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Molecular Aspects of Functional Differences between Alcohol and Sorbitol Dehydrogenases†

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ABSTRACT: The amino acid sequence of sheep liver sorbitol dehydrogenase has been fitted to the high-resolution model of the homologous horse liver alcohol dehydrogenase by computer graphics. This has allowed construction of a model of sorbitol dehydrogenase that provides explanations why sorbitol is not a substrate for alcohol dehydrogenase, why ethanol is not a substrate for sorbitol dehydrogenase, and what determines its specificity for polyols. An important feature of the model is that one of the ligands to the active site zinc atom is a glutamic acid residue instead of a cysteine residue, which is the corresponding ligand in the homologous alcohol dehydrogenases. This is one component of the structural change that can be related to the different substrate specificities, showing how altered enzymic activity might be brought about by structural changes of the kind that it is now possible to introduce by site-directed mutagenesis and recombinant DNA techniques.

Functionally related molecules that exhibit some degree of sequence homology have, in a number of cases, been shown also to have closely similar tertiary structures [see Creighton (1983)]. From the three-dimensional structure of one member of a group of such related molecules, plausible models can be constructed from amino acid sequences for the others by computer graphics, e.g., Bedarkar et al. (1977), Isaacs et al. (1978), Blundell et al. (1978, 1983), and Eklund et al. (1984a). This approach is now gaining increasing importance because of the rapidity with which gene sequences can be determined and the possibility of site-directed mutagenesis.

Alcohol and polyol dehydrogenases with long protein chains (around 350 residues) and with a catalytically active zinc atom are members of one such family of related molecules (Jörnvall et al., 1981). The tertiary structure of only one member, horse liver alcohol dehydrogenase (LADH),[1] is known (Eklund et al., 1976, 1981). From this structure, and the amino acid sequence of yeast alcohol dehydrogenase (YADH), a plausible model for the subunit structure of the latter was developed (Jörnvall et al., 1978). YADH exhibits 23% sequence identity to LADH. Conserved functional features of these alcohol dehydrogenase members are the ligands to the catalytic zinc atom (two cysteine residues and one histidine residue) and a hydrophobic substrate binding pocket. Other mammalian alcohol dehydrogenase sequences (Bühler et al., 1984; Hempel et al., 1984) as well as the maize sequence (MADH) (Dennis et al., 1984), which all have more positional identities to LADH, also show these conserved features.

Sheep liver sorbitol dehydrogenase (SDH) also belongs to this family of alcohol/polyol dehydrogenases (Jörnvall et al., 1981, 1984a). The amino acid sequence homologies reveal about 25% positional identities between LADH and SDH (with higher homology in long segments of the protein chain) and 20% between YADH and SDH. This enzyme has also been shown to contain the expected catalytic zinc atom, but it was simultaneously found to contain only one zinc per subunit (Jeffery et al., 1984a,b) in contrast to the two Zn atoms per subunit found in LADH (Åkeson, 1964). Another overall difference between these enzymes is the quaternary structure; SDH is tetrameric like YADH and not dimeric like LADH and MADH.

There are also significant functional differences between SDH and the alcohol dehydrogenases. SDH selectively oxidizes a secondary alcohol of sorbitol and does not oxidize primary alcohols. Alcohol dehydrogenases on the other hand oxidize a range of primary alcohols, and the broad substrate specificity of the liver enzyme extends to some secondary alcohols (Dutler & Brändén, 1981).

Using criteria to maximize sequence identities while preserving the active site zinc ligands, Jörnvall et al. (1984a) have aligned the amino acid sequence of SDH with those of the alcohol dehydrogenases. This sequence alignment clearly demonstrates that the main features of the domain structures

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[1] Abbreviations: ADH, alcohol dehydrogenase; LADH, horse liver alcohol dehydrogenase; YADH, yeast alcohol dehydrogenase; MADH, maize alcohol dehydrogenase; SDH, sheep liver sorbitol dehydrogenase.

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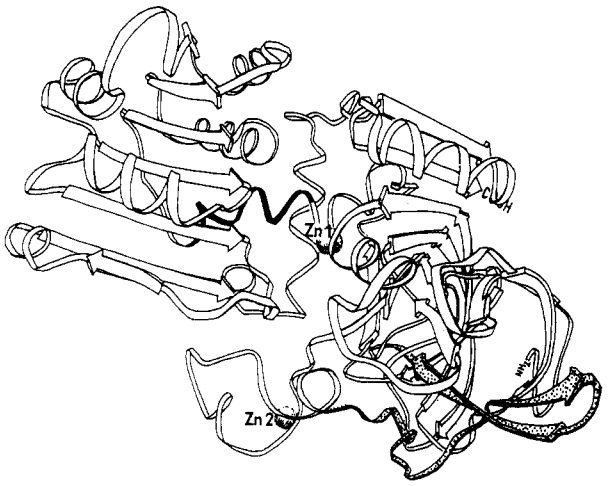


FIGURE 1: Schematic drawing showing the three-dimensional structure of the horse liver alcohol dehydrogenase subunit. The locations of gap regions for different possible alignments with the sorbitol dehydrogenase sequence are indicated: (black) the gap in the "ligand alignment" corresponding to helix αA; (stippled) the gap in the "conformation alignment" corresponding to a surface loop.

of LADH are also present in SDH. It further shows that three of the four cysteine residues which are ligands to the second (noncatalytic) zinc atom in LADH are absent from SDH, in agreement with the different zinc stoichiometries in SDH and LADH. However, the sequence homology between the two proteins is low in the central region, and the comparisons imply large changes close to the active site zinc liganding Cys-174 in LADH. The alignment chosen conserved this ligand. The variable structures in the central region are in contrast to the conserved regions around the other two ligands (Cys-46 and His-67 in LADH) to the active site zinc atom.

