Disruption of the Coenzyme Binding Site and Dimer Interface Revealed in the Crystal Structure of Mitochondrial Aldehyde Dehydrogenase “Asian” Variant*

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Mitochondrial aldehyde dehydrogenase (ALDH2) is the major enzyme that oxidizes ethanol-derived acetaldehyde. A nearly inactive form of the enzyme, ALDH2*2, is found in about 40% of the East Asian population. This variant enzyme is defined by a glutamate to lysine substitution at position 487 located within the oligomerization domain. ALDH2*2 has an increased Km for its coenzyme, NAD+, and a decreased kcat, which lead to low activity in vivo. Here we report the 2.1 Å crystal structure of ALDH2*2. The structure shows a large disordered region located at the dimer interface that includes much of the coenzyme binding cleft and a loop of residues that form the base of the active site. As a consequence of these structural changes, the variant enzyme exhibits rigid body rotations of its catalytic and coenzyme-binding domains relative to the oligomerization domain. These structural perturbations are the direct result of the inability of lysine 487 to form important stabilizing hydrogen bonds with arginines 264 and 475. Thus, the elevated Km for coenzyme exhibited by this variant probably reflects the energetic penalty for reestablishing this site for productive coenzyme binding, whereas the structural alterations near the active site are consistent with the lowered Vmax.

Mitochondrial aldehyde dehydrogenase (ALDH2) is best known for its role in ethanol metabolism, oxidizing acetaldehyde to acetate (1). More recently, an additional role for ALDH2 has been described as the initiator of nitroglycerin bioactivation (2). About 40% of the East Asian population carries a semidominant polymorphism of the ALDH2 gene, ALDH2*2 (3). The associated glutamate to lysine substitution at position 487 causes the enzyme to be nearly inactive in vivo.

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¶¶ The abbreviations used are: ALDH2, mitochondrial aldehyde dehydrogenase; ALDH2*2, semidominant polymorphism of ALDH2; MCAD, medium chain acyl-CoA dehydrogenase; ACES, N-(2-Acetamido)-2-aminothanesulfonic acid.

The resulting phenotype is characterized by an aversive response to ethanol consumption, which may include facial flushing, nausea, and tachycardia, with more severe reactions observed in those individuals who are homozygous for the polymorphism (4). This adverse effect has been linked to a lower frequency of alcoholism among those both hetero- and homozygous for the variant enzyme. Even so, ALDH2*2 has been linked to alcoholic liver disease as well as oropharyngeal and esophageal cancers in alcoholic patients (5, 6).

The kinetic properties of human ALDH2*2 have been determined using protein expressed and purified from Escherichia coli (7). The variant enzyme was found to be active, but it exhibits a 200-fold increased Km for NAD+ and a diminished kcat. The enzyme Km for NAD+ exceeds the available concentration in the cell by 15-fold, which, combined with the 10-fold lower kcat value, would lead to an approximately 100-fold lower activity in vivo (7).

The three-dimensional structure of ALDH2 has been solved and described previously (8, 9). The enzyme is a tetramer of four identical subunits, each consisting of 500 amino acid residues (Fig. 1). This tetramer can be regarded as a dimer of dimers in that the interface between monomers that form a dimer is different and more extensive than the interface between the two dimers that form the tetramer. Each subunit is composed of three main domains: the catalytic domain, the coenzyme or NAD+-binding domain, and the oligomerization domain (8) (Fig. 2). Glu487 is located within the oligomerization domain, which is important for both dimer and tetramer formation. In wild-type ALDH2, Glu487 forms hydrogen bonds with Arg264 of the same subunit and with Arg475 of the adjacent dimer partner. Consequently, the disruption of these interactions by the presence of Lys487 was projected to perturb the structure of both its own subunit as well as its dimer partner (8). The interaction across the dimer interface has been thought to be responsible for the dominant effects of this mutation (8).

