Human liver mitochondrial aldehyde dehydrogenase: a C-terminal segment positions and defines the structure corresponding to the one reported to differ in the Oriental enzyme variant

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A C-terminal segment of mitochondrial human liver aldehyde dehydrogenase was characterized. The results prove that a central part of this segment largely but not completely agrees with a structure of a tryptic peptide previously reported for the same isoenzyme. This part corresponds to a segment that contains the exchanged residue in the functionally deficient Oriental variant of mitochondrial aldehyde dehydrogenase [(1984) Proc. Natl. Acad. Sci. USA 81, 258–261]. The data suggest important functions for the C-terminal region of aldehyde dehydrogenase, clarify previously inconsistent results, and establish this structure in the typical enzyme, including the position corresponding to the mutation in the functional variant.

Mitochondrial isoenzyme *Amino acid sequence* *Isoenzyme difference* *Structure-function relationship*

1. INTRODUCTION

The low tolerance to ethanol often observed among Oriental individuals [1–3] can be correlated with elevated levels of acetaldehyde after ethanol consumption [2–3]. Such increased levels may be facilitated by two enzyme variants frequently occurring in Oriental livers and caused by allelic variations. Thus, the presence of a more active variant of alcohol dehydrogenase [4,5], an inactive variant of the mitochondrial isoenzyme of aldehyde dehydrogenase [6], or both may explain the intolerance. The molecular alteration in the variant alcohol dehydrogenase ('atypical' alcohol dehydrogenase) is due to an allele at the ADH_2 locus [7] coding for β_2 chains. Such alcohol dehydrogenase subunits from Oriental and Caucasian populations (β_2-Oriental and β_2-Bern chains, respectively) have been characterized and shown to be identical [5,8], reflecting only relative differences in the gene occurrence between the two populations. The other molecular alteration, in the mitochondrial human liver aldehyde dehydrogenase, has also been studied and a mutation suggested [6].

The established difference in the Oriental alcohol dehydrogenase can be functionally interpreted since the structure of the corresponding protein is well known [9]. The increased enzyme activity and aldehyde concentration are explained by a His/Arg substitution, affecting coenzyme-binding and therefore the rate-limiting step in aldehyde formation [5,8]. The difference suggested for aldehyde dehydrogenase [6] has not been correspondingly interpreted in functional terms because of lack of knowledge of the whole enzyme structure. Now, however, characterization and further interpretation of the difference in human mitochondrial aldehyde dehydrogenase are possible. Thus, the first primary structure of a liver

