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Alcohol and Polyol Dehydrogenases

HANS JÖRNVALL,[1] MATS CARLQUIST AND JONATHAN JEFFERY

Departments of Chemistry I and Biochemistry II, Karolinska Institutet, S-104 01 Stockholm 60, Sweden and Department of Biochemistry, University of Aberdeen, Marischal College, Aberdeen AB9 1AS, U.K.

JÖRNVALL, H., M. CARLQUIST AND J. JEFFERY. *Alcohol and polyol dehydrogenases.* PHARMACOL BIOCHEM BEHAV **18**: Suppl. 1, 67–71, 1983.—"Long" and "short" alcohol dehydrogenases with different structures and catalytic mechanisms exist and the same sub-grouping appears to apply to polyol dehydrogenases. Mammalian liver sorbitol dehydrogenase is clearly related to "long" alcohol dehydrogenases and has structural properties intermediate between those of mammalian and yeast alcohol dehydrogenases. The amino acid sequence of a large segment of the N-terminal part of a liver sorbitol dehydrogenase is now determined and shown to be strictly homologous with the long alcohol dehydrogenases. Seventeen of 93 positions have identical residues among all enzymes compared, defining residues of particular functional significance. Proline and glycine residues suggest largely similar conformations between N-terminal parts of sorbitol dehydrogenase and "long" alcohol dehydrogenases, cysteine and histidine residues suggest a conserved zinc atom at the active site, and other residues correlate with structures of special importance.

Sorbitol dehydrogenase Alcohol dehydrogenase Dehydrogenases Amino acid sequence Homology Isozymes Structure-function relationships

DIFFERENT types of alcohol and polyol dehydrogenases exist. However, both types show structural interrelationships between the two enzyme specificities [6]. On the one hand, sorbitol dehydrogenase (studied from sheep liver) has regions homologous to mammalian and yeast alcohol dehydrogenases with "long" subunits. These three proteins form a group of zinc-containing enzymes, where the sorbitol dehydrogenase is intermediate in structure between the alcohol dehydrogenases, thus linking several protein forms. On the other hand, a different alcohol dehydrogenase of insects, and a bacterial polyol dehydrogenase appear to form a second group of enzymes with "short" subunits and without zinc.

The two groups, namely the "long" and "short" alcohol dehydrogenases, are not closely related, but they may have distant connections in the form of common, smaller building units. They catalyze similar reactions in apparently different ways with and without zinc at the active site, respectively. The two groups therefore seem likely to illustrate parallel evolution with convergence towards the same substrates. At the same time, the different enzyme activities (towards alcohols and polyols) within each group demonstrate divergence. Together, therefore, the enzymes of these groups offer possibilities of linking successive changes in catalytic mechanisms, substrate specificities, quaternary structure, and other properties.

Finally, these structural and mechanistic aspects relate to functional and metabolic roles. Thus, alcohol and sorbitol dehydrogenases both occur in a pathway of glucose metabolism that bypasses regulatory steps in glycolysis and the pentose phosphate shunt [4]. This pathway may have special relevance in relation to alcoholism and diabetes. It furthermore involves reductases that are possibly related, and illustrates occurrence of isozymes of several of the proteins concerned [4].

These relationships have hitherto been based on observations of particular peptide homologies between sorbitol dehydrogenase and "long" alcohol dehydrogenases [3]. Further structural studies, positioning these peptides in the whole protein of sorbitol dehydrogenase, were essential to establish the relationships firmly and to allow detailed correlations. The studies now reported position the reactive cysteine residue at the active site of sorbitol dehydrogenase (as residue 43) and show the structure of a large N-terminal region of the protein. The findings confirm the homologies, reveal that the residue numerical positions are similar, and provide further correlations.

METHOD

Sorbitol dehydrogenase from sheep liver was ^{14}C-carboxymethylated under denaturing conditions and treated with CNBr as described [3]. CNBr-fragments were purified by successive steps of Sephadex G-50 exclusion chromatography and reverse phase high performance liquid chromatography [9]. Enzymatic redigestions were carried out as previously reported [3], and resulting peptides were again separated by reverse phase high performance liquid chromatography. For sequence analysis of peptides, the manual DABITC method was used [10], as well as degradations in a modified [11] Beckman 890C liquid phase sequencer. Residues from the DABITC degradations were identified by thin-layer chromatography utilizing secondary

[1]Requests for reprints should be addressed to Hans Jörnvall, Department of Chemistry I, Karolinska Institutet, S-104 01 Stockholm 60, Sweden.

