC number
1.1.1.296

Systematic name
menth-8-en-2-ol:NAD^+ oxidoreductase

Recommended name
dihydrocarveol dehydrogenase

CAS registry number
287179-19-5

2 Source Organism

<1> *Rhodococcus erythropolis* (NAD^+-dependent dihydrocarveol dehydrogenase activity is high in carveol- and dihydrocarveol grown cells [1]) [1]

3 Reaction and Specificity

Catalyzed reaction
menth-8-en-2-ol + NAD^+ = menth-8-en-2-one + NADH + H^+

Substrates and products
S (1R,2R,4R)-dihydrocarveol + NAD^+ <1> (Reversibility: ?) [1]
P (1R,4R)-dihydrocarvone + NADH + H^+
S (1R,2S,4R)-neo-dihydrocarveol + NAD^+ <1> (Reversibility: ?) [1]
P (1R,4R)-dihydrocarvone + NADH + H^+
S (1S,2R,4R)-neoisodihydrocarveol + NAD^+ <1> (Reversibility: ?) [1]
P (1S,4R)-iso-dihydrocarvone + NADH + H^+
S (1S,2S,4R)-iso-dihydrocarveol + NAD^+ <1> (Reversibility: ?) [1]
P (1S,4R)-iso-dihydrocarvone + NADH + H^+

References

[1] van der Werf, M.J.; Boot, A.M.: Metabolism of carveol and dihydrocarveol in Rhodococcus erythropolis DCL14. Microbiology, **146**, 1129-1141 (2000)

D. Schomburg and I. Schomburg (eds.), *Class 1 Oxidoreductases: EC 1*,
Springer Handbook of Enzymes S8, DOI 10.1007/978-3-642-36265-1_2,

limonene-1,2-diol dehydrogenase 1.1.1.297

1 Nomenclature

EC number
1.1.1.297

Systematic name
menth-8-ene-1,2-diol:NAD^+ oxidoreductase

Recommended name
limonene-1,2-diol dehydrogenase

2 Source Organism

<1> *Rhodococcus erythropolis* [1]

3 Reaction and Specificity

Catalyzed reaction
menth-8-ene-1,2-diol + NAD^+ = 1-hydroxymenth-8-en-2-one + NADH + H^+ (general reaction)
1. (1R,2R,4S)-menth-8-ene-1,2-diol + NAD^+ = (1R,4S)-1-hydroxymenth-8-en-2-one + NADH + H^+
2. (1S,2S,4R)-menth-8-ene-1,2-diol + NAD^+ = (1S,4R)-1-hydroxymenth-8-en-2-one + NADH + H^+

Natural substrates and products
S Additional information <1> (<1> enzymatic activity is involved in the limonene degradation pathway [1]) (Reversibility: ?) [1]
P ?

Substrates and products
S (1R,2R,4S)-limonene-1,2-diol + NAD^+ <1> (Reversibility: ?) [1]
P (1R,4S)-1-hydroxy-2-oxolimonene + (1R,2S,4S)-limonene-1,2-diol + NADH + H^+
S (1S,2S,4R)-limonene-1,2-diol + NAD^+ <1> (Reversibility: ?) [1]
P (1S,4R)-1-hydroxy-2-oxolimonene + NADH + H^+
S Additional information <1> (<1> enzymatic activity is involved in the limonene degradation pathway [1]) (Reversibility: ?) [1]
P ?

D. Schomburg and I. Schomburg (eds.), *Class 1 Oxidoreductases: EC 1*,
Springer Handbook of Enzymes S8, DOI 10.1007/978-3-642-36265-1_3,

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[1] van der Werf, M.J.; Swarts, H.J.; de Bont, J.A.: Rhodococcus erythropolis DCL14 contains a novel degradation pathway for limonene. Appl. Environ. Microbiol., **65**, 2092-2102 (1999)

3-hydroxypropionate dehydrogenase ($NADP^+$) 1.1.1.298

1 Nomenclature

EC number
1.1.1.298

Systematic name
3-hydroxypropionate:$NADP^+$ oxidoreductase

Recommended name
3-hydroxypropionate dehydrogenase ($NADP^+$)

Synonyms
3-HIBADH <1> [2]
3-hydroxyisobutyrate dehydrogenase <1> [2]
malonate semialdehyde reductase (NADPH) <3> [4]
malonyl-CoA reductase <2> [3]

CAS registry number
150386-09-7

2 Source Organism

<1> *Bacillus cereus* [2]
<2> *Chloroflexus aurantiacus* [1,3]
<3> *Metallosphaera sedula* [4]

3 Reaction and Specificity

Catalyzed reaction
3-hydroxypropionate + $NADP^+$ = malonate semialdehyde + NADPH + H^+

Natural substrates and products
S 3-hydroxypropanoate + $NADP^+$ <1> (Reversibility: r) [2]
P 3-oxopropanoate + NADPH + H^+
S 3-hydroxypropionate + $NADP^+$ <2> (Reversibility: r) [1]
P malonate-semialdehyde + NADPH + H^+
S malonate-semialdehyde + NADPH + H^+ <2> (Reversibility: r) [1]
P 3-hydroxypropionate + $NADP^+$
S Additional information <1,2> (<2> enzyme is part of an autotrophic CO_2 fixation pathway in which acetyl-CoA is carboxylated and reductively converted via 3-hydroxypropionate to propionyl-CoA. Propionyl-CoA is car-

D. Schomburg and I. Schomburg (eds.), *Class 1 Oxidoreductases: EC 1*,
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boxylated and converted via succinyl-CoA and CoA transfer to malyl-CoA. Malyl-CoA is cleaved to acetyl-CoA and glyoxylate. Thereby, the first CO, acceptor molecule acetyl-CoA is regenerated, completing the cycle and the net CO, fixation product glyoxylate is released [1]; <1> MmsB from Bacillus cereus exhibits 3-hydroxyisobutyrate dehydrogenase, EC 1.1.1.31, as well as 3-hydroxypropionate dehydrogenase activity [2]) [1,2]

P ?

Substrates and products

S 3-hydroxypropanoate + $NADP^+$ <1> (Reversibility: r) [2]
P 3-oxopropanoate + NADPH + H^+
S 3-hydroxypropionate + NAD^+ <1> (Reversibility: ?) [2]
P malonate-semialdehyde + NADH + H^+
S 3-hydroxypropionate + $NADP^+$ <1,2> (<1> 3-hydroxyisobutyrate dehydrogenase, EC 1.1.1.31, additionally exhibits 3-hydroxypropionate dehydrogenase activity [2]) (Reversibility: r) [1,2]
P malonate-semialdehyde + NADPH + H^+
S malonate semialdehyde + NADPH <2,3> (Reversibility: ?) [1,4]
P 3-hydroxypropionate + $NADP^+$
S Additional information <1,2> (<2> enzyme is part of an autotrophic CO_2 fixation pathway in which acetyl-CoA is carboxylated and reductively converted via 3-hydroxypropionate to propionyl-CoA. Propionyl-CoA is carboxylated and converted via succinyl-CoA and CoA transfer to malyl-CoA. Malyl-CoA is cleaved to acetyl-CoA and glyoxylate. Thereby, the first CO, acceptor molecule acetyl-CoA is regenerated, completing the cycle and the net CO, fixation product glyoxylate is released [1]; <2> bifunctional enzyme which catalyzes the two-step reduction from malonyl-CoA to malonate semialdehyde and from malonate semialdehyde to 3-hydroxypropionate [3]; <1> MmsB from Bacillus cereus exhibits 3-hydroxyisobutyrate dehydrogenase, EC 1.1.1.31, as well as 3-hydroxypropionate dehydrogenase activity [2]) [1,2,3]
P ?

Inhibitors

EDTA <2> (<2> 90% inhibition [3]) [3]
Zn <1> (<1> 0.2 mM, 60% inhibition [2]) [2]
Additional information <1> (<1> not inhibitory: EDTA and ethylene glycol, up to 0.2 mM [2]) [2]

Cofactors/prosthetic groups

NAD^+ <1> (<1> $NADP^+$ is preferred over NAD^+ [2]) [2]
$NADP^+$ <1,2> (<1> $NADP^+$ is preferred over NAD^+ [2]) [1,2]
NADPH <1,2,3> [1,2,3,4]
Additional information <2> (<2> no cofactor: NADH [3]; <2> no cofactor: NAD^+ [1]) [1,3]

Metals, ions

Ca^{2+} <2> (<2> stimulation [3]) [3]
Fe^{2+} <2> (<2> stimulation [3]) [3]

Mg^{2+} <2> (<2> stimulation [3]) [3]
Additional information <1> (<1> no metal ion requirement [2]; <1> up to 0.2 mM, no effect on activity: $MnSO_4$, $CuSO_4$, $CaCl_2$, $MgSO_4$, and $FeSO_4$ [2]) [2]

Turnover number (s^{-1})
0.21 <1> (3-hydroxypropanoate, <1> pH 8.5, 37°C [2]) [2]

Specific activity (U/mg)
0.002 <2> (<2> substrate 3-hydroxypropionate, pH 9.0, 45°C [1]) [1]
0.04 <2> (<2> substrate malonate-semialdehyde, pH 9.0, 45°C [1]) [1]
1.5 <3> (<3> 65°C [4]) [4]
8.7 <1> (<1> pH 8.5, 37°C [2]) [2]

K_m-Value (mM)
0.25 <1> ($NADP^+$, <1> pH 8.5, 37°C [2]) [2]
2.4 <1> (NAD^+, <1> pH 8.5, 37°C [2]) [2]
16.8 <1> (3-hydroxypropanoate, <1> pH 8.5, 37°C [2]) [2]
Additional information <1> (<1> steady-state kinetic analysis, overview [2]) [2]

pH-Optimum
7.8 <2> [3]
8.8 <1> [2]
8.8-9 <1> [2]

pH-Range
6.5-8.5 <2> (<2> more than 50% of maximal acitivty within [3]) [3]
7-10 <1> (<1> activity range [2]) [2]
8.2 <1> (<1> about 30% of maximum acticity [2]) [2]
9 <1> (<1> about 65% of maximum activity [2]) [2]

Temperature optimum (°C)
37 <1> [2]

Temperature range (°C)
35-45 <1> (<1> 90% of maximal activity within this range [2]; <1> more than 90% of maximum activity [2]) [2]

4 Enzyme Structure

Molecular weight
300000 <2> (<2> gel filtration [3]) [3]

Subunits
? <1> (<1> x * 32000, SDS-PAGE [2]) [2]
dimer <2> (<2> 2 * 145000, SDS-PAGE [3]) [3]

5 Isolation/Preparation/Mutation/Application

Purification

<1> (native enzyme 100fold by ammonium sulfate fractionation, and hydrophobic interaction and anion exchange chromatography) [2]
<2> [3]

Cloning

<1> (MmsB gene, overexpression in Escherichia coli strain BL21, subcloning in strain DH5α) [2]

6 Stability

Temperature stability

45 <1> (<1> 30 min, stable [2]; <1> 30 min, purified enzyme, completely stable [2]) [2]
55 <1> (<1> 3 min, about 40% residual activity [2]; <1> 3 min, purified enzyme, significant denaturation and inactivation [2]) [2]

General stability information

<2>, enzyme is oxygen insensitive but sensitive to repeated freezing and thawing [3]

Storage stability

<2>, -20°C, presence of 10% (vol/vol) glycerol, stable for weeks [3]

References

[1] Strauss, G.; Fuchs, G.: Enzymes of a novel autotrophic carbon dioxide fixation pathway in the phototrophic bacterium Chloroflexus aurantiacus, the 3-hydroxypropionate cycle. Eur. J. Biochem., **215**, 633-643 (1993)
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[4] Berg, I.A.; Kockelkorn, D.; Buckel, W.; Fuchs, G.: A 3-hydroxypropionate/4-hydroxybutyrate autotrophic carbon dioxide assimilation pathway in Archaea. Science, **318**, 1782-1786 (2007)

malate dehydrogenase [NAD(P)$^+$] 1.1.1.299

1 Nomenclature

EC number
1.1.1.299

Systematic name
(S)-malate:NAD(P)$^+$ oxidoreductase

Recommended name
malate dehydrogenase [NAD(P)$^+$]

Synonyms
MDH II <1> (<1> uses both NADP$^+$ and NAD$^+$ [2]) [2]

CAS registry number
9001-64-3 (cf. EC 1.1.1.37)

2 Source Organism

<1> *Methanothermobacter thermautotrophicus* [2]
<2> *Flavobacterium frigidimaris* [1]

3 Reaction and Specificity

Catalyzed reaction
(S)-malate + NAD(P)$^+$ = oxaloacetate + NAD(P)H + H$^+$ (<2> ordered bi-bi mechanism [1])

Substrates and products
- **S** (S)-malate + NAD$^+$ <1,2> (Reversibility: ?) [1,2]
- **P** oxaloacetate + NADH + H$^+$
- **S** (S)-malate + NADP$^+$ <1,2> (Reversibility: ?) [1,2]
- **P** oxaloacetate + NADPH + H$^+$
- **S** oxaloacetate + NADH <1,2> (Reversibility: ?) [1,2]
- **P** L-malate + NAD$^+$
- **S** oxaloacetate + NADPH <1> (Reversibility: ?) [2]
- **P** L-malate + NADP$^+$
- **S** Additional information <1,2> (<1> no substrate: pyruvate [2]; <2> enzyme shows pro-R stereospecificity for hydrogen transfer at the C_4 position of the nicotinamide moiety of the coenzyme. No substrates are: D-malate, malonate, L-glutamate, L-aspartate, DL-2-hydroxybutanoate, DL-3-

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hydroxybutanoate, citrate, maleate, succinate, L-tartrate, L-threonine, L-serine, L-hydroxymalonate, D-glutamate, 2-oxocaproate, 2-oxoisovalerate, glyoxylate, 2-oxoglutarate, 2-oxobutanoate [1]) [1,2]

P ?

