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Structural Studies of Horse Liver Alcohol Dehydrogenase: Coenzyme, Substrate and Inhibitor Binding

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EKLUND, H. *Structural studies of horse liver alcohol dehydrogenase: Coenzyme, substrate and inhibitor binding.* PHARMACOL BIOCHEM BEHAV **18**: Suppl. 1, 73–81, 1983.—Alcohol dehydrogenase from horse liver has been thoroughly investigated with crystallographic methods. Four different crystal forms of the enzyme have been solved and refined. They show that the enzyme exists in two predominant forms. The open form is found in the absence of coenzyme and has two long deep clefts cutting the enzyme in three units. In the closed form of the enzyme these clefts are closed around the coenzyme and substrate/inhibitor. Although there are large conformational changes in the enzyme, they are mainly restricted to relative movements of the separate domains. The internal structure of these domains is virtually identical in the open and closed forms. The coenzyme is the main cause of the conformational change and binds with a large number of interactions to the enzyme. About 4% of the enzyme surface is covered by the bound coenzyme. The nicotinamide ring is not bound to the active site zinc atom, but puts one surface of the ring in contact with the zinc coordinated cystein sulphur atoms. The oxygen atom of the substrate binds directly to the zinc atom with the rest of the substrate close to the nicotinamide of the coenzyme. Large substrates extend into a 15–20 Å long hydrophobic channel which opens up towards the solution. The widely used inhibitor pyrazole binds as a bridge between the zinc atom and the nicotinamide ring. Pyrazoles substituted in the 4-position are generally strong inhibitors. This can be properly related to the organization of the substrate channel of the enzyme.

Alcohol dehydrogenase Horse liver Structure-function relationships Binding sites

MUCH interest has been devoted to the study of the enzymes that participate in alcohol metabolism. Especially alcohol dehydrogenase from mammalian livers has been extensively studied since this enzyme is rate limiting for the oxidation of alcohol in vivo. The investigations made on this enzyme have been done by a number of different methods and thus the enzyme has become a common used system for general investigations in enzymology.

The aim of a crystallographic investigation of an enzyme is to be able to relate the structural features of an enzyme to its biological function. The three-dimensional architecture of liver alcohol dehydrogenase was essentially determined around 1974 [2,7] and published subsequently [8]. A number of available biochemical investigations were then possible to relate to this structure and a number of plausible events of the catalytic action could be suggested [3].

The possibilities for defining structure-function relationships of alcohol dehydrogenase has improved considerably during the last few years. The reasons for this are that the originally solved unligated enzyme structure has been refined and that a new crystal form of the enzyme which binds coenzyme and substrate or inhibitor in ternary complexes has been solved and refined (Table 1). This makes possible the study of a number of different ternary complexes of the enzyme.

This paper summarizes a number of crystallographic investigations carried out in recent years. The main points are to describe how the structure of the alcohol dehydrogenase enzyme molecule is built up so as to be able to achieve its activity and the structural basis for the inhibition of the enzyme.

COENZYME BINDING

Alcohol dehydrogenase is generally regarded as a two-substrate enzyme, where the coenzyme NAD is one of the substrates and alcohol (or aldehyde) the other. The main purpose of such an enzyme is to provide binding sites for the two substrates which force the two reactants together in an environment which is favorable for the chemical reaction to occur. The alcohol dehydrogenase molecule is built in such a way that it is divided into three parts which are separated from each other by two long, rather deep clefts (Fig. 1). Each subunit is divided clearly into two domains, where the smaller domain has a structural organization found in other dehydrogenases and serves as the principle coenzyme binding unit. The larger domain contains most of the groups involved in the catalytic action. The two coenzyme binding domains are bound together to form one central unit of the molecule with the catalytic domains as wings on either side.

TABLE 1

DIFFERENT CRYSTAL FORMS OF ALCOHOL DEHYDROGENASE INVESTIGATED WITH X-RAY DIFFRACTION

Type of complex	Crystal form	Resolution Å	Content of assymetric unit (D)	Crystallographic R-factor	Reference
Unligated	Orthorhombic C 222_1	2.4	40.000	0.21	Eklund *et al.* [8] Jones, unpublished
Ternary	Triclinic Pl	2.9	2×40.000 +2 NADH	0.26	Eklund *et al.* [9] Eklund and Jones, unpublished
	Monoclinic $P2_1$	2.9	2×40.000 +2 NADH	0.29	Samama *et al.* unpublished results
Lysine modified enzyme	Monoclinic C2	3.2	42.000	0.29	Plapp *et al.* [18]

Since the enzyme is a dimer with two independent active sites, each of these two clefts is a binding site for the two substrates, i.e., NAD^+ and alcohol. The simplest structural arrangement for making these substrates meet and react is that the substrates bind to different parts of the cleft, with the active site at the crossing point of these binding sites (Fig. 2). In the center of the cleft lies one of the two Zn atoms of the subunit. This zinc atom, which has been related to the catalytic activity of the enzyme in a number of studies, is located deep inside the protein.

When the coenzyme binds, it triggers a large change of conformation of the enzyme. A substantial rearrangement of the relation between the three parts of the molecule takes place [9]. This is schematically shown in Fig. 3 where the catalytic domains rotate 10 degrees around an axis through the center of the domain. The catalytic domains move 3–4 Å closer to the coenzyme binding domain and therefore the residues of the coenzyme binding domain, which are in the domain-domain interface, must move away to allow the rotation of the catalytic domain. The catalytic and coenzyme binding domains have two covalent contacts on the back side of the cleft, while there are only a few weak van der Waals contacts on the front side. These interactions must change to allow this rotation of the catalytic domains as shown in Fig. 4. The first event during this change of enzyme conformation is that the coenzyme comes into van der Waals contact with a short loop of about 6 amino acids. This triggers the changes of the weak interactions between the domains.

