ELSEVIER

Molecular and Cellular Endocrinology 241 (2005) 80–87

MCE
Molecular and Cellular Endocrinology

www.elsevier.com/locate/mce

Coenzyme specificity in fungal 17β-hydroxysteroid dehydrogenase

Katja Kristan[a], Jure Stojan[a], Gabriele Möller[b], Jerzy Adamski[b], Tea Lanišnik Rižner[a,*]

[a] Institute of Biochemistry, Medical Faculty, University of Ljubljana, Vrazov trg 2, 1000 Ljubljana, Slovenia
[b] GSF–National Research Center for Environment and Health, Institute of Experimental Genetics, Genome Analysis Center, Ingolstädter Landstraße 1, 85764 Neuherberg, Germany

Received 18 March 2005; received in revised form 20 May 2005; accepted 20 May 2005

Abstract

The 17β-hydroxysteroid dehydrogenase from the fungus *Cochliobolus lunatus* is an NADP(H)-dependent member of the short-chain dehydrogenase/reductase superfamily (SDR) that belongs to the cP1 classical subfamily. Here, we have created several mutants by site-directed mutagenesis, and through these we have studied the amino acid residues that are responsible for coenzyme binding and specificity. The Thr202Val and Thr202Ile mutants were inactive, thus confirming the importance of Thr202 for the appropriate orientation of the coenzyme that enables the hydride transfer. The Ala50Arg and Asn51Arg mutants had increased rates of NADPH dissociation, and thus an enhanced substrate oxidation with $NADP^+$, while the Asn51Arg mutant also showed an increased rate of $NADP^+$ dissociation, and thus an enhanced substrate reduction with NADPH. Addition of a negatively-charged amino acid residue at the first position after the second β-strand (Tyr49Asp) affected the coenzyme specificity and turned the enzyme into an NAD^+-dependent oxidase resembling the cD1d subfamily members.
© 2005 Elsevier Ireland Ltd. All rights reserved.

Keywords: 17β-Hydroxysteroid dehydrogenase; Short-chain dehydrogenase/reductase; Site-directed mutagenesis; Enzyme kinetic; Coenzyme specificity

1. Introduction

The protein superfamily of short-chain dehydrogenases/reductases (SDRs) includes a large number of prokaryotic and eukaryotic enzymes. To date, about 3000 members of this superfamily have been reported in different species (Oppermann et al., 2003). The SDR proteins function as NAD^+-dependent oxidases or NADPH-dependent reductases, and they are involved among others in steroid hormone metabolism, fatty acid oxidation and biotransformation of xenobiotics (Oppermann et al., 2003). Although the sequence identities between different SDR proteins are low, varying from 15% to 30%, all of the available three-dimensional (3D) structures display a highly similar α/β folding pattern, where the β strands form a layer that is surrounded by three α helices on each side (Oppermann et al., 2003).

The SDRs have been divided into five families (classical, extended, intermediate, divergent and complex) with different motifs in the coenzyme binding and active-site regions, and different chain lengths (Kallberg et al., 2002). The classical and extended SDR families have been further divided into subfamilies based on their patterns of charged coenzyme-binding residues (Kallberg et al., 2002). The classical family includes oxidoreductases, such as steroid dehydrogenases and carbonyl reductases. In the NADP(H)-preferring classical SDRs, the two negative charges of the 2′-phosphate group of the coenzyme are compensated for by one or two positively charged residues of the enzymes. The first of these is found in the Gly-X-X-X-Gly-X-Gly coenzyme binding motif towards the N-terminal, and is positioned before the second glycine. The second basic residue is situated in the first loop position after the second β-strand. The proteins with the basic residue in the first position fall into the cP1 subfamily, while those with the basic residue in the second position belong to the cP2 subfamily. The cP3 subfamily consists of proteins that have basic residues in both positions (Kallberg et al., 2002; Persson et al., 2003) (Table 1).

The presence of an acidic residue at the C-terminus of the second β-strand in classical SDRs is a key determining

* Corresponding author. Tel.: +386 1543 7657; fax: +386 1543 7641.
E-mail address: Tea.Lanisnik-Rizner@mf.uni-lj.si (T.L. Rižner).

0303-7207/$ – see front matter © 2005 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.mce.2005.05.007

Table 1
The alignment of the amino acid sequences around the coenzyme binding fold