In addition to the dominance of ALDH2*2, other examples of intersubunit communication have been observed in ALDH2. Half-site reactivity for ALDH2 was first demonstrated in wild-type horse liver ALDH2 where there was a pre-steady-state burst of NADH formation equivalent to 2 mol of NADH per ALDH2 tetramer (10). Wild-type rat ALDH2 demonstrated the same pre-steady-state burst, and the rate-limiting step was determined to be the deacylation step (7). However, ALDH2*2 does not demonstrate the pre-steady-state burst of NADH formation, suggesting that for the variant enzyme, the rate-limiting step occurs before NADH formation, possibly involving thiohemiacetal formation (7).

Based on studies conducted with wild-type ALDH2*2 heterotetramers, the current model of half-site reactivity is one
where only one subunit per dimer pair is active in wild-type ALDH2 (11). Whether activity alternates between active sites or remains fixed in a particular active site is not yet understood. These kinetics studies support a model of dominance where a variant subunit would inactivate its wild-type dimer partner but would have no effect on the subunits in the dimer pair across the tetramer interface.

Numerous aldehyde dehydrogenase crystal structures have shown that the nicotinamide portion of NAD(P) is flexible and occupies two main conformations (9, 12–15). These conformations are likely to play a functional role in catalysis where the extended conformation is primarily occupied by NADH and is conducive to hydride transfer, and the other, more contracted conformation is primarily occupied by NADPH and is conducive to acyl-enzyme hydrolysis (12). Magnesium is important for stabilizing these two conformations, thereby elucidating its documented role in elevating $V_{\text{max}}$.

To further study the variant ALDH2 enzyme, we have solved the apoenzyme crystal structure of ALDH2*2 to 2.1 Å resolution. This structure reveals that mutation of Glu487 to lysine in the apoenzyme crystal structure of ALDH2*2 to 2.1 Å resolution. This structure reveals that mutation of Glu487 to lysine in the apoenzyme crystal structure of ALDH2*2 to 2.1 Å resolution...
**Data Collection, Processing, and Model Refinement**—Data were collected at the Advanced Photon Source at Argonne National Laboratory (Argonne, IL) on beamline SBC-CAT 19ID. The beamline was equipped with the APS-1.3 × 3 array CCD detector. The x-ray beam wavelength for data collection was 12.4 keV. Data were indexed, integrated, and scaled with the HKL2000 program suite (19).

Phases were solved using the molecular replacement method with the human ALDH2 coordinates as a starting model. Molecular replacement was executed using the program AMoRe as implemented in the CCP4 program package (20, 21). The resulting models were refined using the Crystallography and NMR System (CNS) suite (22). For the refinement of the apoenzyme model, rigid body refinement was followed by simulated annealing and minimization that were performed with NCS restraints applied at a weight of 100 kcal/mol Å² for the main-chain atoms and 10 kcal/mol Å² for the side-chain atoms. This was followed by individual restrained isotropic temperature factor refinement. Model rebuilding was performed using the program O (23). To reduce model bias, composite annealed omit maps were calculated. Only energy minimization and temperature factor refinements were utilized in subsequent rounds with residues 246–264 and 466–476 excluded from NCS restraints during minimization. In the final round of refinement, the NCS restraint weights were reduced to 50 kcal/mol Å² for main-chain atoms with no NCS restraints imposed on the side-chain atoms. In this final round, residues 244–271, 424–425, and 463–478 were excluded from the main-chain NCS restraints. R_pfree was evaluated using a randomly chosen 5% of the crystallographic data.

**Structure Analysis**—Hinge axis determination for positional domain differences between ALDH2 and ALDH2*2 were analyzed using the program DynDom as implemented in the CCP4 package (20, 21). Tetramer, subunit, and domain alignments were performed using the program LSQKAB as implemented in the CCP4 package (20, 25). Root mean square deviations were calculated for the Cα atoms of each alignment. Residues 244–271, 424–425, and 463–478 were omitted for all pairings. To determine the extent of domain reorientation relative to wild type, the cofactor-binding domain was established as the point of reference. This domain was aligned to apoenzyme wild type. The rotated ALDH2*2 subunit was then realigned to wild type using either the catalytic or oligomerization domain. The extent of domain rotation is thus expressed as the amount of rotation necessary to align the domain of interest relative to the common positions of the coenzyme-binding domains.