Inhibitors

$CuCl_2$ <2> (<2> 1 mM, 10% residual activity [1]) [1]
$HgCl_2$ <2> (<2> 1 mM, no residual activity [1]) [1]
N-ethylmaleimide <2> (<2> 1 mM, no residual activity [1]) [1]
NADH <1> (<1> inhibitory above 0.2 mM [2]) [2]
NADPH <1> (<1> inhibitory above 0.2 mM [2]) [2]
$ZnCl_2$ <2> (<2> 1 mM, 27% residual activity [1]) [1]
iodoacetate <2> (<2> 1 mM, 16.4% residual activity [1]) [1]

Cofactors/prosthetic groups

NAD^+ <1,2> (<2> relative activity of NAD^+ to $NADP^+$ is 43.9% [1]) [1,2]
NADH <1,2> [1,2]
$NADP^+$ <1,2> (<2> relative activity of NAD^+ to $NADP^+$ is 43.9% [1]) [1,2]
NADPH <1> [2]
Additional information <2> (<2> no cofactor: NADPH [1]) [1]

Turnover number (s^{-1})

41 <2> (NAD^+, <2> 10°C [1]) [1]
41.3 <2> (L-malate, <2> 10°C [1]) [1]
48.4 <2> (L-malate, <2> 20°C [1]) [1]
48.6 <2> (NAD^+, <2> 20°C [1]) [1]
83.2 <2> (L-malate, <2> 30°C [1]) [1]
83.4 <2> (NAD^+, <2> 30°C [1]) [1]
138 <2> (L-malate, <2> 40°C [1]) [1]
139 <2> (NAD^+, <2> 40°C [1]) [1]

Specific activity (U/mg)

1 <1> (<1> pH 7.6, oxidation of malate, cosubstrate NAD^+ [2]; <1> pH 7.6, oxidation of malate, cosubstrate $NADP^+$ [2]) [2]
52 <1> (<1> pH 7.6, reduction of oxaloacetate, cosubstrate $NADP^+$ [2]) [2]
60 <1> (<1> pH 7.6, reduction of oxaloacetate, cosubstrate NAD^+ [2]) [2]
176 <2> (<2> 30°C [1]) [1]

K_m-Value (mM)

0.028 <2> (NAD^+, <2> 10°C [1]) [1]
0.029 <2> (NAD^+, <2> 20°C [1]) [1]
0.03 <2> (NAD^+, <2> 30°C [1]) [1]
0.038 <2> (NAD^+, <2> 40°C [1]) [1]
0.09 <1> (NADH, <1> pH 7.6 [2]) [2]
0.09 <1> (NADPH, <1> pH 7.6 [2]) [2]
0.09 <1> (oxaloacetate, <1> pH 7.6 [2]) [2]
0.269 <2> (L-malate, <2> 20°C [1]) [1]
0.288 <2> (L-malate, <2> 30°C [1]) [1]
0.5 <1> (NAD^+, <1> pH 7.6 [2]) [2]

0.543 <2> (L-malate, <2> 10°C [1]) [1]
0.74 <2> (L-malate, <2> 40°C [1]) [1]
1 <1> (malate, <1> pH 7.6 [2]) [2]

pH-Optimum
8 <2> (<2> reduction of oxaloacetate [1]) [1]
10.5 <2> (<2> oxidation of malate [1]) [1]

pH-Range
4-10 <2> (<2> reduction of oxaloacetate [1]) [1]
6.5-12 <2> (<2> oxidation of malate [1]) [1]

Temperature optimum (°C)
40 <2> (<2> highest initial velocity [1]) [1]

Temperature range (°C)
5-60 <2> [1]

4 Enzyme Structure

Molecular weight
123000 <2> (<2> gel filtration [1]) [1]

Subunits
? <1> (<1> x * 36000, SDS-PAGE, x * 36700, calculated [2]) [2]
tetramer <2> (<2> 4 * 32000, SDS-PAGE [1]) [1]

5 Isolation/Preparation/Mutation/Application

Purification
<1> [2]

6 Stability

pH-Stability
4-10.5 <2> [1]

Temperature stability
35 <2> (<2> half-life 26 min [1]) [1]
40 <2> (<2> half-life 3 min [1]) [1]

References

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[2] Thompson, H.; Tersteegen, A.; Thauer, R.; Hedderich, R.: Two malate dehydrogenases in Methanobacterium thermoautotrophicum. Arch. Microbiol., **170**, 38-42 (1998)

NADP-retinol dehydrogenase 1.1.1.300

1 Nomenclature

EC number
1.1.1.300

Systematic name
retinol:$NADP^+$ oxidoreductase

Recommended name
NADP-retinol dehydrogenase

Synonyms
CG2065 <22> [22]
CG2070 <21> [22]
CG30491 <23> [22]
CG3842 <24> [22]
NADP(H)-dependent retinol dehydrogenase/reductase <16,17,18,19> [17]
$NADP^+$-dependent all-trans-retinol dehydrogenase <2> [23]
NDRD <15> [14]
NRDR <16,17,18,19> [17]
NRDRA1 <19> (<19> alternatively spliced variant with a complete deletion of exons 3-6 [17]) [17]
NRDRB1 <17> (<17> alternatively spliced variant with a complete deletion of exon 3 [17]) [17]
NRDRB2 <18> (<18> alternatively spliced variant with a complete deletion of exons 3 and 6 [17]) [17]
RDH <5> [13]
RDH10 <2> [23]
RDH11 <2,9,11> [4,7,18,23]
RDH12 <1,2,7,12> [4,8,10,12,18,20,23]
RDH13 <20> [16]
RDH14 <8> [4,10]
RDH5 <10> [10]
RHD8 <2> [24]
RalR1 <2> [2,3]
Retinol dehydrogenase <1,5> [12,13]
all-trans retinal reductase <15> [14]
all-trans-retinol dehydrogenase <2> [1]
hRDH5 <11> [10]
hRoDH <2> [5]
mRDH11 <9> [10]
mouse RDH11 <9> [10]

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peroxisomal NADP(H)-dependent retinol dehydrogenase-reductase <15> [14]
photoreceptor retinol dehydrogenase <2> [24]
photoreceptor-associated retinol dehydrogenase <6,14> [26]
photoreceptor-specific retinol dehydrogenase <1> [9]
prRDH <1,6,14> [9,26]
retSDR1 <2,13> [10]
retinal reductase <2,15> [14,23]
retinal reductase 1 <2> [2,3]
retinol dehydrogenase 12 <7> [8]
retinol dehydrogenase 13 <20> [16]
retinol dehydrogenase [$NADP^+$] <15> [14]
short-chain dehydrogenase/reductase RRD <1> [21]
Additional information <16,17,18,19> (<16,17,18,19> enzyme additionally displays high aldehyde reductase activity in retinoic acid metabolism [17]) [17]

CAS registry number

90033-53-8 (cf. EC 1.1.1.105)

2 Source Organism

<1> *Mus musculus* [9,11,12,18,19,21,23]
<2> *Homo sapiens* (aldo-keto reductase family 1 member C2, AKR1C2 [4]) [1,2,3,4,5,6,8,10,15,19,23,24]
<3> *Rattus norvegicus* [23]
<4> *Bos taurus* [11]
<5> *Ambystoma tigrinum* [13]
<6> *Danio rerio* [26]
<7> *Homo sapiens* (UNIPROT accession number: Q96NR8) (aldo-keto reductase family 1 member C2, AKR1C2 [4]) [4,8,10]
<8> *Homo sapiens* (UNIPROT accession number: Q9HBH5) (aldo-keto reductase family 1 member C2, AKR1C2 [4]) [4,10]
<9> *Mus musculus* (UNIPROT accession number: Q9QYF1, isoenzyme Rdh11 [25]) [10,18,25]
<10> *Homo sapiens* (UNIPROT accession number: Q92781) [10]
<11> *Homo sapiens* (UNIPROT accession number: Q8TC12) [3,7,10]
<12> *Mus musculus* (UNIPROT accession number: Q8BYK4, isoenzyme Rdh12 [25]) [18,20,25]
<13> *Homo sapiens* (UNIPROT accession number: O75911) [10]
<14> *Homo sapiens* (UNIPROT accession number: Q9NYR8) [26]
<15> *Oryctolagus cuniculus* (UNIPROT accession number: Q9GKX2) [14]
<16> *Homo sapiens* (UNIPROT accession number: Q9BTZ2) [17]
<17> *Homo sapiens* (UNIPROT accession number: Q9BTZ2-4, isoform of NRDR without residues 85-118 [17]) [17]
<18> *Homo sapiens* (UNIPROT accession number: Q9BTZ2-5, isoform of NRDR without residues 85-118 and 160-204 [17]) [17]

<19> *Homo sapiens* (UNIPROT accession number: Q9BTZ2-6, isoform of NRDR without residues 1-203 [17]) [17]
<20> *Homo sapiens* (UNIPROT accession number: Q8NBN7) [16]
<21> *Drosophila melanogaster* (UNIPROT accession number: Q8MZG9) [22]
<22> *Drosophila melanogaster* (UNIPROT accession number: Q7JYX2) [22]
<23> *Drosophila melanogaster* (UNIPROT accession number: Q7JUS1) [22]
<24> *Drosophila melanogaster* (UNIPROT accession number: Q9W404) [22]

3 Reaction and Specificity

Catalyzed reaction

retinol + $NADP^+$ = retinal + NADPH + H^+

Natural substrates and products

S 11-cis-retinal + NADPH + H^+ <7> (Reversibility: ?) [8]
P 11-cis-retinol + $NADP^+$
S 11-cis-retinol + $NADP^+$ <7> (<7> possibly involved in the production of 11-cis-retinal from 11-cis-retinol during regeneration of the cone visual pigments [4]) (Reversibility: r) [4]
P 11-cis-retinal + NADPH + H^+
S 9-cis-retinol + $NADP^+$ <7> (<7> possibly involved in the first step of 9-cis-retinoic acid production [4]) (Reversibility: r) [4]
P 9-cis-retinal + NADPH + H^+
S all-trans retinal + NADPH + H^+ <7> (Reversibility: r) [8]
P all-tans-retinol + $NADP^+$
S all-trans-retinal + NADPH + H^+ <15> (Reversibility: r) [14]
P all-tans-retinol + $NADP^+$
S all-trans-retinal + NADPH + H^+ <2,12,20> (<2> involved in the regeneration of bleached visual pigments in photoreceptor cells, involved in retinol metabolism outside of photoreceptor cells [1]; <20> greater catalytic efficiency in the reductive than in the oxidative direction. Localization of RDH13 at the entrance to the mitochondrial matrix suggests that it may function to protect mitochondria against oxidative stress associated with the highly reactive retinaldehyde produced from dietary β-carotene [16]; <12> RDH12 is dispensable in support of the visual cycle but appears to be a key component in clearance of free all-trans-retinal, thereby preventing accumulation of N-retinylidene-N-retinylethanolamine (a toxic substance known to contribute to retinal degeneration) and photoreceptor cell death [20]) (Reversibility: ?) [1,15,16,20]
P all-trans-retinol + $NADP^+$ <2> [1]
S all-trans-retinol + $NADP^+$ <2,7,11> (<2> the enzyme plays a role in retinoid metabolism [2]; <11> involved in retinoid homeostasis in the prostate [3]; <7> possibly involved in the first step of all-trans-retinoic acid production [4]) (Reversibility: r) [2,3,4]
P all-trans-retinal + NADPH + H^+

- **S** n-nonanal + NADPH + H^+ <7> (<7> might play a role in detoxification of lipid peroxidation products [8]) (Reversibility: r) [8]
- **P** n-nonanol + $NADP^+$
- **S** retinol + $NADP^+$ <16,17,18,19> (<16,18,19> important for the maintenance of retinoid homeostasis [17]; <17> important for the maintenance of retinoid homeostasis, low activity of the NRDRB1 splice variant possibly contributes to a disturbed retinoid homeostasis leading to abnormal differentiation and high susceptibility to human papilloma virus in the cervical epithelium [17]) (Reversibility: r) [17]
- **P** retinal + NADPH + H^+
- **S** Additional information <2,9,12> (<2> although bi-directional in vitro, in living cells, RDH12 acts exclusively as a retinaldehyde reductase, shifting the retinoid homeostasis toward the increased levels of retinol and decreased levels of bioactive retinoic acid. The retinaldehyde reductase activity of RDH12 protects the cells from retinaldehyde-induced cell death [15]; <9> the low and constant expression of RDH11 suggests a housekeeping function for this enzyme in retina [18]; <12> the onset of RDH12 expression during the maturation of photoreceptor cells suggests a function related to the visual process. The light-induced rapid decrease of RDH12 protein, preceding the decrease of the mRNA, suggested a specific degradation of the protein rather than a regulation of gene expression [18]; <2> RDH10 is essential for retinoic acid biosynthesis during embryogenesis [23]) (Reversibility: ?) [15,18,23]
- **P** ?

