THE STRUCTURE OF HORSE LIVER ALCOHOL DEHYDROGENASE

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1. Introduction

The LADH molecule (EC 1.1.1.1) is a dimer of molecular weight 80 000. The identical subunits are composed of 374 amino acids in one chain [1] plus two firmly bound zinc atoms. From crystallographic studies to 2.9 Å resolution [2] we found that each subunit is divided into two domains separated by a deep active-site cleft. One of these domains binds the coenzyme and is structurally similar to corresponding domains in LDH (EC 1.1.1.27) [3], s-MDH (EC 1.1.1.37) [4] and GAPDH (EC 1.2.1.12) [5]. The second domain binds the two zinc atoms and provides the catalytic center.

We now report an extension of these studies to 2.4 Å resolution which has enabled us to position all the side chains and derive a plausible mechanism of action for the enzyme.

2. Methods

The crystallographic data for the native protein and the three previously used heavy atom derivatives [2] were collected at 4°C on a computer controlled four-circle diffractometer to a resolution of 2.4 Å. Data to 2.9 Å were collected for a fourth derivative, $K_2Pt(CN)_4$ + ethylmercurythiosalicylate and for a complex with the coenzyme analogue ADP-ribose. Data to 4.5 Å resolution were measured for inhibitor complexes with 1,10-phenanthroline, 8 Br-ADP-ribose and 5-iodo-salicylate. Heavy atom positional, thermal and occupancy parameters were refined. The mean figure of merit averaged over 10 300 reflexions was 0.77. A model of one of the subunits of the enzyme has been built with the Kendrew-type models in an optical comparator. Atomic coordinates are available on request. A stereo diagram of the 748 α-carbon atoms and 4 zinc atoms of the whole molecule is given in fig. 1.

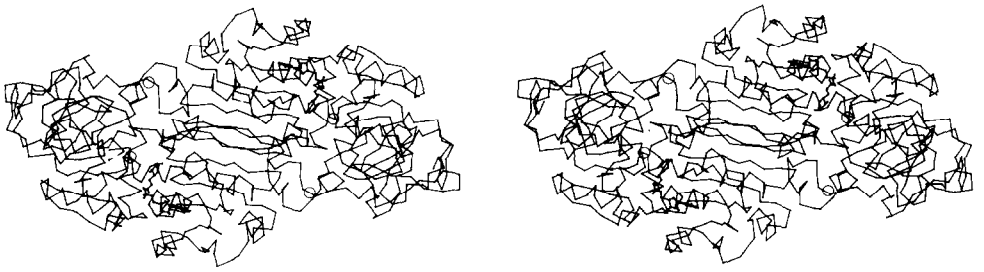

Fig. 1. Stereo diagram of the 748 α-carbon atoms and the four Zn-atoms of the LADH molecule. In addition the two bound ADP-ribose molecules are depicted.

2.1. *Coenzyme-binding domain and comparison with other dehydrogenases*

The coenzyme-binding domain is built up by residues 176 to 318, these are in the form of six parallel strands of pleated sheet which are flanked by helices. The general structure of this domain has been described earlier [2]. The folding of this domain as well as the sequential arrangement of these secondary structure elements have been found earlier to be the same in LADH [2], LDH [3], s-MDH [4] and GAPDH [5].

We have now made a more detailed comparison of these structures [6]. The method used was to define a set of parameters within a rotation matrix that brings the C α-atoms of the parallel pleated sheet of one structure into coincidence with those of the second structure. The positions of the β-carbon atoms of the side-chains within these sheets were used to align structurally equivalent residues [7] which were found to occupy very different positions within the sequence of LADH compared to the other dehydrogenases. Table 1 lists these alignments for the pairwise comparisons of LADH, LDH and GAPDH showing the excellent agreement between the C α-atoms within the pleated sheet structure.

Applying the same rotation parameters tc the bound dinucleotides and to the remaining parts of the domains (helices and loops) gave the following results. The conformations of the bound dinucleotides and their mode of binding to the protein are virtually identical. The similarities and differences of the protein chains are illustrated in fig. 2. The central region of the domains at the carboxyl ends of the strands have almost identical positions for their C α-atoms. Helices αB and αE [2–5] occupy very similar positions. These regions define the crevice where the coenzyme is bound.