In this paper, we describe a model for the tertiary structure of the SDH subunit based on model building and substrate docking with computer graphics. This model building utilizes another alignment that largely conserves the conformation instead of all zinc ligands in the central region of the proteins where the homology is low. Consequences of the model for details of coenzyme, metal, and substrate binding to SDH are given.

Experimental Procedures

The sorbitol dehydrogenase amino acid sequence was compared with four widely different but clearly related alcohol dehydrogenase sequences: the horse liver alcohol dehydrogenase E isozyme (Jörnvall, 1970), the alcohol dehydrogenase from maize (Dennis et al., 1984), the alcohol dehydrogenase from the budding yeast *Saccharomyces cerevisiae* (Jörnvall, 1977), and the alcohol dehydrogenase from the fission yeast *Schizosaccharomyces pombe* (Russell & Hall, 1983). The alignment between the alcohol dehydrogenases was as described earlier (Jörnvall et al., 1978; Dennis et al., 1984). However, a new sequence alignment of SDH was carried out by keeping as many identities as possible and introducing as few insertions/deletions as possible, while conserving conformational aspects. No attempt was made to selectively superimpose a particular residue or residue type. Instead, emphasis was placed on keeping the important elements of secondary structure of the LADH subunit, and the main gap was introduced where there is a surface loop in LADH (see Figure 1).

The refined coordinates of the structure of the ternary complex of LADH (Eklund et al., 1981, 1984b) were used as a starting model for the construction of a three-dimensional structure of one subunit of sorbitol dehydrogenase. The model-building program FRODO (Jones, 1978, 1982) was used with a Vector General VG 3404 interactive graphics display on a Digital Vax 11/750. The structure was modified by replacing residues in the LADH structure with the corresponding residues in the SDH sequence, keeping the structure as close to the original structure as possible. The final coordinate set of SDH was energy-minimized by the EREF program (Jack & Levitt, 1978) to optimize the bonded and nonbonded interactions. The coordinates are deposited in the Brookhaven Protein Data Bank.

Models of sorbitol and substrate analogues were introduced into the display data set with ideal bond distances and angles with the program TOM (Cambillau et al., 1984). This program was then used to find a possible, productive position for the sorbitol molecule. The interaction potential, used in the energy-minimization procedure FIT, was the sum of three terms, a van der Waals potential between nonbonded atoms, the Coulomb interaction, and a modified van der Waals potential (with shorter radii) for the 1–4 interactions (Åkvist et al., 1985). The parameters of the potential (radii and partial charges of the protein atoms) were taken from the Groningen molecular dynamic system GROMOS (Hermans et al., 1984). The partial charges on the sorbitol atoms were estimated with the same criteria as in GROMOS. The van der Waals radius of the Zn atom was taken short enough to allow contacts to 2.2 Å with the sulfur atom at Cys-46.