Substrates and products

- **S** (E)-4-hydroxy-2-nonenal + NADPH + H^+ <9,12> (<12> Rdh12 is able to efficiently detoxify 4-hydroxynonenal in cells, most probably through its ability to reduce it to a nontoxic alcohol [25]) (Reversibility: ?) [25]
- **P** (E)-4-hydroxy-2-nonenol + $NADP^+$
- **S** 11-cis-retinal + NADH + H^+ <7> (<7> NADH much less efficient than NADPH [8]) (Reversibility: r) [8]
- **P** 11-cis-retinol + NAD^+
- **S** 11-cis-retinal + NADPH + H^+ <2,7,8,9,11> (<9> The reverse reaction, oxidation of all-trans-retinol, is not catalyzed by mRDH11 [10]) (Reversibility: ?) [4,8,10]
- **P** 11-cis-retinol + $NADP^+$
- **S** 11-cis-retinol + $NADP^+$ <10> (Reversibility: ?) [10]
- **P** 11-cis-retinal + NADPH
- **S** 11-cis-retinol + $NADP^+$ + H^+ <7> (<7> possibly involved in the production of 11-cis-retinal from 11-cis-retinol during regeneration of the cone visual pigments [4]) (Reversibility: r) [4]
- **P** 11-cis-retinal + NADPH + H^+
- **S** 13-cis-retinal + NADPH <7> (Reversibility: r) [4]
- **P** 13-cis-retinol + $NADP^+$ + H^+
- **S** 13-cis-retinal + NADPH + H^+ <1> (<1> 4fold lower activity than with all-trans-retinal [21]) (Reversibility: ?) [21]

P 13-cis-retinol + $NADP^+$
S 13-cis-retinol + $NADP^+$ <2> (Reversibility: r) [3]
P 13-cis-retinal + NADPH + H^+
S 9-cis-retinal + NADPH <7> (Reversibility: r) [4]
P 9-cis-retinol + $NADP^+$
S 9-cis-retinal + NADPH + H^+ <1> (<1> 60fold lower activity than with all-trans-retinal [21]) (Reversibility: ?) [21]
P 9-cis-retinol + $NADP^+$
S 9-cis-retinol + $NADP^+$ <2,7> (<7> possibly involved in the first step of 9-cis-retinoic acid production [4]) (Reversibility: r) [3,4]
P 9-cis-retinal + NADPH + H^+
S all-trans retinal + NADH + H^+ <21,22,23,24> (<21,22,23,24> prefers $NADP^+$ and NADPH as cofactors [22]) (Reversibility: r) [22]
P all-trans-retinol + NAD^+
S all-trans retinal + NADH + H^+ <7> (<7> NADH much less efficient than NADPH [8]) (Reversibility: r) [8]
P all-tans-retinol + NAD^+
S all-trans retinal + NADPH + H^+ <7> (Reversibility: r) [8]
P all-tans-retinol + $NADP^+$
S all-trans retinal + NADPH + H^+ <21,22,23,24> (<21,22,23,24> prefers $NADP^+$ and NADPH as cofactors [22]) (Reversibility: r) [22]
P all-trans-retinol + $NADP^+$
S all-trans-3-hydroxyretinal + NADH + H^+ <21,22,23,24> (<21,22,23,24> catalytic efficiency towards all-trans-3-hydroxyretinal is lower than that towards all-trans retinal, prefers $NADP^+$ and NADPH as cofactors [22]) (Reversibility: ?) [22]
P all-trans-3-hydroxyretinol + NAD^+
S all-trans-3-hydroxyretinal + NADPH + H^+ <21,22,23,24> (<21,22,23,24> catalytic efficiency towards all-trans-3-hydroxyretinal is lower than that towards all-trans retinal, prefers $NADP^+$ and NADPH as cofactors [22]) (Reversibility: ?) [22]
P all-trans-3-hydroxyretinol + $NADP^+$
S all-trans-retinal + NAD(P)H + H^+ <2> (Reversibility: ?) [1]
P all-trans-retinol + $NAD(P)^+$ <2> [1]
S all-trans-retinal + NADH + H^+ <6,14> (Reversibility: ?) [26]
P all-trans-retinol + NAD^+
S all-trans-retinal + NADPH + H^+ <1,2,7,8,11,12,13,20> (<2> involved in the regeneration of bleached visual pigments in photoreceptor cells, involved in retinol metabolism outside of photoreceptor cells [1]; <20> greater catalytic efficiency in the reductive than in the oxidative direction. Localization of RDH13 at the entrance to the mitochondrial matrix suggests that it may function to protect mitochondria against oxidative stress associated with the highly reactive retinaldehyde produced from dietary β-carotene [16]; <12> RDH12 is dispensable in support of the visual cycle but appears to be a key component in clearance of free all-trans-retinal, thereby preventing accumulation of N-retinylidene-N-retinylethanolamine (a toxic substance known to contribute to retinal degeneration) and

photoreceptor cell death [20]; <20> prefers NADPH to NADH as a cofactor. Activity in presence of 1 mM NADPH is about 20fold greater than that in the presence of 1 mM NADH [16]) (Reversibility: ?) [1,4,8,10,15,16, 20,21]

P all-trans-retinol + $NADP^+$ <2> [1]
S all-trans-retinal + NADPH + H^+ <2,9,15> (<9> The reverse reaction, oxidation of all-trans-retinol, is not catalyzed by mRDH11 [10]) (Reversibility: r) [10,14]
P all-tans-retinol + $NADP^+$
S all-trans-retinol + NAD^+ <11> (<11> low activity with NAD^+ as cofactor [3]) (Reversibility: r) [3]
P all-trans-retinal + NADH + H^+
S all-trans-retinol + $NADP^+$ <2,7,11> (<2> the enzyme plays a role in retinoid metabolism [2]; <11> involved in retinoid homeostasis in the prostate [3]; <7> possibly involved in the first step of all-trans-retinoic acid production [4]; <11> more efficient in the reductive direction [7]) (Reversibility: r) [2,3,4,7]
P all-trans-retinal + NADPH + H^+
S cis-6-nonenal + NADPH + H^+ <2> (<2> good substrate of RDH11 and RDH12, while RHD10 has very low activity towards this substrate [23]) (Reversibility: ?) [23]
P ?
S estrone + NADH + H^+ <6,14> (<6> no substrate for wild-type isoforms prRDH1 and prRDH2, but substrate for mutants M146G of prRDH1 and M147G of $prRDH_2$ [26]; <14> no substrate for wild-type, but substrate for mutant M144G [26]) (Reversibility: ?) [26]
P estradiol + NAD^+
S n-nonanal + NADPH + H^+ <7> (<7> might play a role in detoxification of lipid peroxidation products [8]) (Reversibility: r) [8]
P n-nonanol + $NADP^+$
S n-nonanol + NADPH + H^+ <2> (<2> substrate of RDH11 and RDH12 [23]) (Reversibility: ?) [23]
P n-nonanol + $NADP^+$
S retinal + NADH <2> (Reversibility: ?) [15]
P retinol + NAD^+
S retinal + NADPH + H^+ <2> (Reversibility: ?) [23]
P retinol + $NADP^+$
S retinol + NAD^+ + H^+ <2> (Reversibility: ?) [5]
P retinal + NADH
S retinol + $NADP^+$ + H^+ <2> (Reversibility: ?) [5]
P retinal + NADPH
S retinol + $NADP^+$ <16,17,18,19> (<16,18,19> important for the maintenance of retinoid homeostasis [17]; <17> important for the maintenance of retinoid homeostasis, low activity of the NRDRB1 splice variant possibly contributes to a disturbed retinoid homeostasis leading to abnormal differentiation and high susceptibility to human papilloma virus in the cervical epithelium [17]) (Reversibility: r) [17]

P retinal + NADPH + H^+
S retinol bound to cellular retinol binding protein + $NADP^+$ <2> (Reversibility: ?) [5]
P retinal bound to cellular retinol binding protein + NADPH
S trans-2-nonenal + NADPH + H^+ <2> (<2> good substrate of RDH11 and RDH12, while RHD10 has very low activity towards this substrate [23]) (Reversibility: ?) [23]
P ?
S Additional information <1,2,8,9,11,12,20> (<8> clear specificity for pro-S hydrogen of NADPH and for pro-R-hydrogen on C15 of the retinols, no steroid dehydrogenase activity [4]; <2> prefers $NADP^+$ over NAD^+ [5]; <2> although bi-directional in vitro, in living cells, RDH12 acts exclusively as a retinaldehyde reductase, shifting the retinoid homeostasis toward the increased levels of retinol and decreased levels of bioactive retinoic acid. The retinaldehyde reductase activity of RDH12 protects the cells from retinaldehyde-induced cell death [15]; <1> dihydrotestosterone is not a substrate for mouse isoform RDH12 [19]; <2> isoform RDH12 additionally cataylzes the reduction of dihydrotestosterone to androstanediol [19]; <9> the low and constant expression of RDH11 suggests a housekeeping function for this enzyme in retina [18]; <12> the onset of RDH12 expression during the maturation of photoreceptor cells suggests a function related to the visual process. The light-induced rapid decrease of RDH12 protein, preceding the decrease of the mRNA, suggested a specific degradation of the protein rather than a regulation of gene expression [18]; <20> no significant conversion of 17β-, 3α- and 11β-hydroxysteroids [16]; <1> recombinant RRD functions with both unbound and CRBP(I) (cellular retinol-binding protein)-bound retinal [21]; <11> the enzymes utilizes retinol bound to cellular retinol binding protein type I at a much lower rate than free retinol [7]; <2> RDH10 is essential for retinoic acid biosynthesis during embryogenesis [23]) (Reversibility: ?) [4,5,7,15,16,18,19,21,23]
P ?

