Human Glutathione-Dependent Formaldehyde Dehydrogenase. Structural Changes Associated with Ternary Complex Formation†

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ABSTRACT: Human glutathione-dependent formaldehyde dehydrogenase plays an important role in the metabolism of glutathione adducts such as *S*-(hydroxymethyl)glutathione and *S*-nitrosoglutathione. The role of specific active site residues in binding these physiologically important substrates and the structural changes during the catalytic cycle of glutathione-dependent formaldehyde dehydrogenase was examined by determining the crystal structure of a ternary complex with *S*-(hydroxymethyl)glutathione and the reduced coenzyme to 2.6 Å resolution. The formation of the ternary complex caused the movement of the catalytic domain toward the coenzyme-binding domain. This represents the first observation of domain closure in glutathione-dependent formaldehyde dehydrogenase in response to substrate binding. A water molecule adjacent to the 2′-ribose hydroxyl of NADH suggests that the alcohol proton is relayed to solvent directly from the coenzyme, rather than through the action of the terminal histidine residue as observed in the proton relay system for class I alcohol dehydrogenases. *S*-(Hydroxymethyl)glutathione is directly coordinated to the active site zinc and forms interactions with the highly conserved residues Arg114, Asp55, Glu57, and Thr46. The active site zinc has a tetrahedral coordination environment with Cys44, His66, and Cys173 as the three protein ligands in addition to *S*-(hydroxymethyl)glutathione. This is in contrast to zinc coordination in the binary coenzyme complex where all of the ligands were contributed by the enzyme and included Glu67 as the fourth protein ligand. This change in zinc coordination is accomplished by an ∼2.3 Å movement of the catalytic zinc.

Glutathione-dependent formaldehyde dehydrogenase is a class III alcohol dehydrogenase that metabolizes glutathione adducts such as *S*-(hydroxymethyl)glutathione (HMGSH)[1] and *S*-nitrosoglutathione (GSNO) more efficiently than primary alcohols and aldehydes (*1*−*3*). HMGSH is a spontaneously formed thioacetal adduct between glutathione and formaldehyde and constitutes a cellular strategy for sequestering and metabolizing highly toxic formaldehyde (*4*). FDH oxidizes HMGSH to *S*-formylglutathione with the stoichiometric reduction of NAD^+ to NADH. GSNO, a *S*-nitrosothiol, is believed to be an important carrier form of nitric oxide within cells. FDH catalyzes the reduction of GSNO to its hydroxysulfenamide derivative by a process similar to NADH-dependent aldehyde reduction (*2*, *3*). This unusual substrate specificity and the random kinetic mechanism characteristic of FDH (*5*) are in contrast to those of the class I alcohol dehydrogenases which prefer aliphatic alcohols as substrates and typically obey an ordered kinetic mechanism with the coenzyme binding first (*6*).

Our previous structure−function studies on FDH explored the structural basis for the random addition of substrates and its substrate preference (*7*, *8*). FDH, like members of the ADH family, functions as a homodimer with each of its subunits being made up of two domains: a coenzyme-binding domain at the subunit interface and a distal catalytic domain. The active site is located in the cleft between the two domains. In horse class I ADH, binding of the coenzyme induces the catalytic domain to move toward the coenzyme-binding domain and narrow the active site cleft. This domain movement aligns the alcohol or aldehyde binding residues optimally for binding of the substrate. The two domain conformations of horse class I ADH thus are described as "open" (in the apoenzyme) and "closed" (in the binary and ternary complexes). The ability of FDH to form both the FDH·NAD(H) and FDH·alcohol complexes in a kinetically random fashion was attributed to its "semiopen" catalytic domain position that exhibited minimal changes in the tertiary structure upon binding of these substrates. In addition, the active site zinc was observed to change its coordination environment in different enzyme−ligand complexes. This observation suggested a role for zinc movement in substrate binding and release. In the apoenzyme, the zinc was tetrahedrally coordinated to a water molecule and three protein ligands (Cys44, His66, and Cys173). However, in both the FDH·NAD(H) binary complex and the FDH·NAD^+· dodecanoic acid ternary complex, Glu67 was added to the coordination sphere and the bound solvent molecule became more loosely held. Thus, the changes in the coordination environment of the active site zinc appeared to facilitate the release of the zinc-bound water molecule.

