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Structural Relationships Among Aldehyde Dehydrogenases

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HEMPEL, J., H. VON BAHR-LINDSTRÖM AND H. JÖRNVALL. *Structural relationships among aldehyde dehydrogenases.* PHARMACOL BIOCHEM BEHAV **18:** Suppl. 1, 117–121, 1983.—Two functional regions of liver aldehyde dehydrogenase were characterized before; other structures of homologous parts from isoenzymes have now been determined to obtain further information on the isoenzyme relationships. In a 22-residue region from the horse cytoplasmic and mitochondrial isoenzymes, substitutions occur at 12 positions, including a continuous six-residue portion characterized by non-conservative changes. In contrast, the same structure from the cytoplasmic isoenzyme shows exchanges at only three positions when compared to its counterpart from human cytoplasm. A similar estimate of substitution frequency between species is obtained from a larger sampling at 236 positions. Thus, the isoenzyme difference between aldehyde dehydrogenases from the same species is about five-fold greater than the species difference between corresponding isoenzymes. Hence, the relationship between cytoplasmic and mitochondrial aldehyde dehydrogenases, while recognizable, is distant. This is compatible with the fact that a property such as high sensitivity to disulfiram is a characteristic of only the cytoplasmic isoenzyme.

Aldehyde dehydrogenase Structure-function relationship Amino acid sequence comparison
Peptide purification Disulfiram DABITC

NAD^+-linked liver aldehyde dehydrogenase acts at the second step in the clearance of ingested ethanol. In mammals, two isoenzymes predominante, one cytoplasmic and one mitochondrial [3,4], but their relative roles is somewhat unclear. The cytoplasmic isoenzyme is highly sensitive to inactivation by the alcohol aversive agent disulfiram (Antabuse), and the inactivation, causing acetaldehyde levels to rise, is the apparent basis of the therapeutic effectiveness of this compound [12]. However, deficiency of the mitochondrial isoenzyme also results in sensitivity to alcohol, suggesting that this isoenzyme, as well, is necessary for normal clearance of acetaldehyde [5].

Cytoplasmic and mitochondrial aldehyde dehydrogenases may be distinguished by their K_m values for acetaldehyde. This value is lower for the mitochondrial isoenzyme from man and horse by at least a factor of 10, but gross structural properties are similar; the molecular weights of these native enzymes are around 220,000 and those of the subunits about 55,000. Peptide mappings suggest that all molecules are homotetramers and furthermore show both differences and similarities in primary structure between the two types of isoenzyme subunits [8,13]. Immunological cross-reactivity also suggests a structural relatedness between the bovine isoenzyme pair [11].

During investigations on the structure of aldehyde dehydrogenases from human and horse liver, we have characterized peptides sufficient to enable us to draw some conclusions concerning relationships between aldehyde dehydrogenases from different subcellular compartments and from different species.

METHOD

Proteins

Cytoplasmic and mitochondrial aldehyde dehydrogenase isoenzymes were purified [4,8]. Enzyme SH groups were alkylated with iodoacetamide or iodoacetate and the proteins subsequently fragmented with CNBr or trypsin as described [6,15].

Peptide Purification

Fragments from these proteins were separated into groups by chromatography on Sephadex G-50 and further purified by methods including ion exchange chromatography on CM-52 cellulose (Whatman) or DEAE BioGel (Calbiochem), and reverse phase high performance liquid chromatography [7, 10, 13]. In some cases paper chromatography and high voltage paper electrophoresis were also used. Conditions for proteolytic redigestions of large fragments were as described before [10].

Sequence Analysis

Fragments isolated were checked for purity by analysis of compositions and end groups. Manual sequence degradations were performed by the DABITC method as described [2] using byproducts to assist the identifications [14]. Alternatively, some peptides were degraded by the dansyl-Edman method using polyamide thin-layer chromatography for identification and electrophoresis to establish amide groups. Liquid-phase sequencer degradations were carried out in a

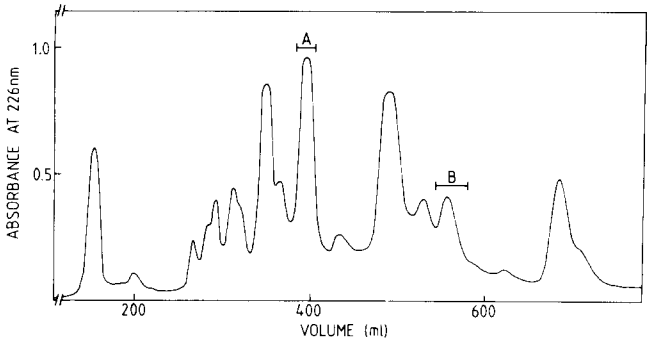

FIG. 1. Chromatography of tryptic fragments from human cytoplasmic aldehyde dehydrogenase on CM-52 cellulose. A Sephadex G-50 fraction, containing the fragments analyzed (A,B, cf. Fig. 2I) was applied. The column was equilibrated in 20 mM acetic acid, 2 M urea (pH 3.65 with NaOH), and developed with a linear gradient of 0-110 mM NaCl (total volume=900 ml) in the same buffer.