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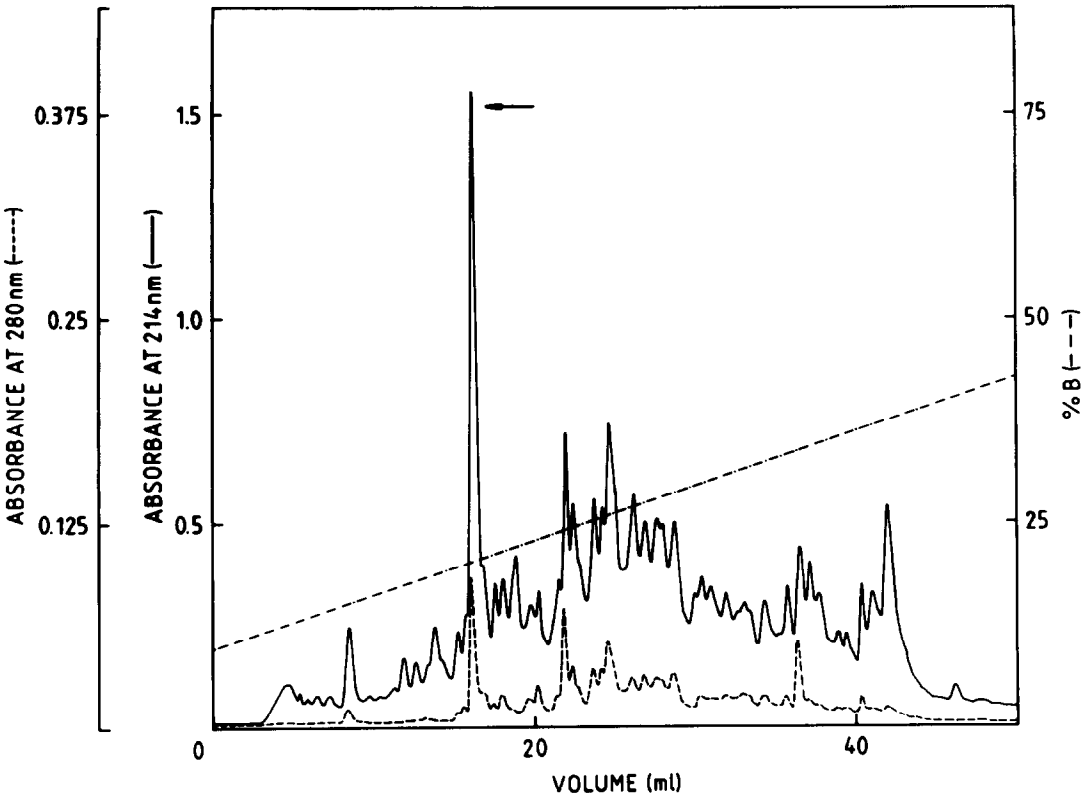
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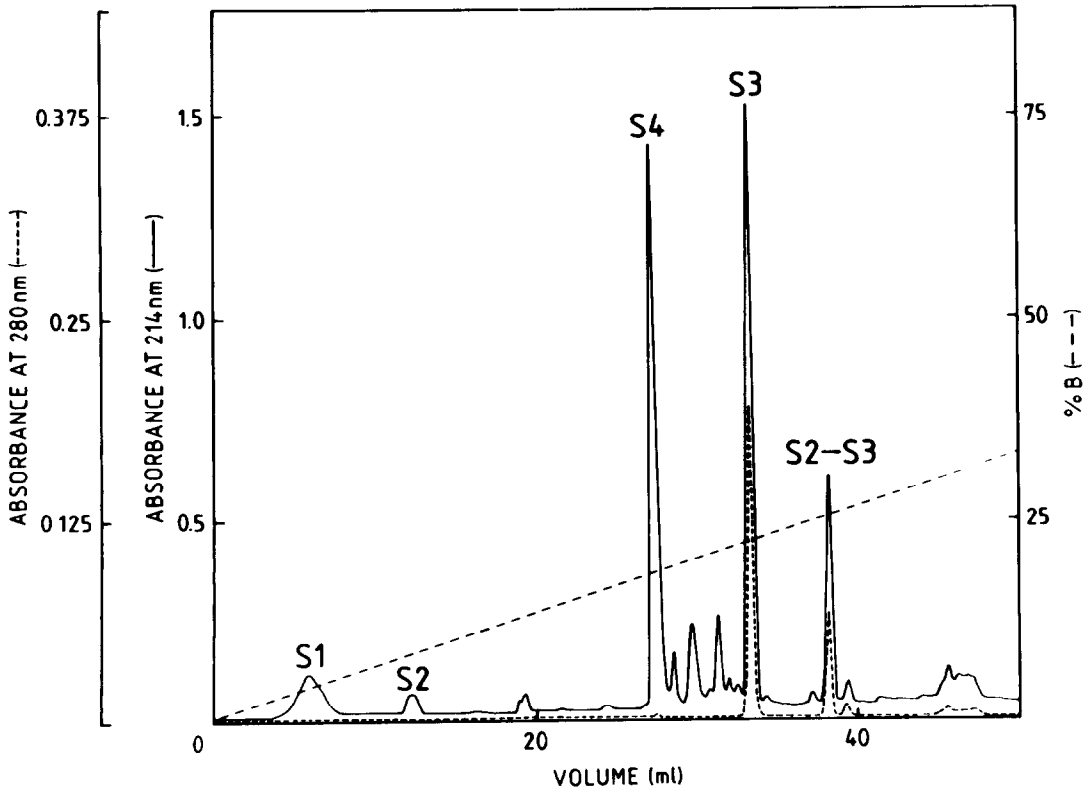
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aldehyde dehydrogenase (the human cytoplasmic isoenzyme) has recently been determined [10], chemical modifications have correlated two segments with enzyme activity [11–13], segments possibly forming the $\beta\alpha\beta$ coenzyme-binding fold have been identified [10], and comparatively distant relationships between the mitochondrial and cytoplasmic isoenzymes have been established [14].