The extent of the conformational change is demonstrated when the alfa carbons of the coenzyme binding domains of the two forms of the enzyme, which have very similar structures, are superimposed. Some parts of the catalytic domain are very differently located in space with a difference of up to 7 Å. The domain-domain interface residues from the coenzyme binding domain are also very different. On the other hand, if the catalytic domains of the two forms are compared separately, the differences are small. The internal structure of the two catalytic domains is thus very similar showing that the movement of the catalytic domain is essentially a rigid body rotation.

The main cause for this large conformational change is the binding of the coenzyme; this conformational change has never been observed in the absence of coenzyme. To be able to bind a large molecule like NAD^+, the enzyme must have an open structure to allow the coenzyme to enter into its binding site without any sterical hindrance. This is the situation in the unliganded form of alcohol dehydrogenase, whereas this binding site is not available in the closed form.

The binding of coenzyme is obtained by a large number of interactions summarized in a schematic drawing (Fig. 5) [12]. The adenine ring binds in a hydrophobic slit between two isoleucine side chains. An important part of the adenosine binding site is Asp 223 which forms two hydrogen bonds to the ribose oxygens. Lys 228 hydrogen bonds to the O3′ oxygen and also interacts with the negative charges of Asp 223 and the phosphates of the coenzyme. The adenosine binding site plays an important part in this initial binding as a recognition site. This is reflected in drastic changes of the velocity of binding when this part is modified [19]. The phosphates are bound by two arginine sidechains. They also bind between the two helices which provide NH-groups at their amino ends for hydrogen bonding to phosphate oxygens. The dipole moment of these contribute to the charged interactions. The nicotinamide ribose is firmly anchored in a narrow part between the two domains and has one hydrogen bond to the main chain of the coenzyme binding domain and two sidechains in the catalytic domain: Ser 48 and His 51. The nicotinamide moiety is bound by hydrogen bonds to main chain atoms plus a number of van der Waals contacts.

When a large ligand like the coenzyme binds to a protein there is a loss of translational and rotational freedom of the ligand which must be compensated for with the binding energy. As Janin and Chothia [13] have pointed out in their study of coenzyme binding to lactic dehydrogenase and glyceraldehyde-3-phosphate dehydrogenase, the burying of the solvent accessible surfaces is a driving force for the binding of cofactors. This is true also for alcohol dehydrogenase. The surface that is accessible to solvent can be calculated as Lee and Richards [15] have shown by the moving of a water probe along the van der Waal surface of the protein. The calculation for alcohol dehydrogenase shows that 0.5% of the accessible surface of the protein is buried due to the large conformational change, while 4.5% is buried when the coenzyme is also bound. The total amount of surface which is buried is similar to what has been found for other dehydrogenases. The energy gain by this burying of accessible surface has been calculated to be in the same energy range as the loss of rotational and transitional energy. Thus, the clos-