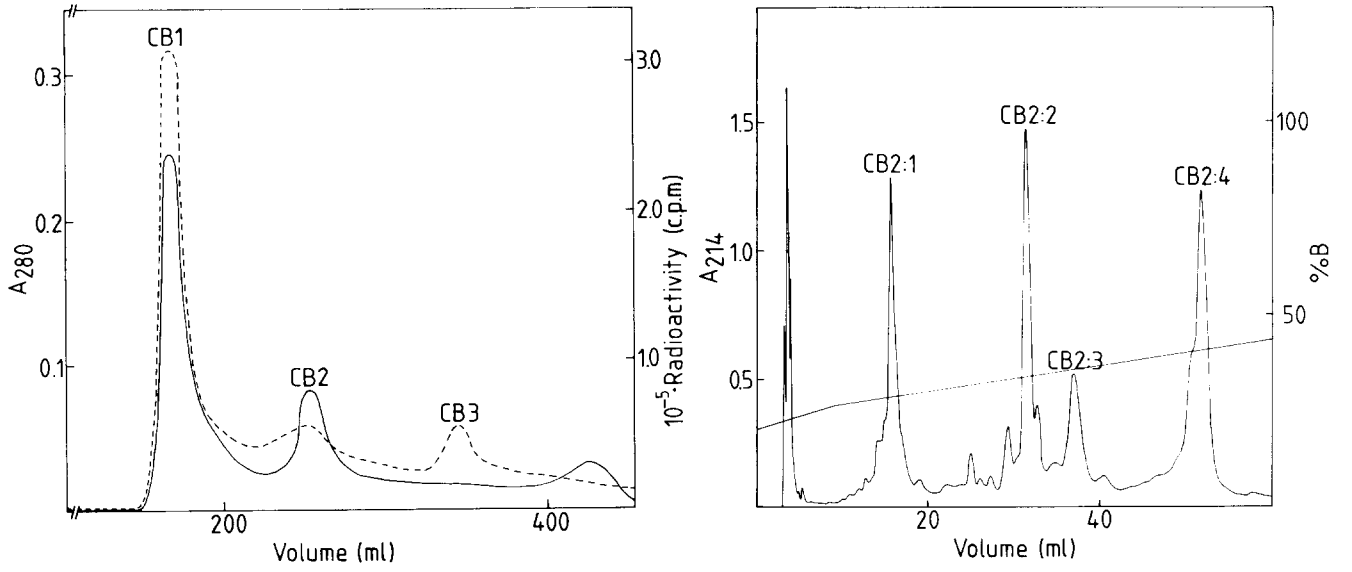


FIG. 1. Left: Separation of the CNBr-fragments of ^{14}C-carboxymethylated sorbitol dehydrogenase into three groups (CB1, 2, 3) by chromatography on Sephadex G-50. Right: Purification of four fragments (CB2:1–4) from group CB2 (Fig. 1, left) by reverse phase high performance liquid chromatography on μ-Bondapak C_{18} in 0.1% trifluoroacetic acid with a gradient (%B) of acetonitrile.

spots as aids in the identifications [10]. Residues from the sequencer degradations were identified by high performance liquid chromatography [12]. Total compositions after acid hydrolysis were determined with a Beckman 121M analyzer.

