Pergamon

0960-0760(95)00210-3

J. Steroid Biochem. Molec. Biol. Vol. 55, No. 5/6, pp. 589–600, 1995

0960-0760/95 $9.50 + 0.00

Structures Important in Mammalian 11β- and 17β-Hydroxysteroid Dehydrogenases

Igor Tsigelny[1] and Michael E. Baker[2]*

[1]Department of Chemistry and Biochemistry, 0654 and [2]Department of Medicine, 0623B, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0623, U.S.A.

We have used the X-ray crystallographic structures of rat and human dihydropteridine reductase and *Streptomyces hydrogenans* 20β-hydroxysteroid dehydrogenase to model parts of the 3-dimensional structure of human 11β- and 17β-hydroxysteroid dehydrogenases. We use this information along with previous results from studies of *Drosophila* alcohol dehydrogenase mutants to analyze the structures in binding sites for NAD(H) and NADP(H) in 11β-hydroxysteroid dehydrogenase-types 1 and 2. We also examine the structure of an α-helix at catalytic site of 17β-hydroxysteroid dehydrogenase-types 1, 2, 3, and 4. This α-helix contains a highly conserved tyrosine and lysine. Adjacent to the carboxyl side of this lysine is a site proposed to be important in subunit association. We find that 11β- and 17β-hydroxysteroid dehydrogenases-type 1 have the same residues at the "anchor site" and conserve other stabilizing features, despite only 20% sequence identity between their entire sequences. Similar conservation of stabilizing structures is found in the 11β- and 17β-hydroxysteroid dehydrogenases-type 2. We suggest that interactions of the dimerization surface of α-helix F with proteins or membranes may be important in regulating activity of hydroxysteroid dehydrogenases.

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INTRODUCTION

Human 11β-hydroxysteroid, 17β-hydroxysteroid, and 15-hydroxyprostaglandin dehydrogenases belong to a large family of oxidoreductases—over 60 different enzymes are present in the release 30 of the Swiss Protein database [1–4]. These enzymes metabolize a diverse group of secondary alcohols or ketones [1–5]. Based on this substrate specificity, we have called these enzymes *sec*-alcohol dehydrogenases [5]; they are also known as short chain alcohol dehydrogenases [2, 4].

These oxidoreductases are found in bacteria, plants, and animals, where they perform many different functions. Some *sec*-alcohol dehydrogenases metabolize sugars for use as energy and a carbon source; others are important in antibiotic synthesis by soil bacteria [6], fatty acid synthesis and degradation in animals, plants and bacteria; chlorophyll synthesis in plants [5], resistance of the protozoan parasite *Leishmania* to methotrexate [7, 8], and regulation of concentrations of glucocorticoids, estrogens, androgens, and prostaglandins E_2 and $F_{2\alpha}$ in humans. The widespread occurrence and diverse functions of *sec*-alcohol dehydrogenases has stimulated research to understand their catalytic mechanism and the basis for recognition of different substrates.

SEQUENCE ANALYSES, MUTAGENESIS STUDIES, 3-DIMENSIONAL STRUCTURES, AND EVOLUTION

In this paper we combine three diverse approaches—sequence analysis, mutagenesis studies, and 3-dimensional modeling of sequences on templates of homologs—in the context of an evolutionary paradigm [1, 9] to understand structures important in hydroxysteroid dehydrogenases and other *sec*-alcohol dehydrogenases.

The availability of a large data set of *sec*-alcohol dehydrogenase sequences from phylogenetically diverse organisms provides a valuable resource for identifying catalytically important domains and residues on these dehydrogenases. Alignment of sequences from bacteria, fungi, invertebrates, and vertebrates identifies conserved residues and domains [1–5, 9], which are likely to be functionally important based on the notion that sites conserved for at least 2 billion years since

Proceedings of the Workshop on the Molecular and Cell Biology of Hydroxysteroid Dehydrogenases, Hannover, Germany, 19–22 April 1995.
*Correspondence to M. E. Baker.

eukaryotes and bacteria diverged from a common ancestor have an important role in catalysis. This approach has been useful in identifying residues for mutagenesis in *Drosophila* alcohol dehydrogenase (ADH) [10–16], human 11β-hydroxysteroid dehydrogenase (11β-HSD) type 1 [17], human 17β-hydroxysteroid dehydrogenase (17β-HSD) type 1 [18], and human 15-hydroxyprostaglandin dehydrogenase [19] to elucidate residues important in binding of nucleotide cofactors or hydride transfer. Moreover, knowledge of conserved residues and domains is useful for analyzing 3D structures determined by X-ray crystallographic analysis of rat [20, 21] and human [22] dihydropteridine reductase and *Streptomyces hydrogenans* 20β-hydroxysteroid dehydrogenase [23, 24], as well as for constructing molecular models of homologous proteins [15, 16, 25–27] using known 3D structures as templates.

CONSERVATION OF 3D STRUCTURES IN HOMOLOGOUS PROTEINS

Central to the validity of constructing a model of a protein that has less than 30% sequence identity with a template protein is that the tertiary structure is better conserved than the amino acid sequence; that is, the tertiary structure changes more slowly than the amino acid sequence in diverging proteins. In recent years, as the number of solved 3D-structures of proteins in the Brookhaven database has increased, there has been ample confirmation of this hypothesis. In fact, two proteins can have as little as 20–25% sequence identity and still have very similar 3D structures, especially in α-helices and β-strands [28–31]. Variation is found in the loops and coiled structures.

A relevant example for this paper is the comparison of the tertiary structure of rat dihydropteridine reductase [21] and *Streptomyces hydrogenans* 20β-hydroxysteroid dehydrogenase (20β-HSD) [23, 24]. As noted by Varughese *et al.* [21], despite less than 20% sequence identity between dihydropteridine reductase and 20β-HSD, the root mean square deviation for the two tertiary structures is 2 Å over 160 C_α carbon atoms. Moreover, the 3D structures of α-helix E and F in the two proteins are very similar [21]. This evidence for the conservation of 3D structure in two homologous proteins with divergence amino acid sequences is the core axiom upon which modeling of other *sec*-alcohol dehydrogenases by others [15, 25, 26] and us [16, 27] is based.

In this paper, we discuss what is known about the NAD(H) and NADP(H) binding site and the catalytic site of *sec*-alcohol dehydrogenases from evolutionary analyses, mutagenesis studies and solved 3D structures of two *sec*-alcohol dehydrogenases and apply this information to construct and analyze a 3D model of mammalian 11β-HSD and 17β-HSD.

METHODS

Molecular modeling

The structures of rat [20, 21] and human [22] dihydropteridine reductase and *S. hydrogenans* 20β-HSD [24] were taken from the Brookhaven database. The rat and human dihydropteridine reductase are resolved to 2.3 and 2.6 Å, respectively. Both enzymes contain NADH. The two dihydropteridine reductase sequences are over 95% identical and their 3D structures superimpose. *S. hydrogenans* 20β-HSD consists of a tetramer resolved to 2.6 Å. NADH was inserted into the structure of 20β-HSD by extraction from dihydropteridine reductase after it was superimposed onto 20β-HSD.

