ELSEVIER

Human type 10 17β-hydroxysteroid dehydrogenase: Molecular modelling and substrate docking

Erik Nordling,*† Udo C.T. Oppermann,* Hans Jörnvall,* and Bengt Persson*†

**Department of Medical Biochemistry and Biophysics, and †Stockholm Bioinformatics Centre, Karolinska Institutet, Stockholm, Sweden*

17β-hydroxysteroid dehydrogenases catalyze the oxidoreduction of hydroxy/oxo groups at position C17 of steroid hormones, thereby constituting a prereceptor control mechanism of hormone action. At present, 11 different mammalian 17β-hydroxysteroid dehydrogenases have been identified, catalyzing the cell- and steroid-specific activation and inactivation of estrogens and androgens. The human type 10 17β-hydroxysteroid dehydrogenase (17β-HSD-10) is a multifunctional mitochondrial enzyme that efficiently catalyzes the oxidative inactivation at C17 of androgens and estrogens. However, it also mediates oxidation of 3α-hydroxy groups of androgens, thereby reactivating androgen metabolites. Finally, it is involved in β-oxidation of fatty acids by catalyzing the L-hydroxyacyl CoA dehydrogenase reaction of the β-oxidation cycle. These features and expression profiles suggest a critical role of 17β-HSD-10 in neurodegenerative and steroid-dependent cancer forms. Since no three-dimensional structure of 17β-HSD-10 is available, homology modelling was carried out to understand the molecular basis of these substrate specificities. The structure obtained displays the properties of a one-domain, α/β fold enzyme of the SDR family. The active site is located within a large, hydrophobic cleft, which forms optimal contacts with the different steroid surfaces. The data provide explanations for the substrate specificities toward the various classes of sex steroid hormones. The model is suitable to explore substrate and inhibitor characteristics that may be used in the development of novel strategies in the treatment of degenerative or malignant diseases. © 2001 by Elsevier Science Inc.

Keywords: short-chain dehydrogenases/reductases, 17β-hydroxysteroid dehydrogenase, molecular modelling, steroid hormone, metabolism; substrate docking

INTRODUCTION

Hydroxysteroid dehydrogenases (HSDs) constitute important determinants in steroid hormone physiology by mediating the reversible NAD(P)(H)-dependent oxidoreduction of hydroxy/oxo groups at defined positions of the steroid nucleus. They represent a prereceptor control mechanism, achieved by catalyzing the "switch" between receptor-binding ligands and non-binding hormone metabolites.[1–3] Hydroxysteroid dehydrogenases important in human physiology are enzymes acting at positions 3, 11, 17, and 20 (3α-HSD, 3β-HSD, 11β-HSD, 17β-HSD, 20α-HSD, and 20β-HSD) of steroid hormones. Most of the vertebrate HSDs characterized to date belong to the protein superfamilies of short-chain dehydrogenases/reductases (SDRs) or to the aldo-keto reductases (AKRs), with the majority of HSDs grouped into the SDR family.[4–6] The two families differ fundamentally in structure and stereospecificity of hydride transfer from the nucleotide cofactor. Interestingly, they constitute examples of convergent evolution by displaying highly conserved and nearly superimposable key residues at the active sites, with a tyrosine residue acting as the catalytic base.[1,5] 17β-HSDs fulfill essential roles in sex steroid metabolism by catalyzing the conversion between ligands and non-receptor metabolites for the estrogen (conversion between estradiol and estrone) and androgen receptor (conversion between testosterone and androstenedione, and 5α-dihydrotestosterone and androstanedione, respectively).[3] At present, 11 different types of 17β-HSDs have been described, differing in their substrate specificities, tissue, developmental and subcellular distribution patterns, and the preferred in vivo

The coordinates are deposited in the PDB database with accession number 1F67.

Color Plates for this article are on pages 591–593.

Corresponding author: Udo C.T. Oppermann, Department of Medical Biochemistry and Biophysics, Karolinska Institutet, S-171 77 Stockholm, Sweden. Fax: + 46 8 33 74 62. *E-mail:* Udo.Oppermann@mbb.ki.se (U. Oppermann)