The extent of secondary structural shifts was determined by aligning the cofactor-binding and catalytic domains together to wild-type enzyme. Therefore, these values are expressed as the amount of shift within the context of these domains and are determined independently of domain shifts.

Residues were considered ordered and were included in the final coordinates if the electron density maps contoured at one S.D. of the map indicated their presence.

**RESULTS**

ALDH2*2 crystallized in the triclinic space group with three tetramers in the asymmetric unit. The crystal diffracted to 2.1 Å resolution, and the structure was refined to R_pfree = 23.8% and R_work = 20.4%. Like wild-type ALDH2, ALDH2*2 is a tetramer of four identical subunits. Ordered solvent molecules included 2649 water molecules, 21 ethylene glycol molecules, 8 guanidines, and 12 sodium ions. All data collection and refinement statistics are summarized in Table I.

**Interactions Surrounding Lys487**—The majority of residue Lys487 is well ordered and clearly present in the electron density maps contoured at one S.D. (Fig. 3a). The loss of hydrogen bonds among residues 487, 475, and 264 is accompanied by changes in side-chain conformation of these residues (Fig. 4). No favorable interactions were observed between Lys487 and Arg475 in the dimer partner. Lys487 points away from the dimer interface and forms no new hydrogen bonds. Lys487 (Fig. 2) makes van der Waals contacts with the side chain of Tyr105 (when observed) in the neighboring subunit as well as with the peptide carbonyl oxygen of Pro159 and the main chain and side chain of Val159 within its own subunit. Arg475 can only be modeled in seven of the 12 subunits. In each of the seven instances, residue 475 occupies the same conformation as wild type with the Nα atom within hydrogen bonding distance of the main-chain carbonyl of 488 in the neighboring subunit. The five remaining subunits do not display interpretable electron density for Arg475. Although not identical, all observed conformations for Arg264 are rotated away from Lys487.
Table II

<table>
<thead>
<tr>
<th>Subunit</th>
<th>α helix</th>
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</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>257–263</td>
<td>424–425</td>
<td>463–478</td>
</tr>
<tr>
<td>B</td>
<td>244–264</td>
<td>424–425</td>
<td>468–473</td>
</tr>
<tr>
<td>C</td>
<td>246–262</td>
<td>424–425</td>
<td>468–474</td>
</tr>
<tr>
<td>D</td>
<td>248–263</td>
<td>424–425</td>
<td>469–476</td>
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<tr>
<td>E</td>
<td>246–263</td>
<td>424–425</td>
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<tr>
<td>F</td>
<td>246–262</td>
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<td>G</td>
<td>246–262</td>
<td>424–425</td>
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<td>H</td>
<td>246–262</td>
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<tr>
<td>I</td>
<td>246–263</td>
<td>424–425</td>
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</tr>
<tr>
<td>J</td>
<td>246–263</td>
<td>424–425</td>
<td>469–475</td>
</tr>
<tr>
<td>K</td>
<td>246–271</td>
<td>424–425</td>
<td>466–475</td>
</tr>
<tr>
<td>L</td>
<td>245–263</td>
<td>424–425</td>
<td>466–476</td>
</tr>
</tbody>
</table>

DISORDER AT THE INTERFACE—The most striking feature of the ALDH2*2 crystal structure is the disordered region surrounding Lys487 including residues 244–272 and 463–478 (Table II). Adjacent to 469 in the catalytic domain, residues 424 and 425 are also disordered. Residues 469–473 are disordered in all subunits of the asymmetric unit. In 11 of the 12 subunits within the asymmetric unit, residues 248–262 are not visible in electron density maps contoured at one S.D. (Fig. 3b). These residues comprise the αG helix located at the interface between adjacent subunits within a dimer pair. This helix also comprises part of the adenine binding pocket for NAD+. In subunit A, αG is more ordered with only residues 257–263 missing. Although this helix is observed, it has shifted 3 Å, relative to the wild-type structure (Fig. 2b). In the remaining subunits, the general path of αG appears to shift in the same manner as in subunit A; however, the residues are insufficiently defined in the electron density maps for confident placement.