Human 11β-HSD type 1 [4] and 2 [32] and 17β-HSD type 1 [32], 2 [34] and 3 [35] and porcine 17β-HSD [36] were aligned with *S. hydrogenans* 20β-HSD for modeling. Some of these hydroxysteroid dehydrogenases have an extra segment at either the amino terminus and/or carboxyl terminus. Previously reported alignments [1–4, 9] were used to find the core structure consisting of about 255 residues in 11β-HSD types 1 and 2 that is structurally similar to the template. In this alignment, the first 190 residues of the 255 residues are reasonably well conserved among the hydroxysterioid dehydrogenases. Alignment of the C-terminal 65 residues is less certain, as this part contains gaps and insertions. We have confined our analysis of 11β-HSD type 1 and 2 to the part that aligns well with the template.

The Homology program (Biosym Technologies Inc., San Diego, California, 1995) was used to model a 255 segment of 11β-HSD type 1 and 2 on the *S. hydrogenans* 20β-HSD template. To construct a model of the α-helix F interface of the four 17β-HSDs, each sequence was aligned to the template. The modeled monomer molecules were energetically minimized separately to avoid artifactual adjustment of the side chains in the dimer interface. Minimization was done for 1000 iterations of the conjugate gradient using the Discover program of Biosym Technologies Inc., 1995. If the unsolved enzyme has a structure similar to that of the template then the modeled structure would be a reasonable approximation to the true structure. In this paper, we only discuss side chain interactions that are strong enough so that they would be expected to be present in the true structure.

RESULTS AND DISCUSSION

NAD(P)(H) binding site

Several lines of evidence—sequence analysis, mutagenesis studies, and the solved 3D structure of two *sec*-alcohol dehydrogenases—indicate that *sec*-alcohol dehydrogenases have a nucleotide binding site with many similarities to that of other classes of dehydrogenases. For many dehydrogenases, the nucleotide

binding domain consists of a β-strand, α-helix, β-strand in a fold that provides a hydrophobic pocket for the AMP part of the nucleotide cofactor [30, 37, 38]. In *sec*-alcohol dehydrogenases, this βαβ fold is at the amino terminus. The turn between the first β-strand and the α-helix is a glycine-rich segment of the form Gly–X–X–X–Gly–X–Gly. This glycine-rich segment forms a hydrophobic pocket that allows close association of adenosine monophosphate (AMP) part of the cofactor.

*Studies with Drosophila alcohol dehydrogenase**

Studies with *Drosophila* ADH, which is homologous to 17β-HSD type 1 [1–5, 39], have revealed important information about the structure of the cofactor binding site and the catalytic site. In 1981, Thatcher sequenced an inactive ADH mutant and found that glycine-15 is replaced with aspartic acid [40]. Thatcher proposed that the glycine is in the turn in the βαβ fold and that aspartic acid side chain interfered with binding of NAD^+. A series of elegant studies by Dr Georgia Chen [10, 11, 13, 16], who at the time was a graduate student in Professor Simon Chang's laboratory, confirmed and extended Thatcher's observation. Glycine was mutated to alanine [10] and the catalytic activity was reduced by 30%, due to a lower affinity of NAD^+ for ADH. Chen *et al.* also showed that an ADH mutant with a valine at position 15 is almost inactive. These results suggested that the side chain of alanine-15 and valine-15 interfered with NAD^+ binding. Most importantly, Dr Chen's measurements showed that catalytic efficiency of the alanine-15 mutant with respect to NAD^+ is lowered by about 5-fold, while catalytic efficiency with respect to the 2-propanol substrate is essentially unaltered, suggesting that the changes due

*For *Drosophila* ADH, we use sequence numbering for the recombinant enzyme, in which methionine is the first amino acid and serine is the second. Enzyme isolated from flies has serine as the first amino acid.

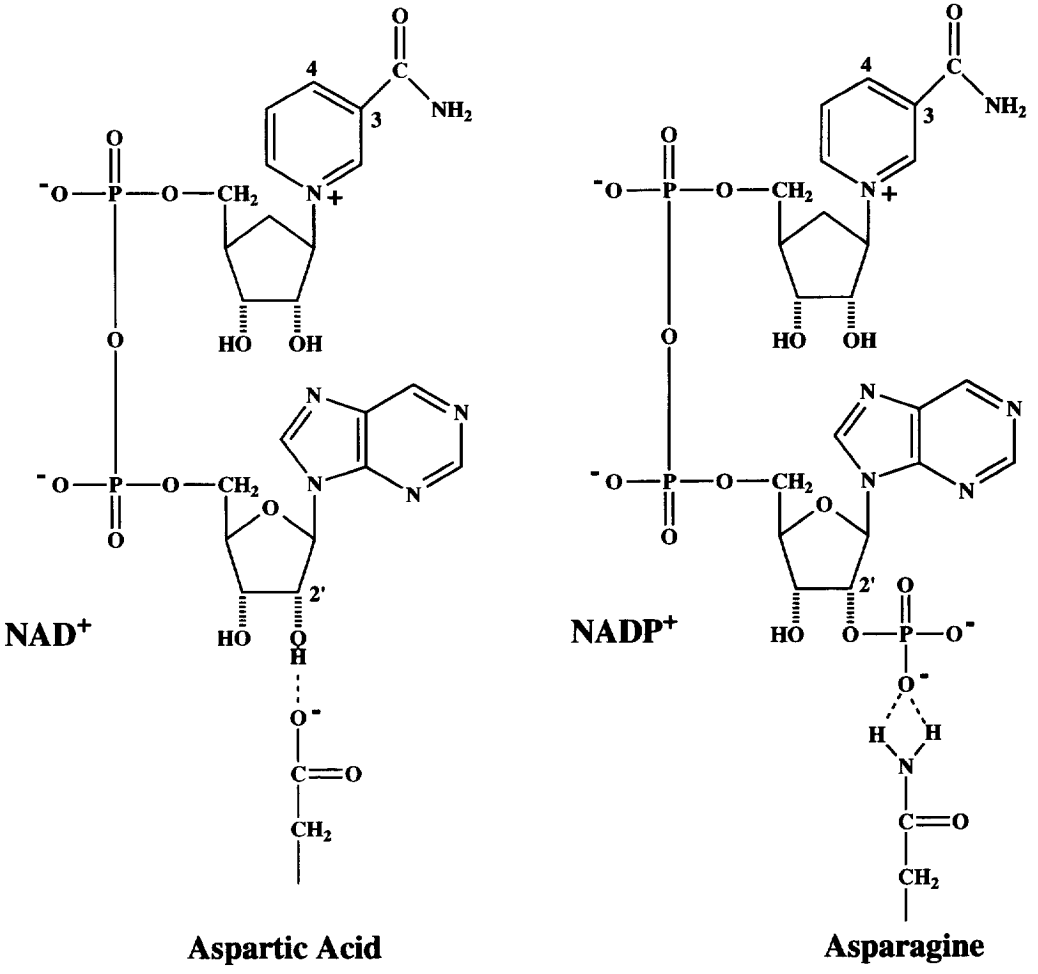

Fig. 1. Schematic of the interaction of aspartic acid-39 with NAD^+ and asparagine-39 with $NADP^+$ in *Drosophila* ADH. Aspartic acid can hydrogen bond with the 2′ hydroxyl on NAD^+. However, there is an unfavorable coulombic repulsion between aspartic acid and the 2′-phosphate on $NADP^+$, which is proposed to explain the preference of *Drosophila* ADH for NAD^+ [11, 16]. Adding a nearby amino acid with a positively charged side chain, such as arginine at position 45 to *Drosophila* ADH can compensate for the negative charge on aspartic acid-39 [16]. Alternatively, aspartic acid can be replaced by asparagine [11], which can hydrogen bond to the phosphate moiety, yielding a mutant that has about 600-fold better activity with $NADP^+$ than wild type ADH.

to this mutation in the AMP binding domain are localized, with little effect on the substrate binding domain.

