Volume 277, number 1,2, 115-118 FEBS 09246 December 1990

Class IV mammalian alcohol dehydrogenase

Structural data of the rat stomach enzyme reveal a new class well separated from those already characterized

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Received 30 October 1990

The stomach form of alcohol dehydrogenase has been structurally evaluated by peptide analysis covering six separate regions of the rat enzyme. Overall, this new structure differs widely (32–40% residue differences) from the structures of three classes of alcohol dehydrogenase characterized before from the same species. Consequently, this novel enzyme constitutes a true fourth class of mammalian alcohol dehydrogenase. In particular, differences are extensive also towards class II, although enzymatic and physicochemical properties initially suggested overall similarities with class II. The new structure establishes the presence of one further alcohol dehydrogenase mammalian gene, extends the enzyme family derived from repeated gene duplications, and confirms tissue-specific expressions.

Alcohol dehydrogenase; Enzyme class; Amino acid sequence; Gene duplication; Homology

1. INTRODUCTION

Mammalian alcohol dehydrogenase (EC 1.1.1.1) constitutes a complex system. Three classes were established for the human liver enzyme from different properties [1] and have been structurally characterized. In particular, classes I and III are well known in structure from several species, demonstrating wide differences between the classes and suggesting that they constitute separate enzymes [2–5]. They also have different genetic regulations [6], and studies on sub-mammalian forms trace distant gene duplications reflecting class origins [7,8].

However, class II forms are less well known, as are the relationships for the enzyme from tissues outside liver. The rat stomach enzyme was tentatively assigned to class II [9], and an alcohol dehydrogenase, with class II characteristics, typical of mammalian eye and stomach has been reported [10,11]. Kinetic and physicochemical properties of a human alcohol dehydrogenase of the stomach mucosa but absent in liver were recently described, and the stomach enzyme family was suggested to constitute a gastric class II type different from the liver type [12]. Independently, it has been reported from a partial cDNA sequence that human stomach alcohol dehydrogenase represents a new class [13]. Similarly, a cDNA sequence has been determined for a rat enzyme with apparent class II properties (Höög and Jörnvall, unpublished). However, no peptide data have been obtained for the stomach enzyme from any species, thus preventing definite assignments.

We have now studied peptides from rat stomach alcohol dehydrogenase. The results confirm the presence of a fourth class of mammalian alcohol dehydrogenase, reveal it to be highly different from all the three classes characterized before, surprisingly also from the class II enzyme, and suggest the presence of additional gene duplications and separate enzymes in the family of long-chain, zinc-containing dehydrogenases.

2. MATERIALS AND METHODS

Alcohol dehydrogenase was purified from whole stomach of Sprague-Dawley rats as described [9]. In agreement with recent characterizations of the human stomach enzyme [12], the protein has overall physicochemical properties like other zinc-containing, mammalian alcohol dehydrogenases, i.e. is a dimer of 40 kDa subunits with two zinc atoms per monomer [9]. The pure protein was [^{14}C]carboxymethylated by solubilization in 8 M urea, 0.4 M Tris, 2 mM EDTA, pH 8.15, reduction with dithiothreitol, and treatment with ^{14}C-labelled iodoacetate [2].

Samples of the carboxymethylated protein were cleaved with *Lysobacter* Lys-specific protease and *Pseudomonas* Asp-specific protease. In both cases, digests obtained were directly fractionated by reverse phase high performance liquid chromatography on Vydac C4 and Ultropac C18 as described [8]. Pure peptides obtained were analyzed for amino acid sequence by degradations in an ABI 477A

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Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/90/$3.50

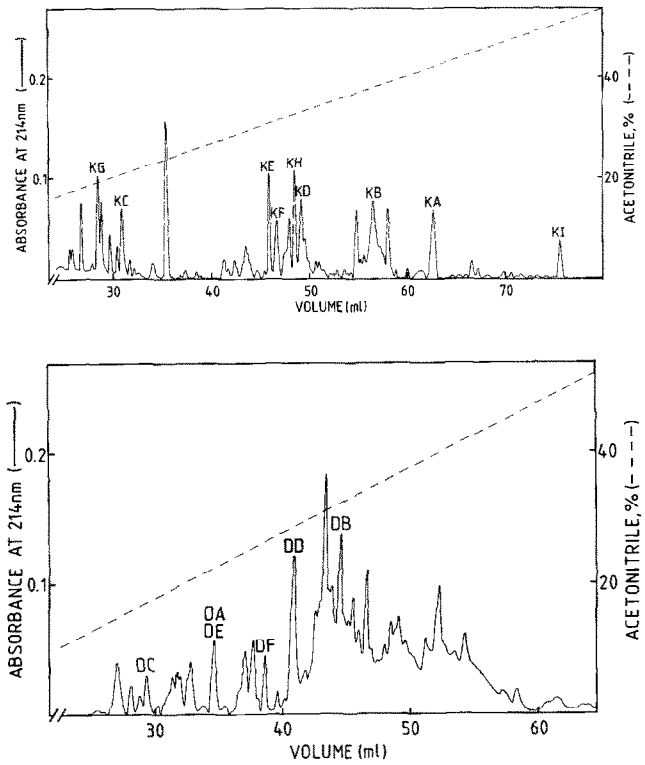

Fig. 1. Separation by reverse-phase high-performance liquid chromatography un Ultropac C18 of the peptides obtained by cleavages of carboxymethylated rat stomach alcohol dehydrogenase with the Lys-specific protease (top) and on Vydac C4 of those from cleavages with the Asp-specific protease (bottom). Peptides indicated are those now analyzed as shown in Fig. 2.

sequencer with an on-line 120A analyzer, and a MilliGen/Biosearch Prosequencer 6600, also with on-line detection.