RESULTS

Structure of N-Terminal Region of Sorbitol Dehydrogenase

First CNBr-fragment in the protein chain. Exclusion chromatography of the CNBr-fragments of ^{14}C-carboxymethylated sorbitol dehydrogenase separates the peptides into three size groups, as shown in Fig. 1 (left). The middle fraction is further separated into four peptides by reverse phase high performance liquid chromatography, as shown in Fig. 1 (right). Analysis for N-terminal residues on material from each fraction in Fig. 1 revealed that only the peptide corresponding to peak CB2:2 in Fig. 1 (right) was blocked, that is failed to yield a free N-terminus. Since the intact protein chain also lacks a free α-amino group, the single blocked peptide is the first CNBr-fragment of the chain. In order to generate a free N-terminus accessible to direct sequence analysis, the peptide was cleaved with trypsin. Two large fragments were obtained and separated by high performance liquid chromatography (Fig. 2), one (T1) containing the blocked N-terminus. Different samples of this peptide were further cleaved by treatment with Glu-specific Staphylococcal extracellular protease I or by partial acid hydrolysis. Fragments were again separated by high performance liquid chromatography, as shown in Fig. 3. Sequence analysis of all the fragments isolated revealed the structures to be as given in Table 1A, in agreement with the total compositions shown in Table 2.

Second CNBr-fragment in the protein chain. The C-terminal structure in Table 1A shows that the second CNBr-fragment in the whole protein is preceded by a Lys-Met structure. This structure has already been found to precede the CNBr-fragment containing the reactive cysteine residue at the active site [3]. The latter corresponds to peak CB2:1 in Fig. 1 and its structure has been fully determined [3]. It is in turn known to be followed by a CNBr-fragment starting with the N-terminal structure Val-Leu [3].

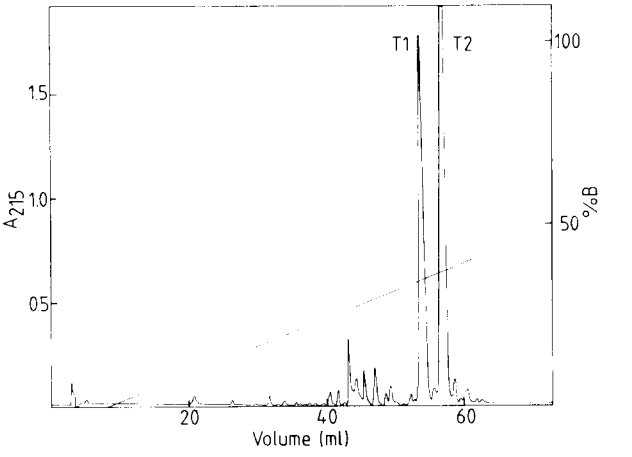
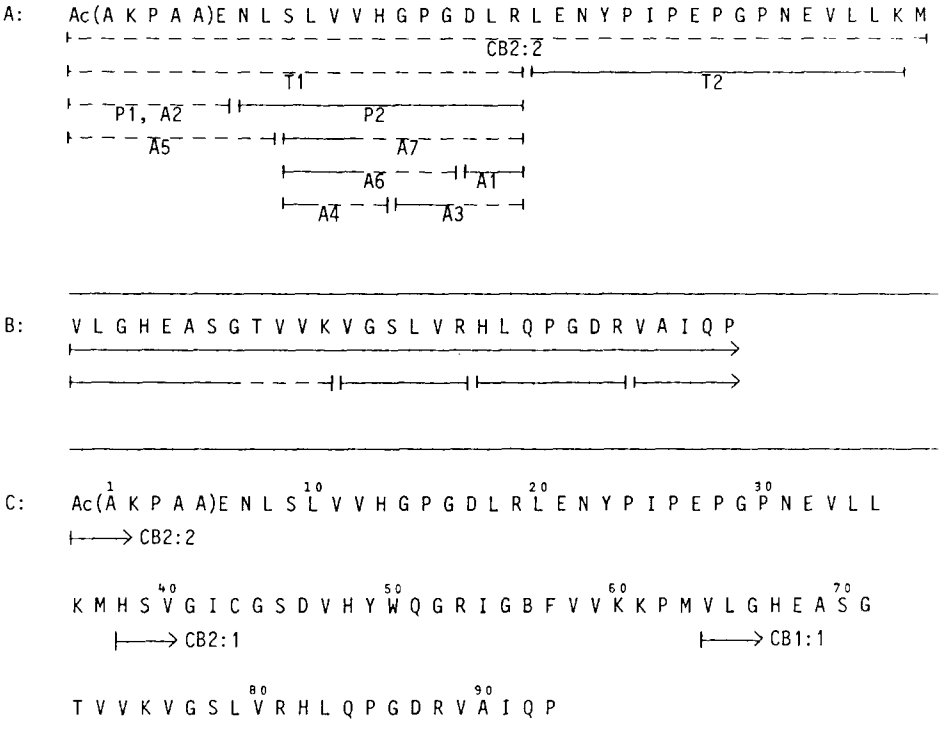


FIG. 2. Purification by high performance liquid chromatography as in Fig. 1 (right) of two tryptic peptides (T1–2) obtained from the α-amino blocked CNBr-fragment CB2:2.