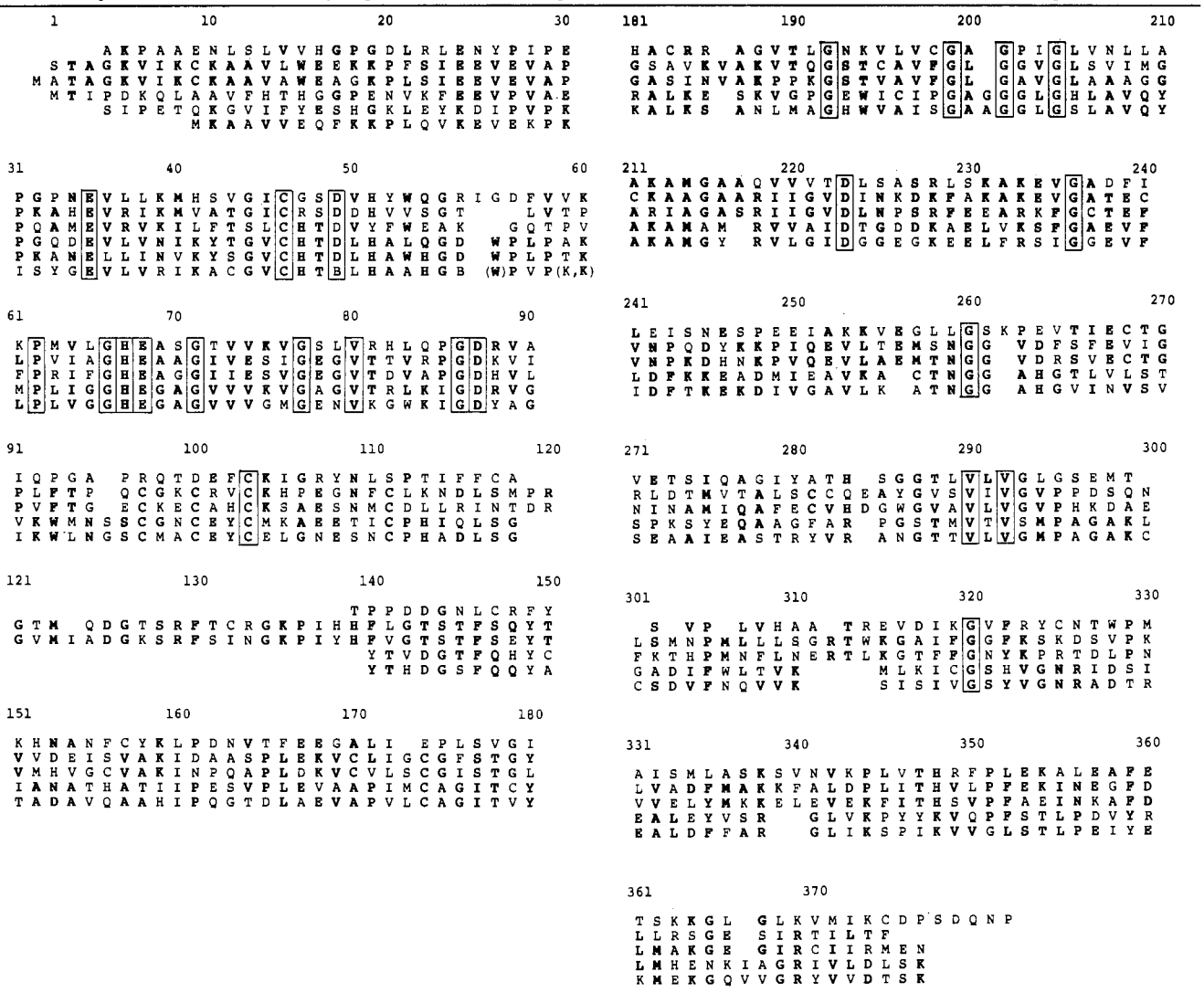
Results and Discussion

Alignment of SDH with ADH and Construction of a Three-Dimensional Model of SDH. The alignment obtained here is shown in Table I. Included are also yeast, plant, and bacterial alcohol dehydrogenases. The amino acid sequence of SDH is easily aligned with the alcohol dehydrogenase sequences in the main parts of both the catalytic and the coenzyme binding domains (Jörnvall et al., 1984a). In the first 95 residues of SDH, only one region shows variation in chain length between SDH and LADH, namely, positions 55–60, where SDH has more residues than LADH, as do other ADH molecules. This correlates with the first gap positions in the alignment with yeast alcohol dehydrogenase (YADH) (Jörnvall, 1977; Jörnvall et al., 1981) and with the position of one intron in the maize gene (Dennis et al., 1984; Brändén et al., 1984) and is an example of location of introns at positions of variable chain length (Craik et al., 1983). There is also a long segment of the sequence of SDH that aligns with residues 185–299 of the coenzyme binding domain of LADH. This segment can be matched so that it contains no insertion/deletion (Jörnvall et al., 1984a). Here, we have chosen an alignment with one insertion (after residue 261 of LADH) and one deletion (residue 284 of LADH). In the carboxyl end part, alignments to residues 313–374 of LADH can be given without any insertion or deletion. The SDH sequence, like the other ADH sequences, is longer at the carboxyl end than LADH.

There are two stretches of the amino acid sequence of SDH that clearly differ from that of LADH. The first is around positions 300–315, which constitutes the main subunit–subunit interaction area of LADH. Here, the five-residue shorter sequence of the tetrameric SDH is analogous to what is found for tetrameric alcohol dehydrogenases (YADH) and may be related to the quaternary structure (see below).

The other stretch of clear differences between SDH and LADH concerns the part corresponding to residues 110–186. The alignment shown in Table I has three gaps in the region 110–186. Two of these gaps are essentially the same as for

Table I: Comparison of the Sorbitol Dehydrogenase Amino Acid Sequence with Liver, Maize, and Yeast ADH Amino Acid Sequences[a]



[a] The alignment shown is in the central region the one based on the conformational similarities now outlined rather than on the conservation of zinc ligands previously considered. Sequence data are from Jörnvall (1970, 1977), Bridgen et al. (1973), Jeck et al. (1979), Russel & Hall (1983), Dennis et al. (1984), and Jeffery et al. (1984b). Numbers refer to the LADH amino acid sequence. For *Bacillus stearothermophilus* ADH, data are incomplete (ending at the position corresponding to residue 60 in LADH) and tentative for some positions (from 49 onward, as indicated by B and parentheses). Residues that occur in at least two species are boldfaced, and residues present in all species are enclosed in boxes. (Line 1) Sheep liver sorbitol dehydrogenase; (line 2) horse liver alcohol dehydrogenase; (line 3) maize alcohol dehydrogenase; (line 4) yeast (*Schizosaccharomyces pombe*) alcohol dehydrogenase; (line 5) yeast (*Saccharomyces cerevisiae*) alcohol dehydrogenase; (line 6) *Bacillus stearothermophilus* alcohol dehydrogenase.

the tetrameric yeast alcohol dehydrogenases: a long gap between 118–138 (Table I) and a one-residue gap at residue 186. The alignment thus keeps the subunit structure of the tetrameric SDH close to those of the tetrameric ADH molecules, with gaps and insertions at the same places and of about the same lengths. In this region the alignment differs from that which can be obtained from comparison with the LADH sequence (Jörnvall et al., 1984a). Although the present alignment gives fewer identities with LADH (6 instead of 13, in the region 119–186, Table II), it gives 16 identities compared with 13 between occupied positions of SDH and at least one of the ADH molecules. Coincident gaps are a kind of identity, and if this is taken into account, the present alignment gives further matches.