Similar results were reported by Ribas dePoplana and Fothergill-Gillmore [15] for their mutation of glycine-20 to alanine, which reduced enzyme activity by about 50%. They found that the reduction of activity was due to a lower affinity of NAD^+ for the glycine-20 to alanine mutant. They proposed that the alanine side chain interfered with association of NAD^+ suggesting that glycine-20 in wild type ADH is close to the ribose moiety of AMP and that the methyl group of alanine prevents $NADP^+$ binding.

This interpretation of the ADH mutagenesis studies is consistent with orientation of NADH in the structure of dihydropteridine reductase as solved by X-ray crystallography [22] and the two 3D models of ADH [15, 16].

ADH has a strong preference for NAD^+ over $NADP^+$. It's catalytic efficiency, k_{cat}/K_m, is about 900 fold higher with NAD^+ compared to $NADP^+$; the K_m for NAD^+ is about 60-fold lower than that of $NADP^+$. If the AMP binding site is at the amino terminus, then coulombic repulsion between a negatively charged residue and the 2′-phosphate on $NADP^+$ could account for the low affinity of $NADP^+$ and the high affinity of NAD^+ because there would not be coulombic repulsion with NAD^+, which could hydrogen bond to the 2′-hydroxyl group, as shown schematically in Fig. 1. Examination of the sequence of ADH suggested that aspartic acid-39 could be the residue repelling $NADP^+$. Confirmation of the role of aspartic acid-39 in preference for NAD^+ over $NADP^+$ was accomplished by mutating aspartic acid-39 to asparagine, which increased the catalytic efficiency with $NADP^+$ binding by about 600-fold,