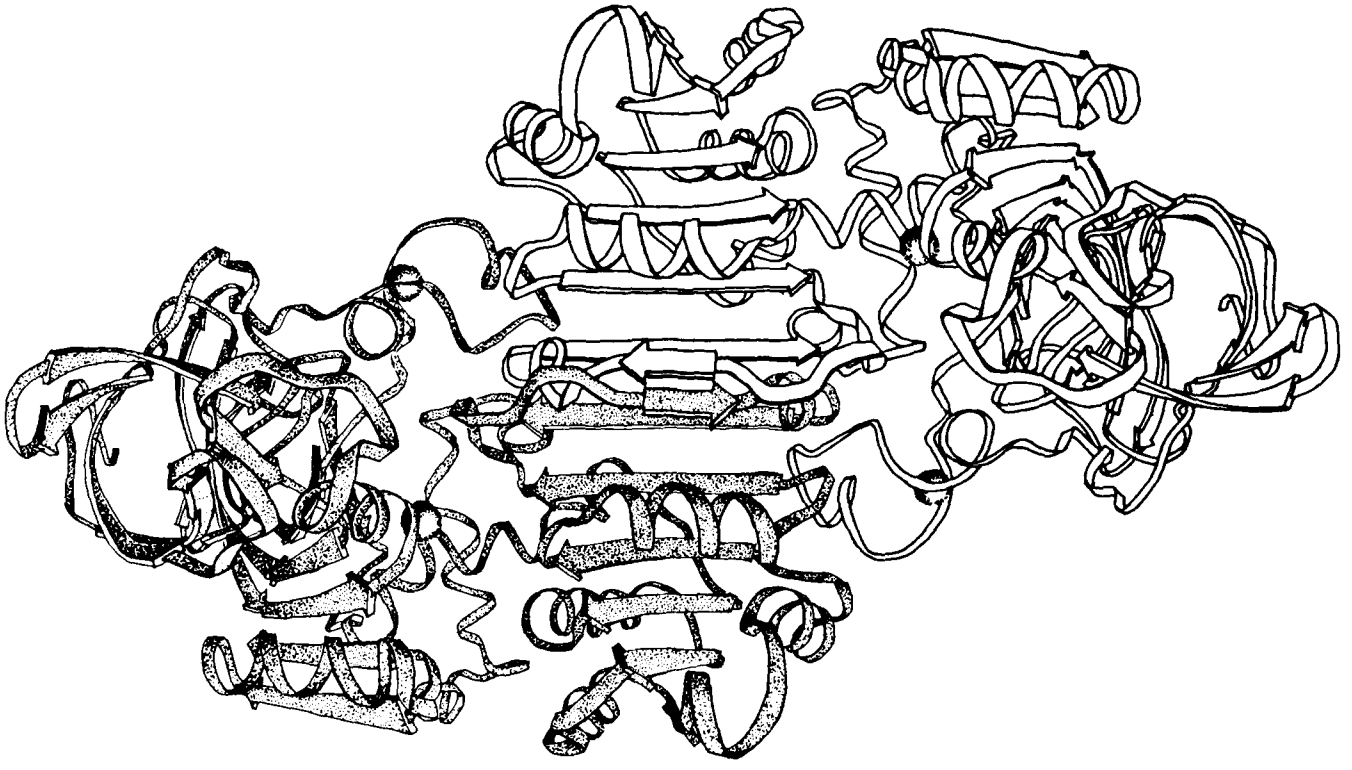

FIG. 1. Schematic drawing of the dimeric horse liver alcohol dehydrogenase molecule. The arrows represent pleated sheet strands. The spheres are zinc atoms. Drawing by Bo Furugren.

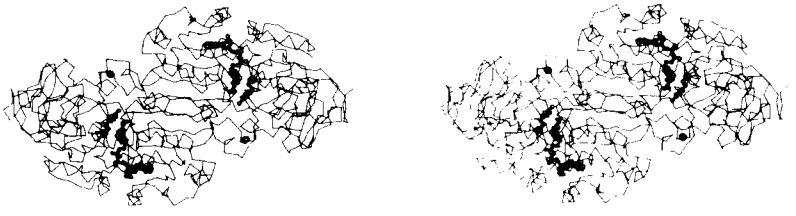

FIG. 2. Stereodiagram of the alpha-carbon backbone of the alcohol dehydrogenase molecule with zinc atoms, coenzyme and bromo benzylalcohol molecules bound in their binding sites.

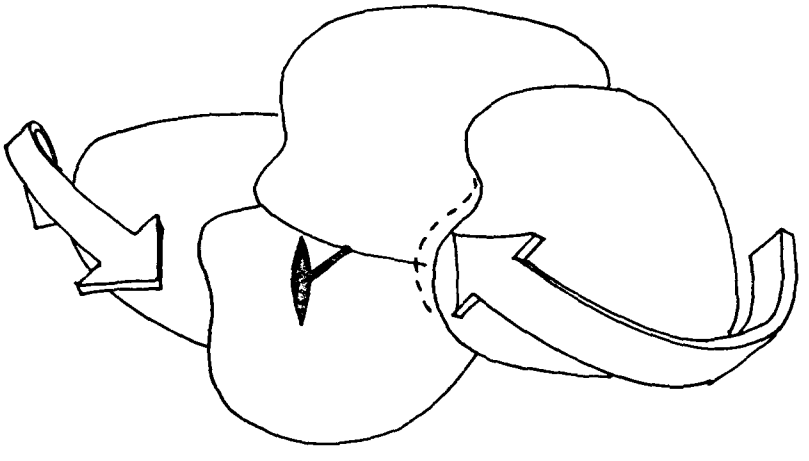

FIG. 3. Schematic drawing of the rotation of the catalytic domains towards the coenzyme binding domains. Drawing by Bo Furugren.

