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Review

Structural and functional features of dimeric dihydrodiol dehydrogenase

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Abstract. Dimeric dihydrodiol dehydrogenase (DD) catalyzes the $NADP^+$-dependent oxidation of *trans*-dihydrodiols of aromatic hydrocarbons to their corresponding catechols. The tertiary structure of dimeric DD concists of a classical dinucleotide binding domain comprising two βαβαβ motifs at the N-terminus, and an eight-stranded, predominantly anti-parallel β-sheet, forming the C-terminal domain The aim of this review is to summarize the biochemical and structural properties of dimeric DD, compare it to enzymes that are structurally similar, and provide an insight into its catalytic mechanism and membership amongst a unique family of monomeric/oligomeric proteins that most likely share a common ancestry.

Keywords. Dihydrodiol dehydrogenase, oxido-reductases, site-directed mutagenesis, crystal structure, molecular modeling.

Introduction

Dihydrodiol dehydrogenase (DD, E. C. 1.3.1.20) was first identified in rabbit liver, where it was found to oxidize benzene dihydrodiol to catechol [1]. This enzyme drew attention because it oxidizes *trans*-dihydrodiols of polycyclic aromatic hydrocarbons (PAHs) such as benzo[*a*]pyrene [2–5], which are formed as intermediate metabolites in their widely accepted pathway of metabolic activation to diol epoxides (ultimate carcinogens) catalyzed by cytochrome P450 1A1/1B1 and epoxide hydrolase [6, 7]. While DD was thought to be involved in metabolic inactivation of PAHs by decreasing concentrations of the *trans*-dihydrodiols (proximate carcinogens) of PAHs [2, 3], it has been recognized to be an important activation enzyme [8, 9]. The oxidation of *trans*-dihydrodiols of PAHs by DD forms the corresponding catechols, which are air-sensitive and undergo autoxidation to produce *o*-semiquinone anion radicals that are then converted to *o*-quinones. The *o*-quinones are reactive electrophiles that can react with cellular glutathione and macromolecules such as DNA and proteins, and form reactive oxygen species *via* their redox cycles using cellular nucleophiles (e.g., NAD(P)H) and molecular oxygen. DD is also regarded as an intoxication enzyme in the metabolism of naphthalene and benzene. The enzyme converts the *trans*-dihydrodiol of naphthalene to 1,2-dihydroxynaphthalene (Fig. 1), which is rapidly autoxidized to cytotoxic 1,2-naphthoquinone, a process thought to be involved in the pathogenesis of naphthalene-induced cataracts [10, 11]. Benzene is metabolized to a *trans*-benzene dihydrodiol by cytochrome P450 and DD,

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and the resultant catechol is one of the metabolites with high genotoxicity and immunotoxicity [12, 13].

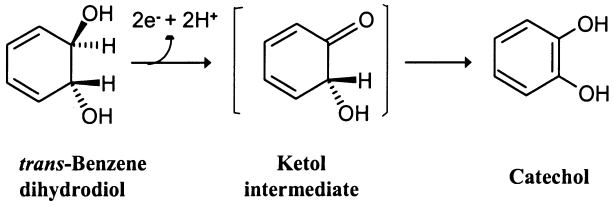

Figure 1. Oxidation of *trans*-benzene dihydrodiol catalyzed by DD. The reaction produces a ketol which then enolizes to the catechol.

DD exists in multiple forms in cytosols of mammalian tissues, and various types of the enzyme, differing in substrate specificity and subunit structure, have been purified [11, 14–26]. The majority of these mammalian enzymes shows specificity for *trans*-dihydrodiols of aromatic hydrocarbons, and thereby differs from bacterial DDs for the *cis*-dihydrodiols that belong to the short-chain dehydrogenase/reductase (SDR) superfamily [27]. Mammalian DDs are divided into two groups, monomeric and dimeric enzymes. Monomeric DDs are identified with aldehyde reductase, aldose reductase and/or several hydroxysteroid dehydrogenases, of which all the enzymes determined by cDNA (complementary DNA) cloning belong to the aldo-keto reductase (AKR) superfamily (see AKR superfamily homepage: http://www.med.upenn.edu/akr/). Therefore, it is proposed that DD simply possesses additional activities of these known enzymes, and that the reaction catalyzed by the enzymes is a two-electron oxidation of one of the hydroxyl groups of *trans*-dihydrodiol to yield an intermediate ketol which then enolizes to the catechols (Fig. 1) [28]. There have been several excellent reviews on the structures and functions of the enzymes in the AKR superfamily [29–32]. On the other hand, the physiological substrates and amino acid sequences of dimeric DDs have recently been identified [33, 34] and the crystal structure of the monkey enzyme recently determined [35]. These studies reveal that dimeric DD is dissimilar to the enzymes of the SDR and AKR superfamilies, but is reminiscent of oxido-reductases found in bacterial species and together form a new and unique family of oxido-reductases. In this review we illustrate the structural similarities of dimeric DDs to the bacterial oxido-reductases, perform amino acid sequence comparisons of mammalian DDs and formulate the biochemical nature of this emergent enzyme family. The name and symbol of dimeric DD approved by the HUGO Gene Nomenclature Committee are dihydrodiol dehydrogenase (dimeric) and DHDH, respectively, and DHDH is used for the abbreviation for this enzyme.