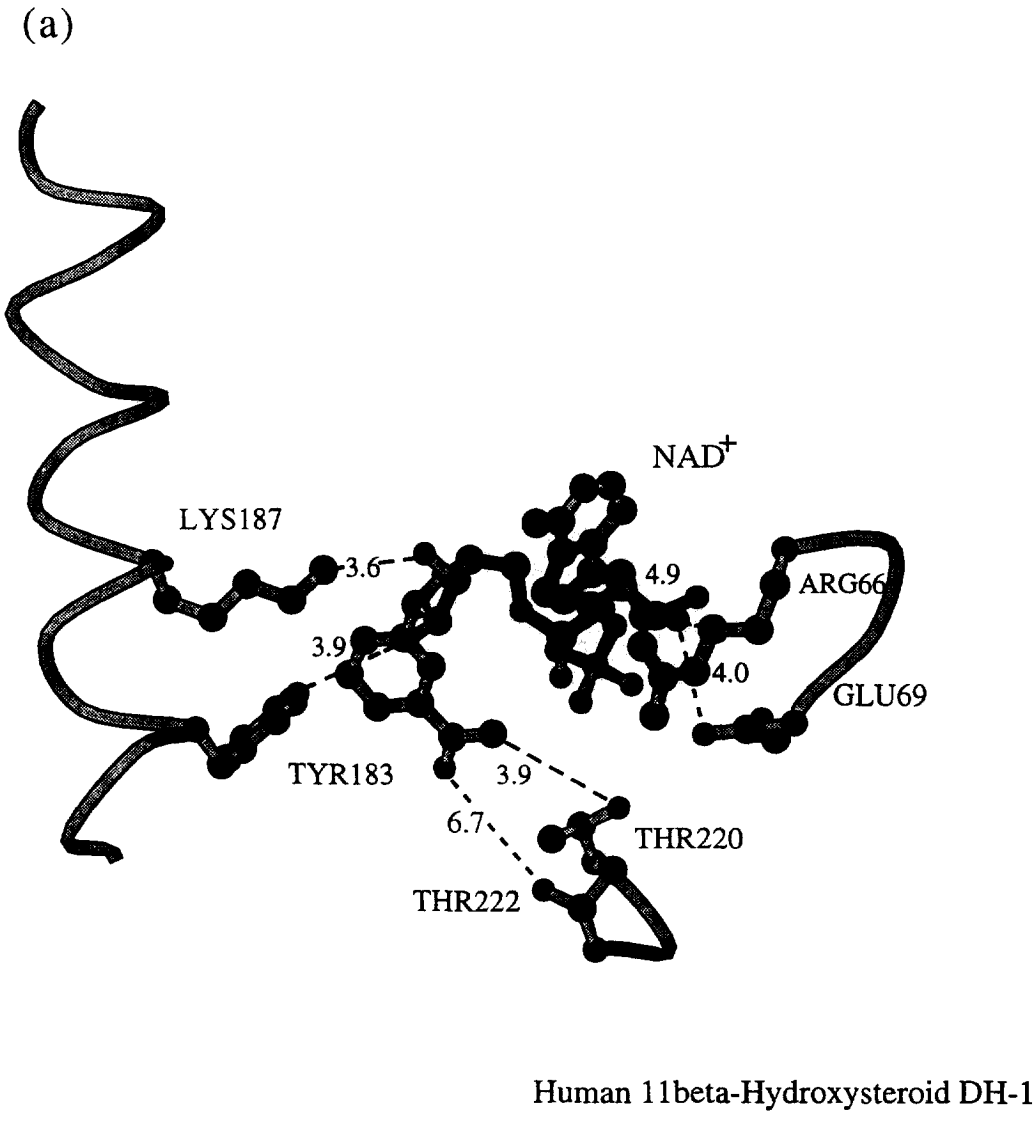

Fig. 2(a)—*legend opposite.*

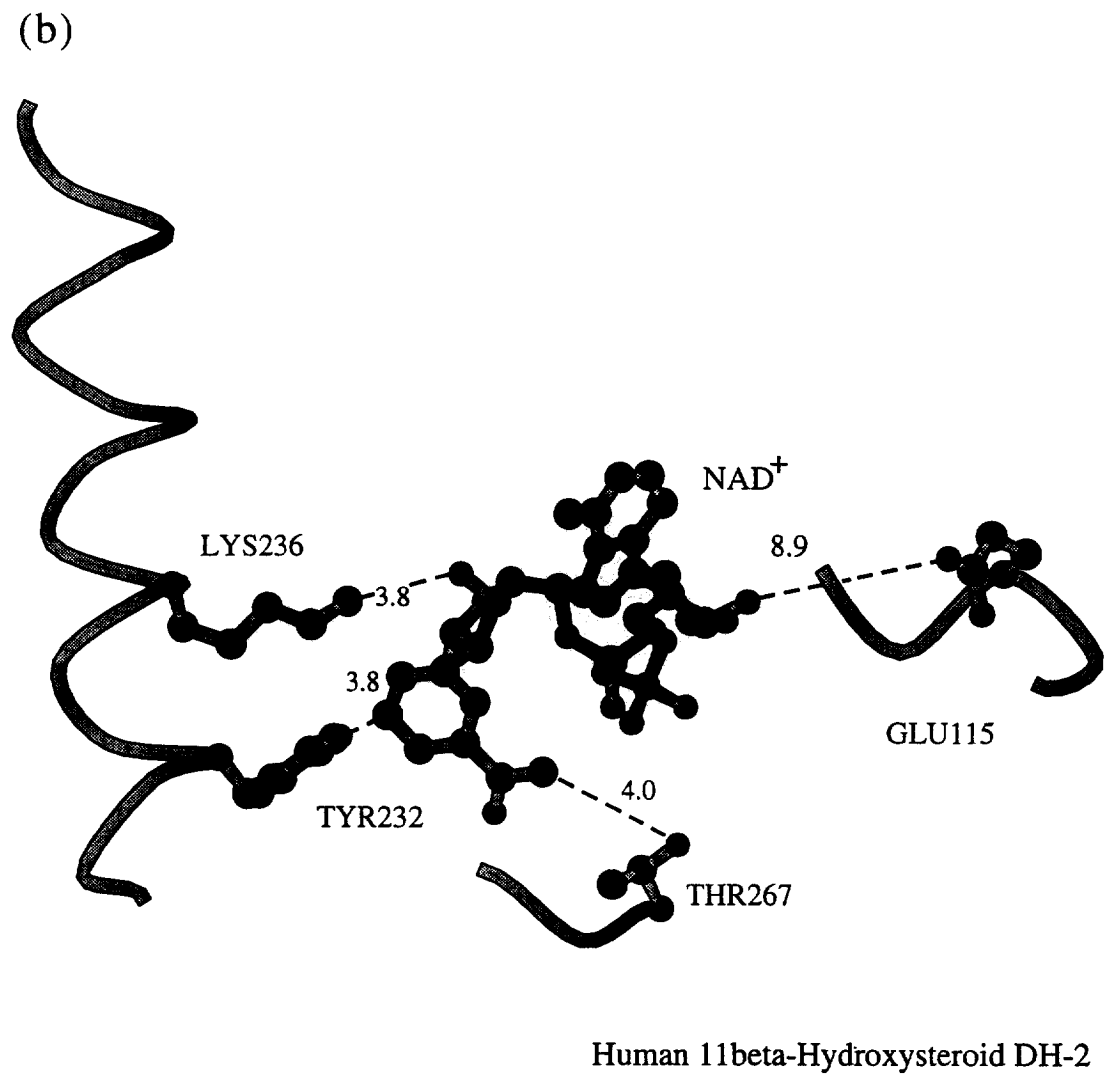

Fig. 2. The nucleotide binding domain at the amino terminus of human 11β-HSD type 1 and 2. The AMP binding domain comprises a βαβ fold that is glycine rich. 11β-HSD type 1(a) has a preference for NADPH over NADH. The type 2 counterpart (b) prefers NAD^+ over $NADP^+$. Arginine-66 in the type 1 enzyme can interact with the 2′-phosphate on NADPH. It can also shield the phosphate group from repulsive interactions with glutamic acid-69. In contrast the type 2 enzyme lacks an arginine that could compensate for the coulombic repulsion between glutamic acid-115 and $NADP^+$. Also shown are threonine residues that could hydrogen bond to nicotinamide's carboxamide moiety.

with little change in the binding and catalytic activity of NAD^+ [11].

This model of the AMP binding site on *Drosophila* ADH suggests that adding a strategically located positive charge would improve $NADP^+$ binding by neutralizing the negatively charged aspartic acid-39 and/or contributing a positive coulombic interaction with the 2′-phosphate group on $NADP^+$. This was accomplished by Chen *et al.* [16] who replaced alanine-46 with arginine and found that catalytic efficiency with $NADP^+$ improved by about 60 fold, while catalytic efficiency with NAD^+ was about 25% of that of wild type ADH. These mutagenesis studies and molecular models of ADH [15, 16] support the notion that the region around the C-terminus of the second β-strand is important for specificity of NAD(H) and NADP(H).

The coenzyme binding domain in human 11β-hydroxysteroid dehydrogenase types 1 and 2

We have used the above information in analyzing the modeled human 11β-HSD types 1 and 2 for determinants for preference for NAD(H) and NADP(H). Figure 2 shows our model of the amino terminal domains on these enzymes. In the type 1 enzyme, arginine-66 could have a favorable coulombic interaction with the 2′-phosphate on $NADP^+$ [Fig. 2(a)]. Arginine's positively charged side chain could also neutralize negative interactions between glutamic

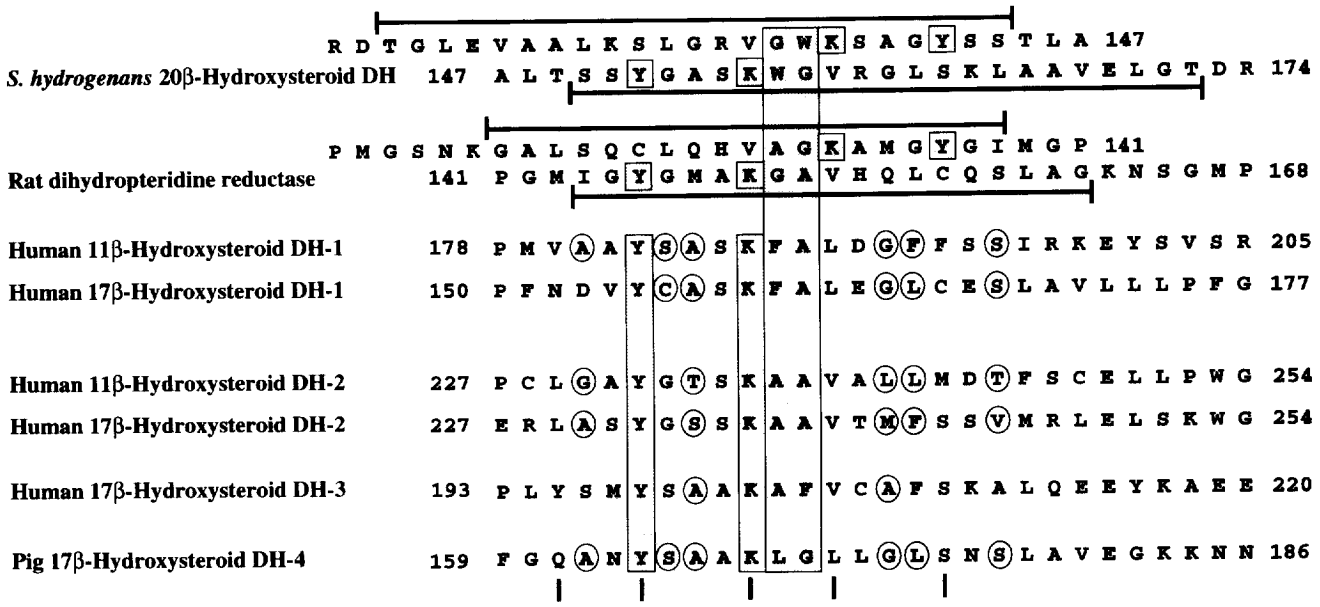

Fig. 3. Alignment of α-helix F in *S. hydrogenans* 20β-HSD and rat dihydropteridine reductase with 11β-HSD and 17β-HSD. The bars over 20β-HSD and dihydropteridine reductase show α-helix F as determined by the X-ray crystallographic analysis. Both strands of α-helix F are shown. The "anchor site" and the conserved tyrosine and lysine residues are in shaded boxes. The proposed anchor is at the midpoint of the overlapping segment of α-helix F from each monomer. Circled residues interact with each other or residues in the anchor site to stabilize the dimer, as discussed in the text. Also circled are residues in 11β-HSD that are important in dimer stability [Tsigelny and Baker, submitted]. Note the importance in dimer stability of residues betwen the conserved tyrosine and lysine residues. A short vertical bar marks residues that have side chains oriented into the substrate and cofactor binding site. The sequences of rat and human dihydropteridine reductase are identical in this segment; the two enzyme sequences are 96% identical.

acid-69 and the 2′-phosphate group. This proposed effect of arginine resembles that found by Chen *et al.* [16] in the *Drosophila* ADH arginine-46 mutant.