We could then align the sequences of about 100 of the residues comprising these domains based on these structural comparisons, permitting a sequence comparison of the three dehydrogenases. No apparent sequence homology was found in spite of the extensive 3-dimensional homology. Only four residues were invariant, corresponding to Gly 199, Gly 203, Asp 223 and Gly 270 in LADH. Corresponding residues in LDH and GAPDH can be deduced from table 1.

The similarities in the main chain configuration of the nucleotide binding domains are paralleled not only by similarities in the conformation of the coenzymes but also in the details of their interaction with the protein. Fig. 3 illustrates the interactions found between LADH and the coenzyme analogue ADP-ribose. The adenine moiety binds in a hydrophobic pocket with its NH_2 group pointing away from the enzyme. The O2′ hydroxyl of the adenosine ribose is hydrogen bonded to Asp 223. The invariance of this residue suggests that this hydrogen bond is an important feature of coenzyme binding. NADP would require a very different mode of binding of this ribose which accounts for the weak binding of NADP to LADH. Hydrophobic coenzyme-

Table 1
Correspondence of the secondary structure elements in the nucleotide binding domains of LADH, LDH and GAPDH

Structural element	Residue numbers (1) LADH	(2) LDH	(3) GAPDH	Root mean square deviation (A) 1–2	1–3	2–3
βA	193–199	22–28	1–7	1.1	2.4	1.4
αB	203–212	32–41	11–20	2.3	2.3	1.9
βB	218–224	48–54	27–33	2.0	2.1	1.4
βC	238–243	78–84*	71–76	1.7	2.5	2.6
βD	263–269	92–98	90–96	1.7	2.0	1.9
αE	275–283	124–132	105–113	6.1	4.3	6.3
βE	287–293	133–139	114–120	2.2	2.1	2.8
βF	312–318	158–164	141–147	3.1	2.4	1.8

* Residue number 82 is nonexistent in the current LDH-numbering system.

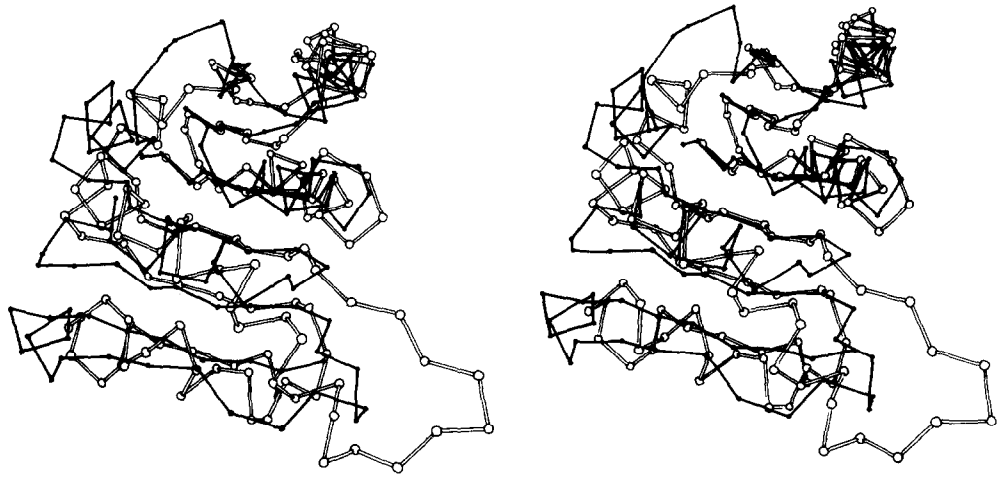

Fig. 2. Stereo diagram of the coenzyme-binding domain of LADH (dark bonds) superimposed on the corresponding region of LDH (open bonds).