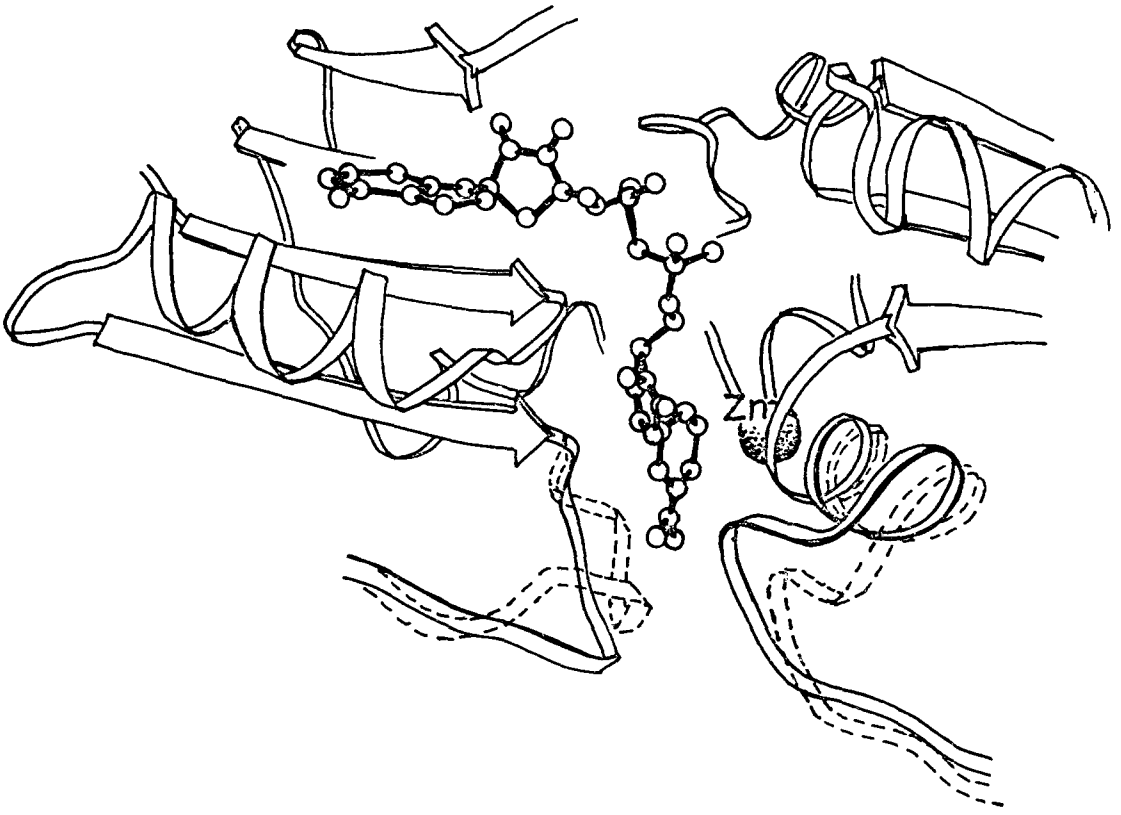

FIG. 4. Schematic drawing showing the rearrangement in the domain-domain interactions when the coenzyme binds. The polypeptide chain in dotted lines represents the situation before the binding of coenzyme. Drawing by Bo Furugren.

ing of the cleft between the domains around the coenzyme provides both energy to allow coenzyme binding as well as to position the protein residues properly for the specific interactions with the coenzyme. For example, Arg 369 is too far from the phosphate binding site in the open form of the enzyme whereas it is a few Å closer to the pyrophosphate site in the closed form to form a hydrogen bond to the oxygen atom of the phosphate on the nicotinamide site.

As a consequence of the conformational change, the protein covers the coenzyme to a very large extent and only a few atoms of the coenzyme are at all accessible to the solvent. All atoms of the nicotinamide ring are completely buried inside the protein in the presence of substrate or inhibitor.

From a kinetic point of view the enzyme binds the coenzyme a bit too well and the dissociation of the reduced coenzyme from the enzyme-coenzyme complex is the rate limiting step in the oxidation of alcohol. Plapp and coworkers [16,25] found that modification of the lysines and more specifically the modification of Lys 228 [6,20] made the enzyme as much as 10 times faster and that the hydride transfer step is rate limiting for the modified enzyme. This enzyme, where most of the lysine side chains are modified, has been studied crystallographically [18]. This enzyme crystallizes in a completely different space group due to the new interactions between molecules because of the modified lysine side chains, but the main chain atomic conformation essentially remains the same as in the native enzyme. The modification on Lys 228 occupies part of the coenzyme binding area and it is obvious that the modification thus affects the coenzyme binding and speeds up its dissociation.

SUBSTRATE BINDING

The reaction catalyzed by alcohol dehydrogenase is the interconversion of alcohol and aldehyde via ternary complexes: enzyme-NAD^+-alcohol $\rightleftharpoons$ enzyme-NADH-aldehyde. Substrate binding has so far been studied crystallographically in three different complexes: (1) Trifluoroethanol which forms a ternary complex on the ethanol side of the reaction which is not converted to aldehyde [17], (2) dimethylaminocinnemaldehyde [4] which forms a stable ternary complex on the aldehyde side with a coenzyme analogue and (3) an equilibrium complex between bromobenzyl alcohol and bromobenzyl aldehyde [10]. In solution the equilibrium favors alcohol and the oxidized coenzyme. We chose to study these three complexes because the first two should give information about the ternary alcohol and aldehyde complexes, and the equilibrium complex would be similar to an active complex.

The substrates and the substrate analogue bind to the active site zinc atom in all three complexes. We chose a bromo substituted benzyl alcohol for our equilibrium studies of substrate binding, because the bromine atom is electron rich and easy to locate in an electron density map. The difference electron density map (Fig. 6) is easily interpreted