We report here a FDH·HMGSH·NADH ternary complex which addresses the zinc coordination and the position of

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[1] Abbreviations: 12-HDDA, 12-hydroxydodecanoic acid; HMGSH, *S*-(hydroxymethyl)glutathione; ADH, alcohol dehydrogenase; FDH, human glutathione-dependent formaldehyde dehydrogenase.

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the catalytic domain when the substrate is present in the ternary complex. Furthermore, the specific roles for active site residues in the binding of HMGSH are identified in this complex.

MATERIALS AND METHODS

All chemicals used in this study were obtained from Sigma Chemical Co.

Crystal Growth and Data Collection. Recombinant human FDH was expressed in *Escherichia coli* and purified to homogeneity (*7*). Crystals were grown by sitting-drop vapor diffusion at 4 °C. A 15−20 mg/mL enzyme solution equilibrated with a mother liquor containing 0.1 M phosphate buffer (pH 7.1), 100 mM $ZnSO_4$, 1 mM DTT, and 12−16% PEG8000 was used for crystal growth. Crystals of FDH grew as rectangular rods after 8 days and reached full size in 2 weeks. Crystals of the FDH·HMGSH·NADH ternary complex were prepared by soaking the crystals of the apoenzyme in the crystallization solution with 5 mM glutathione, 2 mM formaldehyde, and 2 mM NADH. The soaking procedure involved a 3 h soak in this solution, followed by transferring the crystal into a fresh solution for an additional 1 h. For data collection, a rapid two-step soaking procedure was used to introduce 21% (v/v) PEG400 into the crystal before it was flash-frozen at −160 °C in a gaseous nitrogen stream. The data were collected using a Raxis IIc image plate detector and a Rigaku RU 200HB rotating anode generator. The ternary complex was in space group $P4_32_12$ and had the following cell dimensions: $a = 79$ Å, $b = 79$ Å, and $c = 310$ Å. The data were integrated and scaled using Denzo and Scalepack (version 1.96.0).

The structure of the ternary complex was determined by molecular replacement using the program CNS and the FDH apoenzyme as the search model (PDB entry 1M6H). During the initial cycles of refinement, noncrystallographic symmetry restraints were applied to the main chain atoms in the dimer with weights of 100 and 10 kcal/mol for the side chain atoms. These restraints were removed in the final cycles of refinement. The refinement procedure utilized a bulk solvent correction to account for the low-resolution data and individual restrained isotropic temperature factors. The nonbonded ionic radii of the bound zinc ions were reduced from 1.568 to 0.75 Å in the ion parameter file of CNS to maintain the ligand geometry observed in high-resolution structures of the horse liver EE isoenzyme. Visual inspection of the resulting models during refinement was performed using the program O (*9*). Solvent molecules were added as indicated by the presence of strong electron density peaks in $F_o - F_c$ maps within hydrogen bonding distance of protein atoms. The topology and parameter files for HMGSH were created using XPLO2D (*10*).

RESULTS

Structure of the FDH·HMGSH·NADH Ternary Complex. The structure of the FDH·HMGSH·NADH ternary complex was determined at 2.6 Å resolution by molecular replacement using the previously reported apo form of FDH as the search model (Table 1). Within the dimer that comprises the asymmetric unit, HMGSH is present only in the A subunit, while NADH is present in both subunits.

The catalytic domain shows significant movement in both subunits compared to that in the apoenzyme (Figure 1). To determine the extent of domain movement, the coenzyme-binding domain of each subunit was aligned with that of the apoenzyme. The domain movement can be described in terms of degrees by which the catalytic domain in the ternary complex had to be rotated as a rigid body to superimpose it on the catalytic domain in the horse apoenzyme (Table 2). This analysis revealed that the catalytic domain in the A subunit containing HMGSH and NADH had rotated by an additional 2° toward the coenzyme-binding domain, creating a domain orientation more "closed" than that in the apoenzyme and binary complexes of FDH (Figure 1 and Table 2). In the B subunit of this complex (NADH only), the catalytic domain rotated 1° away from the coenzyme-binding domain, creating a domain orientation more "open" than that in the apoenzyme of FDH (Figure 1 and Table 2). The closed catalytic domain position observed in the A subunit of the FDH·HMGSH·NADH ternary complex is similar to the closed conformation observed in binary and ternary com-