Inhibitors

(3β)-3-[(3-carboxypropanoyl)oxy]-11-oxoolean-12-en-30-oic acid <2> (<2> 0.5 mM, 60% of inhibition [5]) [5]
1,2-diheptanoyl-sn-glycero-3-phosphocholine <20> (<20> substitution of the detergent 1,2-diheptanoyl-sn-glycero-3-phosphocholine for Tween-20 results in complete inactivation of the enzyme [16]) [16]
9-cis-retinoic acid <7> [4]
nonanal <2> (<2> inhibits the activity towards retinaldehyde [15]) [15]
phenylarsine oxide <2> (<2> 1 mM, 85% of inhibition [5]) [5]
Additional information <2> (<2> not inhibitory: methylpyrazole, phenylmethylsulfonylfluoride [5]) [5]

Cofactors/prosthetic groups

NAD^+ <2,7,21,22,23,24> (<7> NAD^+ much less efficient than $NADP^+$ [8]; <21,22,23,24> prefers $NADP^+$ and NADPH as cofactors [22]) [5,8,22]

NADH <7,20,21,22,23,24> (<7> NADH much less efficient than NADPH [8]; <21,22,23,24> prefers $NADP^+$ and NADPH as cofactors [22]; <20> prefers NADPH to NADH as a cofactor. Activity in presence of 1 mM NADPH is about 20fold greater than that in the presence of 1 mM NADH [16]) [8,16,22]
$NADP^+$ <1,2,3,4,7,8,9,10,11,16,17,18,19,21,22,23,24> (<11> preferred cofactor [7]; <21,22,23,24> prefers $NADP^+$ and NADPH as cofactors [22]; <2> prefers $NADP^+$ as cofactor [23]) [2,3,4,5,7,8,10,11,15,17,22,23]
NADPH <1,2,3,4,7,8,11,13,15,16,17,18,19,20,21,22,23,24> (<11> preferred cofactor [7]; <1> NADPH is at least 30fold more effective than NADH [21]; <21,22,23,24> prefers $NADP^+$ and NADPH as cofactors [22]; <20> prefers NADPH to NADH as a cofactor. Activity in presence of 1 mM NADPH is about 20fold greater than that in the presence of 1 mM NADH [16]) [1,2,3,4,7,8,10,11,14,15,16,17,21,22,23,24]

Turnover number (s^{-1})

0.00012 <11> (all-trans-retinal, <11> 37°C [7]) [7]
0.0006 <11> (all-trans-retinol, <11> 37°C [7]) [7]
0.18 <2> (all-trans-retinal, <2> wild-type [15]) [15]
0.2 <2> (all-trans-retinol, <2> purified recombinant enzyme [2]) [2]
0.21 <2> (all-trans-retinal, <2> mutant I51N [15]) [15]
0.3 <2> (all-trans-retinal, <2> purified recombinant enzyme [2]) [2]
0.4 <2> (all-trans-retinal, <2> mutant T49M [15]) [15]

Specific activity (U/mg)

0.63 <2> (<2> eluate after purification, the specific activity of the purified enzyme with all-trans-retinal as substrate is 13fold higher than that of the microsomal preparation of wild-type [2]) [2]

K_m-Value (mM)

6e-005 <2> (all-trans-retinal, <2> wild-type [15]) [15]
0.0001 <2> (11-cis-retinal, <2> pH 7.4, 37°C [8]) [8]
0.00012 <2> (all-trans-retinal, <2> purified recombinant enzyme [2]) [2]
0.00014 <2> (9-cis-retinal, <2> pH 7.4, 37°C [8]) [8]
0.0002 <2> (all-trans-retinal, <2> membrane-bound wild-type [2]) [2]
0.00027 <24> (all-trans-retinal, <24> K_m-value is determined for microsomal preparations expressing recombinant Drosophila proteins [22]) [22]
0.00028 <2> (all-trans-retinal, <2> mutant T49M [15]) [15]
0.0004 <2> ($NADP^+$, <2> membrane-bound wild-type [2]) [2]
0.0004 <2,21> (all-trans-retinal, <21> K_m-value is determined for microsomal preparations expressing recombinant Drosophila proteins [22]) [8,22]
0.00041 <2> (all-trans-retinal, <2> mutant I51N [15]) [15]
0.00047 <2> (NADPH, <2> purified recombinant enzyme [2]) [2]
0.00048 <2> (NADPH, <2> membrane-bound wild-type [2]) [2]
0.0006 <23> (all-trans-retinal, <23> K_m-value is determined for microsomal preparations expressing recombinant Drosophila proteins [22]) [22]
0.0006 <2> (all-trans-retinol, <2> purified recombinant enzyme [2]) [2]
0.0007 <2> (all-trans-retinol, <2> membrane-bound wild-type [2]) [2]
0.0007 <2> (NADPH, <2> wild-type [15]) [15]

0.0007 <22> (all-trans-retinal, <22> K_m-value is determined for microsomal preparations expressing recombinant Drosophila proteins [22]) [22]
0.00074 <2> (NADPH, <2> pH 7.4, 37°C [8]) [8]
0.001 <2> ($NADP^+$, <2> purified recombinant enzyme [2]) [2]
0.0012 <2> ($NADP^+$, <2> pH 7.4, 37°C [8]) [8]
0.0012 <2> (NADPH, <2> pH 7.4, 37°C [8]) [8]
0.00126 <14> (all-trans-retinal, <14> wild-type, pH 7.3, 37°C [26]) [26]
0.0015 <20> (NADPH) [16]
0.0016 <2> (11-cis-retinol, <2> pH 7.4, 37°C [8]) [8]
0.0016 <2> (9-cis-retinol, <2> pH 7.4, 37°C [8]) [8]
0.0017 <6> (all-trans-retinal, <6> mutant M146G, pH 7.3, 37°C [26]) [26]
0.0023 <14> (all-trans-retinal, <14> mutant M144G, pH 7.3, 37°C [26]) [26]
0.003 <6> (all-trans-retinal, <6> mutant M147G, pH 7.3, 37°C [26]) [26]
0.0031 <6> (all-trans-retinal, <6> wild-type isoform prRDH1, pH 7.3, 37°C [26]) [26]
0.0032 <2> ($NADP^+$, <2> wild-type [15]; <2> pH 7.4, 37°C [8]) [8,15]
0.0032 <23> (all-trans-3-hydroxyretinal, <23> K_m-value is determined for microsomal preparations expressing recombinant Drosophila proteins [22]) [22]
0.0032 <20> (all-trans-retinal) [16]
0.004 <2,20> (all-trans-retinol, <2> pH 7.4, 37°C [8]; <20> K_m-value for all-trans-retinol is similar to that for retinal however, the rate of retinol oxidation by RDH13 is extremely low [16]) [8,16]
0.0041 <24> (all-trans-3-hydroxyretinal, <24> K_m-value is determined for microsomal preparations expressing recombinant Drosophila proteins [22]) [22]
0.0044 <22> (all-trans-3-hydroxyretinal, <22> K_m-value is determined for microsomal preparations expressing recombinant Drosophila proteins [22]) [22]
0.0044 <6> (all-trans-retinal, <6> wild-type isoform prRDH1, pH 7.3, 37°C [26]) [26]
0.0096 <14> (estrone, <14> mutant M144G, pH 7.3, 37°C [26]) [26]
0.0233 <6> (estrone, <6> mutant M147G, pH 7.3, 37°C [26]) [26]
0.0307 <6> (estrone, <6> mutant M146G, pH 7.3, 37°C [26]) [26]
0.033 <2> (NADPH, <2> mutant T49M [15]) [15]
0.1 <2> ($NADP^+$, <2> mutant T49M [15]) [15]
0.105 <2> (NADPH, <2> mutant I51N [15]) [15]
0.18 <11> (all-trans-retinol, <11> 37°C [7]) [7]
0.19 <2> (9-cis-retinal, <2> pH 7.4, 37°C, determined with $NADP^+$ [3]) [3]
0.23 <2> (NADPH, <2> pH 7.4, 37°C, determined with all-trans-retinal [3]) [3]
0.3 <11> (all-trans-retinal, <11> 37°C [7]) [7]
0.35 <2> ($NADP^+$, <2> mutant I51N [15]) [15]
0.5 <2> (all-trans-retinal, <2> pH 7.4, 37°C, determined with $NADP^+$ [3]) [3]
0.62 <2> (13-cis-retinal, <2> pH 7.4, 37°C, determined with $NADP^+$ [3]) [3]
0.8 <2> ($NADP^+$, <2> pH 7.4, 37°C, determined with all-trans-retinol [3]) [3]
1.3 <2> (all-trans-retinol, <2> pH 7.4, 37°C, determined with NADPH [3]) [3]
2.22 <2> (NADH, <2> pH 7.4, 37°C [8]) [8]
2.42 <2> (NADH, <2> pH 7.4, 37°C [8]) [8]
6 <20> (NADH) [16]
7.75 <2> (NAD^+, <2> pH 7.4, 37°C [8]) [8]

680 <2> (NAD^+, <2> pH 7.4, 37°C, determined with all-trans-retinol [3]) [3]
1300 <2> (NADH, <2> pH 7.4, 37°C, determined with all-trans-retinal [3]) [3]
Additional information <1,21> (<21> CG2070-expessing microsomes do not show saturation with up to 0.008 mM all-trans-3-hydroxyretinaldehyde [22]; <1> recombinant RRD reduces free retinal (not bound with cellular retinol binding protein) with a K0.5 value of 0.0023 mM and a Hill constant of 1.7, and reduces CRBP(I)-bound retinal (2fold molar excess of cellular retinol binding protein(I) at each retinal concentration) with a $K_{0.5}$ value of 0.0086 mM and a Hill constant of 2.1 [21]) [21,22]

K_i-Value (mM)

0.001 <2> (9-cis-retinoic acid, <2> pH 7.4 [4]) [4]

pi-Value

7.67 <16> (<16> calculated from the deduced amino acid sequence [17]) [17]
9.32 <17> (<17> calculated from the deduced amino acid sequence [17]) [17]

4 Enzyme Structure

Molecular weight

35400 <2> [2]
60000 <15> (<15> gel filtration [14]) [14]
365000 <2> (<2> molecular mass of RDH12-His6 [8]) [8]

Subunits

? <16,17> (<17> x * 23917, calculated from the deduced amino acid sequence [17]; <16> x * 27572, calculated from the deduced amino acid sequence [17]) [17]
dimer <15> (<15> 2 * 27000, SDS-PAGE, native mass by gel filtration [14]; <15> 2 * 27368, MALDI-TOF MS, native mass by gel filtration [14]; <15> 2 * 27430, calculated from the deduced amino acid sequence, native mass by gel filtration [14]) [14]

Posttranslational modification

no glycoprotein <2> (<2> the wild-type enzyme is not glycosylated [2]) [2]

5 Isolation/Preparation/Mutation/Application

Source/tissue

HeLa cell <17> [17]
SF-9 cell <2> [2]
brain <1,2,7> (<1> low mRNA expression [21]) [2,4,21]
carcinoma cell <2> (<2> squamos cell carcinoma, SCC tumor [5]) [5]
cervix <16,17,18,19> (<17> detected in 54% of cervical tumor tissue samples but not in normal cervical tissue [17]; <18,19> detected in cervical tumor tissue samples where NRDRB1 could not be detected [17]; <16> normal and neoplastic cervical tissue samples [17]) [17]

embryo <1> (<1> RRD mRNA expression is initiated on embryo day 7 [21]) [21]
epithelial cell <2> [3]
eye <2,7,8,9,10,11,13> (<7> photoreceptor nuclear layer [4]) [4,10]
heart <1,20> (<20> high expression level [16]; <1> high mRNA expression [21]) [16,21]
jejunum <2> [2]
kidney <1,2,7,20> (<20> high expression level [16]; <1> high mRNA expression [21]) [2,4,16,21]
liver <1,2,15> (<1> high mRNA expression [21]) [2,14,21]
lung <1,2,20> (<20> high expression level [16]; <1> low mRNA expression [21]) [2,16,21]
ovary <20> (<20> moderate expression level [16]) [16]
pancreas <8> [4]
photoreceptor <2,7,9> (<9> inner segment [10]; <2> cone photoreceptor cell [1]) [1,4,10]
photoreceptor cell <1> [9]
photoreceptor inner segment <1> [12]
placenta <8> [4]
prostate <20> (<20> moderate expression level [16]) [16]
prostate gland <2> [2,3]
retina <1,9,12> (<1> level of isoform Rdh11 is low and remarkably constant during development and oxidative stress. Rdh12 expression starts at postnatal day 7 and increases until postnatal day 30 to approximately sevenfold higher than Rdh11. Oxidative stress induced by exposure to constant bright light leads to a rapid and significant decrease of Rdh12 protein [18]; <12> RDH12 expression starts at postnatal day and increases until postnatal day 30. Oxidative stress induced by exposure to constant bright light leads to a rapid and significant decrease of RDH12 protein [18]; <9> the RDH11 level is low and remarkably constant during development and oxidative stress [18]; <12> expression in inner segments, cell bodies and synaptic termini of photoreceptors [25]) [18,20,25]
retinal pigment epithelium <7,9,10,11> (<9> in vivo [10]) [10]
skeletal muscle <7> [4]
skin <2> (<2> and foreskin [5]) [5]
spleen <1> (<1> low mRNA expression [21]) [21]
stomach <7> [4]
testis <2,20> (<20> moderate expression level [16]) [2,16]
Additional information <2> (<2> not: skeletal muscle and heart [2]) [2]

Localization

cytosol <15> [14]
endoplasmic reticulum <1,2,11> (<2> isoform RDH12 [19]) [3,19]
membrane <2,11,21,22,23,24> (<11> microsomal membrane [7]; <2> model of enzyme insertion into the membrane [2]; <21,22,23,24> associated with microsomal membranes [22]) [2,7,22]
microsome <2,9,12> [5,8,25]

mitochondrial membrane <20> (<20> RDH13 is localized on the outer side of the inner mitochondrial membrane [16]) [16]
mitochondrion <2> (<2> isoform RDH13 [19]) [19]
peroxisome <1,16> [17,21]
photoreceptor outer segment <1,2,8,13> (<1,13> rod [9,10]) [9,10]
Additional information <17> (<17> not detected in the peroxisome [17]) [17]