Third CNBr-fragment in the protein chain. The Val-Leu structure deduced above to constitute the third CNBr-fragment is the largest CNBr-fragment in the protein, and consequently is a constituent of peak CB1 in Fig. 1 (left). That material was purified further by CM-cellulose chromatography in 8 M urea [2], and the pure peptide obtained was degraded in a liquid phase sequencer for 30 steps, giving the

TABLE 1

STRUCTURE* OF THE FIRST CNBr-FRAGMENT OF THE PROTEIN CHAIN OF SORBITOL DEHYDROGENASE (A), OF THE THIRD FRAGMENT (B) AND OF THE 93 FIRST RESIDUES OF THE ENZYME (C), AS WELL AS POSITIONS OF ALL PEPTIDES ANALYZED



*Residues are shown by the amino acid one-letter abbreviations (Met in CNBr-fragments recovered as homoserine/homoserine lactone). In A and B, solid lines indicate regions passed by Edman degradations and dashed lines show parts of peptides analyzed by total composition only. The blocking group and residues 1–5 in A and C are tentative and deduced from homology. Names of peptides in A refer to the peaks similarly marked in Figs. 1–3. In B, the top line shows the result of sequencer degradation, while bottom lines show the constituent tryptic peptides analyzed.

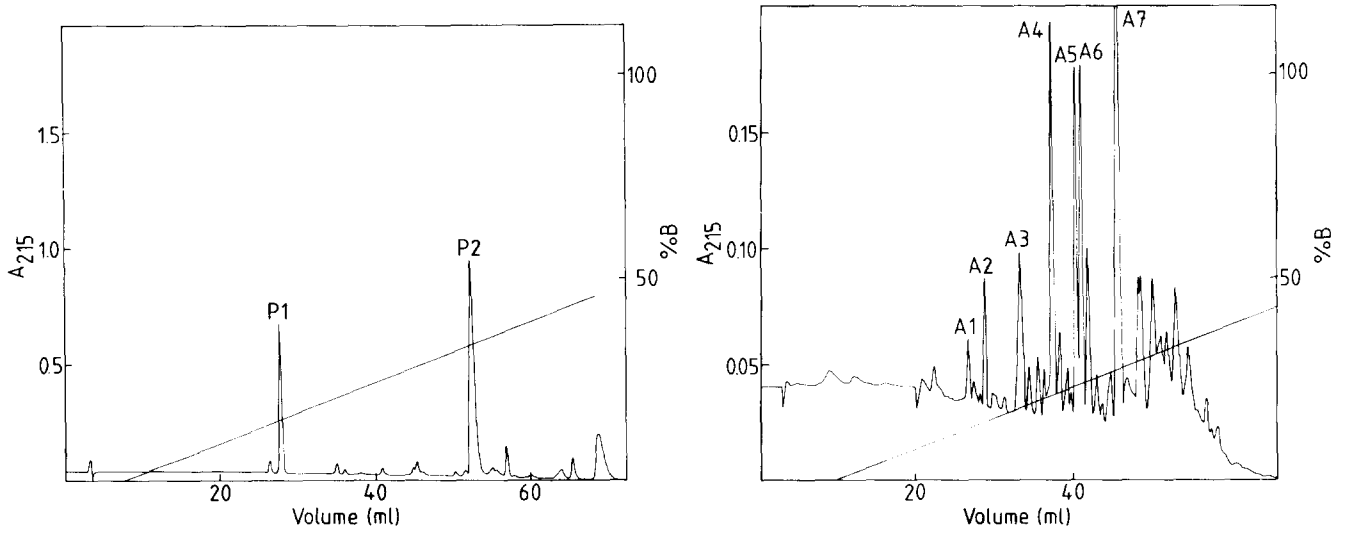


FIG. 3. Purification by high performance liquid chromatography as in Fig. 1 (right) of the peptides derived from the α-amino blocked fragment T1 by digestion (left) with a Glu-specific protease, yielding peptides P1–2, and by limited acid hydrolysis, 9 M HCl, 24 hr, room temperature (right), peptides A1–7.