As shown in Fig. 2(b), human 11β-HSD type 2 contains glutamic acid-115 that would have a coulombic repulsion with the 2′-phosphate on $NADP^-$, which would destabilize binding of $NADP^+$. Mutation of glutamic acid-115 to glutamine would evaluate the contribution of this residue to specificity for NAD^+.

Two other residues appear to be important for coenzyme binding. Threonine-220 in 11β-HSD type 1 and threonine-267 in 11β-HSD type 2 can hydrogen bond to nicotinamide's carboxamide group. We also find that in 11β-HSD type 2, the face of the side chain of phenylalanine-94 is about 2.8 Å from the nicotinamide ring and its carboxamide group (not shown), which would be an important stabilizing interaction. Phenylalanine-94 is between the two canonical glycine residues in the βαβ fold, as discussed earlier in this paper. Interestingly, phenylalanine is not found between the homologous glycines in either 11β-HSD type 1 or 17β-HSD type 1, 2, 3, and 4, which instead have either leucine or isoleucine. Although our analysis of 11β-HSD type 1 indicates that isoleucine-46 has a similar hydrophobic interaction with the carboxamide moiety, the interaction is not as extensive as that of the type 2 enzyme's phenylalanine-94, due to the structural differences between the side chains on isoleucine and phenylalanine. Importantly, analyses of other homologs [2–4] indicate that isoleucine or leucine are the preferred residues for this position, suggesting that the phenylalanine-94 has a special role in catalysis by 11β-HSD type 2. Mammalian β-hydroxybutyrate dehydrogenase [41] and retinol dehydrogenase [42, 43] also have phenylalanine between the two conserved glycines.

Catalytic site

The location of the catalytic site, identification of the active residues and understanding their mechanism of action is a central goal of research on steroid dehydrogenases. As will be discussed below, the catalytic site is also part of the dimerization domain, which adds a constraint to the structure of this site.

As far as the catalytically active residue(s) are concerned, cysteine, histidine, and tyrosine are candidates for residues that could be deprotonated at physiological pH and participate in hydride transfer. A role for cysteine was eliminated by Chen *et al.*'s report that replacing both cysteines in *Drosophila* ADH did not reduce enzyme activity [10]. This focused attention on ADH's tyrosine-153 as a candidate for the catalytically active residue because sequence analyses of ADH and its homologs revealed it was in a highly conserved pentapeptide with the form Tyr–Xaa–Xaa–Xaa–Lys [1–5] near the middle of the primary sequence of the enzymes in this family. Mutagenesis of tyrosine-153 and lysine-157 in ADH [12–14] confirm their

importance in catalysis in agreement with similar studies with 11β-HSD type 1 [17], 17β-HSD type 1 [18], and 15-hydroxyprostaglandin dehydrogenase [19]. Together these studies support the hypothesis that the conserved tyrosine and lysine are at the catalytic site and important for hydride transfer.

As mentioned above, the conserved tyrosine and lysine are part of α-helix F, which is part of the dimer interface in steroid dehydrogenases, which is discussed next.

Dimer interface

Most *sec*-alcohol dehydrogenases are active as either dimers or tetramers. Analysis of rat dihydropteridine reductase by Varughese *et al.* [20] indicates that the dimer interface consists of α-helix E and α-helix F from each monomer arranged in a four α-helix bundle, a structure in which the hydrophobic surfaces on the helices form a core that yields very stable structure in a wide variety of proteins [44–47]. A four helix bundle also appears to stabilize *S. hydrogenans* 20β-HSD, a tetrameric enzyme [24]. α-helix F contains the conserved tyrosine and lysine residue, which adds a constraint to changes in the sequence of this helix. It has at least two functions: stabilizing the dimer and orienting tyrosine and lysine and other residues for optimal interaction with substrate and nucleotide cofactor.

The role of a specific site on the outer hydrophobic surface of α-helix F in dimerization was suggested recently when a *Drosophila* ADH mutant that does not form stable dimers [48] was sequenced [27]. This ADH mutant has alanine-159 replaced with threonine. A 3-D model of *Drosophila* ADH shows alanine-159 on the opposite surface of α-helix F from tyrosine-153 and lysine-157 [27]. Alanine-159 along with alanine-158 form a hydrophobic anchor that stabilizes the dimer interface. These two residues of ADH and the homologous residues in other *sec*-alcohol dehydrogenases have