655 Avenue of the Americas, New York, NY 10010

1093-3263/01/$–see front matter
PII S1093-3263(00)00098-X

reaction direction.[3,7–9] They all belong, except for the type 5 17β-HSD, to the SDR enzyme family.[3] The complex enzymological pattern is further complicated by the fact, that some of these 17β-HSDs display further substrate specificities. Accordingly, type 4 17β-HSD is a peroxisomal enzyme involved in β-oxidation,[3] type 9 17β-HSD has retinol dehydrogenase and 3α-HSD activities,[7] and the mitochondrial 17β-HSD type 10 (17β-HSD-10) has L-hydroxyacyl CoA dehydrogenase activity and displays 3α-HSD activity with several androgen steroid substrates.[8,10] Finally, several of the 17β-HSDs act as xenobiotic phase I enzymes, catalyzing the reduction of carbonyl moieties of several xenobiotic compounds.[11] A role in amyloid β-peptide (Aβ) mediated neurodegeneration has been ascribed to 17β-HSD-10. This enzyme binds to the Aβ-peptide and consequently mediates enhanced apoptosis. For this reason, 17β-HSD-10 was initially termed ERAB (*e*ndoplasmic *r*eticulum associated *A*β *b*inding protein) or type II L-hydroxyacyl CoA dehydrogenase.[8,10,12] To understand the multiple substrate specificities of human 17β-HSD-10, we performed molecular modelling and substrate docking calculations of this isozyme.

METHODOLOGY

Homology Modelling of Human 17β-HSD-10

The amino acid sequence of human 17β-HSD-10 was modelled into the known three-dimensional structure of the homologue 7α-HSD (PDB code 1FMC)[13] from *E. coli* using the ICM software package (version 2.7, Molsoft LLC, San Diego, CA).[14] Initial alignments were created with the ZEGA algorithm[15] implemented in ICM using the Gonnet matrix[16] with normalized gap open/extension penalties of 2.4/0.15. The modelling proceeded in an iterative fashion with manual adjustment of the alignment guided by the multiple alignment and the predicted secondary structure elements using PSIPRED[17] and PHD.[18] The coordinates of the NAD^+ from the template 1FMC were used for the initial placement of the NAD^+. Energy minimization at 300 K was used to relieve unfavorable interatomic interactions in the model. The method used was the conjugate gradient method with a solvation term added to the energy calculations. The solvation energy was evaluated on the basis of the atomic accessible surfaces proposed by Wesson and Eisenberg[19] with a dielectric constant of 4 kcal/mol for the protein interior and 78.5 kcal/mol for the solvent region.[14] After minimization, the energy values for the models were in the same order of magnitude as those for the used template.

Docking Analysis of Substrates

The steroid substrates were retrieved[20] in MDL/SDF format and charges were assigned according to the Merck molecular force field.[21] The docking calculations were performed in ICM with a flexible side-chain technique.[22] The position was arbitrarily chosen, the bond angles of the substrates had full freedom, and the side-chain χ-angles of the enzyme were free within 7 Å from the substrate. The backbone was kept rigid during the docking calculations. Distance restraints (1.8–2.2 Å) were imposed between Tyr168 and the hydroxyl group of the steroid, and between NAD^+ and the hydrogen involved in the hydride transfer. Each docking calculation was repeated three times with random starting positions in the active site channel and they all yielded essentially identical results. Each Monte Carlo simulation was run for 200,000 steps.

Sequence Comparisons

Sequences homologous to ERAB were extracted from the KIND nonredundant database[23] and aligned using ClustalW.[24] The residue identity between 7α-HSD and human type 10 17β-HSD was 29.4% (excluding gaps).

RESULTS AND DISCUSSION

Homology Modelling of Human ERAB/ 17β-HSD-10

A structural model of human type 10 17β-HSD was obtained using the known 1.8-Å-resolution structure of the related SDR enzyme 7α-HSD (1FMC).[13] The model is shown in Color Plate 1 and displays the typical fold of SDR enzymes.[5,25] The subunit of 17β-HSD-10 has a single-domain α/β fold structure with the characteristic dinucleotide cofactor-binding motif (Rossmann fold). This consists of a central twisted, 7-stranded β-sheet flanked on each side by three α-helices. The secondary structure elements within the subunit shown are indicated in the multiple sequence alignment (Figure 1). The 1FMC structure was used also because it is a ternary complex with substrate bound. This consideration is important since several members of the SDR family display an induced fit mechanism with rearrangement of the substrate binding region from disordered to ordered structures during catalysis.[13,25,26]

The C-terminal loop regions of some SDR enzymes (from residues Ala256 to Pro261 in 17β-HSD-10) are important in active-site closure mechanisms or subunit interactions and constitute the most variable segment in SDR enzymes.[5,27] This part cannot be reliably predicted by modelling approaches, and therefore was omitted from final refinements. The N-terminal part of 17β-HSD-10 harbors the noncleavable mitochondrial-targeting signal (unpublished data). During import this segment is likely to adopt a secondary structure that is different from the final folded state.