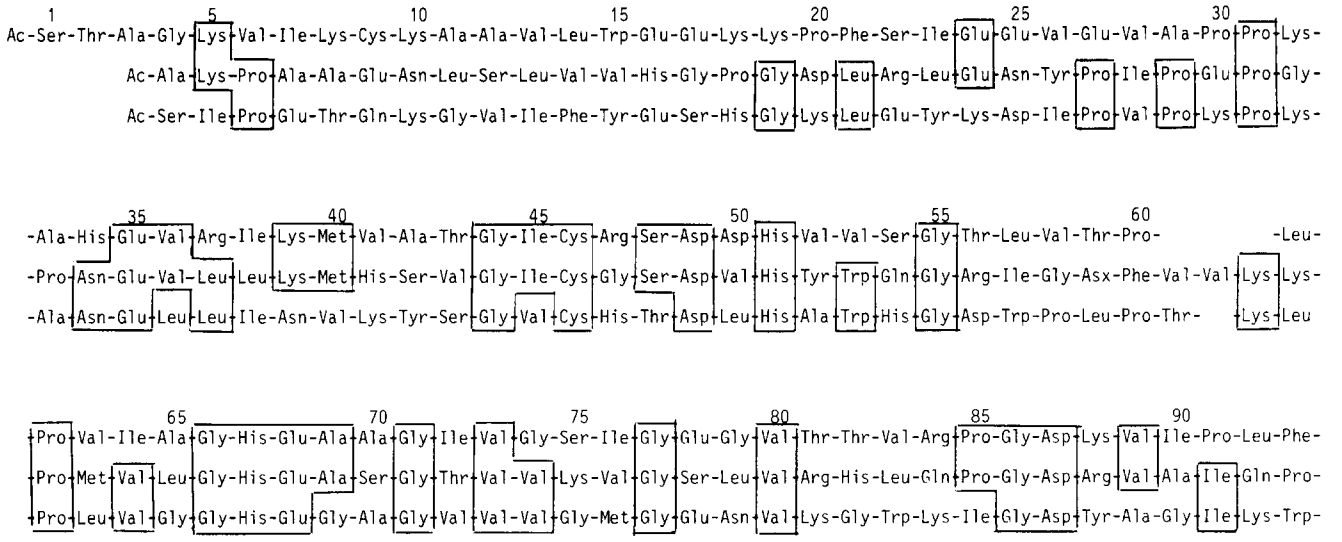


FIG. 4. Alignment of residues 1–93 of sorbitol dehydrogenase presently determined (middle line in each of the three sets forming the continuous sequence) with corresponding regions of horse liver alcohol dehydrogenase (top line) and yeast alcohol dehydrogenase (bottom line). Numerical positions refer to the horse liver alcohol dehydrogenase structure.

structure shown in Table 1B. Combined, the three CNBr-fragments presently analyzed yield the structure of residues 1–93 in sorbitol dehydrogenase, as shown in Table 1C.

Sorbitol Dehydrogenase Is Strictly Homologous with "Long" Alcohol Dehydrogenases

The structure now determined for sorbitol dehydrogenase allows an alignment of N-terminal parts of mammalian alcohol dehydrogenases, yeast alcohol dehydrogenase, and sorbitol dehydrogenase. This is shown in Fig. 4, establishing that the three proteins are clearly related, and that previously suggested homologies are valid and occupy the same positions in all these proteins. Thus, sorbitol dehydrogenase is a perfect link between the two alcohol dehydrogenases. In fact, this sheep liver enzyme is even slightly more similar to yeast alcohol dehydrogenase than the latter is to horse alcohol dehydrogenase. Thus, yeast alcohol dehydrogenase and sorbitol dehydrogenase start at the same position (Fig. 4), contain most of the identities in the structures compared (Table 3A) and are both tetrameric. It is also established from Fig. 4 that the functionally important residues are conserved, like the reactive cysteine, and occupy equivalent positions in all the enzymes.