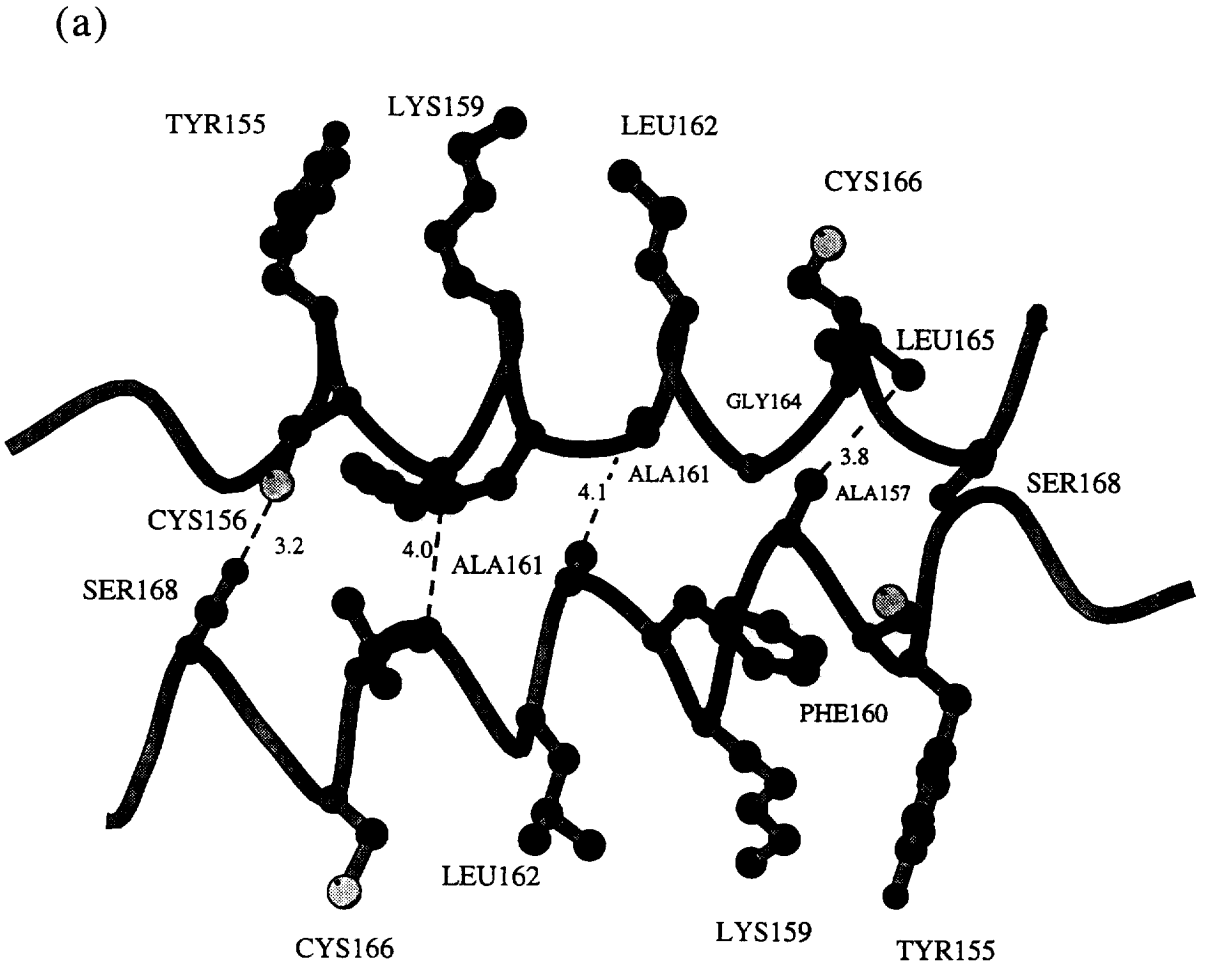

Fig. 4(a)—*legend on p. 598.*

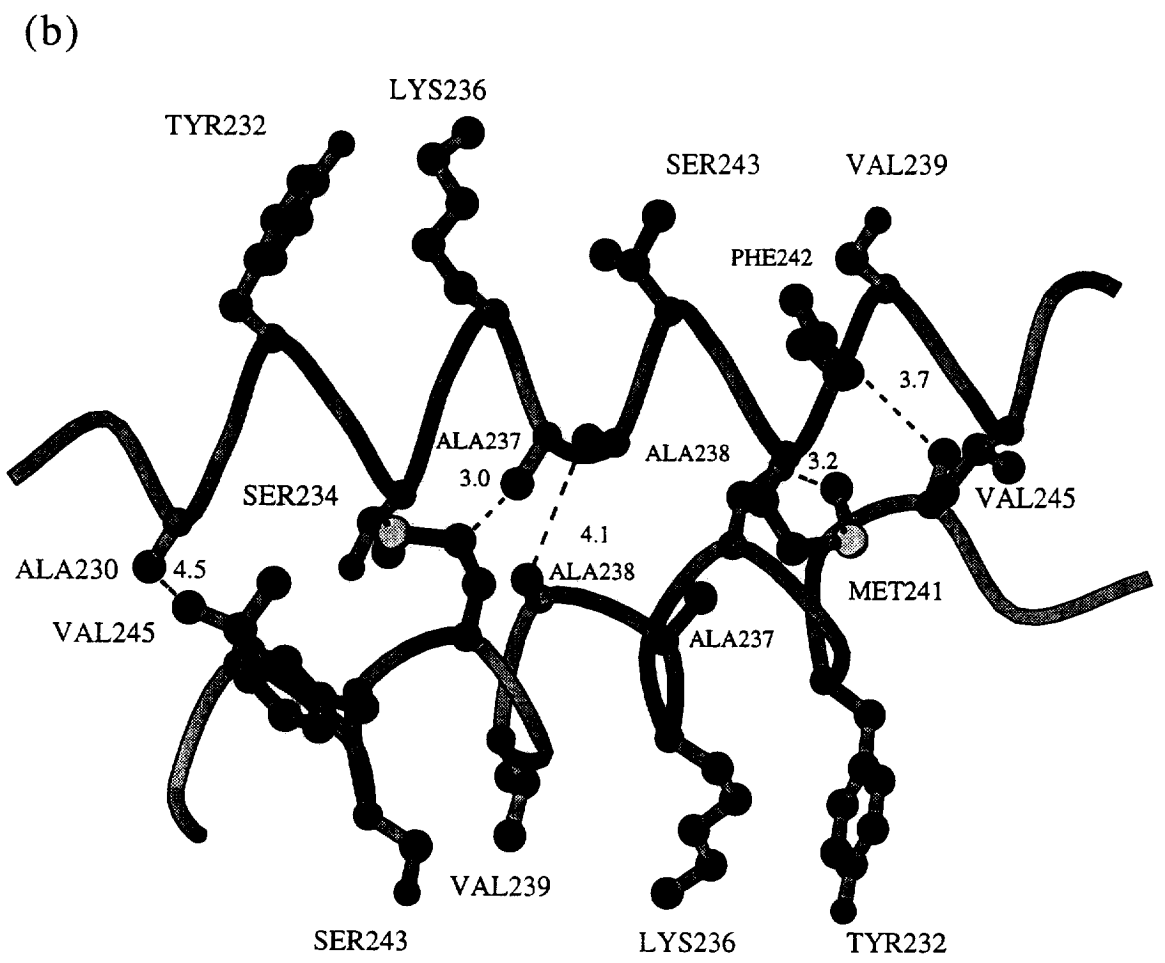

Fig. 4(b)—*legend on p. 598.*

been overlooked in sequence analyses because they are not absolutely conserved. In fact, at least five amino acids are found in these positions among the different *sec*-alcohol dehydrogenases.

Dimer interface and catalytically important residues in 17β-HSDs

With the results from *Drosophila* ADH and our recent analysis of human 11β-hydroxysteroid dehydrogenase-types 1 and 2 [Tsigelny and Baker, submitted] in mind, we modeled α-helix F interface from the four different mammalian 17β-HSDs to gain an insight into stabilizing interactions and how they may affect the catalytic site. Figure 3 shows the sequence alignment of α-helix F on the four 17β-HSDs with rat dihydropteridine reductase and *S. hydrogenans* 20β-HSD, the two templates that we used to model the steroid dehydrogenases. The catalytically important tyrosine and lysine and the anchor site are shaded. Also shown at the bottom of the figure in vertical bars are other residues that have their side chain positioned into the substrate and cofactor binding site. Circled residues have stabilizing interactions across the dimer interface with each other or with one of the anchor site residues as obtained from the 3D structures shown in Fig. 4. Figure 3 highlights our hypothesis [Tsigelny and Baker, submitted] that there is conservation in structure of these enzymes and their homologs for stabilizing the association between the hydrophobic surfaces on α-helix F. For example, 11β- and 17β-HSD type 1 have identical residues at the anchor site, despite the overall divergence of the sequences of these enzymes, which have about 20% identity after insertion of gaps. And the two enzymes conserve other structures that stabilize the dimer.