The previous alignment was chosen to keep a cysteine residue of SDH in the position corresponding to the active site zinc ligand Cys-174 of LADH. This forces several gaps in the SDH sequence to optimize the local fit and to keep the zinc ligand. For the construction of a three-dimensional structure of SDH, one of these gaps creates difficulties: a 10-residue gap in the middle of helix αA, of which only three residues in the beginning and two at the end remain. This helix bridges the two domains, and a large gap here is not compatible with a conserved conformation. A change of this helix to a strand would make the active site open and make the nicotinamide ring accessible to solvent. A model constructed from the present alignment has the advantage that the helix covers the nicotinamide ring from solution. In a recent paper, Read et al. (1984) showed that alignments considering the three-dimensional structure were significantly more correct that those just optimizing the fit of sequences.

A drastic consequence of the present alignment in the region 110–186 is that the third zinc ligand (position 174) is not conserved. Instead of a cysteine residue here, as in all alcohol dehydrogenases (Table I), this alignment gives a glutamic acid residue in SDH. Nothing is yet known about the zinc coordination of SDH, but suggestive evidence that SDH is a zinc enzyme (Jeffery et al., 1981; Jörnvall et al., 1984a) has been verified by metal analysis (Jeffery et al., 1984b). Nothing is known about zinc coordination in other dehydrogenases, but

Table II: Comparison of the Two Different Alignments of SDH with Alcohol Dehydrogenases[a]

	115	120	130	140	150
SDH	F F C A T P P D	D G	N L C R F Y K	H N A N F C Y K L P D N	
LADH	D L S M P R G T M	Q D G T S R F T C R G K P I H		H F L G T S T F S Q Y T	
MADH	R I N T D R G V M I	A D G K S R F S I N G K P I Y		H F V G T S T F S E Y T	
F-YADH	Q L S G			Y T V D G T F Q H Y C	
B-YADH	D L S G			Y T H D G S F Q Q Y A	
SDH	F F C A			T P P D D G N L C R F Y	

	160	170	180	190
	V T F E E G A L I E P L S V G I H A C R R			A G V T
	V V D E I S V A K I D A A S P L E K V C L I G C G F S T G Y G S A V K V A K V T			
	V M H V G C V A K I N P Q A P L D K V C V L S C G I S T G L G A S I N V A K P P			
	I A N A T H A T I I P E S V P L E V A A P I M C A G I T C Y R A L K E			S K V G
	T A D A V Q A A H L P Q G T D L Q E V V P V L C A G I T V Y K A L K S			A N L M
	K H N A N F C Y K L P D N V T F E E G A L I E P L S V G I H A C R R			A G V T

[a] Only the central regions of the subunits, where the alignments differ (top and bottom, respectively), are shown, covering positions 115–190 of horse liver alcohol dehydrogenase. In the region 119–186, the alignment in the top line [cf. Jörnvall et al. (1984a)] gives 13 positional identities with LADH, and this is not increased by inclusion of the other three ADH's in the comparison (boldface). The alignment shown on the bottom line gives only 6 positional identities with LADH, but 16 when the other ADH's are included (underlined). The distribution of the gaps also differs in the two alignments. The maize gene has an intron corresponding to position 179 (third line, indicated by an oblique stroke).

a glutamic acid can act as a zinc ligand in other enzymes and has been found to do so for carboxypeptidase (Rees et al., 1981) and thermolysin (Holmes & Matthews, 1981). Both a cysteine (Schneider & Zeppezauer, 1983) and a glutamic acid residue should be negatively charged as zinc ligands, so the formal net charge of zero for the zinc ligand complex would be the same in either case. The Gap-Glu amino acid sequence in SDH, instead of the Gly-Cys sequence in LADH, should make the environment of the active site zinc atom different in exactly the right place to allow positioning of the primary alcohol group when the alcohol group at C-2 of sorbitol is bound to the zinc atom.

The different zinc ligands in the SDH model and the LADH structure have the consequence that the longer glutamic acid side chain does not ligate in the same way as the corresponding cysteine residue does. However, the deletion in SDH at the preceding position (corresponding to number 173 in LADH) could allow the α-carbon of residue 174 in an otherwise unchanged fold to move slightly away from the zinc atom. The model shows that the glutamic acid side chain could come into a proper position to ligate to the zinc atom with normal coordination distance.

The model also suggests possible further experiments that can be made to test its validity. Investigation of the zinc coordination by different methods clearly merits high priority because of the difference in ligands required by the two alignments discussed here.

Two alternatives were tested in the model building of the region 261–284 in the coenzyme binding domain, one without any deletion/insertion and one with one insertion after position 261 and with residue 284 deleted. Both alternatives give plausible models, but the second alternative has the advantage that a Glu comes at position 267 and can form hydrogen bonds to main-chain nitrogen atoms as in LADH (and probably MADH). Furthermore, this alignment in this region produces four consecutive residues identical with MADH (Table I, positions 267–270), and this is the region involved in binding the adenine moiety of the coenzyme.