Quality of the Model

Different validation tools assessed the quality of the models obtained. We performed calculations of side-chain packing, of main-chain dihedral bond angles, and free energy, using the programs WHATIF[28] and PROCHECK.[29] The results are given in Table 1. The rmsd between the templates and the model were calculated and were 2.55 Å for the backbone of the protein, where the differences in loop regions accounted for the largest part of the difference. Ramachandran plot statistics (using PROCHECK[29]) derived from the coordinates of the human 17β-HSD-10 model showed that 86% of the main-chain dihedral angles are found in the most favorable regions.

Geometry of the Active Site and Substrate Cleft

The putative active site of human 17β-HSD-10 is found near the C-terminal ends of the β-strands that constitute the central β-sheet (Color Plate 2). This region forms a wide and

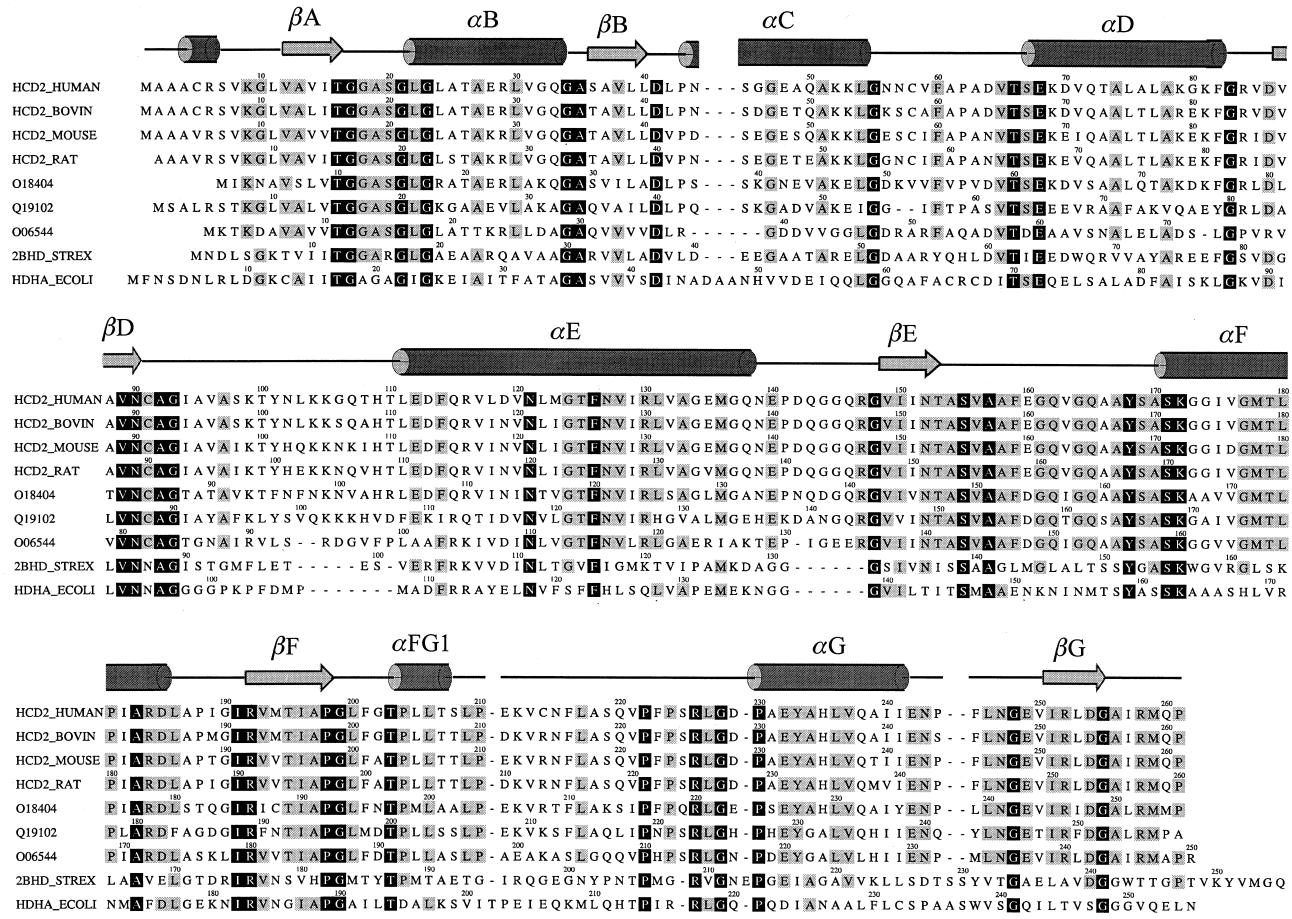

Figure 1. Alignment of mammalian type 10 17β-HSD-10 forms, bacterial hydroxysteroid dehydrogenases and homologues from eukaryotes. 17β-HSD-10 from human (HCD2_HUMAN), bovine (HCD2_BOVIN), mouse (HCD2_MOUSE) and rat (HCD2_RAT) were compared with the two closest homologues with known three-dimensional structure (3α/20β-HSD from Streptomyces hydrogenans (2BHD_STREX) and 7α-HSD from Escherichia coli (HDHA_ECOLI)), and homologous sequences found in the KIND database from Drosophila melanogaster (TrEMBL ID O18404), and Caenorhabditis elegans (Q19102 and O06544). Conserved residues are highlighted by boxing (black: all sequences; gray seven or more sequences). Secondary structure motifs as determined from human 17β-HSD-10 are indicated by cylinders (α-helices) or arrows (β-strands) above the alignment. The alignment figure was created with the program PRALIN.

mainly hydrophobic cleft, composed of secondary structural elements βD, βE, and βF. Variant modes of SDR active-site composition have been described, either by contribution of residues from only one subunit or as active sites formed by residues from different subunits.[13,25] As in 7α-HSD, the catalytic site of 17β-HSD-10 is composed of residues from one subunit. In analogy to other determined SDR structures, this region described can be securely assigned as the active site. Coenzyme binding occurs at the bottom of the channel by association to the central β-sheet of the Rossmann fold. Substrate binding occurs toward the opening of the cleft. A polar region at the bottom of the cleft, composed of the highly conserved residues Ser155, Tyr168, and Lys172, constitutes the triad of catalytically active residues, promoting hydride transfer between substrate and coenzyme. The essential character of these residues has been demonstrated in several studies.