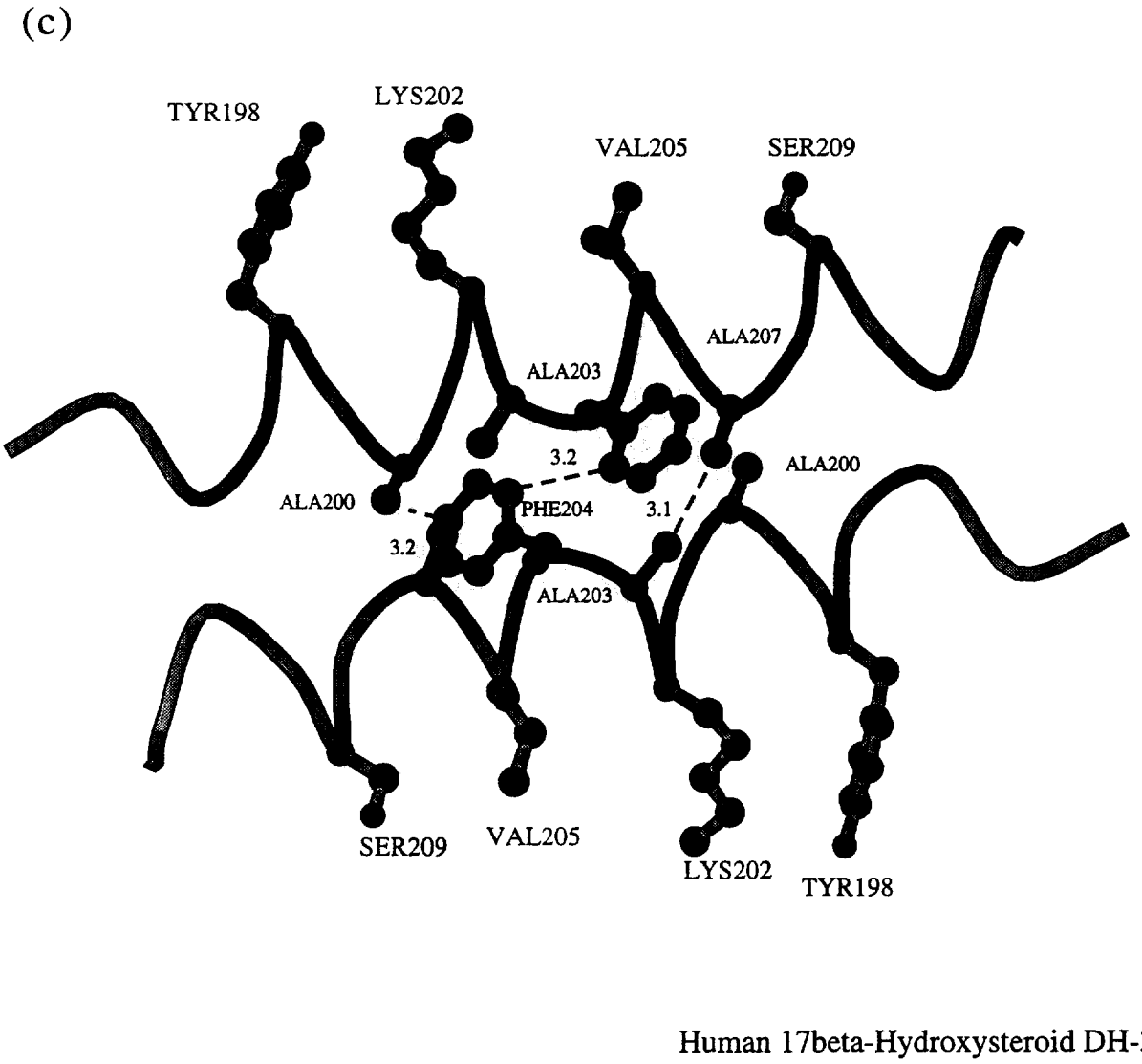

Fig. 4(c)—*legend overleaf.*

Human 17β-HSD type 1

Figure 4(a) shows the modeled α-helix F interface in human 17β-HSD type 1 in which phenylalanine-160 and alanine-161 form an anchor. Both residues have important stabilizing interactions across the dimer interface. Alanine-161 is 4.1 Å from the other subunit's alanine-161. Alanine-161 has a hydrophobic interaction with alanine-157, which is in the segment between the conserved tyrosine-155 and lysine-159. There is a hydrophobic interaction between alanine-157 and leucine-165, which are about 3.8 Å apart. Phenylalanine-160 is 4 Å from glycine-164. There also is a hydrogen bond between cysteine-156 and serine-168, which are 3.2 Å apart. This is an interesting structural property of residues in the segment between the conserved tyrosine and lysine residues: this segment is important in stabilizing dimers. This pattern is repeated in the other 11β- and 17β-HSD (Fig. 3), suggesting conservation of this stabilizing structure, although the residues are not as well conserved as the tyrosine and lysine.

Human 17β-HSD type 2

Figure 4(b) shows the modeled α-helix F interface in human 17β-HSD type 2. Alanine 237 is 3 Å from the hydrophobic part of the side chain of methionine-241 on the other subunit. Met-241 is 3.2 Å from serine-234. Alanine-230 is 3.7 Å from phenylalanine-242 and 4.5 Å from valine-245. Alanine-238, the other anchoring residue, is 4.1 Å from the other subunit's alanine-238.

Human 17β-HSD type 3

Figure 4(c) shows the modeled α-helix F interface in human 17β-HSD type 3. Alanine 203 is 3.1 Å from alanine-207. Phenylalanine-204 is 3.2 Å from the other phenylalanine-204 and alanine-200. These are the only stabilizing interactions that we find in our analysis. Human 17β-HSD type 3 has the weakest interactions