The final three-dimensional model of the subunit structure of SDH has good bonded and nonbonded interactions. The internal core structure is highly hydrophobic and contains no internal, charged residues that are not present in the LADH structure. In the internal cores, a few compensating changes of side-chain size are present, as has been found in other structures [see, for instance, Jörnvall et al. (1978)]. Examples of this are residue pairs 21–65 and 319–328.

Substrate Binding. The substrate specificity of SDH and LADH differ in several respects. While LADH acts on primary alcohols, SDH oxidizes the second alcohol group of sorbitol. This requires that the active site region close to the zinc atom is different in the two enzymes. With respect to the size of the active site cleft, the main difference between the model of SDH and the observed structure of LADH is caused by having a proline residue in SDH corresponding to Phe-93 in LADH. Proline at this position could allow the substrate to come deeper into the protein and could provide space for direct binding of the second alcohol oxygen to the zinc atom. In LADH, it is not possible to position the sorbitol molecule with the second alcohol group bound to zinc. The primary alcohol group would then come too close to the side chain of Phe-93. The replacement of this Phe by a Pro also affects the accessibility of residue 319, such that the lysine side chain at this position in the SDH model can reach the substrate cleft.

When sorbitol is thus positioned for hydride transfer, the primary alcohol would come into a proper position to form hydrogen bonds to Glu-174 (in the numbering system of LADH, cf. Table I) as well as to Lys-319 (Figure 2). A glutamic acid residue as zinc ligand should in this structure thus be favorable compared to a cysteine residue for oxidation of the secondary alcohol group at C-2 rather than the primary alcohol group at C-1.

The size of the substrate channel in which the remaining part of the sorbitol molecule would bind, further away from the zinc atom, is approximately the same in the SDH model as in LADH. The major differences in this area are that Phe-140 and Leu-141 of LADH are both substituted by proline residues in SDH. On the other hand, Leu-116 on the opposite side of the substrate cleft is replaced by a larger Phe in the model of SDH. Another important difference could be the exchange of Val at position 52 in LADH for a Tyr in this model of SDH, because Tyr could provide a hydrogen bond to sorbitol (see below).

Using the SDH model, we have investigated different modes of binding of sorbitol and related six-carbon sugar alcohols, with binding to the Zn atom either through the oxygen at carbon 1 or through that at carbon 2. Using energy minimization, we found that the best docking was obtained with O2 bound to the Zn for all sugar molecules with the same chirality as sorbitol at C2. The resulting distance O2–Zn was

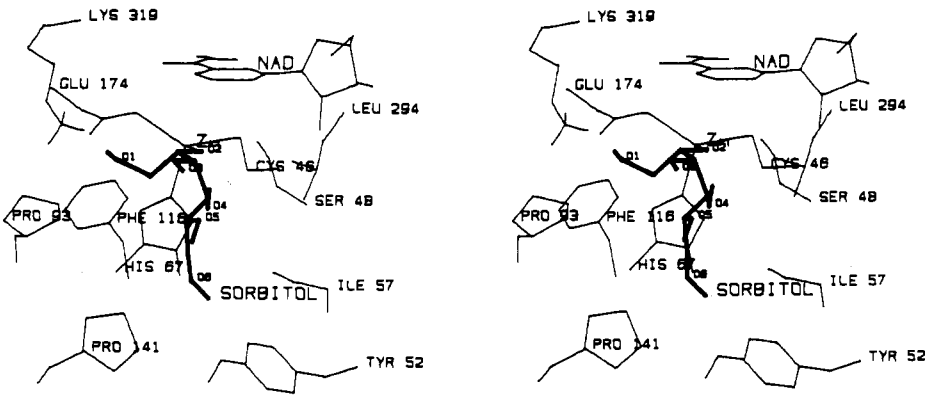


FIGURE 2: Stereo diagram of a sorbitol molecule positioned in the active site of the sorbitol dehydrogenase model.

Table III: Interactions between Enzyme and Sorbitol (Residues with Atoms Closer Than 4 Å)

C1	H67, P93, E174, Zn	C4	S48
O1	H67, P93, E174, K319	O4	S48, H67, Zn
C2	H67, E174, Zn, NAD	C5	F116
O2	C46, S48, H67, E174, Zn, NAD	O5	I57, F116
C3	NAD	C6	I57, P141
O3	F116, I318, NAD, S48, L294	O6	Y52, I57, P141

2.1 Å, which is the same distance as that found for O–Zn interactions in crystallographic studies of substrate and inhibitor complexes of LADH (Eklund et al., 1981, 1982). Furthermore, the positions of O2, C2, and C3 were the same as found in substrate binding studies of LADH for O1, C1, and C2, respectively (Eklund et al., 1982; Horjales & Brändén, 1985).

Free sorbitol crystallized from ethanol solution has an extended conformation with all torsion angles close to 60° (Park et al., 1971). Each oxygen has two intermolecular hydrogen bonds. The conformation of sorbitol that best fits the mainly hydrophobic active site in our model has two internal hydrogen bonds formed between O3–O5, and O5–O6, respectively. This involves a torsion angle O2–C2–C3–O3 of 180° and avoids short contacts with the NAD ring. Further, a torsion angle O3–C3–C4–O4 of 140° adapts the conformation of bound sorbitol to the shape of the active site channel of the model. The remaining angles of enzyme-bound sorbitol in the model are similar to the values for the crystals of free sorbitol.