Table 1: Data Collection and Refinement Statistics

	FDH·HMGSH·NAD(H)
space group	$P4_32_12$
cell parameters (a, b, c) (Å)	78.7, 78.7, 311.4
no. of observations (total/unique)	117217/27884
completeness (%)	88.2 (70.2)
R_{merge}	3.5 (14.1)
I/σ_I	31.2 (5.9)
resolution (Å)	49−2.6
no. of residues	746
no. of zinc ions	4
no. of water molecules	395
no. of phosphate molecules	3
no. of potassium ions	1
no. of coenzyme molecules	2
no. of HMGSHs	1
R (R_{free})	19.7 (24.9)
rmsd for bonds (Å)	0.006
rmsd for angles (deg)	1.5
Ramachandran, most favored (%)	86.3
Ramachandran, allowed (%)	13.3

Table 2: Comparison of the Extent of Domain Closure in Class I and Class III ADH Subunits

enzyme complex	closure (deg)[a]	PDB entry
class I ADH		
apo form of horse class I ADH	−	8ADH
ternary complex of horse class I ADH with NAD^+ and *p*-bromobenzyl alcohol	10	1HLD
binary complex of human $\gamma 2$ ADH with NAD(H)	10	1HTO
class III ADH (FDH)		
apoenzyme	6	1M6H
binary complex with 12-HDDA	6	1M6W
binary complex with NAD(H)	5	1TEH
binary complex with NADH (B subunit of the FDH·HMGSH·NADH ternary complex)	5	1MC5
ternary complex with NAD^+ and dodecanoic acid	6	1MA0
ternary complex with NADH and HMGSH	8	1MC5

[a] The extent of domain closure was calculated using the strategy described earlier. A two-step alignment procedure using the LSQKAB program in the CCP4 program suite was used. Initially, the A subunit of the test ADH was superimposed on the apo form of horse class I ADH via its coenzyme-binding domain (Cα atoms of residues 180−242, 252−288, and 307−320 were aligned). The initially superimposed molecules were again superimposed on each other by their catalytic domains (Cα atoms of residues 13−49, 61−180, and 322−360 were aligned). The closure was calculated as the degrees by which the catalytic domains in the aligned molecules must be rotated to superimpose them.

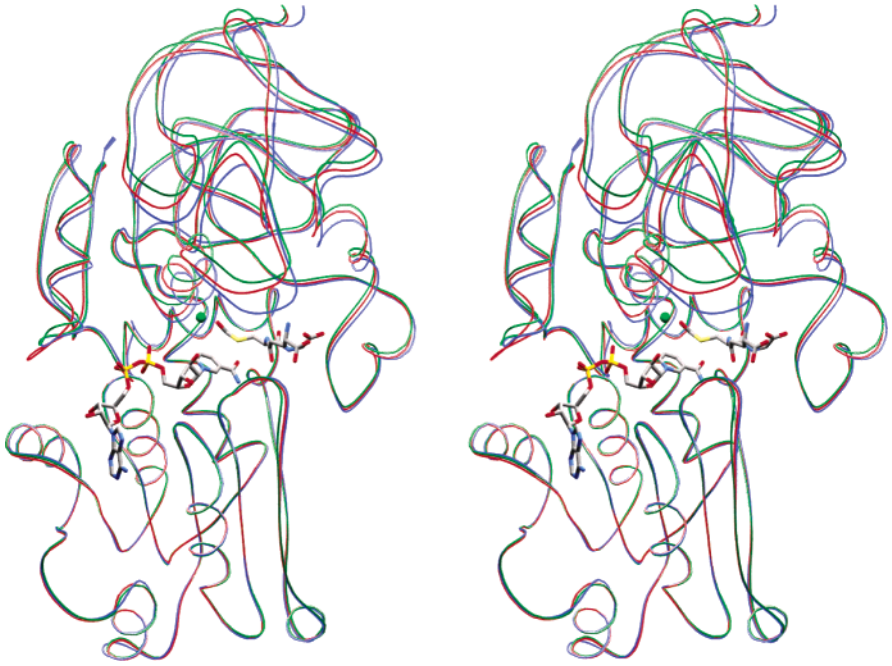


FIGURE 1: Structural changes in FDH upon binding of ligands. Residues 180−289 and 301−320 of the coenzyme-binding domain in the A subunit (blue) and B subunit (green) of the FDH·HMGSH·NADH ternary complex were aligned with the corresponding residues in the apoenzyme (red) using the LSQKAB routine of the CCP4 program. Active site zinc, NADH, and HMGSH are shown in the active site. This figure was generated using Swiss-Pdb Viewer (*19*) and rendered using POV-Ray for windows (www.povray.org).