[5,30–32] Accordingly, Tyr168 acts as the catalytic base, facilitated by lowering the Tyr-OH pK_a through the proximity of the Lys172 side-chain to the Tyr-OH. The Lys side-chain further forms a bifurcated hydrogen bond to the nicotinamide ribose and thus stabilizes coenzyme binding as determined in the ternary complex of 7α-HSD.[13] Thus, essential constraints for catalysis in SDR enzymes are established by hydrogen bonding distances between Tyr-O_η and substrate, and between Ser-H_γ and substrate, the latter to stabilize intermediate states or to position the substrate.[30,31,33] The conserved Ser appears to be essential in SDR enzymes, mediating reduction and oxidation reactions of carbonyls and alcohols, respectively.[31–33] However, the conserved Ser is missing in some SDR enzymes, like dihydropteridine reductase.[34] This subset of enzymes specifically catalyzes the reduction of intracyclic C—N bonds between tetrahydrobiopterin and the "quinonoid" dihydrobiopterin, a reaction apparently requiring a modified SDR reaction mechanism without the catalytic Ser.[34] In other enzymes, such as prokaryotic 3α-HSD,[35] the apparent lack of a "conserved" Ser is compensated by another Ser at a different position, highlighting the essential

Table 1. Quality of main-chain and side-chain parameters of modeled human 17β-HSD-10. The model is verified at 2 Å resolution.

	Comparison values				
Stereochemical parameter	No. of data pts	Parameter value	Typical value	Band width	No. of bandwidths from mean
Stereochemistry of main-chain					
%-tage residues in A, B, L	216	85.6	83.8	10.0	0.2
Omega anglest dev	260	8.4	6.0	3.0	0.8
Bad contacts 100 residues	3	1.1	4.2	10.0	−0.3
Zeta angle st dev	230	1.6	3.1	1.6	−0.9
H-bond energy st dev	173	0.7	0.8	0.2	−0.3
Overall G-factor	261	−0.1	−0.4	0.3	1.1
Stereochemistry of side-chain					
Chi-1 gauche minus st dev	15	13.2	18.1	6.5	−0.8
Chi-1 trans st dev	76	15.5	19.0	5.3	−0.7
Chi-1 gauche plus st dev	91	15.2	17.5	4.9	−0.5
Chi-1 pooled st dev	182	15.2	18.2	4.8	−0.6
Chi-2 trans st dev	57	13.7	20.4	5.0	−1.3

character of Ser residues in alcohol oxidation/carbonyl reduction by SDR enzymes.[36]

Residues, whose side-chains line up the cleft and presumably participate in substrate binding, were found to be Ala95, Ser155, Val156, Ala157, Gln162, Gln165, Tyr168, Leu200, Leu205, Leu206, Leu209, and Pro261. The last residue is part of the opening of the active site channel. The alignment of the species variants of 17β-HSD-10 (Figure 1) shows conservation of all these residues except the changes of Ala95Gly in O06544, Leu205Met in O18404, and Pro261Ala in Q19102.