Beckman 890C sequencer, as well as in one modified with a cold trap, new valves and a conversion cell [16]. Precycled polybrene and a 0.1 M Quadrol peptide program were used for the sequencer degradations.

RESULTS

Human liver aldehyde dehydrogenase was isolated by steps of ion exchange chromatography and affinity chromatography [8]. The cytoplasmic isoenzyme was alkylated first with iodo[^{14}C]acetamide under non-denaturing conditions, primarily alkylating a single enzyme SH group [6,7], and then with unlabelled iodoacetamide in the presence of 6 M guanidine-HCl, alkylating the remaining enzyme SH groups. A tryptic digest of this material was subjected to exclusion chromatography on Sephadex G-50. The early portion of the eluate, containing the fragment selectively ^{14}C-alkylated was further chromatographed on CM-52 cellulose to give five prominent peaks and numerous minor peaks as shown in Fig. 1. The ^{14}C-containing peptide described before [7] corresponds to the peak eluting at 680 ml in the figure. The peptides contained in the remaining peaks were analyzed and one of these (peak A of Fig. 1) was useful for comparisons. It was determined to have the sequence shown in Fig. 2I. Sequencer degradations provided unambiguous identification of most of the residues up to position 19. The entire structure was confirmed by manual DABITC degradations of peptides obtained with Glu-specific Staphylococcal protease, as well as of a peptide resulting from a minor tryptic cleavage at Gln-13 and contained in peak B of Fig. 1; purifications of these fragments were carried out by high performance liquid chromatography. The amino acid compositions are given in Table 1 for fragments necessary to deduce the structures in Fig. 2.

The same tryptic fragment was also purified from horse cytoplasmic aldehyde dehydrogenase. It was purified by DEAE BioGel chromatography after tryptic re-digestion of the appropriate CNBr fragment, and had the composition listed in Table 1. The entire amino acid sequence was determined by sequencer analysis and is given in Fig. 2II.

Finally, the same tryptic fragment was also purified from the horse mitochondrial isoenzyme. It was obtained after two-dimensional mapping on paper (electrophoresis and chromatography) of an appropriate Sephadex G-50 fraction of a tryptic digest. This structure was determined by sequencer degradation of the intact peptide and by manual dansyl-Edman degradations of fragments from redigestions as indicated in Fig. 2III.

DISCUSSION

Reactive Thiol Groups

The structure of a part of aldehyde dehydrogenase associated with coenzyme binding [15] and of a part associated with the disulfiram reaction [7] have each been characterized, although from different species and isoenzymes. Each part contained a reactive cysteine residue, but comparison of the two structures did not reveal any homologies. This indicates that different thiol groups are reactive, dependent on reagents and conditions, and that coenzyme binding and disulfiram reaction affect different cysteine residues.

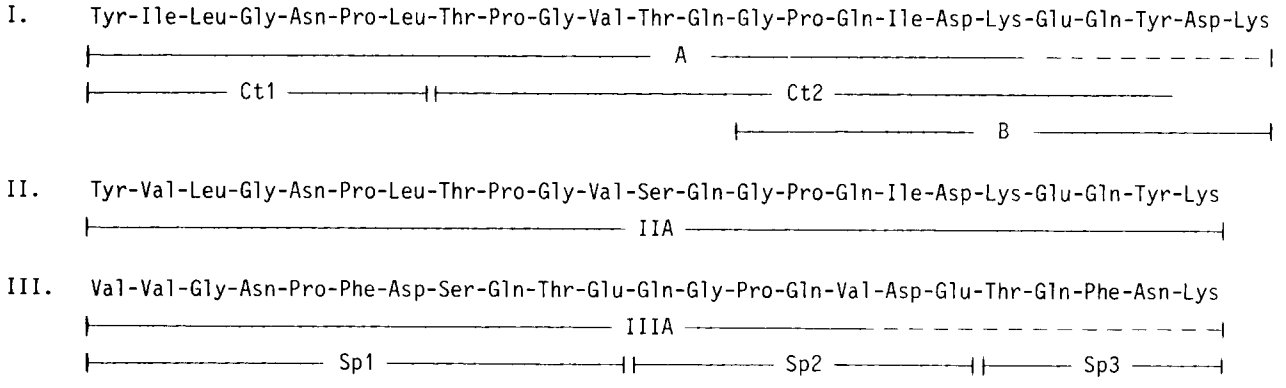

FIG. 2. Structures of tryptic fragments from homologous regions of human cytoplasmic (I), horse cytoplasmic (II), and horse mitochondrial (III) isoenzymes of liver aldehyde dehydrogenase. Fragments necessary to determine the structures were obtained after redigestion with chymotrypsin (Ct) or a Glu-specific Staphylococcal protease (Sp). Amino acid compositions confirming the structures are given in Table 1.