The results of docking the sorbitol molecule into the SDH model were as follows (Table III). O1 was positioned by the energy-minimization procedure in proper hydrogen-bond contacts with OE1 of Glu-174 and with NZ of Lys-319 (2.6 and 2.8 Å, respectively). These bonds stabilize the O2–Zn binding mode. O3 was positioned in the neighborhood of the amide group of NAD, O4 was within 3.0 Å from OG of Ser-48, and O6 was at 3.5 Å from OH of Tyr-52. O5 has no hydrogen bonds and has no contacts shorter than 3.4 Å with other atoms. Thus, in this mainly hydrophobic region, all oxygen atoms in sorbitol except one have hydrogen bonds to the protein or are positioned in a polar region of the SDH model.

Substrate Specificity. Kinetic data for SDH are summarized in Table IV. Polyol substrates have the *S* configuration at C-2 (Figure 3) and the *R* configuration at C-4. The corresponding C-2 and C-4 epimers do not serve as substrates. At C-3, either the *R* or *S* configuration is compatible with reaction, but the 3(*R*) compounds are better substrates. A hydroxyl group at C-3 is not essential; 3-deoxyxylitol is a good substrate. The configuration at C-5 has very little effect on substrate behavior, though occupation of the 5-position by a CHOH moiety is clearly important; D-erythritol scarcely serves as a substrate. The 1-hydroxyl group is also critically important; 1-deoxysorbitol is not a substrate.

In the best model of sorbitol in the active site, the oxygen atom at C-1 of the substrate is hydrogen bonded to Lys-319 and Glu-174 (Table I), and this would stabilize the active conformation at O2. It is also possible in our model to bind

Table IV: Substrate Specificity of Liver Sorbitol Dehydrogenase Preparations[a]

polyol	concn (mM)	pH	rate (relative to sorbitol = 100)
C3			
glycerol	220	7.4	<0.3
(±)-propane-1,2-diol	220	7.4	<0.3
C4			
erythritol	16	8.0	0
C5			
D-arabinol	16	8.0	0
L-arabinol	2–50	7.4–8.0	0–0.8
3-deoxyxylitol	6.6	8.1	148
ribitol	2–50	7.4–8.1	20–49
xylitol	2–16	7.4–8.1	85–169
C6			
allitol	16	8.0	43
1-deoxysorbitol	16	8.0	0
galactitol	16	8.0	0
D-gulitol (L-glucitol)	16	8.0	0
D-iditol			0
L-iditol	6.6	8.1	79
D-*chiro*-inositol	16	8.0	0
L-*chiro*-inositol	16	8.0	0
myo-inositol	16	8.0	0
scyllo-inositol	16	8.0	0
D-mannitol	16	8.0	0
D-talitol	16	8.0	0
C7			
D-*glycero*-D-*galacto*-heptitol	16	8.0	0
D-*glycero*-D-*gluco*-heptitol	6.6–16	8.0–8.1	40–87
L-*glycero*-D-*gluco*-heptitol	16	8.0	50

[a] The table is based on data given in Anderson (1965), Bergmeyer (1974), Jeffery et al. (1981), McCorkindale & Edson (1954), and Smith (1962).

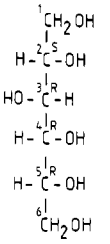


FIGURE 3: Sorbitol, showing the numbering of carbon atoms (superscript left) and absolute configuration (superscript right).

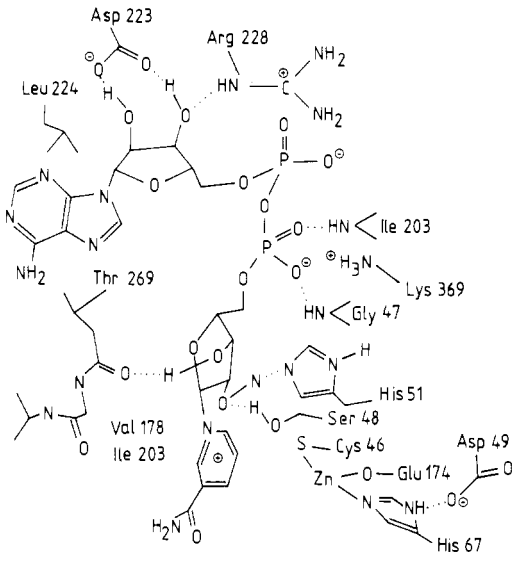


FIGURE 4: Schematic drawing showing the interactions of NAD with residues in the sorbitol dehydrogenase model.

sorbitol in an alternative way. Thus, O1 could bind to the zinc atom, and O2 be hydrogen bonded to Glu-174 and to Lys-319. However, in this mode of binding, the conformations of both sorbitol and several protein side chains are considerably changed. In addition, the C-1 hydrogen atom that might be considered a candidate for the reaction would not be favorably located for hydride transfer. The model therefore suggests explanations why, notwithstanding the structural similarities to ADH, reaction does not occur at C-1 and also why the hydroxyl at C-1 is important for reaction at C-2.