Purification

<2> (recombinant enzyme) [2]
<7> [8]
<11> (purified to homogeneity) [7]
<15> [14]
<17> (recombinant protein from Escherichia coli) [17]
<20> [16]

Cloning

<1> (expression in CHO-K1 cells) [21]
<1> (expression in HeLa cell and 293T cell) [19]
<2> (expression in HEK-293 cell) [15]
<2> (expression in HeLa cell and 293T cell) [19]
<2> (expression in Sf9 cells) [3]
<2> (full-length short-chain dehydrogenase/reductase cDNA expressed in Escherichia coli, truncated cDNA expressed in SF9 insect cells, enzyme belongs to the short-chain dehydrogenase/reductase superfamily) [1]
<2> (production of recombinant His6-tagged enzyme in insect cells) [2]
<6> (expression in HEK-293 cell) [26]
<9> (cloned as a gene regulated by the transcription factor sterol regulatory element binding proteins and previously named SCALD for short chain aldehyde reductase) [10]
<9> (stable transfection of HEK-293 cells) [25]
<11> [7]
<12> (stable transfection of HEK-293 cells) [25]
<14> (expression in HEK-293 cell) [26]
<16> (GFP-fusion protein in HeLa-cells) [17]
<17> (expressed in native, soluble form in Escherichia coli BL21-AI and as GFP-fusion protein in HeLa-cells) [17]
<20> (expression in Sf9 cells) [16]
<21> (expression in Sf9 cells) [22]
<22> (expression in Sf9 cells) [22]
<23> (expression in Sf9 cells) [22]
<24> (expression in Sf9 cells) [22]

Engineering

I51N <2> (<2> site-directed mutagenesis, significant activity in vitro. Dramatically reduced affinity for NADPH results in loss of function within cells [15]) [15]

L99I <2> (<2> site-directed mutagenesis, about 30% of wild-type activity [15]) [15]
M144G <14> (<14> gain-of-function mutant, enables estrone to bind and be reduced as an additional substrate [26]) [26]
M146G <6> (<6> mutation in isoform prRDH1, gain-of-function mutant, enables estrone to bind and be reduced as an additional substrate [26]) [26]
M147G <6> (<6> mutation in isoform prRDH2, gain-of-function mutant, enables estrone to bind and be reduced as an additional substrate [26]) [26]
Q189X <2> (<2> mutation found in an individual affected by autosomal recessive childhood-onset severe retinal dystrophy [6]) [6]
R25G/K26I <2> (<2> The mutation allows the enzyme to flip its orientation in the membrane. The mutant is glycosylated in intact cells. [2]) [2]
R62X <2> (<2> mutation found in an individual affected by autosomal recessive childhood-onset severe retinal dystrophy [6]) [6]
S175P <2> (<2> site-directed mutagenesis, no catalytic activity. Protein is stable and abundantly expressed [15]) [15]
T49M <2,12> (<2> mutation found in an individual affected by autosomal recessive childhood-onset severe retinal dystrophy [6]; <2> site-directed mutagenesis, significant activity in vitro. Dramatically reduced affinity for NADPH results in loss of function within cells [15]; <12> inactive. Mutation is associated with Lebr congenital amaurosis. Mutant is not able to detoxify 4-hydroxynonenal in cells [25]) [6,15,25]
Y226C <2> (<2> mutation present in all individuals affected by autosomal recessive childhood-onset severe retinal dystrophy from three Austrian kindreds, enzyme expressed in COS-7 cells shows diminished activity [6]) [6]
Additional information <2> (<2> transfection with retinol dehydrogenase 12 protects cells against nonanal-induced toxicity but is ineffective against 4-hydroxynonenal [15]) [15]

Application

medicine <2> (<2> enzyme is associated with retinal dystrophy and encodes an enzyme with unique, nonredundant role in the photoreceptor cells [6]; <2> transfection with retinol dehydrogenase 12 protects cells against nonanal-induced toxicity but is ineffective against 4-hydroxynonenal. 4-Hydroxynonenal strongly inhibits the activities of lecithin:retinol acyl transferase and aldehyde dehydrogenase, resulting in decreased levels of retinyl esters and retinoic acid and accumulation of unesterified retinol [15]) [6,15]

6 Stability

Temperature stability

37 <20> (<20> partially inactivated after 20 min [16]) [16]

Oxidation stability

<20>, requires reducing conditions to stay active [16]

Storage stability

<2>, -80°C, buffer containing 1 mM 1,2-diheptanoyl-sn-glycero-3-phosphocholine, stable for several months [2]

<2>, 8°C, in a refrigerator, the enzyme is nearly fully active for at least one month [2]

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D-arabitol-phosphate dehydrogenase 1.1.1.301

1 Nomenclature

EC number
1.1.1.301

Systematic name
D-arabitol-phosphate:NAD^+ oxidoreductase

Recommended name
D-arabitol-phosphate dehydrogenase

Synonyms
APDH <1,2> [1,2]
D-arabitol 1-phosphate dehydrogenase <1> [1]
D-arabitol 5-phosphate dehydrogenase <1> [1]
D-arabitol-phosphate dehydrogenase <1> [1]

2 Source Organism

<1> *Enterococcus avium* (UNIPROT accession number: Q8KQL2) [1]
<2> *Enterococcus avium* [2]

3 Reaction and Specificity

Catalyzed reaction
D-arabitol 1-phosphate + NAD^+ = D-xylulose 5-phosphate + NADH + H^+

Natural substrates and products
S Additional information <1> (<1> participates in arabitol catabolism via the arabitol phosphate route [1]) (Reversibility: ?) [1]
P ?

Substrates and products
S D-arabitol 1-phosphate + NAD^+ <1> (Reversibility: r) [1]
P D-xylulose 5-phosphate + NADH + H^+
S D-arabitol 1-phosphate + $NADP^+$ <1> (Reversibility: r) [1]
P D-xylulose 5-phosphate + NADPH + H^+
S D-arabitol 5-phosphate + NAD^+ <1> (Reversibility: ?) [1]
P D-ribulose 5-phosphate + NADH + H^+
S D-arabitol 5-phosphate + $NADP^+$ <1> (Reversibility: ?) [1]
P D-ribulose 5-phosphate + NADPH + H^+

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S D-xylulose 5-phosphate + NADH <2> (Reversibility: ?) [2]
P D-arabitol 1-phosphate + NAD^+
S Additional information <1> (<1> participates in arabitol catabolism via the arabitol phosphate route [1]; <1> no activity with: xylitol 5-phosphate, D-sorbitol, D-mannitol, xylitol, erythrose 4-phosphate and ribose 5-phosphate. APDH kinetics are consistent with a ternary-complex mechanism [1]) (Reversibility: ?) [1]
P ?

Inhibitors

EDTA <1> (<1> xylulose 5-phosphate does not protect the enzyme from EDTA inactivation. Addition of Mn^{2+} at concentrations of up to 2 mM results in complete reactivation of APDH [1]) [1]
Hg^{2+} <1> (<1> 2 mM, complete inactivation [1]) [1]
PHMB <1> (<1> 2 mM, complete inactivation [1]) [1]
Zn^{2+} <1> (<1> 2 mM, complete inactivation [1]) [1]

Cofactors/prosthetic groups

NAD^+ <1> (<1> the rates of both reductive and oxidative reactions with NAD^+ and NADH as cofactors are about 14 times higher than with $NADP^+$ and NADPH [1]) [1]
NADH <1,2> (<1> the rates of both reductive and oxidative reactions with NAD^+ and NADH as cofactors are about 14 times higher than with $NADP^+$ and NADPH [1]) [1,2]
$NADP^+$ <1> (<1> the rates of both reductive and oxidative reactions with NAD^+ and NADH as cofactors are about 14 times higher than with $NADP^+$ and NADPH [1]) [1]
NADPH <1> (<1> the rates of both reductive and oxidative reactions with NAD^+ and NADH as cofactors are about 14 times higher than with $NADP^+$ and NADPH [1]) [1]

Metals, ions

Mn^{2+} <1> (<1> required. Pure APDH contains 4.05 ions of Mn^{2+} [1]) [1]
Additional information <1> (<1> no requirement for Zn^{2+} [1]) [1]

K_m-Value (mM)

0.021 <1> (NADH, <1> 20°C, pH 7.2, cosubstrate: D-xylulose 5-phosphate [1]) [1]
0.23 <1> (D-xylulose 5-phosphate, <1> 20°C, pH 7.2, cofactor: NADH [1]) [1]
0.24 <1> (NADPH, <1> 20°C, pH 7.2, cosubstrate: D-xylulose 5-phosphate [1]) [1]
0.63 <1> (D-arabitol 5-phosphate, <1> 20°C, pH 8.5, cofactor: NAD^+ [1]) [1]
0.65 <1> (D-xylulose 5-phosphate, <1> 20°C, pH 7.2, cofactor NADPH [1]) [1]
0.71 <1> (NAD^+, <1> 20°C, pH 8.5: cosubstrate: D-arabitol 5-phosphate [1]) [1]
0.8 <1> (NAD^+, <1> 20°C, pH 8.5, cosubstrate: D-arabitol 1-phosphate [1]) [1]
2.7 <1> ($NADP^+$, <1> 20°C, pH 8.5, cosubstrate: D-arabitol 1-phosphate [1]) [1]
2.9 <1> (D-arabitol 1-phosphate, <1> 20°C, pH 8.5, cofactor: NAD^+ [1]) [1]
3.6 <1> (D-arabitol 1-phosphate, <1> 20°C, pH 8.5, cofactor: $NADP^+$ [1]) [1]

pH-Optimum

6.8-7.4 <1> (<1> reduction of D-xylulose 5-phosphate with NADH or NADPH [1]) [1]

8.3-8.6 <1> (<1> oxidation of D-arabitol 1-phosphate with NAD^+ or $NADP^+$ [1]) [1]

pi-Value

6.4 <1> (<1> isoelectric focusing [1]) [1]

4 Enzyme Structure

Molecular weight

160000 <1> (<1> non-denaturing PAGE [1]) [1]

Subunits

tetramer <1> (<1> 4 * 41000, SDS-PAGE [1]) [1]

5 Isolation/Preparation/Mutation/Application

Purification

<1> [1]

Cloning

<1> [1]

<2> (expression in Bacillus subtilis. Expression of the D-arabitol phosphate dehydrogenase gene of Enterococcus avium in the D-ribulose- and D-xylulose-producing strain results in a strain of Bacillus subtilis capable of converting D-glucose to D-arabitol with a high yield (28%) and little by-product formation) [2]

Application

biotechnology <2> (<2> expression of the D-arabitol phosphate dehydrogenase gene of Enterococcus avium in the D-ribulose- and D-xylulose-producing strain results in a strain of Bacillus subtilis capable of converting D-glucose to D-arabitol with a high yield (28%) and little by-product formation [2]) [2]

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[2] Povelainen, M.; Miasnikov, A.N.: Production of D-arabitol by a metabolic engineered strain of Bacillus subtilis. Biotechnol. J., **1**, 214-219 (2006)

2,5-diamino-6-(ribosylamino)-4(3H)-pyrimidinone 5′-phosphate reductase 1.1.1.302

1 Nomenclature

EC number
1.1.1.302

Systematic name
2,5-diamino-6-(5-phospho-D-ribosylamino)pyrimidin-4(3H)-one:NAD(P)$^+$ oxidoreductase

Recommended name
2,5-diamino-6-(ribosylamino)-4(3H)-pyrimidinone 5'-phosphate reductase

Synonyms
2,5-diamino-6-ribosylamino-4(3H)-pyrimidinone 5'-phosphate reductase <1> [3]
MJ0671 <1> (<1> gene name [2,3]) [2,3]
MJ0671 pyrimidine reductase <1> [2]
MjaRED <1> [3]

2 Source Organism

<1> *Methanocaldococcus jannaschii* [2,3]
<2> *Methanosarcina thermophila* [2]
<3> *Eremothecium gossypii* [1]

3 Reaction and Specificity

Catalyzed reaction
2,5-diamino-6-(5-phospho-D-ribitylamino)pyrimidin-4(3H)-one + NAD(P)$^+$ = 2,5-diamino-6-(5-phospho-D-ribosylamino)pyrimidin-4(3H)-one + NAD(P)H + H$^+$