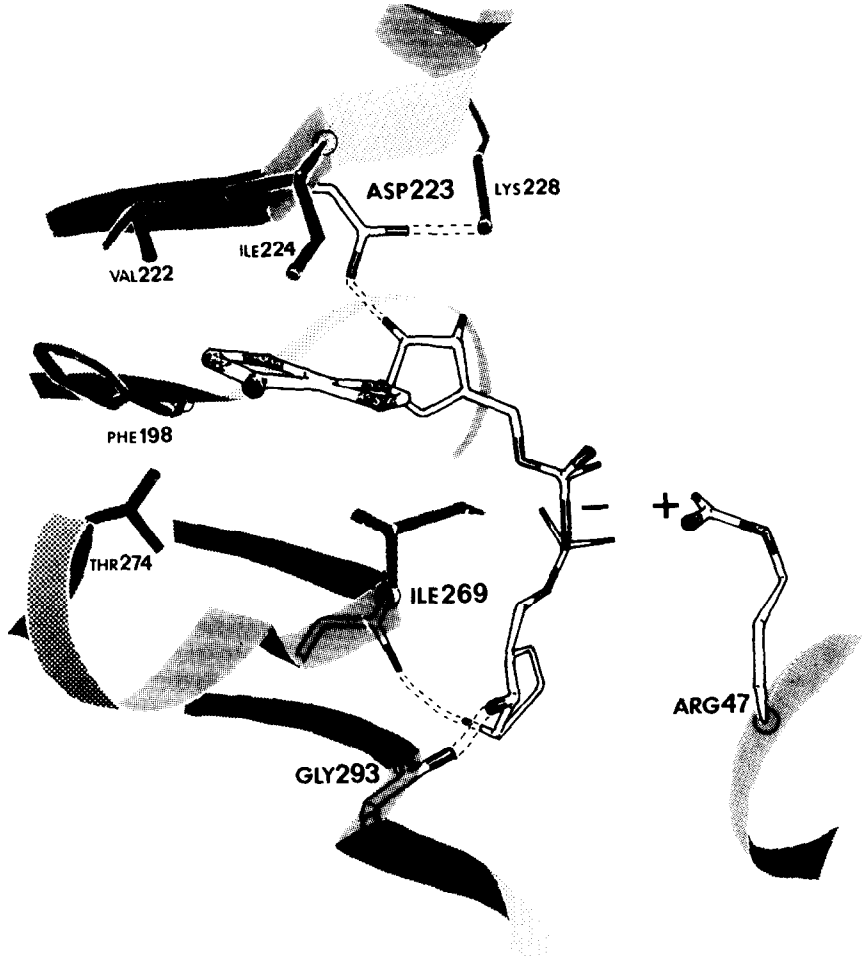

Fig. 3. Schematic diagram illustrating the interactions between ADP-ribose and LADH. We are indebted to Bo Furugren who designed this drawing.

competitive inhibitors like salicylate and 8-anilinonaphthalene sulfonate, ANS, bind in the adenosine binding pocket simulating adenosine binding [8]. The terminal ribose of ADP-ribose is bound by two hydrogen bonds from O3′ and O2′ to the main chain carbonyl oxygen atoms of Ile 269 and Gly 293. Very similar interactions with equivalent residues have been found for NAD binding to LDH [9].

The guanidinium group of Arg 47 provides a positive charge for an ionic interaction with the pyrophosphate moiety. Anions like chloride ions and $Pt(CN)_4^{2-}$ also bind to this general anion binding site. This site is important for the specific carboxymethylation of Cys 46 by iodoacetic acid. Iodoacetate first binds reversibly to this site and is thereby in an excellent position for an irreversible attack on the sulphur atom of the zinc-ligand Cys 46.

3.2. *The catalytic domain*

The catalytic domain comprises residues 1–175 and 319–374. The two zinc atoms of the subunit are bound to ligands from this domain. A stereodiagram of the α-carbon positions and the zinc atoms is given in fig. 4.

The structure of this domain is built up by a complicated network of β-structures. There are three main regions of pleated sheet βI, βII and βIII. The strands of these sheets are mainly antiparallel. Region βI comprises six strands which form a twisted wall through the top half of this domain with the catalytic zinc atom bound at one end of the sheet. On one side of this wall are mainly residues from the carboxy-terminal part of the chain. On the other side are the remaining residues of the amino-terminal half of the chain. These can be divided into three separate regions; the two remaining pleated sheet regions, βII and βIII, and a lobe which binds the second zinc atom. There are only four short helical regions in this domain.

The catalytic zinc atom has been identified from the binding of 1,10-phenanthroline [2]. This zinc atom is situated at the bottom of a deep pocket between the two domains. It has three protein ligands; two sulphur atoms from Cys 46 and Cys 174 and one nitrogen atom from His 67. A water molecule or hydroxyl ion, depending on the pH, completes a distorted tetrahedral coordination. The zinc-bound water molecule forms an internal hydrogen bond to Ser 48 which in turn is within hydrogen bonding distance to one of the nitrogen atoms of His 51. The second nitrogen of His 51 points towards the solution. This system of hydrogen bonds might provide a framework for the proton release of the overall reaction which is induced by NAD^+ binding.