Biochemical nature of DHDH

DHDHs were isolated and characterized from Japanese monkey, pig, dog and rabbit tissues [11, 23, 24, 34, 36–38]. The purified enzymes are composed of identical subunits with molecular weights of 32–36 kDa. The cDNAs for the enzymes of the above animal species, *Cynomolgus* monkey and humans were cloned, and their deduced amino acid sequences reveal that their subunits consist of 334 or 335 amino acids [33]. These studies indicate a species-specific tissue distribution of DHDH. The enzyme is ubiquitously distributed in pigs [23] and dogs [37], whereas it is specifically expressed in kidney of monkeys [38], lens and intestine of rabbits, and intestine of humans [33]. DHDH has not been detected in mouse liver and kidney [15, 18, 39]. But it is detected in the lens [24], and the cDNA for mouse DHDH (accession number AK005050) is deposited in the databases. Although the enzyme has not been characterized in other animal species, cDNAs or genes for proteins that are similar in total amino acids (333–335) and sequence identity (77–96% with Japanese monkey DHDH) are isolated or identified in the chimpanzee, orangutan and cow (accession numbers are XM_512808, CR860863 and BC118168, respectively). The amino acid sequences of mammalian DHDHs exhibit no homology to the enzymes of the SDR and AKR superfamilies (as discussed in the sequence analysis section). Thus, DHDH is probably expressed in almost all mammals. While the roles of human monomeric DDs in the metabolism of PAHs are well documented [8, 9, 32], the reactivity of DHDH for *trans*-dihydrodiols of PAHs has not been studied yet. However, DHDH is the DD which is involved in the pathogenesis of naphthalene-induced cataracts in rabbits [10,11]. In addition, DHDH shows strict stereospecificity in the oxidation of *trans*-dihydrodiols of benzene and naphthalene [40]. It oxidizes the (−)-[1*R*,2*R*]-dihydrodiols, which are predominantly produced in the *in vivo* metabolism of aromatic hydrocarbons. The stereospecificity is the same as that of human aldehyde reductase for PAH *trans*-dihydrodiols [9], but is in contrast to other monomeric DDs that oxidize the (+)-[1*S*,2*S*]-isomer of benzene dihydrodiol [40] or do not exhibit stereospecificity for PAH *trans*-dihydrodiols [9]. This suggests that DHDH plays a role in the metabolism of PAHs. DHDH is inactive towards hydroxysteroids, but oxidizes several endogenous pentoses and hexoses, including D-glucose, and has been identified as $NADP^+$-dependent D-xylose 1-

dehydrogenase (EC 1.1.1.179) [34, 41]. This finding suggests that the enzyme plays a role in the metabolism of dietary sugars and the concomitant generation of NADPH from $NADP^+$, especially in hyperglycemia (K_m values for D-glucose = 19–58 mM). DHDH efficiently reduces reactive carbonyl compounds, such as methylglyoxal and 3-deoxyglucosone, as well as several hydrophilic carbonyl compounds, such as camphorquinone (CQ) and nitrobenzaldehydes [23, 38, 42]. This ability to reduce 3-deoxyglucosone suggests a role for DHDH as a detoxification enzyme preventing the eventual glycation of proteins, which has been linked to the development of diabetic complications [43].

Initial velocity analysis of the reaction catalyzed by DHDH demonstrates that it follows an ordered bi bi mechanism in which $NADP^+$ binds to the enzyme first and expels NADPH last [37, 44, 45]. The dehydrogenase activity of DHDH is competitively inhibited with respect to *trans*-dihydrodiols of naphthalene and cyclohexane by ascorbates such as L-ascorbic acid [porcine DHDH; IC_{50} (50% inhibitory concentration) = 0.45 mM] and its epimer isoascorbic acid (porcine DHDH; IC_{50} = 0.043 mM) [37, 44]. The enzyme is more potently inhibited by 4-hydroxyphenylketones such as 4-hydroxyacetophenone (Fig. 2), which is also a competitive inhibitor (Japanese monkey DHDH; IC_{50} = 0.97 μM), binding to the enzyme-$NADP^+$ complex [45]. The activity of DHDH is apparently activated by the addition of magnesium chloride and salts of phosphate and sulphate, but kinetic analysis in the presence of these salts show a concentration-dependent inhibition in some cases [44], with a decreased affinity (K_m) for the coenzyme accompanied by an increase in the V_{max} value [34, 46]. This occlusion of binding of the coenzyme to the enzyme by salts was characterized in the crystal structures of Japanese monkey DHDH which had been crystallized using buffers containing high concentrations of salt [35, 47]. Molecules of sulphate and phosphate were found to occupy and form a number of strong hydrogen or electrostatic bonds with several polar and charged residues within the coenzyme binding domain instead of $NADP^+$, accounting for the decrease in the affinity for the coenzyme. The effect of salts also increases K_m values for the substrates, and it is recommended to employ low concentrations of Tris-HCl buffer for the kinetic analysis of the reaction catalyzed by DHDH [34].

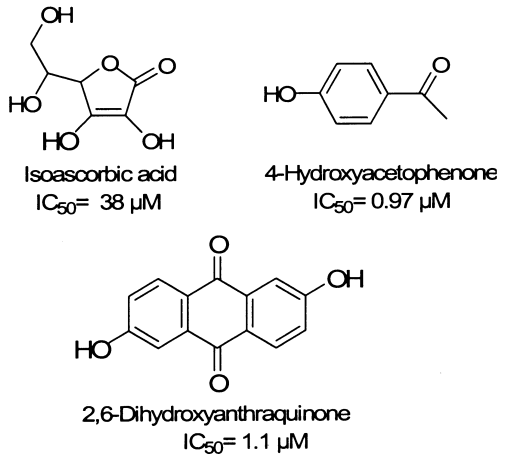

Figure 2. Chemical structures of potent inhibitors of mammalian DHDHs.