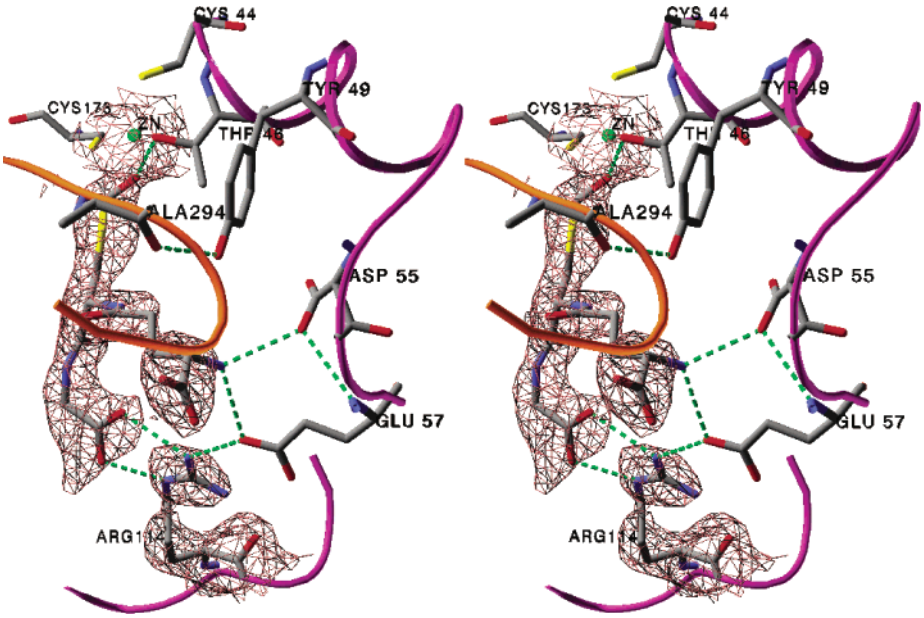


FIGURE 2: Interactions of HMGSH in the A subunit of the FDH·HMGSH·NADH ternary complex. The hydroxyl group of HMGSH interacts with active site zinc (green) and Thr46. The glycinyl carboxyl group of HMGSH interacts directly with Arg114, and the α-amino group of the glutamyl residue of HMGSH hydrogen bonds with Asp55 and Glu57. The interaction of Arg114 with Glu57 and that of Tyr49 with Ala294 appear to assist in domain closure by forming a bridge between the residues of the catalytic domain (purple ribbon) and the coenzyme-binding domain (orange ribbon). This figure was generated using Swiss-Pdb Viewer (*19*) and rendered using POV-Ray for windows (www.povray.org).

plexes of all class I and IV ADH structures (*11*−*13*). The more open catalytic domain position observed in the B subunit is similar to the one observed in the FDH·NAD(H) binary complex (Table 2).

Interactions between HMGSH and the enzyme shown in Figure 2 are similar to interactions between the enzyme active site and bound substrates in ternary complexes of class I ADH (*11*, *12*). The hydroxyl group of HMGSH interacts with both the catalytic zinc and Thr46 (Figures 2 and 3A). HMGSH is positioned appropriately for hydride transfer where its hydroxymethyl carbon is ∼3 Å from the C-4 position of the coenzyme. The *pro-S* hydrogen of the hydroxymethyl group, which is equivalent to the *pro-R* hydrogen of a primary alcohol, is oriented toward the C-4 position of the coenzyme. However, unlike primary aliphatic alcohols, HMGSH is a branched molecule with polar carboxyl and amine groups that enable it to form a number of hydrogen bonds within the active site. The carboxyl group of the shorter glycinyl branch of HMGSH interacts directly with Arg114 in a bidentate fashion (Figure 2) and indirectly