Docking of Substrates

Substrate docking experiments were carried out to investigate the structure–function relationships of the model. For that reason, ternary structure simulations with various substrates were performed as described in the Methodology section. The data obtained are given in Table 2 and explain the functional experimental characteristics[8,10,37–41] of the enzyme. Steroids used for docking calculations were different molecules of the androstane, estrogen and bile acid classes.

As indicated above, the main constraints for catalysis to

Table 2. Results from docking calculations for human 17β-HSD-10. Distances shown are between the different substrates and catalytic residues Ser155, Tyr168 and coenzyme in the model. Kinetic parameters are from the literature.[10,38,39,41] Abbreviations: ND: not determined; NA: no activity.

	Distances (Å)			Kinetic Parameters
Activity/Substrate	H-Tyr168 O_η	H-NAD C_4	O-Ser155 H_γ	K_m (μM)
3α-HSD activity				
3α,5α-androstane,17-one	1.89	2.23	1.96	45 ± 9.3[10]
3β,17β-diol,5α-androstane	2.08	2.24	4.31	ND
3β,5α-androstane,17-one	2.20	2.24	4.28	ND
UDCA (3α-OH)	2.21	2.23	4.52	NA[39]
*iso*UDCA (3β-OH)	2.21	2.24	4.26	NA[39]
17β-HSD activity				
3β,17β-diol,5α-androstane	2.10	2.24	1.89	34 ± 2.4[10]
dihydrotestosterone	2.28	2.24	1.89	112 ± 18[10]
estradiol	2.20	2.25	1.81	43 ± 2.1[38]
testosterone	2.20	2.24	1.92	ND
3-hydroxyacyl CoA dehydrogenase activity				
L-3-hydroxybutyryl-CoA	1.81	2.23	1.88	68.9[41]
D-3-hydroxybutyryl-CoA	2.20	2.22	2.86	ND

take place are specific hydrogen bonds formed between substrate OH/oxo group and essential enzyme residues (Tyr168 and Ser155), which result in hydride transfer to the specifically oriented reduced/oxidized coenzyme. Using the steroids indicated, site- and stereo-specific conversions were simulated. The reaction resulting in 3α-OH/3-oxo conversion can be predicted to occur with 5α reduced steroids such as 3α-ol,5α-androstane,17-one, with optimal distances for the 3α-OH hydrogen bonding to the tyroxyl O_η 168 of 1.89 Å, to C_4 of NAD^+ of 2.23 Å, and a distance between 3α-OH oxygen to Ser155 side-chain H_γ of 1.96 Å. Oxidation of the 3β-hydroxyl as in 3β-ol,5α-androstane,17-one, or 3β-ol,5α-androstane,17β-ol is not possible due to a predicted distance of over 4 Å between the 3β-OH oxygen and the Ser155 side chain. This distance will not allow the hydrogen bond necessary for catalysis. Docking simulations with 5β reduced steroids, such as bile acids (*iso*UDCA and UDCA) reveal again unfavorable distances to the Ser155, thus preventing catalysis to take place. These results are supported by experimental studies.[8,39] The 17β-OH dehydrogenase reaction is possible with steroids of the androgen and estrogen classes, yielding favorable geometry and distances for 3β,17β,5α-androstane-diol, testosterone, 5α-dihydrotestosterone, and estradiol, as indicated in Table 2. These predicted properties again correlate with experimental data.[8]

The substrate-interacting residues of the active site allow accommodation of the different configuration of the steroid substrates and surfaces as demonstrated in Color Plate 2 and Color Plate 3. The residues involved in binding to the steroid surface stabilize the substrate position for catalysis to take place. These residues are mainly hydrophobic residues Val156, Ala157, Leu200, Leu205, Leu206 and Leu209, which form the necessary contacts to the hydrophobic steroid surfaces.