TABLE 2

TOTAL COMPOSITIONS*OF PEPTIDES NECESSARY TO DETERMINE THE STRUCTURE OF THE FIRST CNBr-FRAGMENT OF THE PROTEIN CHAIN OF SORBITOL DEHYDROGENASE

Peptide	CB2:2	T1	T2	P1	P2
Residue					
Asx	4.1 (4)	2.1 (2)	2.1 (2)	—	2.2 (2)
Ser	1.2 (1)	1.0 (1)	—	—	0.9 (1)
Glx	4.4 (4)	1.1 (1)	3.0 (3)	1.2 (1)	—
Pro	6.0 (6)	2.1 (2)	3.9 (4)	1.0 (1)	0.9 (1)
Gly	2.8 (3)	2.2 (2)	1.3 (1)	—	2.1 (2)
Ala	2.6 (3)	3.0 (3)	—	3.0 (3)	—
Val	2.6 (3)	1.8 (2)	1.0 (1)	—	1.6 (2)
Ile	1.0 (1)	—	1.0 (1)	—	—
Leu	5.8 (6)	3.0 (3)	2.9 (3)	—	2.7 (3)
Tyr	1.0 (1)	—	1.0 (1)	—	—
Lys	2.0 (2)	1.0 (1)	1.0 (1)	0.9 (1)	—
His	0.8 (1)	1.0 (1)	—	—	1.0 (1)
Arg	0.7 (1)	1.0 (1)	—	—	0.8 (1)
Sum	37†	19	17	6	13

*Values shown are molar ratios from acid hydrolysis without corrections for slow release, destruction or contaminants. Numbers within parentheses show the values derived from the sequence analyses.

†Including the C-terminal methionine-derivative recovered as homoserine/homoserine lactone.

DISCUSSION

Evolutionary Relationships

As noted above, the identities shown in Fig. 4 and summarized in Table 3, establish a strong homology between sorbitol dehydrogenase and "long" alcohol dehydrogenases. The structure determined therefore supports the evolutionary model previously suggested from data on shorter peptides. It is clear that two groups of alcohol dehydrogenases occur and that one of these groups also includes sorbitol dehydrogenase. Both groups may contain further members (for example, a 15-hydroxyprostaglandin dehydrogenase may belong to the "short" group [7]) and are possibly also linked by still more distant common building units. The discovery that sorbitol dehydrogenase structurally inter-relates the well-known "long" alcohol dehydrogenases, together with the recognized occurrence of several isozymes of

TABLE 3

SUMMARY OF RESIDUE IDENTITIES IN ALIGNMENTS OF THE N-TERMINAL 93 RESIDUES OF SORBITOL DEHYDROGENASE (SDH), HORSE LIVER ALCOHOL DEHYDROGENASE (LADH) AND YEAST ALCOHOL DEHYDROGENASE (YADH) AS SHOWN IN FIGURE 4

A		B	
Enzymes compared	Identities	Residues	Identities
SDH/LADH	27	Any residue	17
SDH/YADH	29	Pro, Gly	8
LADH/YADH	27	Cys, His	3
		Glu, Asp, Val	6

A: Number of residue identities between pairs of the enzymes compared.

B: Number of residues identical among all three of the enzymes compared.

mammalian alcohol dehydrogenases, make the multiplicity of great interest. Presently, at least six genes appear necessary to account for all known types of protein chain. Since sorbitol dehydrogenase and two different alcohol dehydrogenases [1,8] were recent and unexpected additions, still further forms are not excluded. The multiplicities and new enzymes also illustrate the existence of alternative metabolic pathways that may be of importance in normal and pathological (including alcoholic and diabetic) conditions [4].

Conformation

The existence of successive changes also offer possibilities of detailed conclusions on the molecular structure-function relationships. For positioning of residues, it is then first necessary to know if the molecules have overall similar conformations. In the case of the yeast and horse liver alcohol dehydrogenase subunits, this appears highly likely, as shown before [5]. In the case of sorbitol dehydrogenase, final conclusions have to await the determination of the complete amino acid sequence, but already now the known identities can be taken to suggest a related N-terminal conformation. Thus, of the residues unchanged, about half affect glycine and proline (Table 3B). These residues have special conformational consequences, and glycine is often the most conserved residue when distantly related structures with similar conformations are compared. Thus, present data indicate that sorbitol dehydrogenase at least in the N-terminal part may also have a fold largely typical for similar regions of alcohol dehydrogenases.