X-ray crystal structures

Japanese monkey DHDH was crystallized, and crystal structures of the enzyme-inhibitor complexes were determined by the multiple isomorphous replacement method. The subunit of the enzyme consists of two structural domains, an N-terminal coenzyme-binding domain and a novel C-terminal domain. The N-terminal domain forms a classic dinucleotide-binding motif of six β-strands and four α-helices with the two βαβαβ formations linked via an external (short) helix [35] (Fig. 3). The larger C-terminal domain forms an anti-parallel β-sheet consisting of eight rigid β-strands which forms the backbone of the monomer, and six α-helices. The dimeric complex is maintained via a number of the intersubunit contacts formed by the β-sheet and loops at the C-terminus of each monomer (Fig. 4), most significantly by the long β-strands labeled A–D. Each external β-strand forms a number of apolar contacts in the form of a patchwork of closely associated, stacked residues away from any solvent interaction and a vast number of polar contacts. The defining contact, the most important being the hydrogen-bonding network formed in part by a parallel stacking interaction by residues Arg148 and Arg202 of opposing monomers and the hydrogen bond interactions by Asp210 and the side chain nitrogens (NE and NH2) of Arg148. Double mutations of Arg148 and Arg202 demonstrated a propensity for the dimer to form active monomers but in turn displayed significant temperature sensitivity and consequently irreversible inactivation. Therefore, stabilization of the dimeric complex is imperative for peak enzymatic function and long-term enzyme viability, a feature evident in a number of enzymes of differing oligomeric states [48, 49]. The loss of DHDH viability must therefore be a consequence of the loss of a stabilizing and rigid force afforded by the dimer via the intersubunit C-terminal β-sheet association and as monomers expose a number of hydrophobic residues

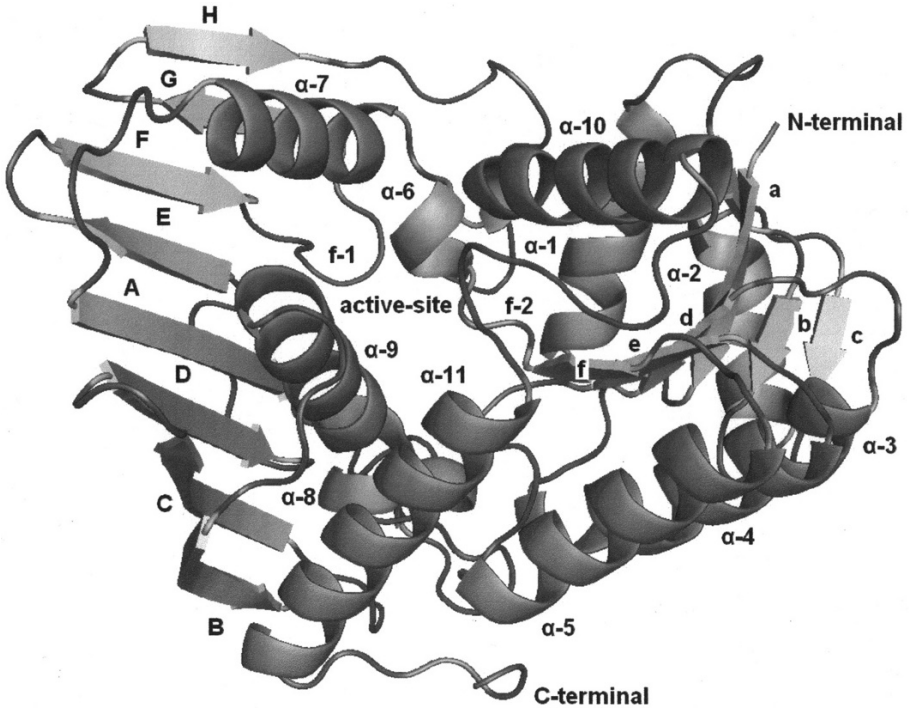

Figure 3. The monomer from DHDH shown in ribbon form. α-Helices are indicated by α-1 to α-11. The β-strands are labeled in lowercase (a–f) for the dinucleotide binding domain and uppercase (A–H) for the predominantly anti-parallel β-sheets. The active site is clearly labeled, as are the loop domains of F1 and F2 [68].