Besides the above described computer-assisted probing with steroids, docking of L-3-hydroxyacyl-CoA and D-3-hydroxyacyl-CoA was performed to the active site of the model. This simulation indicates that the L-form can be preferentially accepted as substrate with excellent distances to the active site residues (Tyr168 O_η 1.81 Å, NAD C_4 2.23 Å, and Ser H_γ 1.88 Å), whereas the D-form displays unfavorable geometry and distances to the active site Ser. Furthermore, the acyl group proximal to the sulphur binds to Tyr168 besides the D3-hydroxyl group, indicating that the reaction of this stereo isomer is not likely to occur. These data support the experimental observation that human 17β-HSD-10 also acts as L-OH-acyl CoA dehydrogenase.[39,41] Thus, the enzyme is involved in the third step of the mitochondrial β-oxidation cycle of fatty acids, besides its ability to participate in the metabolic conversion of different classes of steroid hormones.

CONCLUSIONS

Structure Prediction by Homology Modelling

Homology modelling is becoming increasingly important in predicting the fold of proteins having homologues with known three-dimensional structures. Although we are still far away from replacing high-resolution structures by prediction methods, the approach presented in this study has its value in explaining the structural basis for steroid substrate recognition and specificity of a defined member of the SDR family. Also, as prediction methods are improving and the numbers of available three-dimensional structures increase this approach will be more and more used in the future. The well-established close-to identical basic folding pattern of SDR enzymes allows the modelling of essential parts of the enzyme, in this case including coenzyme binding and active sites. The results obtained clearly allow us to correlate and explain experimental data and molecular properties of the enzyme. The model structure obtained will be instrumental and of value for further experimental projects. These may include predicting further substrate and inhibitor specificities or binding modes, explaining possible effects of genetic variants, and will form a rational basis for design of site-directed mutagenesis work to explore further the biological function.

Biological and Medical Implications

Human 17β-HSD-10 is a mitochondrial enzyme with several enzymatic functions. These are apparently related to its ability to promote neuronal apoptosis and neurodegeneration.[12,39,40] This enzyme displays 17β-HSD activity toward estrogens and androgens, 3α-HSD activity toward androgens and acts as L-hydroxyacyl CoA dehydrogenase in the β-oxidation of fatty acids. 17β-HSD-10 is widely expressed and found in high levels in brain, liver, and gonads. Whereas the biological features and properties of the enzyme are investigated in detail in several laboratories, structure determination and correlation to enzyme activity have not been reported. In this respect, ternary complexes with coenzyme and substrate will be helpful to fully characterize the enzyme molecule. At present, no specific inhibitors for 17β-HSD-10 are available, which could aid in the interpretation of structure–function relationships, as demonstrated with 3α/20β-HSD and carbenoxolone.[42] We have presented a molecular model of the enzyme, of value in the interpretation of substrate characteristics and useful for future inhibitor development approaches in several human diseases. These may include novel treatment strategies in unrelated diseases such as Alzheimer's disease or sex-steroid-dependent cancer forms.

Note added in proof

While this paper was in print, the crystal structure of rat 17β-HSD-10 was reported by Powell et al., *J. Mol. Biol.* 2001, **303,** 311–327.

ACKNOWLEDGMENTS

This project was supported by grants from the European Commission (Bio4-CT97-2123), the Swedish Medical Research Council (13X-3532 and 13X-12564), the Swedish Foundation for Strategic Research, Karolinska Institutet, Magn. Bergvall Foundation, Åke Wiberg Foundation, and Loo och Hans Ostermann Foundation.

REFERENCES

1 Penning, T.M. Molecular endocrinology of hydroxysteroid dehydrogenases. *Endocr. Rev.* 1997, **18**, 281–305
2 Stewart, P.M., and Krozowski, Z.S. 11 β-Hydroxysteroid dehydrogenase *Vitam. Horm.* 1999, **57**, 249–324
3 Peltoketo, H., Luu-The, V., Simard, J., and Adamski, J. 17β-hydroxysteroid dehydrogenase (HSD)/17-ketoster-