Using this background information to position the 14-residue segment suggested an explanation for the difference in the Oriental variant of inactive mitochondrial aldehyde dehydrogenase [6]: it is found that the altered segment appears to correspond to the region Glu-476––Lys-489 near the C-terminus of the 500-residue subunit of the cytoplasmic isoenzyme. However, the structure reported for the segment suggested to be altered in the mitochondrial enzyme is somewhat different and does not exactly fit the primary structure of the cytoplasmic [10] or mitochondrial (unpublished) enzymes. Consequently, the suggested alteration [6] is unclear and assignments not established. Therefore, the 30-residue C-terminal CNBr fragment of human mitochondrial liver aldehyde dehydrogenase was here isolated and fully characterized. The results clarify previous inconsistencies and also define the structure in the typical enzyme that locates the previously reported substitution in the variant enzyme [6] at position −14 from the C-terminus.

2. MATERIALS AND METHODS

Human liver mitochondrial aldehyde dehydrogenase was prepared as described [15]. Carboxymethylation, CNBr cleavage, prefractionation on Sephadex G-50 fine, high-performance liquid chromatography (HPLC) on μBondapak (Waters), redigestion with the Glu-specific Staphylococcal protease (Miles), acid hydrolysis, liquid phase sequencer analysis and manual sequence degradation by the dimethylaminoazobenzene (DABITC) double-coupling method [16,17] were carried out exactly as described for the cytoplasmic isoenzyme [10].

3. RESULTS

The CNBr fragments from carboxymethylated human liver mitochondrial aldehyde dehydrogenase were applied to Sephadex G-50 fine. Fractions at an elution volume corresponding to fragments of about 30 residues contained the original C-terminus (unpublished) and were pooled and re-purified by reverse-phase high performance liquid chromatography (RP-HPLC) (fig.1A). The largest peak, eluting at 31% acetonitrile, contained material which was sufficiently pure to provide the amino acid sequence of the first 29 residues in the fragment by direct, manual DABITC degradation (fig.2). However, small amounts of a contaminant beginning with alanine were also present and especially disturbed the analysis for total composition (yielding too high Asp and Ala). Repurification by RP-HPLC using a Novapak (Waters) column was therefore necessary and yielded a pure product with a composition in full agreement with the amino acid sequence determined. The composition is given in table 1.

For confirmation of the structure and final establishment of all residues, the pure fragment was redigested with the Glu-specific staphylococcal protease. The resultant peptides were also separated by RP-HPLC (fig.1B) and fully degraded for sequence analysis. Total compositions are listed in table 1, and the structures are given in fig.2. Peptides S1 and S2 were analyzed by the manual DABITC method, S3 and S4 by liquid phase sequencer analysis, in which case further details are given in table 2. Combined, all results define the 30-residue C-terminal CNBr fragment of mitochondrial human liver aldehyde dehydrogenase to have the structure shown in fig.2.

←

Fig.1. RP-HPLC separation of peptides analyzed. (A) Separation of the C-terminal CNBr fragment (←—) after prefractionation on Sephadex G-50 fine of a whole CNBr digest of human liver mitochondrial aldehyde dehydrogenase. Material applied: pooled eluate between 220–250 ml from a 1.5 × 190 cm Sephadex column. HPLC conditions: gradient (% B) of acetonitrile as shown, against 0.1% trifluoroacetic acid. Column packing: μBondapak RP-18 (Waters). (B) Separation of peptides obtained after redigestion with staphylococcal Glu-specific protease of the fragment indicated by (—→) in A. Chromatographic conditions as in A, peptide designations as in fig.2.

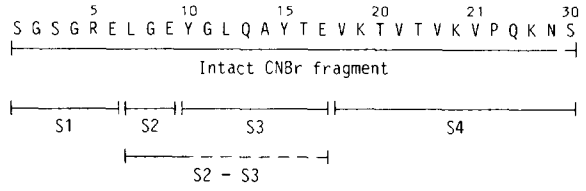

Fig.2. The amino acid sequence of the C-terminal CNBr fragment isolated as in fig.1, together with positions of all peptides used to determine the structure. Peptides S1–S4 denote products obtained after redigestion with Glu-specific staphylococcal protease (an uncleaved product, S2–S3, was also recovered, as indicated). Solid lines show residues identified in a peptide by manual DABITC degradations (intact CNBr fragment, S1, S2) or liquid phase sequencer degradations (S3 and S4, details in table 2).

