Alcohol Dehydrogenase

nicotinamide mononucleotide; Tes, N-\{tris\[hydroxymethyl\]methyl-2-amino\) ethanesulfonic acid.

therefore be hereby marked “advertisement” in accordance with 18

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ications with the enzyme, a crystallographic investigation of

their conformation and orientation within the molecule

whereas the catalytic domains rotate and narrow the
cleft between the domains. The active site becomes

shielded from the solution by a combination of this

rotation, local movements of a loop from residues 53 to

57 and coenzyme and substrate binding. Both subunits

bind coenzyme and inhibitor to the same extent. The

nicotinamide ring of the coenzyme is positioned close

to the active zinc atom and the inhibitor is bound to

this zinc atom. The difference between the two crystal

lographically independent subunits is small. The pro-

posed mechanisms of action for the enzyme based on

the apoenzyme structure are confirmed by the present

investigation.

The three-dimensional structure of a ternary com-

cplex of horse liver alcohol dehydrogenase with reduced

nicotinamide adenine dinucleotide and the inhibitor
dimethyl sulfoxide has been determined to 4.5 Å resolu-

tion independently of the apoenzyme structure. The

electron density maps of both structures have been

compared. The two coenzyme binding domains which

form the center of the dimer molecule have retained

their conformation and orientation within the molecule

but we have chosen the complex which reproducibly gave the

best quality triclinic crystals. These crystals diffract beyond

2 Å resolution and are more stable than the orthorhombic

apoenzyme crystals.

MATERIALS AND METHODS

Horse liver alcohol dehydrogenase, kindly supplied by Dr. Å.

Akeson, was crystallized from 1M protein solutions in the presence of

NADH and Me,SO (5%) by dialysis against 2-methyl-2,4-pentane diol

of increasing concentration in 0.05 M Tes/NHg buffer at pH 7.0. The

crystals are triclinic with the cell dimensions a = 51.2 Å, b = 44.5 Å,
c = 94.3 Å, α = 104.5°, β = 102.0°, and γ = 70.9°. The unit cell (which

is the asymmetric unit) contains the dimer of molecular weight 82,000.

Three derivatives were used: uranyl oxalate (10−3 M), methyl

mercurythiosalicylate (10−3 M, in the absence of Me,SO) and Hg(CN)2

(1 to 3 x 10−4 M).

4746 independent x-ray intensities corresponding to a resolution of

4.5 Å were measured on a Stoe-Phillips four-circle diffractometer and

processed as described earlier (4). The derivative data were scaled by

minimizing the sum of the differences between their F-values and the

native ones. Anisotropic temperature factors were applied to each
derivative crystal in the scaling procedure.

The main heavy atom sites of the derivatives were located from
difference Fourier maps using phases calculated from the apoenzyme

model. The coordinates of this model were oriented and positioned in

the triclinic cell by the rotation function (13) and two mercury

positions common to both structures. Additional sites were found

from difference and double difference Fourier maps, using isomor-

phous phase angles. The heavy atom parameters were subjected to

about 30 cycles of the usual least squares refinement procedure (14).

The present refinement parameters are given in Tables I and II. A

mean figure of merit for the 4591 reflections included in the refine-

ment was 0.73, using best phases during the refinement (15).

The electron density map was plotted perpendicular to the non-
crystallographic molecular 2-fold axis found by the rotation function

(κ = 180°, ψ = 30°, and φ = 17°). An orthorhombic electron density

map at the same resolution was plotted in the same orientation.

a carbon coordinates and main chain tracing of the apoenzyme model

were plotted in the orthorhombic map to facilitate the interpretation.

Details of the structure determination will be reported in a later

paper.

RESULTS AND DISCUSSION

The electron density map obtained was of good quality (Fig.