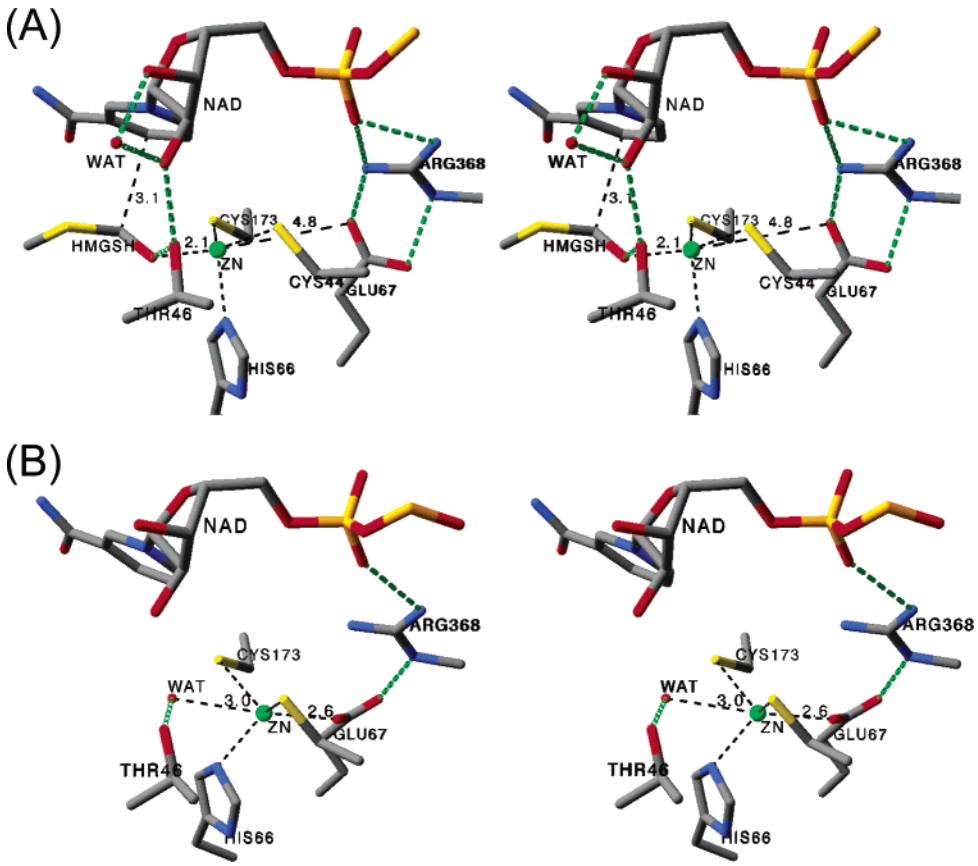


FIGURE 3: Active site zinc coordination environment in the A subunit and B subunit of the FDH·HMGSH·NADH ternary complex. (A) In the A subunit, the active site zinc is coordinated to the hydroxyl group of HMGSH in addition to Cys44, His66, and Cys173. Glu67 is 4.8 Å from the zinc and makes a bidentate interaction with Arg368. The hydroxymethyl carbon is 3.1 Å from C4 of the coenzyme. The proton relay pathway in FDH involves the hydroxyl group of HMGSH, Thr46, ribosyl hydroxyl groups of the coenzyme, and the solvent water molecule (WAT). (B) In the B subunit, only NADH is the ligand present in the active site. The active site zinc is coordinated to Glu67 in addition to the other three ligands. Glu67 is 2.6 Å from the active site zinc and also interacts with Arg368. A solvent water molecule is 3 Å from the zinc. The ribose hydroxyl group of the coenzyme is 3.9 Å from Thr46. This figure was generated using Swiss-Pdb Viewer (*19*) and rendered using POV-Ray for windows (www.povray.org).

with Gln111 via a water molecule (not shown). Arg114 interacts with Glu57 near the mouth of the active site and may help stabilize the closed catalytic domain position. The carboxyl group of the longer γ-glutamyl end of HMGSH stretches away from the catalytic zinc toward residues 49−57 (Figure 2). The α-amino group from the γ-glutamate residue forms hydrogen bonds with Asp55 and Glu57. Tyr49 appears to aid the binding of HMGSH by holding residues 49−57 in its closed position through hydrogen bonding interactions with the backbone oxygen of Ala294 (Figure 2). Such interactions for residues 49−57 in the B subunit might have been adversely affected by nearby lattice contacts and prevented the binding of HMGSH to this subunit.