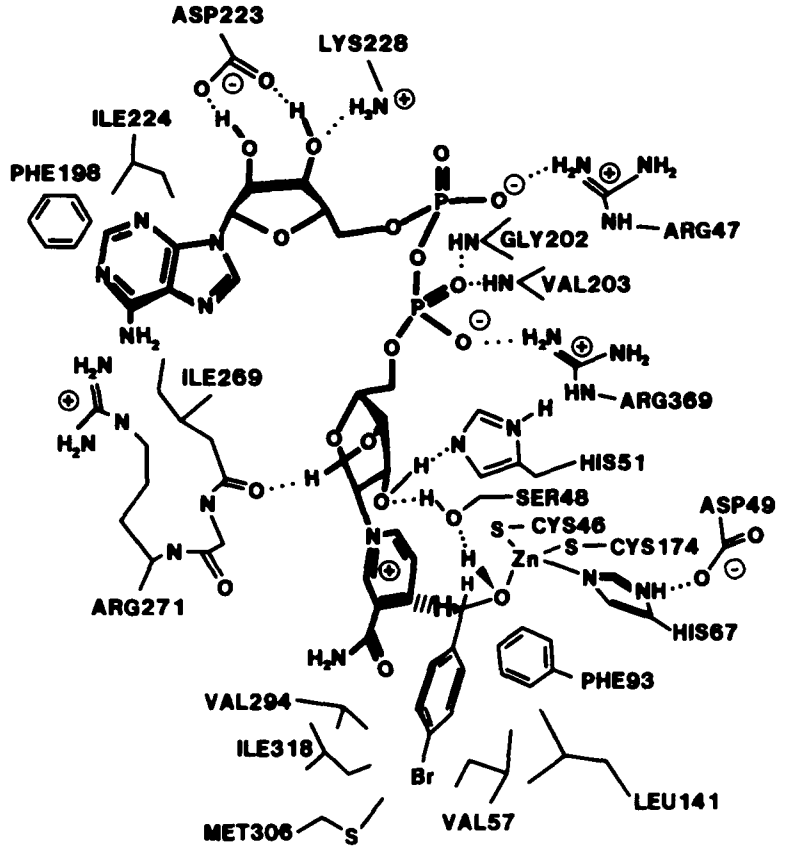

FIG. 5. Schematic drawing illustrating the interactions between the enzyme and coenzyme. The position of a bromobenzyl molecule is also indicated.

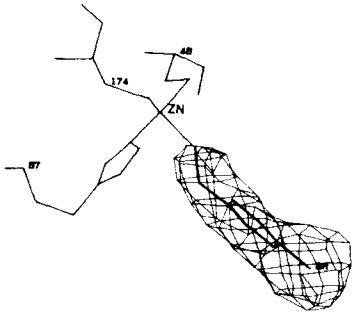

FIG. 6. Bromobenzyl alcohol placed in its electron density. The picture also illustrates the tetrahedral coordination of the zinc atom.

with the highest density in a spherical feature connected to a flat density. The position of the oxygen in the electron density map is compatible only with direct binding to the zinc atom. Binding via a water molecule would shift the position of the substrate about 2.7 Å which is completely incompatible with electron density maps. This direct binding to zinc is also observed in the crystallographic studies with DMSO [9], imidazole [1] and pyrazole [11]. The observed coordination of the active site zinc atom is that of a slightly distorted tetrahedron. The largest deviation for the protein ligands is that the angle between the two sulphur atoms in Cys 46 and Cys 174 is larger than a tetrahedral angle. The free ligand also normally deviates significantly from tetrahedral coordination due to geometrical constraints at the active site, depending mainly on the relation of the free ligand to Ser 48.

When the coenzyme binds to the enzyme, the active site is blocked from one direction. From the other direction there is a long tunnel from the surface of the enzyme. This tunnel is very hydrophobic and is surrounded in its main part by leucines, phenylalanines, isoleucine and valine. This substrate binding site is narrow close to the active site where Phe 93 and Ser 48 limit the space. A bit further away the binding site is more open forming a barrel (Fig. 7). It is limited in this region by the nicotinamide in one direction and protein sidechains in the other directions. When bromobenzyl alcohol binds to the enzyme, the aromatic ring is the correct size to fit into the cleft, as seen in Fig. 8.

In the mode of binding that we observe, the bromobenzyl alcohol molecule is not positioned for hydride transfer to the nicotinamide ring and the hydrogen atoms point away from the nicotinamide ring [10]. It is, however, easy to position the substrate for direct hydride transfer by a simple rotation around the Zn-oxygen bond. The predominant species which is the most energetically favorable species is the one we could observe in a X-ray diffraction study. This species is thus not the transition state, but a state very close to it. The

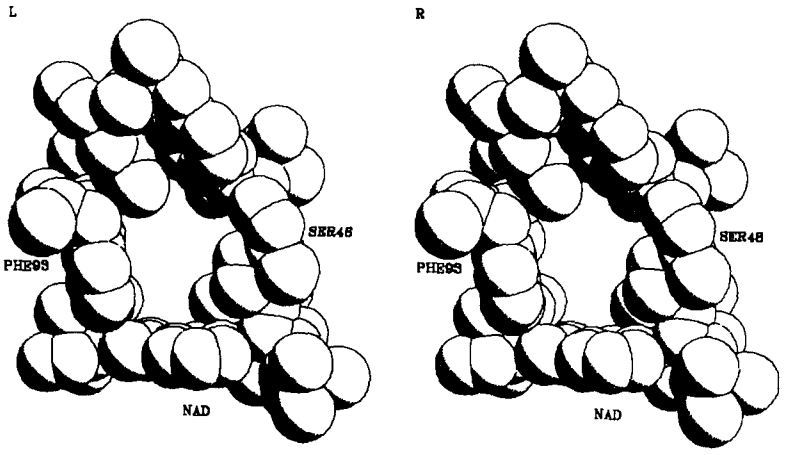

FIG. 7. The hydrophobic barrel forming the main part of the substrate binding site. The view is down the barrel from the zinc atom position.

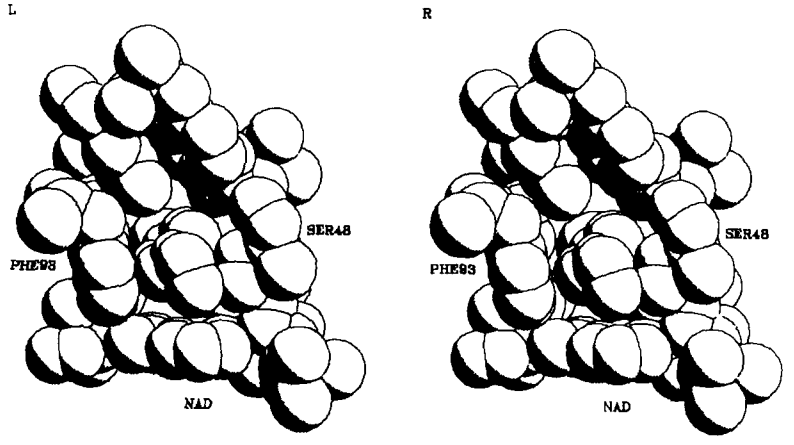

FIG. 8. Bromobenzyl alcohol in its observed position in the hydrophobic barrel.

energy necessary for the rotation of the substrate within the hydrophobic barrel should be very small. Dimethylaminocinnemaldehyde is positioned in a similar way [4], and must be rotated from the observed position to accept a hydride from the coenzyme.