An assumed position of the nicotinamide moiety has been deduced from the similarities of coenzyme conformation and binding described above by rotating the NAD molecules as bound to LDH into the LADH structure, so that the ADP-ribose parts overlap. We then found that the C4 atom of the nicotinamide ring was positioned 4.5 Å from the catalytic zinc with the A-

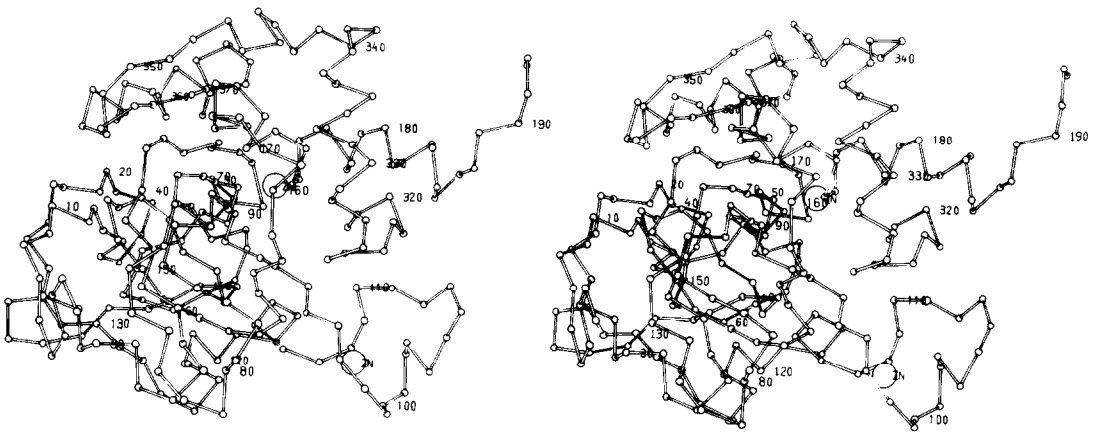

Fig. 4. Stereo diagram of the α-carbon and zinc atoms of the catalytic domain.

side of the ring facing the water ligand.

Without any adjustments we could also build in a substrate molecule with the alcoholate oxygen coordinated to zinc substituting the hydroxyl and with the hydrogen atom which is to be transferred, held in a suitable position for direct hydride transfer to C4 of the nicotinamide moiety.

The active site pocket is lined almost exclusively by hydrophobic residues; Phe 93 and 110, Leu 57, 116 and 141, Pro 296, Ile 318, Ser 48 and 117 and Thr 178 from the same subunit that binds zinc and Met 306, Leu 309 and Ser 310 from the other subunit. Ser 48 and Thr 178 are the only polar residues in the part of this pocket where the catalytic reaction occurs. There are thus no histidines, tryptophans, cysteines, aspartic acids or glutamic acids that can directly participate in the catalytic action as deduced from the apoenzyme structure. It also seems impossible to bring such residues into the active center by conformational changes except by a complete change of the folding of the catalytic domain. This is a very unlikely event.

In view of these structural results, the only plausible mechanism for alcohol oxidation is that of electrophilic catalysis mediated by the active site zinc atom. The structure determination strongly supports the mechanism proposed by Theorell [10] where the zinc-bound water molecule plays a crucial role. Binding of NAD^+ perturbs the pK_a value of this water molecule to lower pH with a concomitant proton release corresponding to the proton liberated in the overall reaction. Alcohol then binds to zinc as the alcoholate ion, displacing the hydroxyl ion. The formation of alcoholate is mediated by the zinc bound hydroxyl that acts as a base and combines with the proton of the hydroxyl group of the alcohol to form a water molecule.

The zinc atom polarizes the alcoholate so that direct hydrogen transfer and subsequent rearrangement to aldehyde can occur.

Residues 95 to 113 form a lobe that binds the second zinc atom of the subunit. This lobe projects out from the catalytic domain having few side chain interactions with the remaining parts of the subunit. This zinc is liganded in a distorted tetrahedral arrangement by four sulphur atoms from cysteine residues 97, 100, 103 and 111. The coordination of this zinc atom in LADH is similar to the tetrahedral arrangement of four cysteine ligands around iron in rubredoxin. Even more striking is the sequential similarity with the four cysteine ligands around the iron clusters in the bacterial ferredoxins. Possible functional and evolutionary correlations are currently being investigated.

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