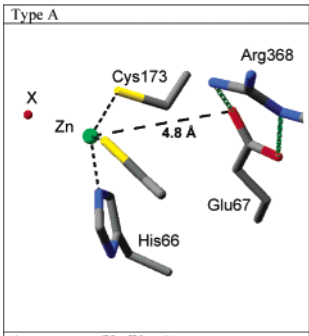
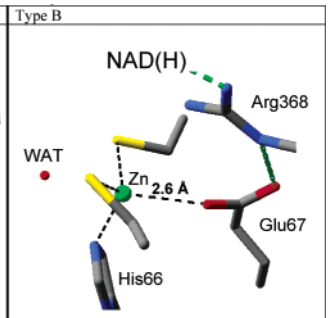
Significant differences exist in the interactions between the coenzyme and active site residues in the two subunits of this structure. The closed domain position in the A subunit enables Thr46 to form a hydrogen bond with the 2′-hydroxyl group of the nicotinamide ribose ring (Figure 3A), while a similar interaction between Thr46 and the coenzyme is absent in the B subunit (Figure 3B) where the more open catalytic domain conformation is observed. As in binary FDH complexes (*7*, *8*), His45 is disorderd in the B subunit, but becomes ordered and interacts with the adenosine phosphate of NADH in the ternary complex observed in the A subunit (not shown). A water molecule that may represent the terminal proton acceptor during catalysis is hydrogen-bonded to the 2′-hydroxyl of the nicotinamide ribose moiety in the A subunit (Figure 3A), but is not observed in the B subunit.

The coordination environment of active site zinc is also different in the two subunits (Figure 3). In the A subunit, where HMGSH is bound, the catalytic zinc is coordinated by Cys44, His66, Cys173, and the hydroxyl group of HMGSH and its position is identical with that in the apoenzyme (Figure 3A and type A zinc coordination in Table 3). Glu67 and the hydroxyl group of HMGSH are 4.6 and 2.1 Å away from the zinc, respectively (Figure 3A). Glu67 in subunit A interacts with the guanidinium group of Arg368. In the binary complex present in the B subunit, the active site zinc is displaced by ∼2.3 Å toward Glu67 relative to the HMGSH complex in the A subunit (Figure 3B and type B zinc coordination in Table 3). As a result, the zinc is coordinated by four ligands from the enzyme that includes Glu67, which is 2.6 Å from zinc. The water molecule from the solvent is 3.0 Å from the active site zinc (Figure 3B).

DISCUSSION

Substrate Binding Site. Kinetic studies show that FDH catalyzes the oxidation and reduction of a wide variety of substrates differing in size, shape, and hydrophobicity (*1*, *14*, *15*). However, larger substrates such as HMGSH and long chain alcohols are catalyzed much more efficiently than smaller substrates such as ethanol (*1*). The poor substrate

Table 3: Zinc Coordination Environment in FDH Complexes[a]

Type A	Type B
	
Apoenzyme (X= Water)	FDH•NAD(H)[b]
FDH•12-HDDA (X=12-HDDA)	FDH•NAD^+•Dodecanoic acid[c]
FDH•HMGSH•NADH (A-subunit) (X=HMGSH)	FDH•HMGSH•NADH (B-subunit)[d]

[a] The active site zinc environments in the A and B subunits of the FDH·HMGSH·NADH ternary complex are shown as type A and B, respectively, in the table. [b] Observed by Yang et al. (*8*). [c] In this complex, the water molecule is absent. [d] This subunit has only NADH present in the active site, and hence, it is an FDH·NADH binary complex.

activity of smaller alcohols is explained by the limited interactions available for these substrates in the open active site of FDH, which undergoes no closure upon binding of the coenzyme. In contrast, class I ADH isoenzymes promote the binding of these small alcohols through a domain closure that occurs upon the binding of the coenzyme and narrows the alcohol-binding site (Table 2). As summarized in Table 2, class I ADHs exhibit approximately 10° of domain closure relative to the apoenzyme upon binding of the coenzyme while the apo form of FDH is semiopen (6° of closure), and there is no domain closure induced upon NAD(H) binding (*7*).