Using our docking program (Cambillau et al., 1984), we have investigated models of several six-carbon sugar alcohols bound to the Zn atom through either the oxygen atom at carbon 1 or that at carbon 2. D-Mannitol differs from sorbitol in the chirality at carbon 2. We find that D-mannitol would bind in a nonproductive orientation if O2 is bound to Zn and O1 is hydrogen-bonded to Glu-174. The hydrogen at carbon 2 (which should be the reactive hydrogen) points in the wrong direction, away from the NAD ring. Trying other possible orientations as described above, we did not find any productive binding mode for this molecule. This is in agreement with kinetic results.

Docking experiments show that the model allows binding in an active conformation whether the configuration at C-3 is *R* or *S*, but the NAD ring comes into short contacts with O3 when docking molecules with 3(*S*) configuration. When these steric hindrances are removed through energy minimization, a higher internal energy of the substrate is obtained. Therefore, the activation energy barrier would be higher for the 3(*S*) epimers, which could account for the lower activity observed in kinetic experiments.

Docking studies on molecules with the 4(*S*) configuration indicate that the model might allow productive complex formation but that, in contrast to the situation with sorbitol as substrate, O4 would no longer be hydrogen bonded to O2 and would not be in contact with OG of Ser-48. Instead, the 4-hydroxyl would be situated in a hydrophobic region, which is energetically unfavorable. It therefore follows from the model that the 4(*S*) epimers should not be well utilized, though the docking studies alone do not exclude such molecules from serving as substrates. The model seems to allow the possibility that the 4-deoxy molecule could function as substrate. The fact that the 4(*R*) configuration is essential suggests that the 4-hydroxyl group has a role that is not yet clear from the model. The reasons why the presence of a C-5 grouping is so important, and the hydrogen bond of the substrate to Tyr-52, may be related to this. Docking studies show that the model predicts similar substrate behavior for the 5(*R*) and 5(*S*) epimers. This is what was found in kinetic experiments.

Ethanol and small primary alcohols are not substrates for SDH. When a molecule of this kind is docked in the SDH model, there remains enough space to allow a water molecule to be situated near to position 319 (Lys) and 174 (Glu). The model indicates that the distance between the water molecule and the C-1 carbon of the bound alcohol would be about 3.2 Å, and it is known that hydride-transfer reactions of this type do not occur in the vicinity of water (Tapia et al., 1982). Furthermore, the water molecule would be in van der Waals contact to the alcoholate oxygen atom and could destabilize its interaction with the zinc atom by acting as a proton donor. The model therefore indicates that, despite the similarity of SDH and ADH, small primary alcohols should not be substrates for SDH. Further studies are needed to establish the reason for the abrupt large increase in activity observed in going from the C4 sugar alcohol erythritol (nonsubstrate) to the corresponding C5 sugar alcohol ribitol (substrate).

In summary, deductions based on the conformationally aligned model are in general agreement with reported kinetic findings regarding good substrates, poorer substrates, and nonsubstrates of various kinds.

Coenzyme Binding. Residues involved in binding NAD are to a large extent different between SDH and LADH (Figure 4). [The coenzyme binding to LADH has recently been described in detail by Eklund et al. (1984b).] Only Ser-48 His-51, and Asp-223 are conserved among those residues whose side chains interact with NAD. Two glycine residues (numbers 199 and 201 in LADH) in the proximity of the NAD molecule are conserved, as in other dehydrogenases (Rossmann et al., 1975; Jörnvall et al., 1984b). The largest difference compared with LADH is a loss in SDH of one charged hydrogen-bonding interaction between the side chain of Arg-47 in LADH and one phosphate of NAD. The corresponding residue in SDH is a glycine. Gly is found at the same position in a human ADH (von Bahr-Lindström et al.,

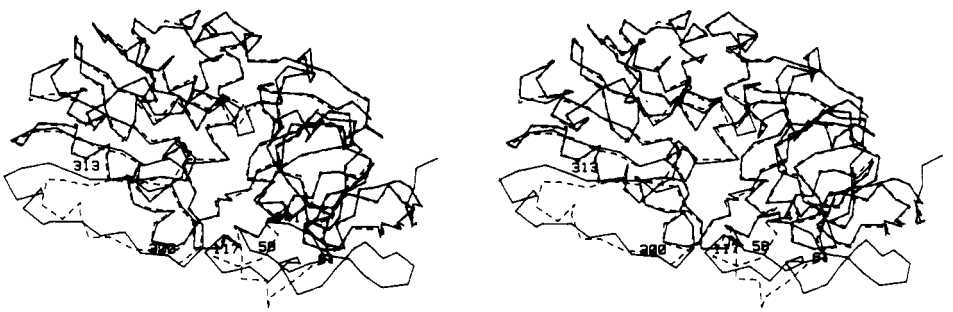


FIGURE 5: Stereo diagram of a subunit of alcohol dehydrogenase (full lines) and the model of sorbitol dehydrogenase (dotted lines).

1985). The positive character of the binding site in LADH is elsewhere also more pronounced than in many coenzyme binding enzymes (Wierenga et al., 1985), including SDH. Thus, Arg-271 in LADH at the rim of the adenine binding pocket is changed to uncharged residues in other ADH molecules and to Val in SDH. No new positively charged residues appear. There have been changes from Lys to Arg at position 228 and from Arg to Lys at position 369.