Natural substrates and products
S 2,5-diamino-6-(1-D-ribosylamino)-4(3H)-pyrimidinone 5'-phosphate + NADH + H$^+$ <2> (<2> step in riboflavin biosynthesis, NADPH and NADH function equally well as reductant [2]) (Reversibility: ?) [2]
P 2,5-diamino-6-(1-D-ribitylamino)-4(3H)-pyrimidinone 5'-phosphate + NAD$^+$

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S 2,5-diamino-6-(1-D-ribosylamino)-4(3H)-pyrimidinone 5'-phosphate + NADPH + H^+ <2> (<2> step in riboflavin biosynthesis, NADPH and NADH function equally well as reductant [2]) (Reversibility: ?) [2]
P 2,5-diamino-6-(1-D-ribitylamino)-4(3H)-pyrimidinone 5'-phosphate + $NADP^+$
S 2,5-diamino-6-ribosylamino-4(3H)-pyrimidinone 5'-phosphate + NADH + H^+ <1> (<1> step in riboflavin biosynthesis, NADPH and NADH function equally well as reductant [2]) (Reversibility: ?) [2]
P 2,5-diamino-6-ribitylamino-4(3H)-pyrimidinone 5'-phosphate + NAD^+
S 2,5-diamino-6-ribosylamino-4(3H)-pyrimidinone 5'-phosphate + NADPH + H^+ <1> (<1> step in riboflavin biosynthesis, NADPH and NADH function equally well as reductant [2]) (Reversibility: ?) [2]
P 2,5-diamino-6-ribitylamino-4(3H)-pyrimidinone 5'-phosphate + $NADP^+$

Substrates and products

S 2,5-diamino-6-(1-D-ribitylamino)-4(3H)-pyrimidinone 5'-phosphate + NAD^+ <2> (Reversibility: ?) [2]
P 2,5-diamino-6-(1-D-ribitylamino)-4(3H)-pyrimidinone 5'-phosphate + NAD^+
S 2,5-diamino-6-(1-D-ribosylamino)-4(3H)-pyrimidinone 5'-phosphate + NADH + H^+ <1,2> (<2> step in riboflavin biosynthesis, NADPH and NADH function equally well as reductant [2]; <1> NADPH and NADH function equally well as reductant [2]) (Reversibility: ?) [2]
P 2,5-diamino-6-(1-D-ribitylamino)-4(3H)-pyrimidinone 5'-phosphate + NAD^+
S 2,5-diamino-6-(1-D-ribosylamino)-4(3H)-pyrimidinone 5'-phosphate + NADPH + H^+ <1,2,3> (<2> step in riboflavin biosynthesis, NADPH and NADH function equally well as reductant [2]; <1,2> NADPH and NADH function equally well as reductant [2]; <1> the substrate, 2,5-diamino-6-ribosylamino-4(3H)-pyrimidinone 5-phosphate, is modeled into the putative active site. The model suggests the transfer of the pro-R hydrogen of C-4 of NADPH to C-1 of the substrate [3]) (Reversibility: ?) [1,2,3]
P 2,5-diamino-6-(1-D-ribitylamino)-4(3H)-pyrimidinone 5'-phosphate + $NADP^+$
S 2,5-diamino-6-ribosylamino-4(3H)-pyrimidinone 5'-phosphate + NADH + H^+ <1> (<1> step in riboflavin biosynthesis, NADPH and NADH function equally well as reductant [2]) (Reversibility: ?) [2]
P 2,5-diamino-6-ribitylamino-4(3H)-pyrimidinone 5'-phosphate + NAD^+
S 2,5-diamino-6-ribosylamino-4(3H)-pyrimidinone 5'-phosphate + NADPH + H^+ <1> (<1> step in riboflavin biosynthesis, NADPH and NADH function equally well as reductant [2]) (Reversibility: ?) [2]
P 2,5-diamino-6-ribitylamino-4(3H)-pyrimidinone 5'-phosphate + $NADP^+$
S Additional information <1> (<1> the enzyme does not catalyze the pyrimidine nucleotide-dependent reduction of 5-amino-6-ribosylamino-2,4(1H,3H)-pyrimidinedione 5-phosphate to 5-amino-6-ribitylamino-2,4(1H,3H)-pyrimidinedione 5-phosphate [2]) [2]
P ?

Cofactors/prosthetic groups

NADH <1,2> (<1,2> NADPH and NADH function equally well as reductant [2]) [2]

NADPH <1,2,3> (<1,2> NADPH and NADH function equally well as reductant [2]; <1> the substrate, 2,5-diamino-6-ribosylamino-4(3H)-pyrimidinone 5-phosphate, is modeled into the putative active site. The model suggests the transfer of the pro-R hydrogen of C-4 of NADPH to C-1 of the substrate [3]) [1,2,3]

Specific activity (U/mg)

0.0008 <1> [3]

pH-Optimum

8 <1> (<1> assay at [3]) [3]

Temperature optimum (°C)

30 <1> (<1> assay at [3]) [3]

4 Enzyme Structure

Molecular weight

50000 <1> (<1> analytical ultracentrifugation, boundary sedimentation [3]) [3]

Subunits

homodimer <1> (<1> 2 * 24906, electrospray mass spectrometry [3]; <1> 2 * 25037, calculated mass of the full-length protein [3]) [3]

5 Isolation/Preparation/Mutation/Application

Purification

<1> (recombinant protein) [3]

Crystallization

<1> (sitting-drop vapour diffusion method. The structure of the enzyme in complex with the cofactor nicotinamide adenine dinucleotide phosphate is determined by X-ray crystallography at a resolution of 2.5 A) [3]

Cloning

<1> (expressed in Escherichia coli using a synthetic gene, the synthetic open reading frame is optimized for expression in Escherichia coli. The hypothetical open reading frame MJ0671 of Methanocaldococcus jannaschii predicts a protein of 224 amino acid residues. Because MJ0671 contains numerous codons that are poorly translated in Escherichia coli, a synthetic gene is designed that is optimized for the Escherichia coli codon usage. Approximately 31% (69 of 224) of the codons are replaced, and 14 singular restriction sites are introduced. The DNA sequence is assembled from 16 synthetic oligonucleotides by a sequence of eight PCR steps. The synthetic gene is transcribed

efficiently in a recombinant Escherichia coli strain, affording approximately 30% of cellular protein) [3]
<1> (overexpressed in Escherichia coli) [2]

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diacetyl reductase [(R)-acetoin forming] 1.1.1.303

1 Nomenclature

EC number
1.1.1.303

Systematic name
(R)-acetoin:NAD^+ oxidoreductase

Recommended name
diacetyl reductase [(R)-acetoin forming]

2 Source Organism

<1> *Saccharomyces cerevisiae* [1]
<2> *Saccharomyces cerevisiae* (UNIPROT accession number: P39714) [2]

3 Reaction and Specificity

Catalyzed reaction
(R)-acetoin + NAD^+ = diacetyl + NADH + H^+

Substrates and products
- **S** 1,2-cyclohexanedione + NADH + H^+ <1> (<1> 5% of the (R)-2,3-butanediol dehydrogenase activity with substrate acetoin [1]) (Reversibility: ir) [1]
- **P** (R)-2-hydroxy-1-cyclohexanone + NAD^+
- **S** 2,3-pentanedione + NADH + H^+ <1> (<1> 7% of the (R)-2,3-butanediol dehydrogenase activity with substrate acetoin [1]) (Reversibility: ir) [1]
- **P** (3R)-3-hydroxy-2-pentanone + NAD^+
- **S** diacetyl + NADH + H^+ <1,2> (<1> 21% of the (R)-2,3-butanediol dehydrogenase activity with substrate acetoin [1]; <2> 51.4% of the (R)-2,3-butanediol dehydrogenase activity with substrate acetoin [2]) (Reversibility: ir) [1,2]
- **P** (R)-acetoin + NAD^+
- **S** Additional information <1> (<1> enzyme is specific for NADH [1]) (Reversibility: ?) [1]
- **P** ?

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Inhibitors

EDTA <1> (<1> inhibition, (R)-2,3-butanediol dehydrogenase activity [1]) [1]
dipicolinate <1> (<1> inhibition, (R)-2,3-butanediol dehydrogenase activity [1]) [1]
o-phenanthroline <1> (<1> inhibition, (R)-2,3-butanediol dehydrogenase activity [1]) [1]

Cofactors/prosthetic groups

NADH <1,2> (<1> enzyme is specific for NADH [1]) [1,2]

Activating compounds

Mg^{2+} <1> (<1> activation, (R)-2,3-butanediol dehydrogenase activity [1]) [1]
Mn^{2+} <1> (<1> activation, (R)-2,3-butanediol dehydrogenase activity [1]) [1]

4 Enzyme Structure

Molecular weight

140000 <1> (<1> gel filtration [1]) [1]

Subunits

tetramer <1> (<1> 4 * 35000, SDS-PAGE [1]) [1]

5 Isolation/Preparation/Mutation/Application

Purification

<1> [1]

References

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diacetyl reductase [(S)-acetoin forming] 1.1.1.304

1 Nomenclature

EC number
1.1.1.304

Systematic name
(S)-acetoin:NAD^+ oxidoreductase

Recommended name
diacetyl reductase [(S)-acetoin forming]

2 Source Organism

<1> *Staphylococcus aureus* [4]
<2> *Geobacillus stearothermophilus* [5]
<3> *Enterobacter aerogenes* [1]
<4> *Columba livia* [2,3]
<5> *Klebsiella pneumoniae* (UNIPROT accession number: Q48436) [6]

3 Reaction and Specificity

Catalyzed reaction
(S)-acetoin + NAD^+ = diacetyl + NADH + H^+ (<4> Theorell-Chance mechanism with NADH as the leading substrate [3])

Substrates and products
- **S** 2,3-pentanedione + NADH + H^+ <3,5> (<5> 77% of the (R)-2,3-butanediol dehydrogenase activity with substrate acetoin [6]; <3> 85.6% of the activity with diacetyl [1]) (Reversibility: ?) [1,6]
- **P** 3-hydroxy-2-pentanone + NAD^+
- **S** 2,3-pentanedione + β-NADH + H^+ <1> (Reversibility: ir) [4]
- **P** L-3-hydroxy-2-pentanone + β-NAD^+
- **S** diacetyl + NADH + H^+ <2,3,4,5> (<5> 87% of the (R)-2,3-butanediol dehydrogenase activity with substrate acetoin [6]) (Reversibility: ir) [1,2,5,6]
- **P** (S)-acetoin + NAD^+
- **S** diacetyl + NADPH + H^+ <4> (Reversibility: ir) [3]
- **P** (S)-acetoin + $NADP^+$
- **S** diacetyl + β-NADH + H^+ <1> (<1> 86.9% of the activity with pentane-2,3-dione [4]) (Reversibility: ir) [4]
- **P** (S)-acetoin + β-NAD^+

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S ethyl pyruvate + NADH + H^+ <3> (<3> 57.7% of the activity with diacetyl [1]) (Reversibility: ?) [1]
P ? + NAD^+
S ethyl pyruvate + β-NADH + H^+ <1> (<1> 38.4% of the activity with pentane-2,3-dione [4]) (Reversibility: ir) [4]
P ? + β-NAD^+
S methyl glyoxal + NADH + H^+ <3> (<3> 11% of the activity with diacetyl [1]) (Reversibility: ?) [1]
P ? + NAD^+
S methyl pyruvate + NADH + H^+ <3> (<3> 49% of the activity with diacetyl [1]) (Reversibility: ?) [1]
P ? + NAD^+
S methyl pyruvate + β-NADH + H^+ <1> (<1> 22.8% of the activity with pentane-2,3-dione [4]) (Reversibility: ir) [4]
P ? + β-NAD^+
S Additional information <1> (<1> no activity with α-NADH or NADPH [4]) (Reversibility: ?) [4]
P ?