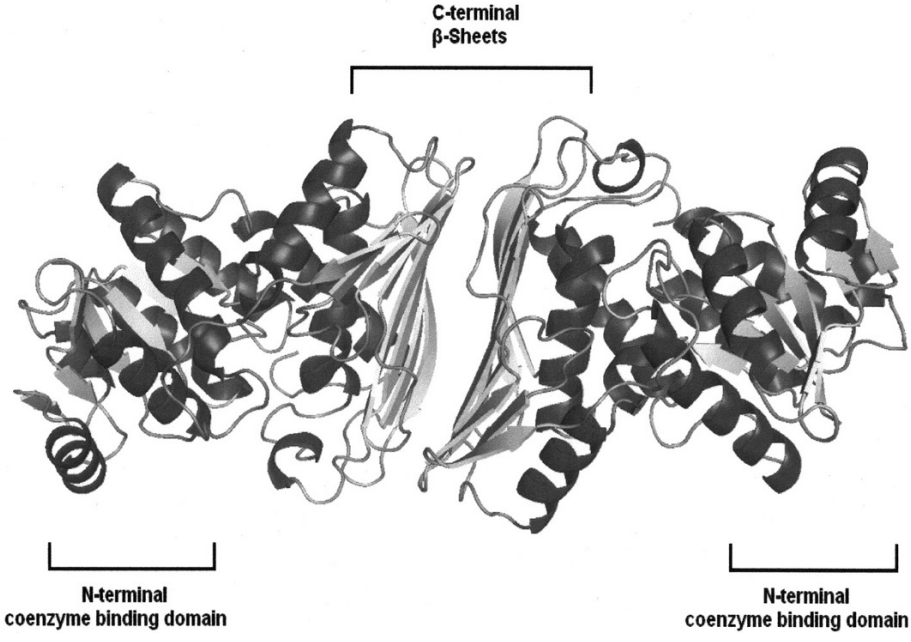

Figure 4. Ribbon representation of mammalian DHDH. The C-terminal β-sheets and N-terminal coenzyme binding domain are indicated [68].

to the solvent, predisposing the enzyme towards aggregation. The strict conservation of a number of residues and sequence motifs within the different species of mammalian DHDH [33] (Fig. 5) would suggest a consistent tertiary structure for the enzyme and a consistent manner with which the dimer is maintained with Arg148, Asp210 and Arg202 in corresponding positions within each mammalian sequence of DHDH.

The active-site/substrate-binding domain of monkey DHDH measures $13 \times 13 \times 9$ Å and is maintained by residues within the C-terminal domain, via the short helix of α-6, the N-terminal end of the centrally positioned helix of α-9, and the short loop labeled F1 and the relatively longer loop of F2. The active site is maintained by a number of predominantly aliphatic residues, including Lys97, Trp125, Phe154, Leu158, Asp176, Tyr180, Trp254 and Phe279 (Figs. 5, 6). These

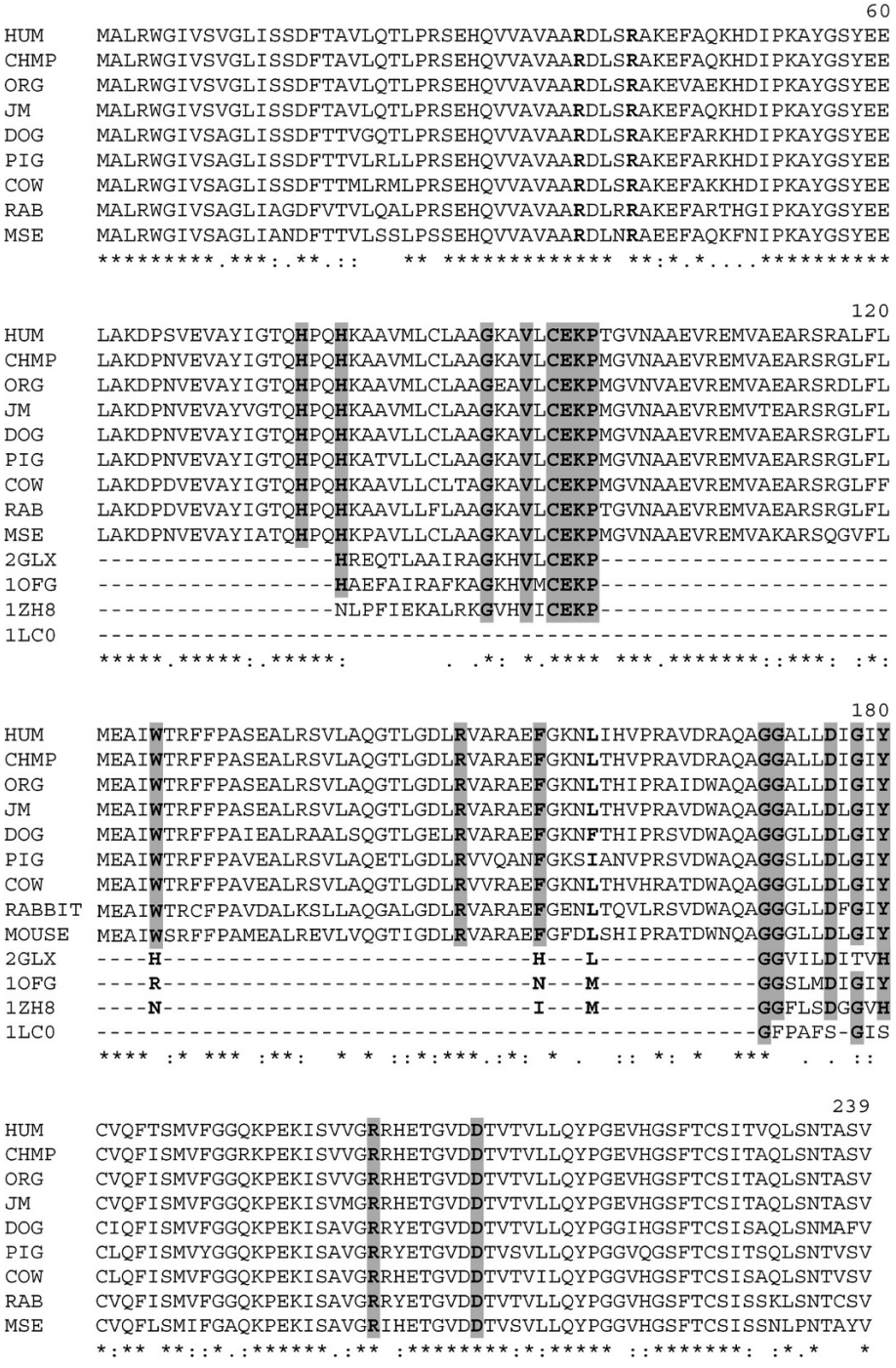

Figure 5. ClustalX [69] amino acid sequence alignment of mammalian DHDHs and structurally related oxido-reductases. Whole sequences are shown for DHDHs of chimpanzee (CHMP), human (HUM), orangutan (ORG), Japanese monkey (JM), dog, cow, pig, rabbit (RAB) and mouse (MSE), in which gaps are indicated with periods. Bold regions indicate catalytic residues, substrate binding residues and residues which maintain dimeric stability. Portions of sequences including, *Z. mobilis* GFO (1OFG), *S. morelense* AFR (2GLX), *T. maritime* TMO (1ZH8) and rat BVR (1LC0) are included with shaded regions indicating residue identity with gaps denoted by dashes.