One hydrogen bond between the phosphate oxygen atom and the main-chain nitrogen of residue 202 cannot be formed in SDH, since this residue is proline. This position is generally not Gly for dehydrogenases. Hydrogen bonds to main-chain nitrogens of residues 203 and 47 can still be formed. Both ribose moieties have three hydrogen bonds to the protein in LADH and the same hydrogen bonds can be formed in the SDH model. The conserved Asp-223 and residue 228 (now Arg instead of Lys) can form the three hydrogen bonds to the adenosine ribose. Residues Ser-48 and His-51 involved in the binding of NMN ribose are conserved, and thus all three hydrogen bonds to this ribose can also be formed in the SDH model.

The adenine ring has only nonspecific van der Waals interactions in LADH. The two isoleucine residues (at positions 224 and 269) mainly responsible for these interactions in LADH are replaced by Leu and Thr, respectively, in the SDH model. These residues can serve the same function, and variations are generally found in these positions among different species at ADH.

The nicotinamide has hydrogen bonds only to main-chain atoms in LADH, which can be formed also in the SDH model. The nicotinamide makes van der Waals contacts with Val-203 and Thr-178 in LADH. The residue corresponding to position 203 is Ile in the SDH model while that corresponding to position 178 is Val. These residues can maintain similar van der Waals interactions with the nicotinamide ring.

The coenzyme can thus be positioned in the SDH model with small adjustments to compensate for the changed residues around the adenine ring and at postion 202. The conformation of NAD to the coenzyme binding domain of dehydrogenases is highly similar (Rossmann et al., 1975; Eklund et al., 1981; Wierenga et al., 1985). The coenzyme binding domain of SDH seems to be highly similar to that of other dehydrogenases. Consequently, the conformation of NAD bound to SDH should be highly similar. The folded conformation suggested by Gronenborn et al. (1984) from NMR measurements is for a SDH–NAD^+ complex. The used method has earlier (Gronenborn & Clore, 1982) been interpreted to show a ribose pucker of NAD bound to LADH, which is not the pucker found from crystallographic studies (Eklund et al., 1984b). Gronenborn et al. do not exclude the possibility that NAD^+ adopts the conventional conformation in the active ternary complex of SDH.

Quaternary Structure. There are distinct differences between the structural organization of the subunits in the dimeric vs. the tetrameric alcohol dehydrogenases. The tetrameric enzymes are slightly longer in the loop at positions 55–60, lack the loop 119–139, and are shorter in the region 305–315 (Table I). At these places, SDH is similar to tetrameric YADH and fits into a general scheme. These regions are all located on one side of the subunit (Figure 5), and this side is probably involved in subunit interactions different from those found in the dimeric enzymes. The side of the subunit opposite from the coenzyme binding clefts has many exposed hydrophobic residues in SDH. This side is also a likely candidate for subunit interactions. With these areas, a roughly spherical tetramer can be formed with the coenzyme binding clefts exposed to the solvent and far apart from each other.

Registry No. SDH, 9028-21-1; ADH, 9031-72-5; NAD, 53-84-9; zinc, 7440-66-6; sorbitol, 50-70-4.

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Alkyldihydroxyacetonephosphate Synthase Mechanism: ^{18}O Studies of Fatty Acid Release from Acyldihydroxyacetone Phosphate†

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ABSTRACT: Alkyldihydroxyacetonephosphate synthase (alkyl-DHAP synthase) catalyzes the exchange of the ester-linked fatty acid of 1-*O*-acyldihydroxyacetone phosphate (1-*O*-acyl-DHAP) for a fatty alcohol that is attached in an ether linkage to form 1-*O*-alkyldihydroxyacetone phosphate (1-*O*-alkyl-DHAP). In our continuing investigation of the mechanism of this enzyme, we have examined the fatty acid released during the reaction. In contrast to the reports of others using whole microsomes, we found that the cleavage of fatty acid by purified preparations of alkyl-DHAP synthase was dependent on the presence of the cosubstrate, fatty alcohol. Furthermore, the amount of fatty acid produced was equivalent to the alkyl-DHAP formed. Our previously proposed detailed mechanism for alkyl-DHAP synthase predicted that the fatty acid should retain both of the carboxyl ester oxygens upon cleavage. Reactions carried out with palmitoyl-[^{18}O]DHAP as substrate yielded [^{18}O]palmitic acid as the product in agreement with this scheme.

Alkyldihydroxyacetonephosphate synthase (alkyl-DHAP synthase)[1] catalyzes the formation of the ether bond found in alkyl and alk-1-enyl glycerolipids. In this reaction, the fatty acid ester of acyl-DHAP is cleaved and replaced with a fatty alcohol in an ether linkage (Hajra, 1970; Wykle et al., 1972). Concurrently, the *pro-R* hydrogen at the carbon of DHAP esterified to the fatty acid (DHAP C-1) is exchanged with the medium (Friedberg et al., 1971, 1972). Another feature of the reaction is the donation of the oxygen in the ether linkage by the fatty alcohol (Snyder et al., 1970); both oxygens in the fatty acyl ester of acyl-DHAP are lost during the formation

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[1] Abbreviations: DHAP, dihydroxyacetone phosphate; alkyl-DHAP synthase, alkyldihydroxyacetonephosphate synthase; alkyl-DHAP, alkyldihydroxyacetone phosphate; acyl-DHAP, acyldihydroxyacetone phosphate; NADPH, reduced nicotinamide adenine dinucleotide phosphate; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride.

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