Inhibitors

2-oxoglutarate <4> (<4> noncompetitive [3]) [3]
diacetyl <1> (<1> substrate inhibition at concentrations above 80-90 mM [4]) [4]
NAD^+ <4> (<4> competitive, product inhibition [3]) [3]
acetoin <4> (<4> noncompetitive, product inhibition [3]) [3]
acetone <4> (<4> competitive for diacetyl, uncompetitive for NADH [3]) [3]
ethyl pyruvate <1> (<1> substrate inhibition at concentrations above 80-90 mM [4]) [4]
hexane-2,5-dione <4> (<4> noncompetitive [3]) [3]
methyl pyruvate <1> (<1> substrate inhibition at concentrations above 80-90 mM [4]) [4]
pentane-3-one <4> (<4> competitive for diacetyl, uncompetitive for NADH [3]) [3]

Cofactors/prosthetic groups

NADH <1,2,3,4> (<1> β-NADH [4]; <3> specific for β-NADH [1]) [1,3,4,5]
Additional information <1> (<1> no activity with α-NADH or NADPH [4]) [4]

Specific activity (U/mg)

71.4 <2> (<2> pH 7.5, 25°C [5]) [5]

K_m-Value (mM)

0.005 <3> (NADH, <3> cosubstrate acetoin, pH 7.0, 25°C [1]) [1]
0.007 <3> (NADH, <3> cosubstrate diacetyl, pH 7.0, 25°C [1]) [1]
0.025 <1> (NADH, <1> 25°C, pH 6.0, cosubstrate 2,3-pentanedione [4]) [4]
0.045 <1> (NADH, <1> 25°C, pH 6.0, cosubstrate diacetyl [4]) [4]
0.087 <4> (NADH, <4> 25°C, pH 5.9 [2]) [2]
0.095 <1> (NADH, <1> 25°C, pH 6.0, cosubstrate methyl pyruvate [4]) [4]

0.1 <4> (NADH, <4> pH 6.1, 25°C [3]) [3]
0.11 <1> (NADH, <1> 25°C, pH 6.0, cosubstrate ethyl pyruvate [4]) [4]
0.116 <4> (NADH, <4> 25°C, pH 6.1 [2]) [2]
0.135 <4> (NADH, <4> 25°C, pH 6.7 [2]) [2]
1.6 <3> (diacetyl, <3> pH 7.0, 25°C [1]) [1]
2.64 <4> (diacetyl, <4> 25°C, pH 6.7 [2]) [2]
2.81 <4> (diacetyl, <4> 25°C, pH 5.9 [2]) [2]
3 <4> (diacetyl, <4> 25°C, pH 6.1 [2]) [2]
3.1 <4> (diacetyl, <4> pH 6.1, 25°C [3]) [3]
6 <1> (2,3-pentanedione, <1> 25°C, pH 6.0 [4]) [4]
6 <3> (pentane-2,3-dione, <3> pH 7.0, 25°C [1]) [1]
15 <1> (diacetyl, <1> 25°C, pH 6.0 [4]) [4]
16 <1> (methyl pyruvate, <1> 25°C, pH 6.0 [4]) [4]
18 <3> (methyl pyruvate, <3> pH 7.0, 25°C [1]) [1]
19 <2> (diacetyl, <2> pH 7.5, 25°C [5]) [5]
20 <3> (ethyl pyruvate, <3> pH 7.0, 25°C [1]) [1]
24 <1> (ethyl pyruvate, <1> 25°C, pH 6.0 [4]) [4]
75 <3> (methyl glyoxal, <3> pH 7.0, 25°C [1]) [1]

K_i-Value (mM)

150 <1> (ethyl pyruvate, <1> 25°C, pH 6.0 [4]) [4]
150 <1> (methyl pyruvate, <1> 25°C, pH 6.0 [4]) [4]
300 <1> (diacetyl, <1> 25°C, pH 6.0 [4]) [4]

pH-Optimum

6 <1> [4]
6.1 <4> [3]

pH-Range

4.8 <4> (<4> 5 min, 60% loss of activity [2]) [2]
5 <4> (<4> 5 min, 30% loss of activity [2]) [2]
5.1 <4> (<4> 5 min, 20% loss of activity [2]) [2]
5.4-7.6 <4> (<4> stable within [2]) [2]

pi-Value

5.9-7.2 <5> (<5> isoelectric focusing [6]) [6]
6.8 <3> (<3> isoelectric focusing [1]) [1]

Temperature optimum (°C)

50 <2> [5]

4 Enzyme Structure

Molecular weight

49000 <2> (<2> gel filtration [5]) [5]
61000 <3> (<3> gel filtration [1]) [1]
68000 <1> (<1> gel filtration [4]) [4]
96000 <5> (<5> gel filtration [6]) [6]

Subunits
dimer <2,3> (<3> 2 * 28000, SDS-PAGE [1]; <2> 2 * 26000, SDS-PAGE [5]) [1,5]
monomer <1> (<1> 1 * 68000, SDS-PAGE [4]) [4]
tetramer <5> (<5> 4 * 26591, calculated [6]) [6]

5 Isolation/Preparation/Mutation/Application

Source/tissue
liver <4> [2,3]

Purification
<1> [4]
<2> [5]
<3> [1]

Cloning
<5> (expression in Escherichia coli) [6]

6 Stability

pH-Stability
7-8 <5> [6]

General stability information
<2>, unstable to dilution, kept diluted at 0°C for ca. 60 min it will lose 62% of activity. This inactivation is almost completely reversed by the addition of NAD^+ [5]

Storage stability
<2>, storage at 0°C in the presence of 20% glycerol, 0.1 mM EDTA, 5 mM 2-mercaptoethanol and 0.6 mM NAD^+ in TEA buffer, pH 7.5, half-life of one month [5]

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UDP-glucuronic acid dehydrogenase (UDP-4-keto-hexauronic acid decarboxylating) 1.1.1.305

1 Nomenclature

EC number
1.1.1.305

Systematic name
UDP-glucuronate:NAD^+ oxidoreductase (decarboxylating)

Recommended name
UDP-glucuronic acid dehydrogenase (UDP-4-keto-hexauronic acid decarboxylating)

Synonyms
ArnA dehydrogenase <1> [3,5]
ArnADH <1> (<1> ArnA is a bifunctional enzyme, ArnADH protein consists of the C-terminal 345 residues of ArnA, starting at Thr-316 converted to an initiating methionine [3]) [3,5]
UDP-GlcUA dehydrogenase <1> [3]

2 Source Organism

<1> *Escherichia coli* [1,2,3,4,5]

3 Reaction and Specificity

Catalyzed reaction
UDP-glucuronate + NAD^+ = UDP-β-L-threo-pentapyranos-4-ulose + CO_2 + NADH + H^+

Natural substrates and products
S UDP-glucuronate + NAD^+ <1> (<1> ArnA is a bi-functional enzyme, the oxidative decarboxylation of UDP-glucuronic acid is catalyzed by the 345-residue C-terminal domain of ArnA. The 304-residue N-terminal domain catalyzes the N-10-formyltetrahydrofolate-dependent formylation of the 4-amine of UDP-L-4-amino-4-deoxy-L-arabinose, generating the sugar nucleotide, uridine 5-diphospho-β-(4-deoxy-4-formamido-L-arabinose). The two domains of ArnA are expressed independently as active proteins in Escherichia coli. Both are required for maintenance of polymyxin resistance and 4-amino-4-deoxy-L-arabinose modification of lipid A. Only the formylated sugar nucleotide is converted in vitro to an undecaprenyl

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phosphate-linked form by the enzyme ArnC [3]; <1> ArnA is a key enzyme in the lipid A modification pathway, and its deletion abolishes both the Ara4N-lipid A modification and polymyxin resistance. ArnA is a bifunctional enzyme. It can catalyze the NAD^+-dependent decarboxylation of UDP-glucuronic acid to UDP-4-keto-arabinose and the N-10-formyltetrahydrofolate dependent formylation of UDP-4-amino-4-deoxy-L-arabinose [1]; <1> modification of the lipid A moiety of lipopolysaccharide by the addition of the sugar 4-amino-4-deoxy-L-arabinose is a strategy adopted by pathogenic Gram-negative bacteria to evade cationic antimicrobial peptides produced by the innate immune system. The bifunctional enzyme ArnA is required for 4-amino-4-deoxy-L-arabinose biosynthesis and catalyzes the NAD^+-dependent oxidative decarboxylation of UDP-glucuronic acid to generate a UDP-4-keto-pentose sugar and also catalyzes transfer of a formyl group from N-10-formyltetrahydrofolate to the 4-amine of UDP-4-amino-4-deoxy-L-arabinose [4]; <1> the modification of lipid A with 4-amino-4-deoxy-L-arabinose allows gram-negative bacteria to resist the antimicrobial activity of cationic antimicrobial peptides and antibiotics such as polymyxin. ArnA is the first enzyme specific to the lipid A-Ara4N pathway. It contains two functionally and physically separable domains: a dehydrogenase domain (ArnA_DH) catalyzing the NAD^+-dependent oxidative decarboxylation of UDP-glucuronic acid, and a transformylase domain that formylates UDP-4-amino-4-deoxy-L-arabinose [5]) (Reversibility: ?) [1,2,3,4,5]

P UDP-β-L-threo-pentapyranos-4-ulose + CO_2 + NADH + H^+

Substrates and products

S UDP-glucuronate + NAD^+ <1> (<1> ArnA is a bi-functional enzyme, the oxidative decarboxylation of UDP-glucuronic acid is catalyzed by the 345-residue C-terminal domain of ArnA. The 304-residue N-terminal domain catalyzes the N-10-formyltetrahydrofolate-dependent formylation of the 4-amine of UDP-L-4-amino-4-deoxy-L-arabinose, generating the sugar nucleotide, uridine 5-diphospho-β-(4-deoxy-4-formamido-L-arabinose). The two domains of ArnA are expressed independently as active proteins in Escherichia coli. Both are required for maintenance of polymyxin resistance and 4-amino-4-deoxy-L-arabinose modification of lipid A. Only the formylated sugar nucleotide is converted in vitro to an undecaprenyl phosphate-linked form by the enzyme ArnC [3]; <1> ArnA is a key enzyme in the lipid A modification pathway, and its deletion abolishes both the Ara4N-lipid A modification and polymyxin resistance. ArnA is a bifunctional enzyme. It can catalyze the NAD^+-dependent decarboxylation of UDP-glucuronic acid to UDP-4-keto-arabinose and the N-10-formyltetrahydrofolate dependent formylation of UDP-4-amino-4-deoxy-L-arabinose [1]; <1> modification of the lipid A moiety of lipopolysaccharide by the addition of the sugar 4-amino-4-deoxy-L-arabinose is a strategy adopted by pathogenic Gram-negative bacteria to evade cationic antimicrobial peptides produced by the innate immune system. The bifunctional enzyme ArnA is required for 4-amino-4-deoxy-L-arabinose biosynthesis

and catalyzes the NAD^+-dependent oxidative decarboxylation of UDP-glucuronic acid to generate a UDP-4-keto-pentose sugar and also catalyzes transfer of a formyl group from N-10-formyltetrahydrofolate to the 4-amine of UDP-4-amino-4-deoxy-L-arabinose [4]; <1> the modification of lipid A with 4-amino-4-deoxy-L-arabinose allows gram-negative bacteria to resist the antimicrobial activity of cationic antimicrobial peptides and antibiotics such as polymyxin. ArnA is the first enzyme specific to the lipid A-Ara4N pathway. It contains two functionally and physically separable domains: a dehydrogenase domain (ArnA_DH) catalyzing the NAD^+-dependent oxidative decarboxylation of UDP-glucuronic acid, and a transformylase domain that formylates UDP-4-amino-4-deoxy-L-arabinose [5]; <1> ArnA is a bi-functional enzyme. The oxidative decarboxylation of UDP-glucuronic acid is catalyzed by the 345-residue C-terminal domain of ArnA. The 304-residue N-terminal domain catalyzes the N-10-formyltetrahydrofolate-dependent formylation of the 4-amine of UDP-4-amino-4-deoxy-L-arabinose, generating the sugar nucleotide, uridine 5-diphospho-β-(4-deoxy-4-formamido-L-arabinose) [3]; <1> ArnA is a bifunctional enzyme. It can catalyze the NAD^+-dependent decarboxylation of UDP-glucuronic acid to UDP-4-keto-arabinose and the N-10-formyltetrahydrofolate dependent formylation of UDP-4-amino-4-deoxy-L-arabinose. The NAD^+-dependent decarboxylating activity is contained in the 360 amino acid C-terminal domain of ArnA. This domain is separable from the N-terminal fragment, and its activity is identical to that of the full-length enzyme. T432, Y463, K467, R619, and S433 are involved in the mechanism of NAD^+-dependent oxidation of the 4-OH of the UDP-glucuronic acid and decarboxylation of the UDP-4-keto-glucuronic acid intermediate [1]; <1> modification of the lipid A moiety of lipopolysaccharide by the addition of the sugar 4-amino-4-deoxy-L-arabinose is a strategy adopted by pathogenic Gram-negative bacteria to evade cationic antimicrobial peptides produced by the innate immune system. The bifunctional enzyme ArnA is required for 4-amino-4-deoxy-L-arabinose biosynthesis and catalyzes the NAD^+-dependent oxidative decarboxylation of UDP-glucuronic acid to generate a UDP-4-keto-pentose sugar and also catalyzes transfer of a formyl group from N-10-formyltetrahydrofolate to the 4-amine of UDP-4-amino-4-deoxy-L-arabinose. Residues Ser433 and Glu434 of the decarboxylase domain are required for the oxidative decarboxylation of UDP-glucuronate. Decarboxylase domain catalyzes both hydride abstraction (oxidation) from the C-4 position and the subsequent decarboxylation [4]; <1> ordered mechanism of substrate binding and product release is proposed. R619 functions as a general acid in catalysis [5]) (Reversibility: ?) [1,2,3,4,5]