Unique to subunit A are the crystal packing contacts between this subunit and the crystallographically related subunit K. The αD helix of subunit K makes contact with and stabilizes αF of subunit A. This results in a 15% lower average temperature factor for αF as compared with the rest of the subunit. αF is positioned on the opposite side of the NAD+ binding cleft from αG, and its increased order supports αG in this subunit.

Adjacent to αG in both primary and tertiary structure is the β11 strand, which includes Arg424 and Glu426. In all subunits of ALDH2*2, the position of the β11 strand is shifted 2 Å in the same direction as αG. The Ca atom of the putative general base, Glu426, has shifted −1.5 Å away from the active site, and its side-chain position is ill defined and not consistent among the subunits. In response to the shifts of both αG and β11, β10 has shifted 0.8 Å in the same direction as αG and β11.

LOOP DISORDER LINKED TO ACTIVE SITE—In wild-type ALDH2, the loop composed of residues 463–478 interacts with residues 269–272 through two hydrogen bonds: one between the peptide carbonyl oxygen of 272 and the main-chain nitrogen of 269 and the other between the side-chain hydroxyl of Ser473 and the peptide carbonyl oxygen of residue 270 (Fig. 4). Residues 269–272 associate with the active site through a hydrogen bond between the peptide nitrogen of 271 and the side chain of Glu399. In cofactor-bound wild-type ALDH2, Glu399 stabilizes the nicotinamide portion of NAD+ by forming hydrogen bonds between its carboxylate and the 2'- and 3'-hydroxyl oxygens of the nicotinamide ribose (hydride transfer conformation). However, in concert with the flexibility of the loop containing residues 463–478 in ALDH2*2, the main chain of residues 269–272 rotates such that the amino nitrogen of 271 no longer stabilizes the side chain of Glu399 in the active site (Fig. 4). Glu399 assumes a new conformation that is rotated away from the active site and is instead stabilized by the peptide nitrogen of 272. Coincident with this change, the side chain of Phe401 rotates into the void created by the movement of Glu399. Phe401 supports the binding of the NMN portion of the coenzyme in all ALDH2 holoenzyme structures.

The Active Site—The catalytic nucleophile, cysteine 302, is well ordered and retains a wild-type-like conformation. Electron density maps surrounding Cys305 reveal binding of an unknown molecule. Kinetics experiments have shown that the crystallization precipitant, polyethylene glycol 6000MW, contains aldehydes at an approximate concentration of 0.5 mM (12). Therefore, this unaccounted for density is probably due to unidentified aldehydes arising from the polyethylene glycol. The resolution of the data is insufficient to fully define the bound molecule; therefore, solvent molecules were modeled into the positive difference peaks to partially account for the unknown molecule.

Asparagine 169, important for stabilizing oxyanion formation in the transition state (8), is also well ordered and is in the same conformation as wild-type enzyme. As mentioned above, the side chain of Glu266 is disordered and not consistent among subunits.

DOMAIN ShiftS—Between the cofactor-binding domain and the oligomerization domain lies a hinge axis about which there is a 2.5° rotation in ALDH2*2 relative to wild type, as determined using the program DynDom (20, 24). Analysis of relative domain positions within the dimer pair reveals residues that form the hinge, which include the three β strands of the oligomerization domain as well as the loop composed of residues 463–478. Individual domain alignments using the tetramer, dimer, and monomer were also performed (Table III). These, too, demonstrate that the cofactor-binding and catalytic domains have changed position within the tetramer in a manner that can best be described as a 2.5° rigid body rotation away from the dimer interfaces (Fig. 5).