	[subfamily]		<-βA> <----αB----> <-βB->
NADP(H)-dependent enzymes			
CR (pig)	[cP3]	9	RALVT**G**AG**KG**I**G**RDTVKALHVS_GARVVAVT**R**T
CR (human)	[cP3]	7	VALVT**G**GN**KG**I**G**LAIVRDLCRLFSGDVVLTA**R**D
20β-HSD (pig)	[cP3]	7	VALVT**G**AN**KG**I**G**FAIVRDLCRKFLGDVVLTA**R**D
11β-HSD/CR (mouse)	[cP3]	36	KVIVT**G**AS**KG**I**G**REMAYHLSKM_GAHVVLTA**R**S
TR-I (*Datura*)	[cP3]	23	TALVT**G**GS**KG**I**G**YAIVEELAGL_GARVYTCS**R**N
TR-II (*Datura*)	[cP3]	11	TALVT**G**GS**RG**I**G**YGIVEELASL_GASVYTCS**R**N
7α-HSD (*Eubacterium*)	[cP3]	7	VILVTAST**RG**I**G**LAIAQACAKE_GAKVYMGA**R**N
PcR (*Arabidopsis*)	[cP2]	90	NVVVT**G**ASS**G**L**G**LATAKALAETGKWNVIMAC**R**D
Ver-1 (*Aspergillus*)	[cP1]	11	VALVT**G**AG**RG**I**G**AAIAVALGER_GAKVVVNYAH
VerA (*Emericella*)	[cP1]	13	VALVT**G**AG**RG**I**G**AAIAVALGQP_GAKVVVNYAN
THNR (*Magnaporthe*)	[cP1]	30	VALVT**G**AG**RG**I**G**REMAMELGRR_GCKVIVNYAN
17β-HSDcl (*C.lunatus*)	[cP1]	20	VALVT**G**SG**RG**I**G**AAVAVHLGRL_GAKVVVNYAN
NAD(H)-dependent enzymes			
3α,20β-HSD (*Streptomyces*)	[cD1d]	8	TVIIT**G**GA**RG**L**G**AEAARQAVAA_GARVVLA**D**VL
7α-HSD (*E.coli*)	[cD1d]	13	CAIIT**G**AGA**G**I**G**KEIAITFATA_GASVVVS**D**IN
DhpR (rat)	[cD1d]	9	RVLVY**G**GRGAL**G**SRCVQAFRAR_NWWVASI**D**VV
15-HPGD (human)	[cD1d]	7	VALVT**G**AAQ**G**I**G**RAFAEALLLK_GAKVALV**D**WN
ADH (*Drosophila*)	[cD1d]	9	VIFVA**G**_LG**G**I**G**LDTSKQLLKRDLKNLVIL**DR**I

Comparison of the sequences around the coenzyme binding fold of the SDR superfamily proteins, grouped according to their coenzyme specificities. The amino acids that determine the coenzyme specificities are boxed (CR: carbonyl reductase; HSD: hydroxysteroid dehydrogenase; TR: tropinone reductase, THNR: tetrahydroxynaphtalene reductase, Ver: versicolorin reductase, PcR: protochlorophyllide reductase, Dhpr: dihydropteridine reductase, 15-HPGD: 15-hydroxyprostaglandin dehydrogenase, ADH: alcohol dehydrogenase).

factor for NAD(H) preference. This residue participates in hydrogen bonding with the 2′-and 3′-hydroxy groups of the adenine ribose moiety of NAD(H), and it repels NADP(H) electrostatically. The members that bind NAD(H) and have an aspartic or glutamic acid at the end of the second β-strand form the cD1d and cD1e subfamilies, respectively. The proteins that instead have a negatively-charged residue in the first or the second positions after the second β-strand belong to the cD2 and cD3 subfamilies, respectively (Kallberg et al., 2002; Persson et al., 2003) (Table 1).

The amino acid residues that determine the coenzyme specificities have been already studied in the following SDR members: NADP(H)-dependent mouse lung carbonyl reductase (cP3 subfamily) (Nakanishi et al., 1996; Nakanishi et al., 1997); type 1 (cP2) (Huang et al., 2001) and type 3 (cP3) (McKeever et al., 2002) human 17β-hydroxysteroid dehydrogenases (HSDs); human carbonyl reductase (cP3) (Sciotti and Wermuth, 2001); rainbow trout carbonyl reductase-like 20β-HSD (cP3) (Guan et al., 2000); as well as in NAD(H)-dependent 15-hydroxyprostaglandin dehydrogenase (cD1d) (Cho et al., 2003); type 2 11β-HSD (cD3) (Arnold et al., 2003); and type 1 and type 2 human 3β-HSD/isomerase (extended SDR) (Thomas et al., 2004).

We have been studying 17β-HSD from the fungus *Cochliobolus lunatus* (17β-HSDcl), which is the only fungal 17β-HSD that has been purified and cloned to date (Lanišnik Rižner et al., 1996, 1999). Although the physiological function of the fungal 17β-HSD has not yet been confirmed, the sequence similarity to human 17β-HSD type 4 and 8 (30% and 29% identity, respectively), the endogenous biosynthesis of androgens (Kastelic-Suhadolc et al., 1994) and the presence of androgen binding proteins in *C. lunatus* (Kastelic-Suhadolc and Lenasi, 1993) indicate 17β-HSDcl might be involved in steroid signalling. Considering homology to fungal reductases 17β-HSDcl might also be involved in the biosynthesis of mycotoxins or it might be a part of fungal detoxification mechanism (Lanišnik Rižner et al., 2001). Further studies uncovering the physiological role of this enzyme are underway.

This 17β-HSD is an oxidoreductase with a molecular mass of 28 kDa that preferentially catalyses reversible oxidoreduction of estrogens and androgens with 4-estrene-3,17-dione and 4-estrene-17β-ol-3-one as the two most preferred substrates. The enzyme has conserved classical SDR motifs, i.e. the Gly^{25}-X-X-X-Gly^{29}-X-Gly^{31} coenzyme binding site in the N-terminal region and the Tyr^{167}-X-X-X-Lys^{171} catalytic site in the central region. It is an NADP(H)-preferring enzyme

Download English Version:

https://daneshyari.com/en/article/9914921

Download Persian Version:

https://daneshyari.com/article/9914921

Daneshyari.com