P UDP-β-L-threo-pentapyranos-4-ulose + CO_2 + NADH + H^+

Cofactors/prosthetic groups

NAD^+ <1> [1,3,4,5]

K_m-Value (mM)

0.054 <1> (UDP-glucuronate, <1> pH 8.0, 37°C, C-terminal domain of ArnA [1]) [1]
0.086 <1> (UDP-glucuronate, <1> pH 8.0, 37°C, full-length enzyme [1]) [1]
0.2 <1> (UDP-glucuronate, <1> pH 7.5, 30°C, S433A decarboxylase mutant [4]) [4]
0.4 <1> (UDP-glucuronate, <1> pH 7.5, 30°C, E434A decarboxylase mutant [4]) [4]
0.57 <1> (NAD^+, <1> pH 8.0, 37°C, C-terminal domain of ArnA [1]) [1]
0.7 <1> (UDP-glucuronate, <1> pH 7.5, 30°C, ArnA decarboxylase domain [4]) [4]
0.76 <1> (NAD^+, <1> pH 8.0, 37°C, full-length enzyme [1]) [1]
1.3 <1> (NAD^+, <1> pH 7.5, 30°C, ArnA decarboxylase domain [4]) [4]
1.4 <1> (NAD^+, <1> pH 7.5, 30°C, S433A decarboxylase mutant [4]) [4]
1.6 <1> (NAD^+, <1> pH 7.5, 30°C, E434A decarboxylase mutant, E434Q decarboxylase mutant [4]) [4]

pH-Optimum

7.5 <1> (<1> assay at [3,4]) [3,4]
8 <1> (<1> assay at [1]) [1]

Temperature optimum (°C)

30 <1> (<1> assay at [3,4]) [3,4]
37 <1> (<1> assay at [1]) [1]

5 Isolation/Preparation/Mutation/Application

Purification

<1> [5]
<1> (recombinant) [3]

Crystallization

<1> (crystal structure of the ArnA decarboxylase domain) [1]
<1> (crystallization of native and Se-Met decarboxylase protein. Good quality crystals are obtained with a precipitant solution of 3.2 M NaCl, 0.1 M Bistris, pH 5.2, using a drop containing 0.004 ml of protein and 0.004 ml of precipitant equilibrated against a reservoir of 0.1 ml of precipitant. Space group as P4(1)3(2), with cell dimensions a = b = c = 149.4 A, $\beta = \gamma = 90°$) [4]
<1> (hanging drop vapor diffusion method, crystal structure of the full-length bifunctional ArnA with UDP-glucuronic acid and ATP bound to the dehydrogenase domain. Binding of UDP-glucuronic acid triggers a 17 A conformational change in ArnA_DH that opens the NAD^+ binding site while trapping UDP-glucuronic acid) [5]

Cloning

<1> [5]
<1> (overexpression of ArnA as a hexahistidine fusion protein, cloning and expression the separate domains in pET28b and pWSK29) [3]

<1> (overexpression of native and selenomethionine decarboxylase and formyltransferase domains of ArnA) [4]

Engineering

E434Q <1> (<1> mutant is inactive, suggesting that chemical rather than steric properties of this residue are crucial in the decarboxylation reaction [4]) [4]
R610M <1> (<1> activity is 800fold lower than wild-type activity [5]) [5]
R619E <1> (<1> no activity [5]) [5]
R619Y <1> (<1> no activity [5]) [5]
S433A <1> (<1> activity is 30fold lower than wild-type activity [5]) [5]
S433T <1> (<1> no activity [5]) [5]

Application

medicine <1> (<1> modification of the lipid A moiety of lipopolysaccharide by the addition of the sugar 4-amino-4-deoxy-L-arabinose is a strategy adopted by pathogenic Gram-negative bacteria to evade cationic antimicrobial peptides produced by the innate immune system. L-Ara4N biosynthesis is therefore a potential anti-infective target [4]) [4]

References

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S-(hydroxymethyl)mycothiol dehydrogenase 1.1.1.306

1 Nomenclature

EC number
1.1.1.306

Systematic name
S-(hydroxymethyl)mycothiol:NAD^+ oxidoreductase

Recommended name
S-(hydroxymethyl)mycothiol dehydrogenase

Synonyms
FD-FA1DH <4> [3]
MD-FALDH
MSH-dependent formaldehyde dehydrogenase <1,2> [4,5]
NAD/factor-dependent formaldehyde dehydrogenase
glutathione-independent formaldehyde dehydrogenase

CAS registry number
192140-85-5

2 Source Organism

<1> *Mycobacterium smegmatis* [4]
<2> *Nocardia sp.* [5]
<3> *Rhodococcus erythropolis* [1]
<4> *Amycolatopsis methanolica* [1,2,3]

3 Reaction and Specificity

Catalyzed reaction
S-(hydroxymethyl)mycothiol + NAD^+ = S-formylmycothiol + NADH + H^+

Reaction type
oxidation
redox reaction
reduction

Natural substrates and products
S formaldehyde + 1-O-(2'-[N-acetyl-L-cysteinyl]amido-2'-deoxy-α-D-glucopyranosyl)-D-myo-inositol + NAD^+ <3,4> (Reversibility: ?) [1,2,3]

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Springer Handbook of Enzymes S8, DOI 10.1007/978-3-642-36265-1_12,

P S-formylmycothiol + NADH <3,4> [1,2,3]

Substrates and products

S 1-butanol + NAD^+ <4> (Reversibility: ?) [3]
P n-butanal + NADH <4> [3]
S 12-hydroxydodecanoic acid + NADH <4> (Reversibility: ?) [3]
P ? + NAD^+ <4> [3]
S S-hydroxymethylmycothiol + NAD^+ <1> (<1> rapid-equilibrium ordered mechanism [4]) (Reversibility: ?) [4]
P formic acid + mycothiol + NADH + ?
S S-nitrosomycothiol + NADH <1> (<1> decomposition with a sequential mechanism, enzyme reduces the nitroso group to the oxidation level of nitroxyl [4]) (Reversibility: ?) [4]
P ?
S formaldehyde + 1-O-(2'-[N-acetyl-L-cysteinyl]amido-2'-deoxy-α-D-glucopyranosyl)-D-myo-inositol + NAD^+ <3,4> (Reversibility: ?) [1,2,3]
P S-formylmycothiol + NADH <3,4> [1,2,3]
S mycothiol + NAD^+ <2> (Reversibility: ?) [5]
P ? + NADH + H^+

Inhibitors

1,10-phenanthroline <4> (<4> 80% inhibition at 5 mM [3]) [3]
2-(cyclohexylamino)ethanesulfonic acid-NaOH <4> (<4> 25 mM at pH 9.0: 80% of the activity compared to 0.1 M diphosphate, pH 9.0 [3]) [3]
Cu^{2+} <4> (<4> complete inhibition at 1 mM [3]) [3]
Hg^{2+} <4> (<4> complete inhibition at 0.1 mM [3]) [3]
KCN <4> (<4> 55% inhibition at 2 mM [3]) [3]
NH_4Cl-NH_3 <4> (<4> 100 mM at pH 9.0: 80% of the activity compared to 0.1 M diphosphate, pH 9.0 [3]) [3]
$Na_2B_4O_7$-HCl <4> (<4> 100 mM at pH 9.0: no activity [3]) [3]
Tris-HCl <4> (<4> 50 mM at pH 9.0: 80% of the activity compared to 0.1 M diphosphate, pH 9.0 [3]) [3]
acetaldehyde <4> (<4> 15% inhibition at 1 mM [3]) [1,3]
glycine-NaOH <4> (<4> 100 mM at pH 9.0: 40% of the activity compared to 0.1 M diphosphate, pH 9.0 [3]) [3]

Cofactors/prosthetic groups

1-O-(2'-[N-acetyl-L-cysteinyl]amido-2'-deoxy-α-D-glucopyranosyl)-D-myo-inositol <3,4> (<3,4> mycothiol [1,2]; <3,4> high specificity for mycothiol [1]) [1,2]
NAD^+ <1,2,3,4> [1,2,3,4,5]

Activating compounds

factor <4> (<4> unidentified factor that is required for maximal activity with formaldehyde as substrate, only 4% activity in its absence, oxidation of alcohols do not require the factor [3]) [3]
formaldehyde <2> [5]
methanol <4> (<4> can replace an unidentified activating factor [3]) [3]

Metals, ions
Zn^{2+} <4> (<4> 6 atoms Zn/enzyme molecule [3]) [3]

Turnover number (s^{-1})
Additional information <1> [4]

Specific activity (U/mg)
0.03 <4> (<4> cell free extract, in the absence of an unknown activating factor [3]) [3]
0.08 <4> (<4> cell free extract, in the presence of an unknown activating factor [3]) [3]
0.23 <4> [1]
6.9 <4> (<4> substrate: ethanol [3]) [3]
15 <4> (<4> substrate: formaldehyde [3]) [3]
22.64 <1> [4]

K_m-Value (mM)
0.0173 <1> (mycothiol, <1> pH 8.7, 30°C [4]) [4]
0.354 <1> (NAD^+, <1> pH 8.7, 30°C [4]) [4]
1.2 <4> (1-octanol) [3]
1.7 <4> (12-hydroxydodecanoic acid) [3]
3.4 <4> (1-hexanol) [3]
9.6 <4> (formaldehyde) [3]
26.5 <4> (1-pentanol) [3]
84 <4> (1-butanol) [3]
324 <4> (1-propanol) [3]
343 <4> (ethanol) [3]

pH-Optimum
8-9.5 <1> (<1> optimal in Na-diphosphate buffer, buffers containing amino groups give poor activity, possibly because of Schiff base formation with formaldehyde [4]) [4]
9 <4> (<4> formaldehyde as substrate [3]) [3]
10.2 <4> (<4> alcohols as substrates [3]) [3]

4 Enzyme Structure

Molecular weight
120000 <4> (<4> gel filtration [3]) [3]

Subunits
? <1> (<1> x * 38263, electrospray mass spectrometry [4]) [4]
trimer <4> (<4> 3 * 40000, SDS-PAGE [3]) [3]

5 Isolation/Preparation/Mutation/Application

Source/tissue

cell culture <1,3,4> (<4> induction of enzyme and factor synthesis only if cells grown in methanol-containing medium [3]) [1,2,3,4]

Purification

<1> [4]
<4> [2]
<4> (to homogeneity) [3]

Application

Additional information <2> (<2> thiol formation and detection of MSH-dependent formaldehyde dehydrogenase activity in cell extracts are relevant to the possible modulation of nitric oxide toxicity generated by strain NRRL 5646 [5]) [5]

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D-xylose reductase 1.1.1.307

1 Nomenclature

EC number
1.1.1.307

Systematic name
xylitol:NAD(P)$^+$ oxidoreductase

Recommended name
D-xylose reductase

Synonyms
CbXR <4> [27]
CtXR <13,19,20> [5,8,10]
D-xylose reductase <3> [4]
D-xylose reductase 1 <6> [21]
D-xylose reductase 2 <6> [21]
D-xylose reductase 3 <6> [21]
NAD(P)H-dependent xylose reductase <11,13> [9,15]
PsXR <11> [24]
Texr <22> [18]
XR1 <6> [21]
XR2 <6> [21]
XR3 <6> [21]
XYL1 <20,23> (<20> gene name [5]) [5,6]
XylR <19> [13]
XyrA <21> [26]
dsXR <14> (<14> Candida intermedia produces two isoforms of xylose reductase: one is NADPH-dependent (monospecific xylose reductase, msXR), and another prefers NADH about 4fold over NADPH (dual specific xylose reductase, dsXR) [17]) [17]
msXR <14> (<14> Candida intermedia produces two isoforms of xylose reductase: one is NADPH-dependent (monospecific xylose reductase, msXR), and another prefers NADH about 4fold over NADPH (dual specific xylose reductase, dsXR) [17]) [17]
xylose reductase <4,5,13> [15,19,22,25,27]

2 Source Organism

<1> *Saccharomyces cerevisiae* [28]
<2> *Neurospora crassa* [7,12]

D. Schomburg and I. Schomburg (eds.), *Class 1 Oxidoreductases: EC 1*,
Springer Handbook of Enzymes S8, DOI 10.1007/978-3-642-36265-1_13,