We have built a model complex where the substrate is positioned for direct hydride transfer and also used it to investigate the reason for stereo selectivity for the enzyme [10]. The coenzyme is known to accept a hydride from only one side of the nicotinamide ring: this stereo selectivity is easily explained since the coenzyme binding area is asymetrical and the nicotinamide can be positioned in only one way in the closed form of the enzyme. Ethanol has also been shown to transfer its pro-R hydride and not the pro-S hydride. An ethanol molecule with its pro-R hydrogen directed towards the nicotinamide ring can easily be constructed from the bromobenzyl alcohol molecule in the productive position. If, however, the molecule is turned with its pro-S hydrogen towards the nicotinamide ring, the methyl group of the ethanol molecule comes too close to Phe 93.

In the overall reaction of the enzyme, both a hydride and a proton are transferred from the ethanol molecule. From the original apoenzyme model we proposed a system which would facilitate proton transfer from the active site in the center of the protein molecule [7]. This consisted of a hydrogen bonded system from the zinc bound oxygen atom to Ser 48 and His 51 to the surrounding solution. The refined structure of the apoenzyme shows that this hydrogen bond system does not exist [14]. The water is hydrogen bonded to Ser 48 but the distance from Ser 48 to His 51 is too long for a hydrogen bond.

From the refined structure of the bromobenzyl alcohol complex we observed another hydrogen bonded system [10] which was first suggested by B. Plapp. Here the oxygen of the alcohol is hydrogen bonded to Ser 48, which is hydrogen bonded to the O2′ of the ribose which is hydrogen bonded to His 51. This system may be involved in the transfer of protons from the active site.

INHIBITORS

The class of inhibitors we have studied most is the het-

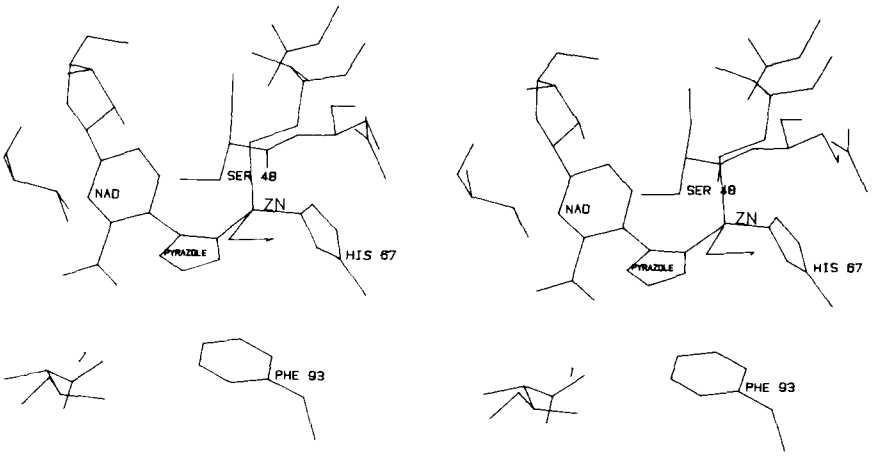

FIG. 9. Stereodiagram showing pyrazole bound as a bridge between the active site zinc atom and the C4-atom of the nicotinamide ring.

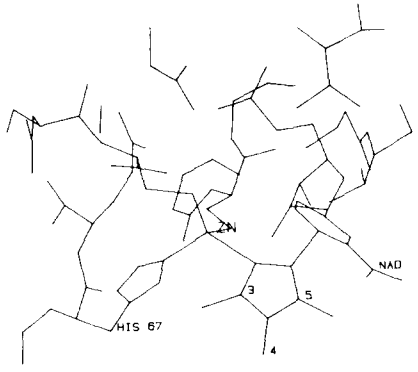

FIG. 10. A 3,4,5-methyl pyrazole molecule constructed from a pyrazole molecule in its observed position. The 3-methyl group comes too close to His 67, the 5-methyl group too close to NAD while the 4-methyl group points into the hydrophobic barrel.

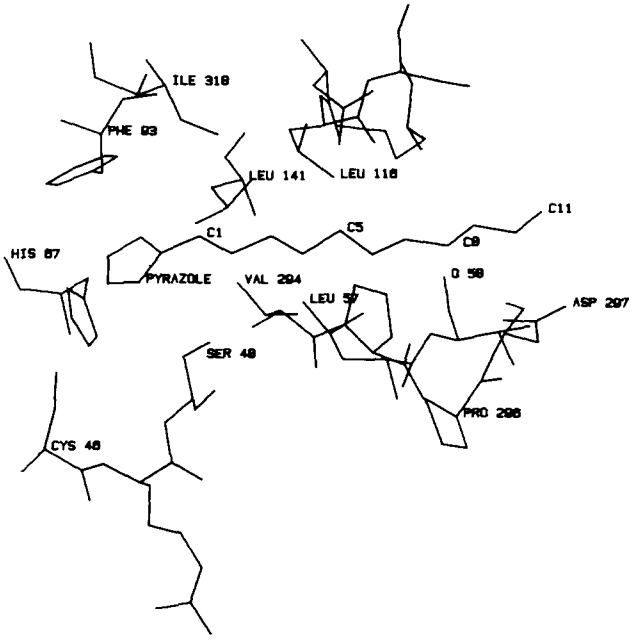

FIG. 11. An alkyl chain attached to a pyrazole molecule in its observed position. The alkyl chain fits nicely into the long hydrophobic tunnel which goes from the active site to the left to the solution to the right.