DISCUSSION

The crystal structure studies of ALDH2*2 have revealed that mutation of glutamate to lysine at residue 487 causes a large structural transition within the variant enzyme. Whereas Lys487 is not itself disordered in the ALDH2*2 crystal structure, its presence at the dimer interface perturbs local secondary structure elements, particularly αG, β10, β11, and the loop consisting of residues 463–478. These structures are either closely or directly involved with NAD+ binding. The disruptions of αG and the aforementioned loop also lead to an unstable dimer interface where the catalytic and coenzyme-binding domains of individual subunits rotate away from the interface. Moreover, the general base, Glu266, is not stably positioned for catalysis, and the active site is further disrupted at residues 399 and 401 through a cascade of changes that appear to originate in the dimer partner. These shifted main-chain and side-chain positions undoubtedly contribute to the decreased activity observed for this ALDH2 variant and also shed light on the variant’s mechanism of allelic dominance.

Disorder of residues 463–478 has been observed in other aldehyde dehydrogenase crystal structures. Lamb and Newcomer have reported these residues missing in the electron density maps of cofactor-bound retinal dehydrogenase type II (RαdH2), which shares about 67% sequence identity with ALDH2 (26, 27). What is unique to ALDH2*2 is the complete loss of both the loop and the αG helix in the electron density maps.

The positional disorder of the αG helix is likely to be the major contributor to the high Km for NAD+ of ALDH2*2. Arg424, located at the C-terminal end of the αG helix, forms a hydrogen bond with Glu487 in wild-type ALDH2, thus helping to anchor the helix. The substitution of lysine for glutamate at 487 leads to the loss of this anchor and the observed flexibility of αG.
No significant differences in main-chain or side-chain positions are found between cofactor-bound and apoenzyme wild-type ALDH2. Therefore, we have compared ALDH2*2 to the coenzyme-bound form of ALDH2 to highlight the potential impact of the structural changes in ALDH2*2 on coenzyme binding. In wild-type enzyme, the adenine ring of NAD⁺ is nestled between αF and αG and makes Van der Waals contacts with Ile²⁴⁹ and Leu²⁵² within αG. The peptide nitrogen and side-chain hydroxyl of Ser²⁴⁶ at the N-terminal end of αG form hydrogen bonds with the adenosine phosphate. In ALDH2*2, Ser²⁴⁶ is only observed in subunits A and D, and Ile²⁴⁹ is only observed in subunit A. In those subunits, Ser²⁴⁶ has shifted 2 Å into the adenine binding site, and Ile²⁴⁹ has shifted about 2.5 Å toward the adenosine phosphate binding site. These positions would sterically interfere with coenzyme binding. In wild-type ALDH2, the carbonyl oxygen of Leu²⁶⁹ stabilizes the amide group of the nicotinamide ring, and in ALDH2*2 it has flipped away from the nicotinamide site and toward the dimer interface.

In order for the adenine portion of NAD⁺ to bind, Ser²⁴⁶, Val²⁴⁹, and Leu²⁵² must be stably and appropriately positioned, and the αG helix must reorder and shift away from helix αF. For proper positioning of the nicotinamide, the main chain at Leu²⁶⁹ would need to rotate to position the carbonyl toward the amide, Phe³⁰¹ would need to rotate out of the nicotinamide ribose binding site, and Glu³⁹⁹ must reposition itself as a hydrogen bond acceptor for the ribose oxygen atoms. A substantial loss in binding energy, such as that actually observed, would be required for such secondary structure and side-chain repositioning.

Deacylation is the rate-limiting step for wild-type ALDH2 for most substrates. In contrast, the rate-limiting step for ALDH2*2 has been proposed to occur prior to NADH formation and is perhaps thiohemiacetal formation. However, these studies on ALDH2*2 have been in the presence of saturating concentrations of NAD⁺. Because the cellular NAD⁺ concentration in humans is only about 500 μM (28), it has been further suggested that in vivo, the binding of NAD⁺ to enzyme may, in fact, be the rate-limiting step of ALDH2*2 oxidation of acetaldehyde (7). This assertion is supported by the presented structural evidence, since at least part of this binding step will also include rate constants associated with the reordering of the binding site for the coenzyme. This effect is most clearly seen where the association rate for NAD⁺ is decreased 3400-fold, whereas the dissociation rate is only accelerated 90-fold (7). These data are consistent with most folding transition data where cooperative folding events are seen; while slow to form, once formed, they are marginally stabilized by the cooperative interactions.