oid reductase (KSR) family; nomenclature and main characteristics of the 17HSD/KSR enzymes. *J. Mol. Endocrinol.* 1999, **23**, 1–11
4 Jez, J.M., Flynn, T.G., and Penning, T.M. A new nomenclature for the aldo-keto reductase superfamily. *Biochem. Pharmacol.* 1997, **54**, 639–647
5 Jörnvall, H., Persson, B., Krook, M., Atrian, S., Gonzàlez-Duarte, R., Jeffery, J., and Ghosh, D. Short-chain dehydrogenases/reductases (SDR). *Biochemistry* 1995, **34**, 6003–6013
6 Oppermann, U.C., Persson, B., Filling, C., and Jörnvall, H. Structure–function relationships of SDR hydroxysteroid dehydrogenases. *Adv. Exp. Med. Biol.* 1997, **414**, 403–415
7 Napoli, J.L. Interactions of retinoid binding proteins and enzymes in retinoid metabolism. *Biochim. Biophys. Acta.* 1999, **1440**, 139–162
8 He, X.Y., Merz, G., Mehta, P., Schulz, H., and Yang, S.Y. Human brain short chain L-3-hydroxyacyl coenzyme A dehydrogenase is a single-domain multifunctional enzyme. Characterization of a novel 17β-hydroxysteroid dehydrogenase. *J. Biol. Chem.* 1999, **274**, 15014–15019
9 Li, K.X., Smith, R.E., and Krozowski, Z.S. Cloning and expression of a novel tissue specific 17β-hydroxysteroid dehydrogenase. *Endocr. Res.* 1998, **24**, 663–667
10 He, X., Merz, G., Yang, Y., Pullakart, R., Mehta, P., Schulz, H., and Yang, S. Function of human brain short chain L-3-hydroxyacyl coenzyme A dehydrogenase in androgen metabolism. *Biochim. Biophys. Acta* 2000, **1484**, 267–277
11 Oppermann, U.C., and Maser, E. Molecular and structural aspects of xenobiotic carbonyl metabolizing enzymes. Role of reductases and dehydrogenases in xenobiotic phase I reactions. *Toxicology* 2000, **144**, 71–81
12 Yan, S.D., Fu, J., Soto, C., Chen, X., Zhu, H., Al-Mohanna, F., Collison, K., Zhu, A., Stern, E., Saido, T., Tohyama, M., Ogawa, S., Roher, A., and Stern, D. An intracellular protein that binds amyloid-beta peptide and mediates neurotoxicity in Alzheimer's disease. *Nature* 1997, **389**, 689–695
13 Tanaka, N., Nonaka, T., Tanabe, T., Yoshimoto, T., Tsuru, D., and Mitsui, Y. Crystal structures of the binary and ternary complexes of 7 alpha-hydroxysteroid dehydrogenase from Escherichia coli. *Biochemistry* 1996, **35**, 7715–7730
14 Cardozo, T., Totrov, M., and Abagyan, R. Homology modelling by the ICM method. *Proteins* 1995, **23**, 403–414
15 Abagyan, R.A., and Batalov, S. Do aligned sequences share the same fold? *J. Mol. Biol.* 1997, **273**, 355–368
16 Gonnet, G.H., Cohen, M.A., and Benner, S.A. Exhaustive matching of the entire protein sequence database. *Science* 1992, **256**, 1443–1445
17 Jones, D.T. Protein secondary structure prediction based on position-specific scoring matrices. *J. Mol. Biol.* 1999, **292**, 195–202
18 Rost, B., Sander, C., and Schneider, R. PHD–an automatic mail server for protein secondary structure prediction. *Comput. Appl. Biosci.* 1994, **10**, 53–60
19 Wesson, L., and Eisenberg, D. Atomic solvation parameters applied to molecular dynamics of proteins in solution. *Protein Sci.* 1992, **1**, 227–235
20 Wagener, M., Sadowski, J., and Gasteiger, J. Autocorrelation of molecular surface properties for modelling corticosteroid binding globulin and cytosolic AH receptor activity by neural networks. *J. Am. Chem. Soc.* 1995, **117**, 7769–7775
21 Halgren, T.A. Merck Molecular Force Field. I.-V. *J. Comp. Chem.* 1995, **17**, 490–641
22 Totrov, M., and Abagyan, R. Flexible protein-ligand docking by global energy optimization in internal coordinates. *Proteins Suppl.* 1997, **1**, 215–220
23 Kallberg, Y., and Persson, B. KIND—a non-redundant protein database. *Bioinformatics* 1999, **15**, 260–261
24 Thompson, J.D., Higgins, D.G., and Gibson, T.J. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.* 1994, **22**, 4673–4680
25 Ghosh, D., Weeks, C.M., Grochulski, P., Duax, W.L., Erman, M., Rimsay, R.L., and Orr, J.C. Three-dimensional structure of holo 3α,20β-hydroxysteroid dehydrogenase: a member of a short-chain dehydrogenase family. *Proc. Natl. Acad. Sci. U.S.A.* 1991, **88**, 10064–10068
26 Benach, J., Atrian, S., Gonzalez-Duarte, R., and Ladenstein, R. The refined crystal structure of Drosophila lebanonensis alcohol dehydrogenase at 1.9 Å resolution. *J. Mol. Biol.* 1998, **282**, 383–399
27 Krozowski, Z. The short-chain alcohol dehydrogenase superfamily: variations on a common theme. *J. Steroid. Biochem. Mol. Biol.* 1994, **51**, 125–130
28 Rodriguez, R., Chinea, G., Lopez, N., Pons, T., and Vriend, G. Homology modelling, model and software evaluation: three related resources. *CABIOS* 1998, **14**, 523–528
29 Laskowski, R.A., MacArthur, M.W., Moss, D.S., and Thornton, J.M. PROCHECK: a program to check the stereochemical quality of protein structures. *J. Appl. Cryst.* 1993, **26**, 283–291
30 Obeid, J., and White, P.C. Tyr-179 and Lys-183 are essential for enzymatic activity of 11 beta-hydroxysteroid dehydrogenase. *Biochem. Biophys. Res. Commun.* 1992, **188**, 222–227
31 Tanabe, T., Tanaka, N., Uchikawa, K., Kabashima, T., Ito, K., Nonaka, T., Mitsui, Y., Tsuru, M., and Yoshimoto, T. Roles of the Ser146, Tyr159, and Lys163 residues in the catalytic action of 7α-hydroxysteroid dehydrogenase from *Escherichia coli*. *J. Biochem.* 1998, **124**, 634–641
32 Oppermann, U.C., Filling, C., Berndt, K.D., Persson, B., Benach, J., Ladenstein, R., and Jörnvall, H. Active site directed mutagenesis of 3β/17beta-hydroxysteroid dehydrogenase establishes differential effects on short-chain dehydrogenase/reductase reactions. *Biochemistry* 1997, **36**, 34–40
33 Winberg, J.O., Brendskag, M.K., Sylte, I., Lindstad, R.I., and McKinley-McKee, J.S. The catalytic triad in *Drosophila* alcohol dehydrogenase: pH, temperature and molecular modelling studies. *J. Mol. Biol.* 1999, **294**, 601–616
34 Varughese, K.I., Skinner, M.M., Whiteley, J.M., Matthews, D.A., and Xuong N.H. Crystal structure of rat liver dihydropteridine reductase. *Proc. Natl. Acad. Sci. U.S.A.* 1992, **89**, 6080–6084
35 Möbus, E., and Maser, E. Molecular cloning, overexpression, and characterization of steroid-inducible 3α-