1) and all significant densities could be assigned to protein,

coenzyme, and inhibitor. The highest peaks in the map cor-

respond to a helices, zinc atoms, and the phosphate atoms of

the coenzyme. The interpretation was greatly aided by com-

parison with the known apoenzyme structure. It was possible

to follow the main chain through the triclinic electron density

even in the most complicated pleated sheet structure of the
catalytic domain. There were breaks at some points in the

electron density, mostly at glycine residues. Furthermore,

many side chains were visible, especially large ones, which

made it easy to relate the density to the model of the enzyme.

The ADP part of the coenzyme molecule binds to and

Structural Differences between Apo- and Holoenzyme of Horse Liver
Alcohol Dehydrogenase*

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* The abbreviations used are: Me,SO, dimethyl sulfoxide; NMN,
nicotinamide mononucleotide; Tes, N-\{tris\[hydroxymethyl\]methyl-2-

amino\) ethanesulfonic acid.
interacts with the enzyme in a similar way as ADP-ribose bound to apoenzyme (6, 7). The nicotinamide ring is close to the zinc atom in the active site pocket (Fig. 2). The electron density for the nicotinamide ring extends to the density of Thr 178 and the carboxamide group is in all probability hydrogen bonded to the side chain of this residue. The densities corresponding to the coenzyme molecule have slightly different positions in the two subunits. However, since these differences are not significant at this resolution we have built a model of bound coenzyme corresponding to the average density (Fig. 2). An unambiguous conformation could not be determined at this resolution. The coordinates of our coenzyme model given in Table III define a probable conformation which positions the various parts in the center of corresponding electron densities.

There is electron density connected with the zinc atom in each substrate pocket. These peaks were interpreted as the direct binding of MeSO to the active site zinc atoms. This interpretation was confirmed by the difference Fourier map of the ethylmercurythiosalicylate derivative, which does not contain MeSO. In addition to the positive peaks due to the mercury complex there were two negative regions significantly lower than the background, one in each subunit, at the MeSO positions. The binding is illustrated in Fig. 2.

The NMN binding cleft, the active site and the substrate channel of each subunit form two extensive clefts which divide the molecule into three parts: a central core containing the two coenzyme binding domains bound tightly together and the two catalytic domains at each side of this core (4) (Fig. 3). The catalytic domains have two covalent connections with the central core, which form the bottom wall of the cleft. The cleft is accessible to molecules from the solution from the two almost perpendicular directions where nicotinamide and substrate are bound. Between these areas there are van der Waals contacts between the domains, residues 295 to 297 of the NAD binding domain are in contact with residues 53 to 57 of the catalytic domain (Fig. 4).

The comparison made here of the orthorhombic and triclinic electron density maps shows that the central parts of the molecule are very similar in the two crystal forms. The coenzyme binding domains, the main subunit interaction area between these domains and parts of the active site clefts are virtually identical.

The catalytic domains, however, show differences in their conformations. The gross change can be described as a rotation of these domains with respect to the central core so that the front sides of the domains move toward the core and the back side away from the core (Fig. 5). The central parts of these domains do not move but the front and back parts move considerably, about 6 to 7 Å.

A necessary requirement for this rotation of the catalytic domains is that there is a change in the domain-domain interaction area at the front of the molecule. Since this area does not involve any strong binding it is easily accommodated. We observe that the loop containing residues 295 to 298 is turned down and allows the loop of residues 53 to 57 to move up as seen in Fig. 4. Residue 55 at the tip of this loop is one of the residues which has the largest movement, about 6 Å. Residues 15 to 18 which are in contact with the loop around residue 55 also move in the same direction. The close contacts between these loops are conserved but both loops are displaced by about 6 Å.

A functionally important result of this conformational change is that the clefts between the domains become narrower. The loop around residue 55 and part of the preceding helix, residues 47 to 53, move closer to the bound coenzyme. At the entrance to the substrate channel the loop formed by residues 115 to 118 narrows the entrance by 1 to 2 Å. The

<table>
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FIG. 1. Seven consecutive sections of the electron density map cut perpendicular to the noncrystallographic molecular 2-fold axis, which is marked in the center of the figure. The broken line encloses 1 molecule. The 2 zinc atoms of the active site cleft are marked.