TABLE 1

AMINO ACID COMPOSITIONS OF FRAGMENTS NECESSARY TO DEDUCE THE STRUCTURES OF ALDEHYDE DEHYDROGENASE SHOWN IN FIG. 2

	Human Cytoplasmic			Horse Cytoplasmic		Horse Mitochondrial			
Peptide:	A	Ct1	Ct2	B	IIA	IIIA	Sp1	Sp2	Sp3
Residue									
Asx	2.9 (3)	1.0 (1)	1.0 (1)	1.9 (2)	2.0 (2)	3.8 (4)	2.1 (2)	1.2 (1)	1.2 (1)
Thr	1.8 (2)		1.8 (2)		1.0 (1)	2.1 (2)	1.0 (1)		0.9 (1)
Ser				0.5 (0)	1.2 (1)	1.0 (1)	1.0 (1)		
Glx	3.9 (4)		3.9 (4)	2.9 (3)	4.2 (4)	5.4 (6)	2.2 (2)	2.8 (3)	1.0 (1)
Pro	2.7 (3)	0.9 (1)	1.8 (2)	1.0 (1)	3.0 (3)	2.2 (2)	1.0 (1)	1.0 (1)	
Gly	3.1 (3)	1.2 (1)	2.3 (2)	1.4 (1)	3.2 (3)	2.0 (2)	1.1 (1)	1.1 (1)	
Val	1.2 (1)		1.1 (1)	0.4 (0)	2.2 (2)	3.2 (3)	1.2 (2)	1.0 (1)	
Ile	1.9 (2)	1.0 (1)	1.1 (1)	0.8 (1)	1.0 (1)				
Leu	2.1 (2)	1.9 (2)		0.4 (0)	1.8 (2)				
Tyr	1.6 (2)	0.9 (1)	1.0 (1)	0.9 (1)	1.7 (2)				
Phe						1.6 (2)	1.0 (1)		1.0 (1)
Lys	1.9 (2)		1.0 (1)	1.7 (2)	1.7 (2)	1.1 (1)			1.0 (1)

Analytical values shown are molar ratios and the numbers shown in parentheses are the values from sequence analysis.

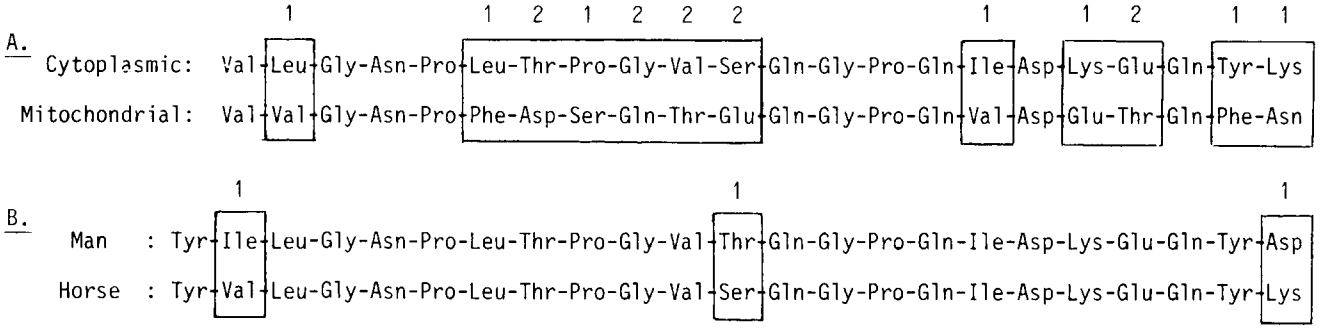

FIG. 3. Comparisons of homologous structures from liver aldehyde dehydrogenases. Exchanges are boxed and numbers show nucleotide base changes minimally required. A: regions of the cytoplasmic and mitochondrial isoenzymes from the horse. B: the same regions of the cytoplasmic isoenzymes from two species.

The non-relatedness of those two structures was not surprising since disulfiram binding and coenzyme binding were not necessarily expected to occur at the same locations. Comparison of equivalent regions, a prerequisite to following successive changes, could therefore not be made from these two fragments. However, we have found related structures in other regions from different isoenzymes of horse and human liver aldehyde dehydrogenase which now make such comparisons possible.