mostly hydrophobic residues are more than indicative of their general importance in a large number of reactions, including facilitating the ability of DHDH to catabolize aromatic hydrocarbons and bind and be inhibited potently by aliphatic compounds. The inhibition and affinity (K_m – oxidation and reduction) for substrate is also affected, mostly by mutants of Trp125Ala, Trp254Ala and Phe279Ala [24, 38, 42, 45, unpublished result].

The coenzyme-binding domain of DHDH has been preliminarily defined with the use of molecular modeling and mutagenesis, indicating that $NADP^+$ binds in an elongated fashion within the enzyme, with the nicotinamide and adenine rings forming a classical anti-conformation [46]. Residues Arg37, Arg41, His76 and His79 contribute greatly to the high affinity for coenzyme (K_m for NADPH/$NADP^+$ is 0.56/0.55 μM) [46] when compared to a structurally equivalent enzyme such as 1,5-anhydro-D-fructose reduc-

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                                                                    298
HUM     SGTKGMVQLLNPCWCPTELVVKGEHKEFLLPPVP.KDCNFDNGAGMSYEAKHVWECLRKG
CHMP    SGTKGMAQLLNPCWCPTELVVKGEHKEFPLPPVP.KDCNFDNRAGMSYEAKHVRECLRKG
ORG     SGTKGMAQLLNPCWCPTELVVKGEHKEFPLPPVP.KDCNFDNGAGMSYEAKHVRECLRKG
JM      SGTKGMAQLLNPCWCPTELVVKGEHKEFLLPPVP.KNCNFDNGAGMSYEAKHVRECLRKG
DOG     SGTKGIGQILDPCWCPTELVLKGEHKEFPLPPAPSKEFNFTNGAGMAYEAKHVRECLRKG
PIG     SGTKGMAQILDPCWCPTELVVKGEHKEFPLPSAPPGEENYTNGMGMCYEAKHVRECLKKG
COW     SGTKGMAQLLDPCWSPTELVVKGEHKEFPLPPAPGKEFNFTNGMGMSYEAKHVRECLQKG
RAB     SGTKGIAQLLEPCWCPTELVVNKERKEFPLAPEENKKFNYRNGMGMSYEAQHVRDCLRKG
MSE     SGTKGMAQIQK.LWAPTELVVNGERKEFP.PPVLGKDYNFVNGSCMLYEANHVRECLRKG
2GLX    -------------T----------------------------------------------
1OFG    -------------T----------------------------------------------
1ZH8    ------------------------------------------------------------
1LC0    ------------------------------------------------------------
        *****: *: .   .*****:: *:***  ..    . *: *   * ***:** :**:**

                                          333
HUM     MKESPVIPLSESELLADILEEVRKAIGVTFPQDKR
CHMP    MKESPVIPLSESELLADILEEVRKAIGVTFPQDKC
ORG     MKESPVIPLSESELLADILEEVRKAIGVTFPQDKR
JM      LKESPVIPLVESELLADILEEVRRAIGVTFPQDKH
DOG     LKESPVIPLAESELLADILEEIRRAIGVTFPQDTR
PIG     LKESPMITLAESELLADILEEVRKAIGVTFPQDKC
COW     LKESPVIPLVESELLADILEEVRKAIGITFPQDKH
RAB     LKESPVIPLAESQLLADILEEVRKAIGVTFPQDKH
MSE     LKESPVVPLAESELLAEILEEARKAIGVTFPQDKR
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Figure 5. *(continued)*