FIG. 2. A tentative conformation of the coenzyme based on the best fit of the models to the electron density. The nicotinamide ring is placed close to the active site zinc atom, which in the figure is shown with parts of the three protein ligands of cysteine 46, histidine 67, and cysteine 174. The binding of the inhibitor Me₂SO to the zinc atom is shown in a tentative conformation.

FIG. 3. A schematic drawing of a section through the molecule based on the van der Waals radii of the atoms of the orthorhombic structure showing the substrate clefts and the active site zinc atoms. The positions of the coenzyme and the Me₂SO molecule are superimposed onto this section.

FIG. 4. The domain-domain interaction in the triclinic structure with the active site and the coenzyme. The parts of the corresponding orthorhombic structure which have different conformation are shown in broken lines. For the nomenclature of strands and helices, see Eklund et al. (4).

FIG. 5. A schematic drawing of the main conformational changes of the alcohol dehydrogenase molecule in the transition from apoenzyme to ternary complex. The arrows describe the movements of the catalytic domains at the ends of the central core of the two coenzyme binding domains. The molecular 2-fold axis which is crystallographic for the apoenzyme but not for the holoenzyme is shown at the center of the molecule. The front side, which faces the viewer, contains the area which binds the 2 coenzyme molecules. We are indebted to Bo Furugren for the drawing of Figs. 4 and 5.

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2 B. K. Lee, unpublished.
combined effect of these conformational changes and coenzyme and substrate binding is to shield off the active site zinc atom and its surroundings from the solution. This effect should play an important role in lowering the activation energy for the reaction mechanism.

A loop movement with a similar effect on the active site has been described for lactate dehydrogenase (16). In that enzyme a loop region in the coenzyme binding domain, residues 103 to 115, moves toward the active site. In a comparison of the structural similarities of the coenzyme binding domains of lactate and alcohol dehydrogenase (17) it was noticed that this important loop was absent in the coenzyme-binding domain of alcohol dehydrogenase. It is now interesting to observe that a loop from the catalytic domain of alcohol dehydrogenase moves to approximately the same region in space. Thus a similar functional effect has been produced by the conformational changes of completely different structural regions in these two enzymes.

Since the active sites are very similar in the triclinic holoenzyme structure and the orthorhombic apoenzyme structure the mechanism of action for the enzyme proposed on the basis of the apoenzyme structure (18, 19) are confirmed by the present study. The assumed position of the coenzyme molecule has been confirmed by the present study and the assumed position of the substrate has been confirmed by a recent study of triclinic crystals containing coenzyme and p-bromobenzyl alcohol (20). It is also important to note that compared to the apoenzyme structure no new residues have been brought into the active site. Furthermore, the positions of the protein groups which have been suggested (18) as important for catalysis and substrate binding have changed only marginally with the possible exception of His 51. However, since local small conformational changes of the order of 1 Å may greatly affect the reactivity of the enzyme, we intend to continue this structure determination to high resolution.

Differences in protein fluorescence upon coenzyme binding have been observed and have been related to the conformational change of the enzyme (21). There are only two tryptophans per subunit and they have very different environments (4). Tryptophans 314 are completely buried in the hydrophobic subunit contact area and do not change conformation or environment as observed at this resolution. Tryptophans 15 of lactate and alcohol dehydrogenase moves to approximately the same region in space. Thus a similar loop movement with a similar effect on the active site cannot exclude the possibility that they are due to errors in phasing or to the low resolution.

By these comparisons we can also see that the coenzyme molecules are bound with similar occupancies in both subunits. Thus, for instance, the peak heights on a relative scale for the pyrophosphate moieties are 2047 and 2095, respectively. Similar occupancies of the inhibitor Me₂SO molecule are apparent from Table I, where sites F and F' correspond to the removal of Me₂SO molecules in the ethylmercurythiosalicylate derivative. These negative occupancies are the same within the limits of the error.

Acknowledgment—We wish to thank Dr. Å. Åkeson for purified enzyme.

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