hydroxysteroid dehydrogenase/carbonyl reductase from *Comamonas testosteroni*. A novel member of the short-chain dehydrogenase/reductase superfamily. *J. Biol. Chem.* 1998, **273**, 30888–30896
36 Maser, E. Personal communication
37 He, X.Y., Schulz, H., and Yang, S.Y. A human brain L-3-hydroxyacyl-coenzyme A dehydrogenase is identical to an amyloid β-peptide-binding protein involved in Alzheimer's disease. *J. Biol. Chem.* 1998, **273**, 10741–10746
38 He, X.Y., Yang, Y.Z., Schulz, H., and Yang, S.Y. Intrinsic alcohol dehydrogenase and hydroxysteroid dehydrogenase activities of human mitochondrial short-chain L-3-hydroxyacyl-CoA. *Biochem. J.* 2000, **345**, 139–143
39 Oppermann, U.C., Salim, S., Tjernberg, L.O., Terenius, L., and Jörnvall, H. Binding of amyloid β-peptide to mitochondrial hydroxyacyl-CoA dehydrogenase (ERAB): regulation of an SDR enzyme activity with implications for apoptosis in Alzheimer's disease. *FEBS Lett.* 1999, **451**, 238–242
40 Yan, S.D., Shi, Y., Zhu, A., Fu, J., Zhu, H., Zhu, Y., Gibson, L., Stern, E., Collison, K., Al-Mohanna, F., Ogawa, S., Roher, A., Clarke, S.G., and Stern, D.M. Role of ERAB/L-3-hydroxyacyl-coenzyme A dehydrogenase type II activity in Aβ-induced cytotoxicity. *J. Biol. Chem.* 1999, **274**, 2145–2156
41 Salim, S., Filling, C., Mårtensson, E., and Oppermann U.C. Lack of quinone reductase activity suggests that amyloid-β peptide/ERAB induced lipid peroxidation is not directly related to production of reactive oxygen species by redoxcycling. *Toxicology* 2000, **144**, 163–168
42 Ghosh, D., Erman, M., Wawrzak, Z., Duax, W.L., and Pangborn, W. Mechanism of inhibition of 3α, 20β-hydroxysteroid dehydrogenase by a licorice-derived steroidal inhibitor. *Structure* 1994, **2**, 973–980