**TABLE III**

<table>
<thead>
<tr>
<th>Domain</th>
<th>All domains</th>
<th>Oligomerization</th>
<th>Coenzyme/catalytic</th>
<th>Coenzyme</th>
<th>Catalytic</th>
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</thead>
<tbody>
<tr>
<td>Wild-type tet1 to 2*2 tet1</td>
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<td>0.41</td>
<td>0.77</td>
<td>0.78</td>
<td>0.71</td>
</tr>
<tr>
<td>Wild-type ab to 2*2 ab</td>
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<td>0.39</td>
<td>0.76</td>
<td>0.67</td>
<td>0.68</td>
</tr>
<tr>
<td>Wild-type a to 2*2 a</td>
<td>0.30</td>
<td>0.34</td>
<td>0.22</td>
<td>0.19</td>
<td>0.22</td>
</tr>
<tr>
<td>2<em>2 a to 2</em>2 b</td>
<td>0.17</td>
<td>0.15</td>
<td>0.14</td>
<td>0.13</td>
<td>0.10</td>
</tr>
</tbody>
</table>

**FIG. 5. The ALDH2*2 enzyme (red) aligned to wild type (blue).** The view shown is rotated about 90° with respect to Fig. 2, a, monomer view; alignment of the coenzyme-binding and catalytic domains. The 2.5° rotation between the coenzyme-binding and oligomerization domains is illustrated by the shift in the oligomerization domain. b, dimer view; alignment of the oligomerization domains. The 2.5° rotation causes the coenzyme-binding and catalytic domains to fall away from the interface at the αG helices.
gen bonds with the carbonyl group of 469. In fact, Arg<sup>264</sup> no longer engages with the dimer partner at all. As expected, no favorable interactions are observed between Lys<sup>487</sup> and Arg<sup>475</sup> in the dimer partner. The lack of these intersubunit ties probably gives rise to the disorder of the 463–478 loop.

Although the loop at 463–478 does not interact directly with coenzyme, its flexibility initiates a cascade of main-chain and side-chain shifts that disturb the nicotinamide binding pocket in the active site, specifically at residues Glu<sup>399</sup> and Phe<sup>401</sup>. This would explain why even with NAD<sup>+</sup> bound, ALDH2*2 still exhibits a low <i>k<sub>cat</sub></i>. NAD<sup>+</sup> may bind, but without these interactions, the nicotinamide portion may not be appropriately constrained for hydride transfer. Mutations of Glu<sup>399</sup> in wild type have been shown to alter the rate-limiting step of ALDH2 and have deleterious effects on activity (29). The mechanism by which a Lys<sup>487</sup> subunit inactivates its dimer partner includes the aforementioned main-chain and side-chain shifts, including Glu<sup>399</sup>, the shift of β11, which contains the general base Glu<sup>268</sup>, and the disorder of the αG helix. Alternatively, NAD<sup>+</sup> binding may reorder these side chains, and the disruptions at the floor of the active site, including Glu<sup>268</sup>, may have a more significant effect on the observed <i>k<sub>cat</sub></i>.

**Intersubunit Communication**—In wild-type ALDH2, the half-site reactivity has been described as an extreme example of negative cooperativity (30). The mechanism of ALDH2*2 dominance has been speculated to be linked to the mechanism of half-site reactivity (8, 11). Although the structure presented here does not immediately describe a mode of half-site reactivity, it does provide clues to which residues might be involved in inter-active site communication.

In ALDH2*2, Arg<sup>264</sup> ceases to form hydrogen bonds with the neighboring subunit, contributing to disorder within that dimer partner. In the seven subunits where Arg<sup>475</sup> is observed, it occupies the same conformation as in wild type. This lack of conformational change may suggest that of the two arginines, Arg<sup>264</sup> may have a more dynamic role in intersubunit communication, particularly through indirect alteration of the Glu<sup>399</sup> conformation and subsequent effects on nicotinamide binding.