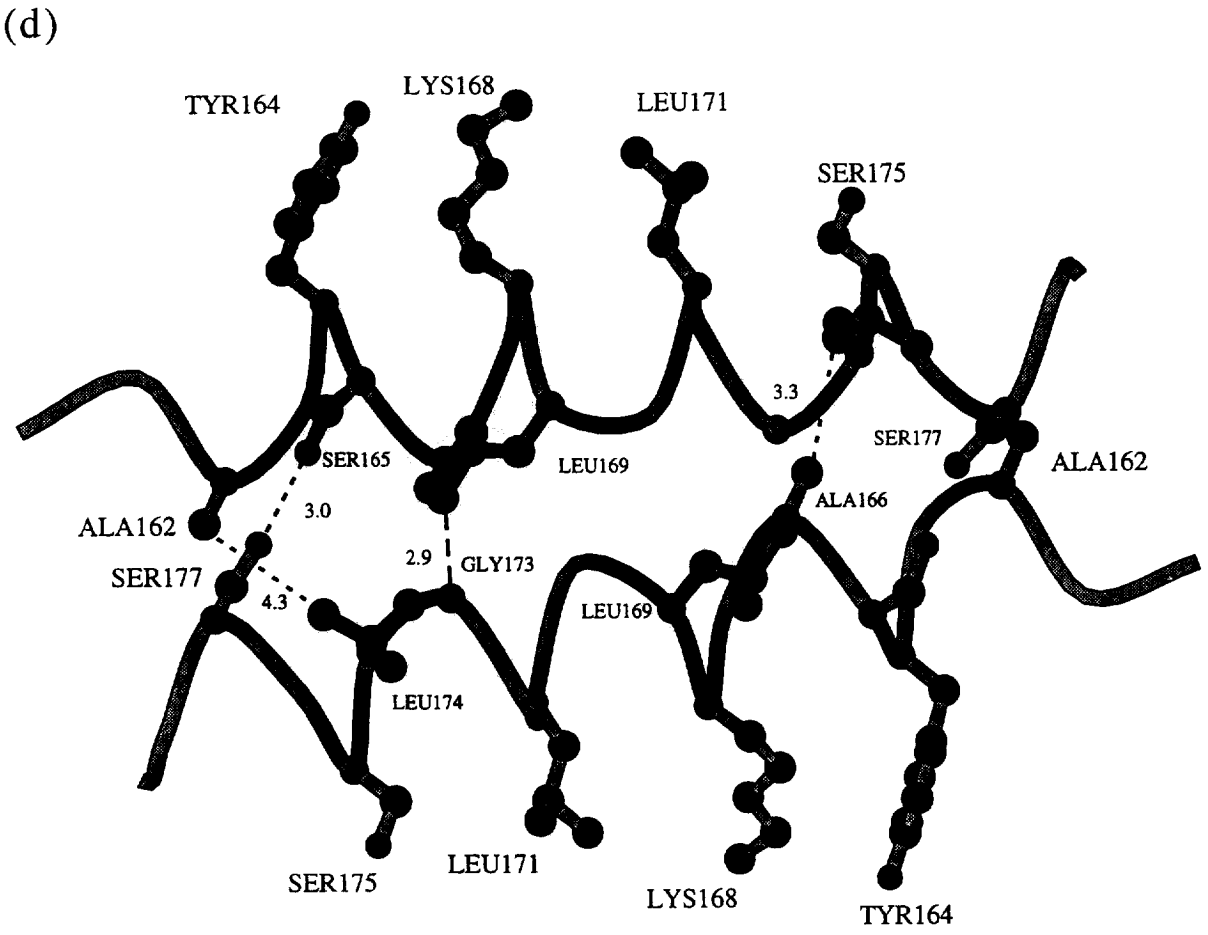

Fig. 4. Structure of α-helix F interface of mammalian 17β-HSDs. The α-helix F part of the dimer interface on 17β-HSDs is shown along with side chains of the highly conserved tyrosine and lysine residues and three other residues that are oriented into the cavity that binds substrate and nucleotide cofactor. (a) Modeled α-helix structure of human 17β-HSD type 1. (b) Modeled α-helix structure of human 17β-HSD type 2. (c) Modeled α-helix structure of human 17β-HSD type 3. (d) Modeled α-helix structure of porcine 17β-HSD type 4.

across the α-helix F interface among the four types of 17β-HSD. The conformation of this part of 17β-HSD type 3 could change upon binding of substrate leading to other stabilizing interactions. And, of course other parts of the protein may have intersubunit interactions that stabilize the dimer. Alternatively, the hydrophobic surface of α-helix F may interact with another protein or a membrane surface, a potentially important regulatory mechanism that we discuss later in this paper.

Pig 17β-HSD type 4

Figure 4(d) shows the modeled α-helix F interface in pig 17β-HSD type 4. Leucine-169 is 2.9 Å from glycine-173. Leucine-174 is 4.3 Å from alanine-162 and 3.3 Å from alanine-166. There also is a hydrogen bond between serine 165 and serine 175, which are 3 Å apart.

Dimer stability and function

Our analysis of the dimer interface indicates why most *sec*-alcohol dehydrogenases are either dimers or tetramers [Tsigelny and Baker, submitted]. A monomer in solution would have the outer hydrophobic surface of α-helix F exposed to solvent, which would be expected to distort the surface and affect the orientation of residues in the active site that bind substrate and catalyze hydride transfer. Our model indicates that an active monomer requires the hydrophobic surface of α-helix F to interact with another hydrophobic surface. In *sec*-alcohol dehydrogenases

that form homodimers or tetramers, this surface is α-helix F in the other subunit.

An important example of an active monomeric *sec*-alcohol dehydrogenase is carbonyl reductase [49]. We have suggested that a nearby segment on carbonyl reductase may interact with the α-helix F to stabilize the active monomer [Tsigelny and Baker, submitted].

Interaction of α-helix F with heterologous hydrophobic surfaces may be a mechanism for localizing the activity of *sec*-alcohol dehydrogenases, for example, by association of the hydrophobic surface of α-helix F with a membrane or a protein in or associated with a membrane. For example, retinol dehydrogenase, a *sec*-alcohol dehydrogenase, is found in microsomal membranes, where it copurifies with a 54 kDa protein [42, 43]; and phosphatidylcholine increases activity of the purified enzyme, as is also found for β-hydroxybutyrate dehydrogenase [50].

Hetero-hydroxysteroid dehydrogenases?

The constraint on α-helix F to have its hydrophobic surface associated with another surface may be important in excluding or promoting formation of hetero-hydroxysteroid dehydrogenases. This possibility is especially relevant for type 1 and type 2 11β- and 17β-HSD. Phylogenetic analysis reveals that mammalian type 2 11β- and 17β-HSD diverged from a common ancestor after their line diverged from the dehydrogenase leading to type 1 11β- and 17β-HSD, leading to the unidirectional enzyme mechanism for regulating steroid hormone action [51]. It is interesting that the residues in the anchor in 11β- and 17β-HSD type 1 are identical, as are the anchors for the homologous type 2 enzymes. Moreover, our previous analysis [Tsigelny and Baker, submitted] and that reported here indicates many similar stabilizing interactions across the α-helix F interface. It will be interesting to know if stable hetero-11β- and 17β-HSDs can form and if they are active.

Summary

Our model of α-helix F 17β-HSD types 1, 2, 3, and 4 indicates that they contain several conserved structures that use hydrophobic interactions and hydrogen bonds to anchor the interface between α-helix F in each subunit. Although there is sequence variation in α-helix F, structural elements for interaction between the subunits are remarkably conserved, even between enzymes where other parts of their sequences have diverged considerably from their common ancestor. For example, the type 1 11β-hydroxy and 17β-HSD have an identical residues in the proposed anchor site and conserve other structures. The same is true for type 2 11β-hydroxy and 17β-HSD.

The proximity of this anchor to the conserved lysine and tyrosine in the proposed catalytic site suggests that sequence variations in this anchor may also be important in substrate specificity and preference for catalyzing oxidation or reduction of substrates.

Our analysis of the polar surface of α-helix F suggests some sites that may be important in enzyme specificity. Our previous analysis [Tsigelny and Baker, submitted] identified phenylalanine-193 on 11β-HSD type 1 and methionine-243 on 11β-HSD type 2 as interesting sites for mutagenesis. The homologous positions in 17β-HSD are cysteine in type 1 and serine in type 2, 3 and 4.

Application of site specific mutagenesis and molecular modeling to six different 11β- and 17β-HSDs has the potential for elucidating how enzyme specificity and preference for oxidation and reduction is accomplished, which should have important application in treating a wide variety of steroid-dependent diseases.

Acknowledgement—The support of the Supercomputer Center of the University of California, San Diego is gratefully acknowledged.

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