Function

Sorbitol dehydrogenase has the two zinc ligands (Cys-46 and His-67 in Fig. 4) conserved in the 1–93 residue region. Together with the known sensitivity of sorbitol dehydrogenase to zinc chelators, its reactivity with thiol reagents, and its directly determined zinc content, sorbitol dehydrogenase is therefore likely also to function mechanistically in the same way as "long" alcohol dehydrogenases, with a zinc atom at the active site.

In addition to the conformationally important glycine and proline residues, and the metal-liganding cysteine and histidine residues mentioned above, a few other residues are also strictly conserved in these proteins (Table 3B, bottom line). Significantly, these are also residues that have been ascribed specific roles in previous interpretations, such as Glu-68 (in a salt bridge), Val-73 and Val-80 (in a hydrophobic core). In summary, present results fully confirm that sorbitol dehydrogenase is a true homologue of "long" alcohol dehydrogenases, and show that complete analyses of this enzyme will excellently illustrate both mechanistic and metabolic functions of alcohol and polyol dehydrogenases.

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REFERENCES

1. Bosron, W. F., T.-K. Li, W. P. Dafeldecker and B. L. Vallee. Human liver π-alcohol dehydrogenase: kinetic and molecular properties. *Biochemistry* **18:** 1101–1105, 1979.
2. Fowler, A. V. Amino acid sequence of β-galactosidase. VII. Isolation of the 24 cyanogen bromide peptides. *J Biol Chem* **253:** 5499–5504, 1978.
3. Jeffery, J., L. Cummins, M. Carlquist and H. Jörnvall. Properties of sorbitol dehydrogenase and characterization of a reactive cysteine residue reveal unexpected similarities to alcohol dehydrogenases. *Eur J Biochem* **120:** 229–234, 1981.
4. Jeffery, J. and H. Jörnvall. Enzyme relationships in a sorbitol pathway that bypasses glycolysis and pentose phosphates in glucose metabolism. *Proc Natl Acad Sci USA*, in press.
5. Jörnvall, H., H. Eklund and C.-I. Brändén. Subunit conformation of yeast alcohol dehydrogenase. *J Biol Chem* **253:** 8414–8419, 1978.
6. Jörnvall, H., M. Persson and J. Jeffery. Alcohol and polyol dehydrogenases are both divided into two protein types, and structural properties cross-relate the different enzyme activities within each type. *Proc Natl Acad Sci USA* **78:** 4226–4230, 1981.
7. Mak, O. T., H. Jörnvall and J. Jeffery. The primary prostaglandin inactivating enzyme of human placenta is a dimeric short-chain dehydrogenase. *Biosci Rep* **2:** 503–508, 1982.
8. Parés, X. and B. L. Vallee. New human liver alcohol dehydrogenase forms with unique kinetic characteristics. *Biochem Biophys Res Commun* **98:** 122–130, 1981.
9. von Bahr-Lindström, H., M. Carlquist, V. Mutt and H. Jörnvall. Analytical and preparative applications of high-performance liquid chromatography (HPLC). Comparisons with other separation methods. In: *Methods of Protein Sequence Analysis*, edited by M. Elzinga. Clifton, NJ: Humana Press, 1982, pp. 455–462.
10. von Bahr-Lindström, H., J. Hempel and H. Jörnvall. Byproducts as an aid in residue identification during peptide sequence analysis with dimethylaminoazobenzene isothiocyanate. *J Protein Chem.*
11. Wittmann-Liebold, B. Microsequencing by manual and automated methods as applied to ribosomal proteins. In: *Chemical Synthesis and Sequencing of Peptides and Proteins*, edited by T.-Y. Liu, A. N. Schechter, R. L. Heinrikson and P. G. Condliffe. Amsterdam: Elsevier/North Holland, 1981, pp. 74–110.
12. Zimmerman, C. L., E. Appella and J. J. Pisano. Rapid analysis of amino acid phenylthiohydantoins by high-performance liquid chromatography. *Anal Biochem* **77:** 569–573, 1977.