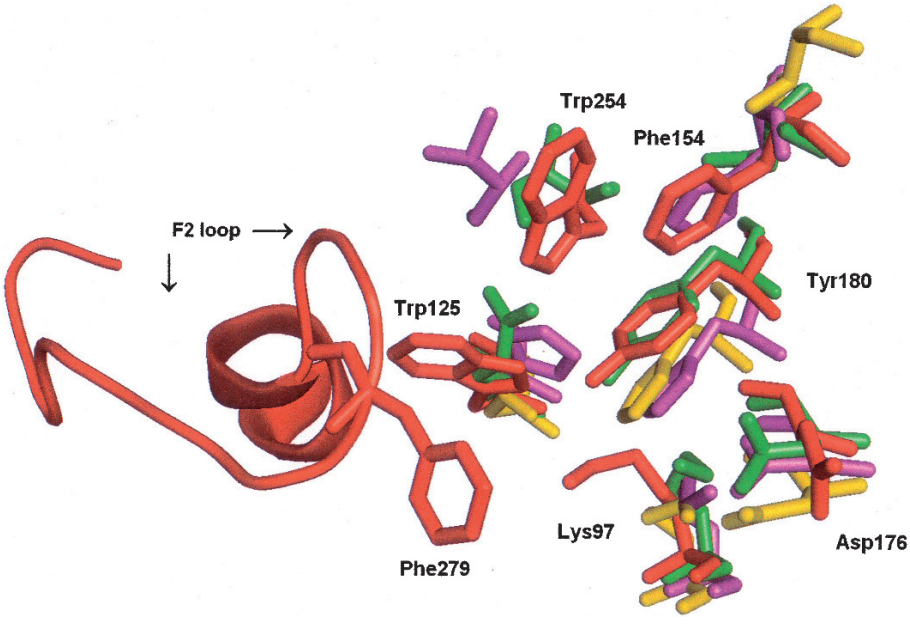

Figure 6. Superimposed active-site residues of Japanese monkey DHDH (red) with TMO (yellow), GFO (green) and AFR (purple) [68]. Residues from DHDH are listed with corresponding residues indicated in the sequence alignment table in Figure 5. The flexible loop F2 of DHDH is represented in cartoon form.

tase (AFR, K_m for NADPH is 60 μM) [50]. The K_d values for the coenzyme by the His76Gln and His79Gln mutations were low (>5-fold for NADPH/$NADP^+$) while also increasing the K_m for substrate (>15-fold for D-xylose) [46, unpublished result], with coenzyme modeling suggesting the proximity of His76 and His79 adjacent to the ribose sugar of NADP(H) and the active site of DHDH. Arg37 and Arg41 are suspected to interact with the 2′-phosphate moiety of NADP(H), in a fashion similar to their interactions with phosphate and sulphate molecules in the coenzyme-binding domain in the crystal structure of DHDH, with the mutation of either residue (Arg37Asp and Arg41Asp) producing an exceptionally large increases in K_d for NADP(H) (>20-fold) [35, unpublished result]. A structurally equivalent enzyme, biliverdin-IXα reductase [51], demonstrated an increase in activity by the addition of inorganic phosphate and NADH as coenzyme, much like DHDH, [52]. Postulated to take on the role of the 2′-phosphate of NADPH, it is thought to interact with Arg44 (in position and orientation similar to Arg41 in

DHDH) and stabilize the interaction between enzyme and coenzyme.

Structural and sequence analysis

Sequence similarity between similar proteins across species is usually high; however, proteins may still belong to the same family in spite of differing enzyme activities and having low sequence homology, entirely based on structural correlation or strict conservation of residues and sequence motifs. Amongst SDR enzymes this can include the coenzyme-binding motif of GlyxxxGlyxGly (where x is any variable amino acid residue), creating a βαβ Rossmann fold [53], or the active-site sequence motif Tyr-X-X-X-Lys [54, 55]. DHDHs have a high degree of interspecies sequence similarity (82–94%), low sequence similarity (17–41%) with 20 putative gene products of micro-organisms, including glucose-fructose oxido-reductase (GFO, EC 1.1.99.28) from *Zymomonas mobilis*, and no significant homology (or structural motif identity) with members of the SDR or AKR enzymes [33, 56]. While the specific sequence motifs indicating the presence of a Rossmann fold in SDRs are not found in DHDH, the tertiary structure clearly indicates its presence, suggesting that this nascent group of enzymes may be best assessed structurally to denote new or potential family members and therefore identify new consensus sequences that would best characterize these proteins. Assessment of the tertiary structure has revealed a number of enzymes similar to DHDH, almost entirely consisting of bacterial oxido-reductases and gene products (Fig. 7) [35]. These most notably include the NADP(H)-dependent glucose-fructose oxido-reductase (GFO, RMSD; 2.6 Å) [56], which along with the oxidized precursor form of the enzyme [57] is a tetrameric enzyme found in the periplasm of the Gram-negative bacterium *Z. mobilis* and is primarily responsible for sorbitol production and small sugar oxidation and reduction [58, 59]. DHDH also possesses a striking secondary structure correlation with another small sugar-metabolizing enzyme, the predominantly monomeric 1,5-anhydro-D-fructose reductase (AFR, RMSD; 1.96 Å) from *Sinorhizobium morelense* [50] and the predicted dehydrogenase/oxido-reductase TM0312 (TMO, RMSD; 2.2 Å) from *Thermotoga maritime* [61]. A single mammalian enzyme from rat, biliverdin reductase, which converts biliverdin to bilirubin also showed a resemblance to DHDH (BVR, RMSD; 2.4 Å) [51]. However, in all cases where there was discernable structural identity, the sequence identity was well below 24% [35].

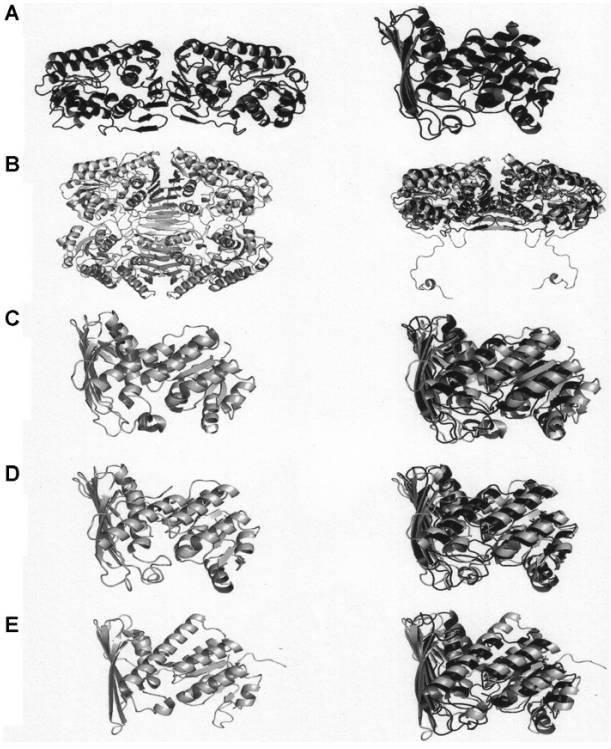

Figure 7. Structures of (*A*) Japanese monkey DHDH monomer and biological dimer (black) superimposed with (*B*) GFO biological tetramer (grey), (*C*) TMO (grey), (*D*) AFR (grey) and (*E*) rat BVR (grey) [68].