rocyclic zinc binding compounds like pyrazole which normally are substrate competetive. The first of these compounds that was studied crystallographically was imidazole [1]. In the absence of coenzyme, pyrazole binds in a similar way [11]. Both these rings bind in the narrow slit that exists between Ser 48 and Phe 93 with one nitrogen bound to the active site zinc atom.

Pyrazole forms a very strong complex with enzyme and oxidized coenzyme [21] and has been extensively studied as an inhibitor for alcohol dehydrogenase. We have studied this complex crystallographically. The ring of pyrazole is easily placed in the difference electron density map. One of its nitrogen atoms is bound to the active site zinc atom: the ring binds in the same slit as in the binary complex (Fig. 9). When the pyrazole molecule is positioned in the electron density, the second nitrogen atom comes 2 Å from the C4 atom of the nicotinamide ring. The free electron pair of this nitrogen is directed towards the C4 atom perpendicular to the plane of the nicotinamide ring.

Substituted pyrazole molecules as inhibitors for alcohol dehydrogenase have been studied extensively [5, 22–24]. It is obvious from the binding of the pyrazole molecule that substitutions in the 3-position interfere with the protein, essentially the zinc ligand His 67, and substitutions in the 5-position interfere with the coenzyme (Fig. 10). This explains the reduced inhibitory power for 3 and/or 5 substituted pyrazole molecules (Table 2). On the other hand, the 4-position of the pyrazole molecule points towards the hydrophobic substrate binding site. As a basis for the study of 4-substituted pyrazole molecules we have studied 4-iodo

TABLE 2

INHIBITORY OF SOME SUBSTITUTED PYRAZOLE MOLECULES*

Position	Substitution	K_i μM
		0.22
4	CH_3	0.13
	CH_2CH_3	0.007
	$(CH_2)_2CH_3$	0.004
	$(CH_2)_3CH_3$	0.0018
	$(CH_2)_4CH_3$	0.0008
	$(CH_2)_5CH_3$	0.0005
	$(CH_2)_6CH_3$	0.0003
4	I	0.02
3,5	CH_3	7100

*See [5, 21–24].

TABLE 3

ENVIRONMENT OF THE CARBON ATOMS IN A LINEAR ALKYL CHAIN ATTACHED IN THE 4-POSITION TO A BOUND PYRAZOLE MOLECULE

Chain position	Interacting amino acid	Interacting atoms
C1	Ser 48	Oγ
C2	Ser 48	Oγ
	Leu 57	Cδ2
	Val 294	Cγ2
C3	Leu 57	Cδ2
	Leu 116	Cγ,Cδ2
C4	Leu 57	Cδ2
	Leu 116	Cδ2
	Val 294	Cγ1,Cγ2
C5	Leu 57	Cδ2
	Leu 116	Cγ,Cδ1,Cδ2
	Val 294	Cγ1
C6	Leu 57	Cα,Cδ2
	Leu 116	Cδ2
C7	Leu 57	Cδ1
	Val 58	N
C8	Val 58	N
	Asp 297	Oδ1
C9	Val 58	O
C10	Asp 297	Cγ,Oδ1,Oδ2
C11	Solvent region starts	

The alkyl chain is positioned by model building. Protein atoms closer than 4 Å are listed.

pyrazole crystallographically. The difference electron density map of this derivative shows a very strong density for the iodine connected to a flat density corresponding to the heterocyclic ring. The 4-iodo-pyrazole molecule could easily be positioned in these densities. The position of the pyrazole ring is practically the same as in the unsubstituted pyrazole complex and the binding situation is the same. The iodine atom is located in the hydrophobic substrate cleft. The substrate binding site is somewhat flexible and adapts to the size of the substrate or inhibitor. For small ligands like DMSO and pyrazole, the side chain of Leu 116 points into the barrel, but for larger ligands like p-bromo-benzyl alcohol, dimethylaminocinnemaldehyde and 4-iodo-pyrazole, this side chain points outwards to make the substrate site larger. The main chain is not changed.

Tolf, Dahlbom and others have shown that 4-alkyl chains on pyrazole are especially potent inhibitors [5, 23, 24]. We have used the 4-iodo-pyrazole molecule as a starting point for model building of 4-substituted alkyl chains [11]. A long alkyl chain was attached in the 4-position in the most extended conformation possible and only small torsions had to be applied at two places to avoid close contact with the protein. This was readily done using computer graphics. The obtained chain is only a tentative suggestion for the binding of an extended alkyl chain using the position of the pyrazole as in the 4-iodo-derivative. This conformation of the alkyl chain fits well in the long hydrophobic tunnel (Fig. 11). As seen in the list of protein neighbors of each of the carbon atoms (Table 3) in the alkyl chain, it is not until carbon number 7–8 that an atom in the chain comes close to the polar groups. These results agree well with the chemical investigations.