(471) (480) (490) (500)
Ser-Gly-**Asn**-Gly-Arg-Glu-Leu-Gly-Glu-Tyr-Gly-**Phe-His-Glu**-Tyr-Thr-Glu-Val-Lys-Thr-Val-Thr-Val-Lys-**Ile-Ser**-Gln-Lys-Asn-Ser

Ser-Gly-Ser-Gly-Arg-Glu-Leu-Gly-Glu-Tyr-Gly-Leu-Gln-Ala-Tyr-Thr-Glu-Val-Lys-Thr-Val-Thr-Val-Lys-Val-Pro-Gln-Lys-Asn-Ser
-30 -20 -10 -1

Glu-Leu-Gly-Glu-(Ala)-Gly-Leu-Gln-Ala-(Asn)-(Val)-(Gln)-Val-Lys

Fig.3. Comparison between the structures of the normal mitochondrial human liver aldehyde dehydrogenase (middle line; this work), the normal cytoplasmic isoenzyme (top line; [10]) and the previously suggested structure of a tryptic peptide from the mitochondrial isoenzyme (bottom line; [6]). Positional numbers below the middle line refer to positions from the protein C-termini at −1. Positional numbers above the sequence refer to the actual numbers in the whole structure of the cytoplasmic isoenzyme chain [10]. The 6 residues which differ between the isoenzymes are bold-faced in the top line. In the case of the previously reported tryptic peptide, the 4 residues which deviate from the present structure are given within parentheses, being derived from the same protein as presently analyzed but reported differently. The underlined residue at position −14 corresponds to the residue that has been reported to differ in the mutant variant of Oriental human liver mitochondrial aldehyde dehydrogenase [6].

Table 1

Amino acid compositions of peptides used in the determination of the structure of the C-terminal CNBr fragment from human liver mitochondrial aldehyde dehydrogenase

Peptide	Intact CNBr fragment	S1	S2	S3	S4
Cys	– –	– –	– –	– –	– –
Asx	1.1 (1)	– –	– –	– –	1.0 (1)
Thr	2.8 (3)	– –	– –	0.9 (1)	1.8 (2)
Ser	2.8 (3)	2.0 (2)	– –	– –	0.9 (1)
Glx	4.9 (5)	1.1 (1)	1.0 (1)	2.0 (2)	1.0 (1)
Pro	1.1 (1)	– –	– –	– –	1.0 (1)
Gly	4.0 (4)	2.2 (2)	1.0 (1)	1.1 (1)	– –
Ala	1.1 (1)	– –	– –	1.0 (1)	– –
Val	3.9 (4)	– –	– –	– –	3.9 (4)
Met	– –	– –	– –	– –	– –
Ile	– –	– –	– –	– –	– –
Leu	2.0 (2)	– –	1.0 (1)	1.0 (1)	– –
Tyr	2.0 (2)	– –	– –	2.0 (2)	– –
Phe	– –	– –	– –	– –	– –
Trp	– –	– –	– –	– –	– –
Lys	2.8 (3)	– –	– –	– –	3.1 (3)
His	– –	– –	– –	– –	– –
Arg	0.9 (1)	1.0 (1)	– –	– –	– –
Sum	30	6	3	8	13
N-terminus	Ser	Ser	Leu	Tyr	Val

Table 2

Results of liquid phase sequencer degradations of peptides S3 and S4. Phenylthiohydantoin identifications were by high-performance liquid chromatography (H) and thin-layer chromatography (T)

Cycle	S3				S4		
	Residue	Method	nmol		Residue	Method	nmol
1	Tyr	HT	6	1	Val	HT	13
2	Gly	HT	3	2	Lys	HT	11
3	Leu	HT	7	3	Thr	HT	6
4	Gln	HT	5	4	Val	HT	8
5	Ala	HT	4	5	Thr	HT	3
6	Tyr	HT	5	6	Val	HT	11
7	Thr	HT	4	7	Lys	HT	10
8	Glu	HT	2	8	Val	HT	12
				9	Pro	H	2
				10	Gln	HT	4
				11	Lys	HT	5
				12	Asn	HT	4
				13	Ser	H	1

4. DISCUSSION

4.1. *Location of a previously reported difference in an aldehyde dehydrogenase variant*

A segment of human liver mitochondrial aldehyde dehydrogenase, reported to differ in the Oriental variant of this isoenzyme, has been described [6], but it could not be positioned in the whole protein chain, which was then uncharacterized. Knowledge of the entire primary structure [10] of the highly different but still clearly homologous [14] cytoplasmic isoenzyme revealed that the segment was similar in structure to the region composed of Glu-476 to Lys-489 in the C-terminal part of the cytoplasmic enzyme. These results suggest that the mutational difference in Oriental aldehyde dehydrogenase should be close to the C-terminus, although data were indirect (based on homology) and not completely consistent since the preliminary structure for the mitochondrial enzyme (unpublished) was not identical to that reported for the segment with a mutational difference.