FDH binary and ternary complexes show the structural basis of its substrate specificity (*1*, *14*, *16*). Structural studies show that FDH active site residues interact with ligands via ion pairing and hydrophobic and hydrogen bonding interactions. An anion-binding pocket at the base of the FDH active site, comprised of residues Gln111, Arg114, and Lys283 (*7*), provides charge complementation for the carboxyl group of substrates. Of these residues, Arg114 has been reported to play an important role in the catalytic efficiency (k_{cat}/K_M) of FDH during the oxidation of 12-HDDA and HMGSH (*17*). The carboxyl groups of 12-HDDA and HMGSH both bind in the anion-binding pocket. However, 12-HDDA and HMGSH differ in their lengths and, consequently, interact differently with this anion-binding pocket. In this close ternary complex, Arg114 extends into the active site and directly interacts with the glycinyl carboxylate group of HMGSH (Figure 2). However, in complexes of FDH, 12-HDDA, and dodecanoic acid, only Gln111 and Lys283 interact directly, while Arg114 plays an indirect role, apparently orienting the side chain of Gln111 (*7*).

Residues 49−57, near the mouth of the active site, interact with the glutamyl arm of HMGSH such that the side chains of Asp55 and Glu57 make hydrogen bonding interactions with the α-amino group of HMGSH. The importance of this interaction is evident from the fact that mutation of Asp55 to Leu impairs the K_M of HMGSH but not that of 12-HDDA or octanol (*18*). The interactions of Arg114 with Glu57 and that of Tyr49 with the backbone oxygen of Ala294 appear to stabilize the interaction of HMGSH in the FDH active site by anchoring the domain closure around HMGSH (Figure 2).

The bulk of the substrate-binding site, lined with nonpolar residues such as Tyr92, Ile93, Leu109, Met140, Val293, Val308, Thr309, and Ala317, facilitates the binding of the hydrocarbon chains of 12-HDDA and dodecanoate (*7*). The hydrogen bonding interaction between the hydroxyl group of the substrate and Thr46 (Figure 2) appears to be essential, as mutation of Thr46 to Ala causes the loss of alcohol oxidation activity and decreased efficiency for oxidation of HMGSH (*18*).

The direct interaction of a ligand with the active site zinc is required for the binding of polar compounds such as HMGSH. This is evident from the fact that glutathione is a poor inhibitor of FDH, while the thioacetal adduct of Cys-Gly is a substrate of FDH, despite its inability to interact with Asp55 (*16*). In contrast, FDH binds the hydrophobic compounds dodecanoate and 12-hydroxydodecanoate with comparable affinities.

Conformational Changes of the Catalytic Domain. The changes in catalytic domain conformation affect the substrate specificity and the kinetic mechanism of ADHs. This can be demonstrated by a comparison of the extent of catalytic domain closure in different FDH complexes relative to the apo form of class I ADH (Table 2). The apo form of class I ADH has a more open domain conformation than FDH and undergoes an approximate 10° closure upon binding of its first substrate, the coenzyme. The domain conformation in the apoenzyme of FDH is intermediate between that of the open and closed complexes of horse class I ADH. FDH undergoes little change in domain closure upon binding of the first substrate [12-HDDA or NAD(H)]. The intermediate domain conformation apparently enables FDH to bind its substrates randomly because a significant number of productive binding interactions occur in the binary complexes without a conformational change.

The FDH·HMGSH·NADH ternary complex examined in this study displays a 1−2° domain closure relative to apo and binary FDH structures (Table 2 and Figure 1). This domain closure likely represents a required step for the formation of the catalytically competent ternary complex. The active site zinc directly coordinates the hydroxyl group of HMGSH and appropriately positions the hydroxymethyl carbon for hydride transfer to the coenzyme (Figure 3A). As observed in the ternary complex of horse class I ADH, the catalytic center of FDH is isolated from solvent in the ternary complex and the 2′-hydroxyl group of the coenzyme ribosyl moiety hydrogen bonds with Thr46, forming the proton relay path (*11*, *12*).

The closed domain conformation of the ternary complex reported here appears to be stabilized by the interactions of Arg114 with Glu57 and of Tyr49 with the carbonyl oxygen of Ala294 (Figure 2). HMGSH could promote domain closure by holding Arg114 in the conformation that enables it to interact with Glu57. However, the formation of a HMGSH binary complex would not appear to induce domain closure by itself since the closed conformation would prevent coenzyme binding without a reopening of the catalytic domain. These structural conclusions are consistent with the random kinetic mechanism of FDH, where either HMGSH or NAD^+ can bind to the enzyme and HMGSH does not display substrate inhibition (*5*). We believe that domain closure occurs following the formation of a catalytically competent ternary complex, since we

observed no closure in either binary complex of FDH (*7*, *8*).