REFERENCES

1. Boiwe, T. and C.-I. Brändén. X-Ray investigation of the binding of 1,10-phenanthroline and imidazole to horse liver alcohol dehydrogenase. *Eur J Biochem* **77:** 173–179, 1977.
2. Brändén, C.-I., H. Eklund, B. Nordström, T. Boiwe, G. Söderlund, E. Zeppezauer, I. Ohlsson and Å. Åkeson. Structure of liver alcohol dehydrogenase at 2.9 Å resolution. *Proc Natl Acad Sci USA* **70:** 2439–2442, 1973.
3. Brändén, C.-I., H. Jörnvall, H. Eklund and B. Furugren. Alcohol dehydrogenases. In: *The Enzymes*, vol. 11. New York: Academic Press, 1975, pp. 103–190.
4. Cedergren-Zeppezauer, E., J.-P. Samama and H. Eklund. Crystal structure determinations of coenzyme analogue and substrate complexes of liver alcohol dehydrogenase. *Biochemistry* **21:** 4895–4908, 1982.
5. Dahlbom, R., B. R. Tolf, Å. Åkeson, G. Lundquist and H. Theorell. On the inhibitory power of some further pyrazole derivatives of horse liver alcohol dehydrogenase. *Biochem Biophys Res Commun* **57:** 549–553, 1974.
6. Dworschak, R., G. Tarr and B. V. Plapp. Identification of the lysine residue modified during the activation by acetimidylation of horse liver alcohol dehydrogenase. *Biochemistry* **14:** 200–203, 1975.
7. Eklund, H., B. Nordström, E. Zeppezauer, G. Söderlund, I. Ohlsson, T. Boiwe and C.-I. Brändén. The structure of horse liver alcohol dehydrogenase. *FEBS Lett* **44:** 200–204, 1974.
8. Eklund, H., B. Nordström, E. Zeppezauer, G. Söderlund, I. Ohlsson, T. Boiwe, B.-O. Söderberg, O. Tapia, C.-I. Brändén and Å. Åkeson. Three-dimension structure of horse liver alcohol dehydrogenase at 2.4 Å resolution. *J Mol Biol* **102:** 27–59, 1976.
9. Eklund, H., J.-P. Samama, L. Wallén, C.-I. Brändén, Å. Åkeson and T. A. Jones. Structure of a triclinic ternary complex of horse liver alcohol dehydrogenase at 2.9 Å resolution. *J Mol Biol* **146:** 561–587, 1981.

10. Eklund, H., B. V. Plapp, J.-P. Samama and C.-I. Brändén. Binding of substrate in a ternary complex of horse liver alcohol dehydrogenase. *J Biol Chem* **257:** 14349–14358, 1982.
11. Eklund, H., J.-P. Samama and L. Wallén. Pyrazole binding in crystalline binary and ternary complexes with liver alcohol dehydrogenase. *Biochemistry* **21:** 4858–4866, 1982.
12. Eklund, H., J.-P. Samama, C.-I. Brändén and T. A. Jones. Coenzyme binding to horse liver alcohol dehydrogenase. Submitted.
13. Janin, J. and C. Chothia. Role of hydrophobicity in the binding of coenzymes. *Biochemistry* **17:** 2943–2948, 1978.
14. Jones, T. A. and H. Eklund. Active site in alcohol dehydrogenase. Submitted.
15. Lee, B. and F. M. Richards. The interpretation of protein structures: Estimation of static accessibility. *J Mol Biol* **55:** 379–400, 1971.
16. Plapp, B. V. Enhancement of the activity of horse liver alcohol dehydrogenase by modification of amino groups at the active sites. *J Biol Chem* **245:** 1727–1735, 1970.
17. Plapp, B. V., H. Eklund and C.-I. Brändén. Crystallography of liver alcohol dehydrogenase complexed with substrates. *J Mol Biol* **122:** 23–32, 1978.
18. Plapp, B. V., H. Eklund, T. A. Jones and C.-I. Brändén. Three dimensional structure of isonicotinimidylated alcohol dehydrogenase. Submitted.
19. Samama, J.-P. and H. Eklund. Structure-activity relationship for coenzyme analogues with liver alcohol dehydrogenase. Submitted.
20. Sogin, D. C. and B. V. Plapp. Activation and inactivation of horse liver alcohol dehydrogenase with pyridoxal compounds. *J Biol Chem* **250:** 205–210, 1975.
21. Theorell, H. and T. Yonetani. Liver alcohol dehydrogenase-DPN-pyrazole complex: A model of a ternary intermediate in the enzyme reaction. *Biochem Z* **338:** 537–553, 1963.
22. Theorell, H., T. Yonetani and B. Sjöberg. On the effects of some heterocyclic compounds on the enzymic activity of liver alcohol dehydrogenase. *Acta Chem Scand* **23:** 255–260, 1969.
23. Tolf, B.-R., J. Piechaczek, R. Dahlbom, H. Theorell, Å. Åkeson and G. Lundquist. Synthetic inhibitors of alcohol dehydrogenase. 4-Substituted alkyl- and cykoloalkylpyrazoles. *Acta Chem Scand* **B33:** 483–487, 1979.
24. Tolf, B. R. 4-Substituted pyrazoles inhibitors of alcohol dehydrogenase. Synthesis and structure-activity relationships. Thesis at Faculty of Pharmacy, Acta Universitatis Upsaliensis, 1981.
25. Zoltobrocki, M., J. C. Kim and B. V. Plapp. Activity of liver alcohol dehydrogenase with various substituents on the amino groups. *Biochemistry* **13:** 899–903, 1974.