GFO, AFR, TMO and BVR all possess a small N-terminal arm forming a classical dinucleotide-binding domain, with the C-terminal portion of GFO comprising a predominantly anti-parallel, nine-stranded β-sheet which forms a tight, extended, biologically active tetramer, hydrogen-bonded by the last strands in each C-terminal domain [56] with N-terminal residues (1–31) forming a tight association with an adjacent subunit most likely to establish oligomer stability and tetramer formation. AFR and TMO have a similar sized eight-stranded C-terminal domain to that of DHDH made up of a number of polar and apolar residues, while BVR forms a smaller six-stranded β-sheet with an external face that is highly polar, indicative of the monomeric nature of the enzyme. While AFR has a limited ability to form oligomers *in vitro,* AFR, TMO and BVR lack the ordered Arg148-Arg202 intersubunit interaction that enables dimerization in DHDH. The majority of differences amongst all the enzymes are related to the length of several α-helices and β-strands and the size of loops that define the active-site domain where DHDH catalyzes the oxido-reduction of large xenobiotic-hydrophobic compounds and some small sugars, while GFO and AFR predominantly catabolize smaller hydrophilic sugars [50, 57, 61, 62]. This

substrate preference is made evident by the overwhelmingly hydrophobic nature of the active site of DHDH in comparison to AFR, GFO and TMO and the unique presence of the active-site loop F2 (between β-strand H and the α-10 helix). Containing the active-site residue Phe279, this flexible loop most likely adjusts to facilitate the binding of larger aliphatic molecules, with mutants of Phe279Ala resulting in a striking increase in K_m for substrate (260-fold) (Fig. 6) [unpublished result].

An inspection of the superimposed proteins and sequence alignment analysis reveals the conservation of several residues in relation to cofactor binding and the catalytic mechanism, a feature of enzymes belonging to the same structural family (Fig. 8). The Rossmann fold amongst DHDHs is characterized with the fingerprint sequence $HX_2HX_{16}EKP$ (residues 76–98) [33], while a consensus sequence involving the bacterial oxido-reductases of GFO, AFR and TMO forms a smaller motif of $GX_2VXCEKP$ (residues 90–98 in DD) resulting from the absence of His76 in AFR, GFO and TMO and His79 in the TMO sequences. Earlier mutagenesis studies based on knowledge of the structure of GFO and the sequence of DHDH from Japanese monkey kidney have identified several conserved catalytic and dinucleotide binding residues [46], including His79, which functions as a coenzyme binding residue, and Tyr180, which is critical for the reaction mechanism. The mutation of His79Gln showed an increase in the kinetic constants (K_d and K_m) for coenzyme in DHDH and a proposed interaction with the ribose sugar of NADP(H), much like AFR and GFO. The functional motif of Glu-Lys-Pro (residues 96–98) is proposed to interact with the nicotinamide ring of NADP(H) in a manner similarly described in GFO and AFR and seen in the crystal structure of TMO, with the formation of a *cis*-peptide by proline maintaining the position of lysine adjacent to both the coenzyme and the catalytic residues of the active site and facilitating the enzyme reaction. Mutation of Lys94 as part of this motif in AFR (and corresponding to Lys129 in GFO) suggested an interaction in the binding of substrate and enzymatic function with significant increases in K_m for substrate and a decrease in k_{cat} [50]. A similar mutational analysis of DHDH revealed an almost complete abrogation in activity by the mutants Lys97Met and Lys97Arg for D-xylose oxidation and $NADP^+$ and very poor activity with camphorquinone reduction and NADPH [unpublished result]. BVR lacks the cofactor-linked Glu-Lys-Pro motif of DHDH, GFO and AFR, perhaps due to its concomitant binding of NADH and NADPH. And while BVR possesses an active-site Tyr (Tyr97), it is not essential for catalysis [51].

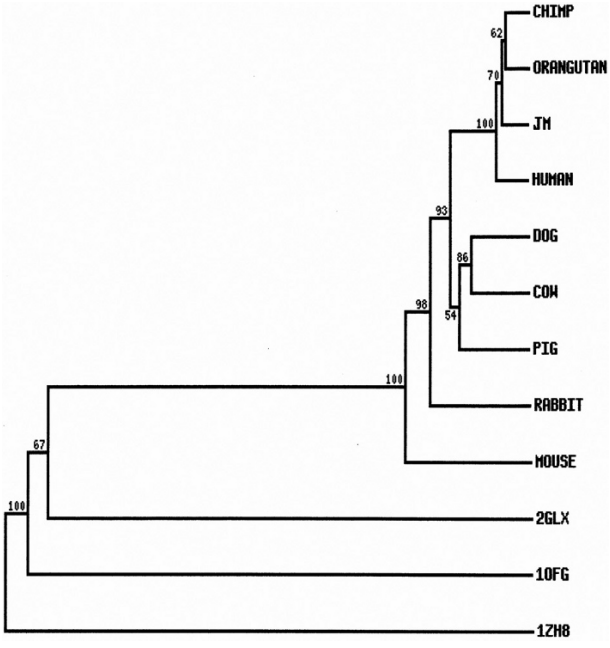

Figure 8. Phylogenetic tree of DHDHs and related dehydrogenases/reductases. The tree with bootstrap confidence values was calculated using the online Gene Bee service [70]. The abbreviations of the enzymes are shown in Figure 5.