Proton Relay. Kinetic studies of horse and human class I ADH suggest that the hydroxyl proton of the alcohol is abstracted and transferred to the solvent by a proton relay pathway prior to hydride transfer (*11*). The components of the class I proton relay are the Thr/Ser residue that hydrogen bonds with the alcohol hydroxyl group (Ser48 in horse class I ADH), the hydroxyl groups of nicotinamide ribose, and a general base in contact with solvent (His51 in horse class I ADH). This proton relay pathway is fully formed in only the closed domain conformation (*11*). The semiopen catalytic domain position present in an FDH·NAD(H) binary complex did not bring the hydroxyl groups of the ribosyl moiety within hydrogen bonding distance of Thr46. The movement of the catalytic domain in the ternary complex reported here brings the ribose hydroxyl groups within hydrogen bonding distance of Thr46 (Figure 3A). However, there are no basic amino acid residues within 3.5 Å of the ribose hydroxyls of the coenzyme in FDH, only water molecules. As a consequence, it would appear that the proton relay terminates at the ribose hydroxyls and occurs directly from the ribose hydroxyl to solvent.

Active Site Zinc Coordination. The number of ligands contributed by FDH to the active site zinc changes from three to four in various complexes (Table 3). In the apoenzyme, the FDH·12-HDDA binary complex, and the FDH·HMGSH·NADH ternary complex, the zinc is liganded to Cys44, His66, and Cys173 (type A coordination, Table 3). In the FDH·NAD(H) binary and FDH·NAD^+·dodecanoic acid ternary complexes, the zinc is liganded by an additional protein residue, Glu67 (type B coordination, Table 3). This change in coordination number is a consequence of a 2.3 Å displacement of the active site zinc toward Glu67, a small rotation of the side chain atoms in Glu67, and a reorientation of the three remaining zinc ligands. The displacement of active site zinc toward Glu67 may promote ligand exchange for catalysis as the distance between the zinc and a solvent ligand is increased.

A comparison of the zinc coordination states in the five different FDH complexes determined to date suggests that the binding of the coenzyme causes the change in coordination. Since the exchange of zinc-bound ligands is an essential step for both the oxidative and reductive reactions, it is conceivable that this process is independent of the oxidation state of the coenzyme. A likely mechanism for initiation of the zinc displacement is the disruption of the interaction between Arg368 and Glu67 in the presence of the coenzyme (Figure 3). Glu67 is bound to Arg368 in the apoenzyme (Table 3). Once the coenzyme binds, the interaction between Arg368 and the pyrophosphate of the coenzyme would permit a stronger interaction between the carboxylate of Glu67 and the active site zinc (Figure 3B).

Following the formation of a catalytically competent ternary complex, the active site zinc reverts to the type A coordination as it is drawn by the proximity of the lone pair electrons present on the hydroxyl oxygen atom of the substrate (Figure 3A and Table 3). At the same time as this change in coordination geometry, the zinc atom promotes the abstraction of the hydroxyl proton by Thr46 (Figure 3A). Following hydride transfer, the collapse of the oxygen electrons onto the aldehydic carbon could promote the displacement of zinc toward Glu67 (type B coordination, Table 3) and facilitate rapid exchange of the product aldehyde. Following release of the product aldehyde, the reduced coenzyme could then diffuse out of the active site and restore the active site zinc to its original coordination state with a water ligand (type A coordination, Table 3). This mechanistic proposal is consistent with the preferred pathway for product release in FDH (*5*).

In summary, the closure of the catalytic domain upon ternary complex formation and the displacement of the catalytic zinc during ligand exchange are unique aspects of the catalytic cycle in FDH relative to other dimeric medium chain ADHs. The stabilization of the semiopen domain conformation in the apoenzyme and both binary complexes of FDH provides a structural explanation for the enzyme's random binding of substrates. Domain closure promotes catalysis in FDH in a manner similar to that described for the classical ADH isoenzymes, by bringing the coenzyme closer to the substrate, isolating the active site from the solvent, and forming the proton relay pathway. However, in FDH this closure is apparently induced only following formation of a catalytically competent ternary complex. The changes in zinc coordination during the catalytic cycle demonstrate the mechanism by which the zinc can achieve substrate−product exchange. It remains to be seen whether zinc displacement and domain movement are similarly coupled in other members of the alcohol dehydrogenase family.

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