Here, the C-terminal CNBr fragment of the mitochondrial enzyme was fully characterized (fig.2). The fragment is known to be derived from the C-terminal region by two independent sets of data. First, it is a CNBr fragment without C-terminal Hsl/Hse, suggesting origin from a protein C-terminus. Secondly, the fragment is closely homologous with the established C-terminal part of the cytoplasmic isoenzyme. As shown in fig.3, all but 6 of the 30 positions in the fragment described here (fig.2) are identical to the C-terminal counterpart from the cytoplasmic isoenzyme [10] and all residue exchanges are compatible with single-base mutations. This region is therefore a more conserved region between the two isoenzymes than another region compared earlier [14], where 11 of 22 positions showed exchanges, many requiring 2-base changes. Thus, the segment reported to differ [6] between Oriental and typical mitochondrial aldehyde dehydrogenases is close to the C-terminus, as summarized in fig.3. The actual mutation reported [6] corresponds to position −14 from the C-terminus (fig.3).

Functionally, an alteration at position −14 resulting in diminished catalytic activity as observed in the Oriental enzyme [6], is compatible with the proximity in primary structure between this region and the segment just upstream from it that contains cysteine residues (at positions 455 and 463 in the human cytoplasmic isoenzyme [10]) suggested to be near the active site from labelling of

the horse liver mitochondrial isoenzyme with a coenzyme analog [11]. Although the Oriental variant enzyme has been reported to have coenzyme-binding parameters nearly identical to those of the Caucasian typical enzyme [18], the Oriental variant enzyme has also been reported no longer to bind NAD or NADH [4]. Since the upstream cysteine-containing region is also implicated in coenzyme binding, the combined findings suggest important functions for the C-terminal region of aldehyde dehydrogenase.

4.2. *Structure of the C-terminal region and nature of the mutational difference*

The results show that the C-terminal region of mitochondrial aldehyde dehydrogenase has the structure shown in fig.3. As mentioned above, this structure contains the tryptic peptide (corresponding to positions −25 to −12 in fig.3) previously suggested to constitute the segment differing in the Oriental enzyme. This tryptic peptide fits nowhere else in the entire protein chain of mitochondrial aldehyde dehydrogenase (unpublished). However, the structure in the previous report [6] is not identical to the present one but has deviations at 4 positions (fig.3). It is likely that the deviations are due to previous misinterpretations. The reason is unknown, but it should be noticed that the isoenzyme studied previously and now is the same and that present analyses have full support by complete agreement, not only between compositions and sequences, but also between the analyses of the intact peptide and the redigested fragments. Consequently, the structure is deduced to be as presently reported. This also applies to the chemically smallest deviation, which actually concerns the residue in the position corresponding to the mutation in the variant Oriental enzyme. This was previously reported as Gln but is now shown to be Glu at position −14 (fig.3). The Glu is proven both by direct identification in manual (fig.2) and liquid phase sequencer (table 2) degradations, and by the specificity of the staphylococcal protease which cleaved after this residue. As a further check against the possibility that a deamidation had occurred, the incomplete cleavage product eluting at 38 ml in fig.1B was analyzed and found to be Leu-7 ⟶ Glu-17 (cf. fig.2).

The mutation in liver mitochondrial aldehyde dehydrogenase which is prevalent among Orientals thus appears not to originate from a structure with Gln as previously reported [6] but from one with Glu (cf. position −14, fig.3). Biochemically, this mutation is still a plausible alternative, since Glu ⟶ Lys is compatible with a single base mutation. As noted above, its position is also compatible with the functional consequences observed.

In summary, the residue at the position corresponding to the reported mutational difference in variant Oriental aldehyde dehydrogenase has been clarified in the typical, fully active mitochondrial isoenzyme. This residue is glutamic acid, occupying position −14 from the C-terminus, and the surrounding structure has been fully characterized.

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