Isoenzyme Differences (Same Species)

As shown in Fig. 3A, equivalent regions of horse cytoplasmic and mitochondrial isoenzymes reveal a substitution frequency of 12/22 residues, or about 50%. Many of these substitutions are functionally conservative; for instance, Leu→Val, Leu→Phe, and Ile→Val replace one hydrophobic residue with another, and Tyr→Phe constitutes an exchange of aromatic residues. These exchanges only require a single nucleotide base change in the genetic code, as indicated in the figure. However, the Thr-Pro-Gly-Val-Ser→Asp-Ser-Gln-Thr-Glu exchange beginning at position 7 (Fig. 3B) is non-conservative both in relation to the residues involved and to the nucleotide base changes required. This indicates either that this region is relatively unimportant in determining the catalytic activity of the enzyme, and hence is not conserved, or alternatively, that it is of special importance in determining the functional differences between the isoenzymes, and hence just therefore not conserved. Proper assessment of these two possibilities must await further data.

From another viewpoint, the fact that nearly half of the substitutions detected require more than a single nucleotide base change indicates that the separation of the two isoenzymes from a common genetic ancestor must be of considerable age. This is in contrast to the situation with horse liver alcohol dehydrogenase in which only six substitutions out of 374 residues have been noted between the subunits from the two homodimeric isoenzyme forms which display notable

TABLE 2

SUBSTITUTIONS OBSERVED BETWEEN PEPTIDES* OF HORSE AND HUMAN CYTOPLASMIC ALDEHYDE DEHYDROGENASES

Minimum Base Changes Required	Horse	Human	Positions Examined: Exchanged	Positions Examined: Total
1	Val	Ile		
1	Ser	Thr		
1	Lys	Asp	3	23
1	Ile	Leu	1	9
1	Phe	Leu		
1	Leu	Ile		
1	Ala	Gly	3	9
1	Gln	Glu		
1	Gln	Glu		
1	Lys	Glu		
1	Gln	Glu		
1	Gln	Glu		
1	Asn	Glu	6	26
2	Thr	Phe		
1	Ile	Leu		
1	Phe	Ser		
2	Ser	Val	4	23
1	Phe	Leu		
1	Glu	Asp	2	9
1	Ile	Val	1	10
1	Val	Ile	1	20
1	Met	Leu	1	22
1	Phe	Tyr		
2	Met	Asn		
1	Gly	Ala		
1	Leu	Ile	4	15
	(Five fragments with no substitutions)		0	70
		Sum:	26	236

*Horizontal lines separate the peptides examined.

differences in substrate specificity [1]. The situation seen here is, however, somewhat reminiscent of the relationship existing between lactate dehydrogenase types (cf. [9]).

Species Differences (Same Isoenzyme)

In contrast, the two corresponding fragments between species but from the same subcellular location differ at only three of 22 positions compared (Fig. 3B). Each substitution is highly conserved, requiring only a single nucleotide change in the genetic code, with an Ile→Val exchange of hydrophobic, β-branched residues, a Thr→Ser replacement of small polar residues, and an Asp→Lys exchange of charged residues.

Thus, a substitution frequency of around 10% appears to be the degree by which these similar isoenzymes from different species differ. This same figure is also obtained from a summary of all substitutions thus far observed between other homologous fragments of human and horse liver cytoplasmic aldehyde dehydrogenase (Table 2). It may also be noticed that most exchanges are conservative both in relation to function and to nucleotide base changes required. Interestingly, although the conformation is not known for any aldehyde dehydrogenase, one of the three replacements detected which requires two base changes, Thr→Phe (Table 2) could perhaps even be structurally compensated by a Phe→Ser replacement since at least they are in the same fragment. Compensation could possibly also apply to some of the isoenzyme differences in Fig. 3A.

In summary, the structural difference between two isoenzymes of aldehyde dehydrogenase from the same species is about five-fold greater than the difference between the analogous isoenzymes from different species. This greater difference is also apparent when the actual substitutions are examined individually. Thus, only in the comparison of isoenzymes within the same species are substitutions involving amino acids of highly different functional properties now detected. Hence, the divergence of cytoplasmic and mitochondrial aldehyde dehydrogenases from a common genetic ancestor appears to have occurred much earlier than the divergence of man and horse. Future, more complete comparisons may reveal regions of additonal homology between cytoplasmic and mitochondrial aldehyde dehydrogenases which could indicate further portions important for catalysis. Also, determination of the phylogenetic point of isoenzyme divergence may aid in the determination of the adaptive significance of two distinct isoenzymes catalyzing the same reaction in different subcellular locations.

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