The active-site consensus sequence observed from structural and sequence superimposition has been identified as $GGX_3DX_3(Y/H)$ [33, 35]. The sequence, indicative of a consistent catalytic mechanism in both the mammalian DHDHs and the bacterial oxido-reductase species, corresponds to a loop and active-site α-helix (formed in part by residues 171–180 in DHDH) which stacks along the C-terminal β-sheet of the respective enzymes through a series of hydrophobic interactions. Tyr180 has been identified as a catalytic residue with the Tyr180Phe mutant of the enzyme nullifying activity. This catalytic tyrosine of DHDH (Tyr217 in GFO) is replaced in AFR and TMO with an active-site catalytic histidine (Figs. 5, 6) [50], with mutagenesis experiments (His180Ala) also showing a similar abrogation of activity in AFR. The relative position of these residues is universal, with either histidine or tyrosine oriented exclusively towards the nicotinamide ring of the coenzyme in each enzyme and immediately adjacent to the second residue consistently repeated in the active-site motif, aspartic acid (Fig. 9). The conservation of aspartic acid suggests a third practical residue that forms a dynamic part of the active-site triad for DHDH, GFO, AFR and TMO. As with lysine and tyrosine, mutants of aspartic acid abrogate enzyme activity in AFR [50], and in crystal structures of preGFO form hydrogen bonds with the substrates, which more than likely helps stabilize the reaction species [57].

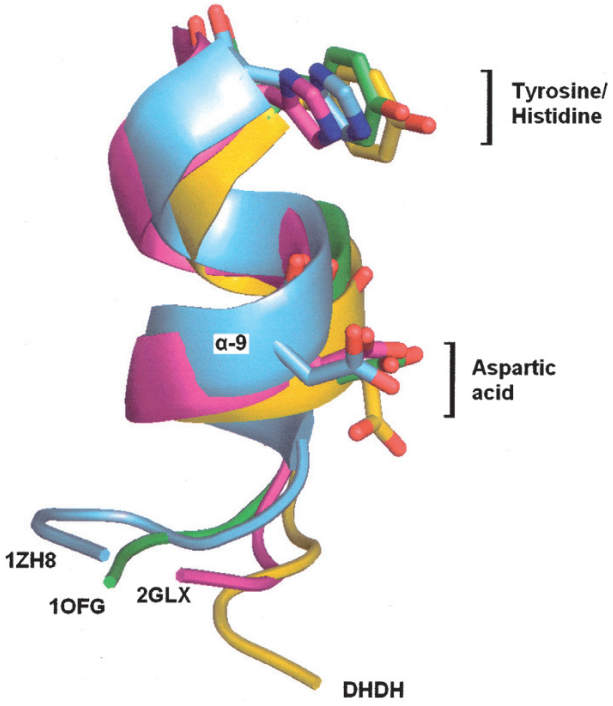

Figure 9. The active-site consensus sequence [GGX3DX3(Y/H)] corresponding to a loop and active-site α-helix in DHDH, GFO (1OFG), AFR (2GLX) and TMO (1ZH8) [68].

The catalytic mechanism

A proposed catalytic action for DHDH can be extrapolated from the observed biochemistry, crystallographic and mutagenesis studies of DHDH, structurally similar enzymes and the kinetic mechanism established for AKR and SDR enzymes. These systems depict a catalytic triad which forms a proton relay/hydrogen transfer system involving tyrosine primarily responsible for hydride transfer, concomitantly with NADPH towards the substrate, with lysine affecting the orientation of the nicotinamide moiety of the coenzyme molecule, and a serine or aspartic acid which are primarily responsible for the orientation of the reaction species [46, 50, 54, 56, 57, 63–66]. We have established that single mutants of Lys97 and Tyr180 severely hamper catalytic efficiency [46, unpublished result], suggesting the same level of catalytic deficiency observed in the Asp176 mutants of DHDH. These results are also reproduced in enzymes with the same catalytic consensus sequence as DHDH, such as AFR [50], and the functional nature of each residue is visually depicted in the crystal structures of GFO bound to substrate [57]. A clear acid-base catalysis involving active-site tyrosine and lysine occurs in GFO, and given their proximity, the formation of an intimate catalytic pair or diad is necessary to facilitate proton transfer. The lysine also maintains coenzyme orientation during catalysis by displaying a definite change in its side-chain orientation (with respect to distance towards the nicotinamide ring) dependant on the oxidation state of the coenzyme and the presence of substrate. The aspartic acid forms a close hydrogen bond contact that may help stabilize the substrate, much like the active-site serine in the SDR enzymes [54, 67]. Based on the proximity of Tyr180, Lys97 and Asp176, a similar reaction process should be expected of DHDH and is largely reminiscent of the catalytic mechanism described for rat liver 3α-hydroxysteroid dehydrogenase, a member of the AKR superfamily [65].

Structural coordinates for Japanese monkey DHDH apoenzyme and its complexes with 4-hydroxyacetophenone and isoascorbic acid are available from the PDB (ID codes 2O4U, 2O48 and 2POQ, respectively).

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