The changes described by the ALDH2*2 structure affect the interface between monomers in a dimer pair. No major changes at the tetramer interface were observed. This evidence supports the current model for dominance where each dimer pair functions as a unit, and only one active site per dimer pair is functioning (11).

Originally, it was thought that half-site reactivity reflected half-site NAD<sup>+</sup> occupancy, and a number of NADH-binding studies were consistent with stoichiometries of less than four per tetramer (30, 31). However, the available holoenzyme ALDH2 structures show full occupancy of coenzyme in all active sites of the tetramer (9, 12, 15). Although no structural differences have been observed between NAD<sup>+</sup>-binding domains within a dimer pair, the two distinct conformations of the nicotinamide half of the coenzyme may influence both the activation/inactivation of a particular active site and the observed stoichiometry of NADH binding using fluorescence measurements. Based solely on proximity to bulk solvent, the two observed conformations of the coenzyme when bound to ALDH would be expected to exhibit different fluorescence properties relative to coenzyme in solution and could confound the analysis of stoichiometry. The conformational differences in how the coenzyme is bound during catalysis could be relayed through the αG helices that form the adenosine binding sites in each dimer partner. The large scale alterations we observe in the ALDH2*2 crystal structure are consistent with a structural connection between changes in the αG helix that forms the adenosine binding cleft in one subunit to changes in the nicotinamide binding site in the dimer partner. However, we have not observed such an activation/inactivation event in wild-type ALDH2 structures, although the molecular averaging inherent in a crystallographic experiment may mask such a difference.

**Medium Chain Acyl-CoA Dehydrogenase (MCAD)**—Single amino acid substitutions in critical subunit interfaces, as discussed here for ALDH2, are also found to exist in other enzymes. For example, a deficiency in MCAD, a mitochondrial enzyme involved in the β-oxidation of straight chain fatty acids, is life-threatening (32), especially during periods of fasting in conjunction with viral infection. Although more than 20 variants of the MCAD gene have been reported, one MCAD enzyme mutation, K304E, is present in more than 90% of alleles in patients with MCAD deficiency. This autosomal recessive mutation is responsible for decreased thermal stability and enzyme activity and may destabilize the active tetramer, once formed. Like Glu<sup>487</sup> in ALDH2, Lys<sup>484</sup> lies at an interface between subunits (33). Although Lys<sup>484</sup> does not make any direct contacts with the neighboring subunit, the residue is located within a helix that makes many contacts across the interface. Perhaps the conversion of lysine to glutamate at 304 within this enzyme perturbs folding at the interface between monomers in a manner similar to glutamate to lysine at 487 in ALDH2.

**Conclusion**—Mutation of residue 487 from glutamate to lysine in ALDH2 leads to an enzyme that is rendered essentially inactive in vivo. Upon mutation to lysine, residue 487 no longer forms hydrogen bonds with arginine 264 in its own subunit and arginine 475 in the dimer partner. The loss of these interactions leads to disorder of the NAD<sup>+</sup>-binding site, particularly in the αG helix at the dimer interface. This positional disorder is a major contributor to the high <i>k<sub>cat</sub></i> observed for NAD<sup>+</sup> binding in ALDH2*2, which leads to its inactivity in vivo. A mechanism of dominance is revealed in the altered main-chain and side-chain positions from Arg<sup>475</sup> to across the dimer interface, leading eventually to the nicotinamide binding site where Glu<sup>399</sup> is not in position to stabilize the nicotinamide ribose. This alteration may contribute to the low <i>k<sub>cat</sub></i> and would predominantly affect the hydride transfer conformation of cofactor, since this position more heavily relies on Glu<sup>487</sup> for nicotinamide stability. However, to fully appreciate the dynamics between Lys<sup>487</sup> and Glu<sup>487</sup> subunits within a ALDH2*2/ALDH2 dimer pair, the crystal structures of heterotetramers would prove most valuable.

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