3. RESULTS

3.1. *Characterization of six peptide segments, covering separate parts of the rat stomach alcohol dehydrogenase subunit*

[^{14}C]Carboxymethylated rat stomach alcohol dehydrogenase was cleaved in separate batches with Lys-specific and Asp-specific proteases, respectively. Direct, one-step reverse-phase high-performance liquid chromatographic separations of the resulting digests are shown in Fig. 1. Fractions obtained were structurally analyzed by sequencer degradations in solid-phase and pulsed liquid phase instruments. Results from peptides recovered sufficiently pure for reliable interpretations are shown in Fig. 2. Together, these structures prove the nature of the residues at 165 positions (44% of the entire subunits) which is more than sufficient for definitive class assignments towards the structures previously known for classes I, II, and III. Conveniently, the latter are previously known for both the rat and human forms, as also shown in Fig. 2.

3.2. *Comparison of structures now obtained with those known before*

The novel structures determined for stomach alcohol dehydrogenase differ from all previously known structures [2,3,14,15], as given in Fig. 2. The overall values show extensive residue differences in all cases, at the 32–40% level towards the other structures from the same species. These values are similar to those that distinguish the three previously characterized classes from each other [3]. In particular, the differences are as large towards class II as towards classes I and III, although the stomach enzyme has electrophoretic properties, pyrazole inhibition patterns, and ethanol oxidation properties somewhat resembling those of the class II enzyme [10–12]. Consequently, the stomach enzyme is structurally proven by direct peptide analysis to represent a new enzyme class distinct from the three known before, and widely separated from all of them. This establishes a fourth alcohol dehydrogenase class at the protein level. Although suggested also from enzyme purification [9,10,12] and cDNA cloning [13], the characterization at the peptide level is important in view of considerable variations, especially in class II of alcohol dehydrogenase (Höög and Jörnvall, unpublished) and the presence of pseudogenes in the enzyme family [16].

4. DISCUSSION

The present results demonstrate unequivocally by peptide data that stomach alcohol dehydrogenase represents a structural type distinct from all three classes previously known [2,3]. The differences are impressive, at the 32–40% level within the same species (Table I), which is exactly the same level as that for the inter-class differences of the three classes previously known [2,3]. Consequently, the stomach enzyme constitutes a separate class, distinct to roughly the same ex-

Fig. 2. Structures of six different peptide segments purified from rat stomach alcohol dehydrogenase as shown in Fig. 1. K and D peptides indicate those derived from the Lys- and Asp-specific cleavages, respectively (cf. Fig. 1). The peptides cover six segments of the subunit. The segments are identified from homology with the class I–III structures, and these regions from rat and human (Hum) enzymes are given for comparison (positional numbers refer to the human class I enzyme). The structure labelled rat class II is surprisingly dissimilar from the one reported for human class II. This may indicate an unexpectedly large species variation within class II, or the presence of still more subforms at the isozyme or class levels. Residues labelled X are not assigned and those positions are excluded from the summaries in Table I. The six structures for comparison are from the human class I γ subunit, the rat class I subunit, the human class II π subunit, the human class III χ subunit, and the rat class III subunit, as reported [2,3,14,15], and for rat class II from a cDNA clone corresponding to a rat cDNA structure obtained by hybridization with a class II human cDNA probe (Höög and Jörnvall, unpublished).

1 (positions 25-33)

```
          ⊢—DA—→
Rat IV    EVEVAPPKA
Rat I     DIEVAPPKA
Hum Iγ    EVEVAPPKA
Rat II    XXXXXXXXX
Hum II    EVEVAPPKA
Rat III   EIEVAPPQA
Hum III   EIEVAPPKA
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2 (positions 61-100)

```
          ⊢————————KA————————⊣⊢——KB——→
                                ⊢——DB——→
Rat IV    FPVIVGHEAVGIVESVGEGVTTVRPGDKVIPLFLPQCRXC
Rat I     LPAVLGHEGAGIVESIGEGVTCVKPGDKVIPLFSPQCGKC
Hum Iγ    LPVILGHEAAGIVESVGEGVTTVKPGDKVIPLFTPQCGKC
Rat II    FPVVLGHECAGIVESVGPGVTNFKPGDKVIPFFAPQCKKC
Hum II    FPVIVGHEAAGIVESIGPGVTNVKPGDKVIPLYAPLCRKC
Rat III   FPVILGHEGAGIVESVGEGVTKLKAGDTVIPLYIPQCGEC
Hum III   FPVILGHEGAGIVESVGEGVTKLKAGDTVIPLYIPQCGEC
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3 (positions 153-184)

```
          ⊢——KC—⊣⊢——KD————→
          ⊢—DC—⊣
Rat IV    DXSSVAKIDAEAPPEKGCLXGCGFXTGYGAAV
Rat I     DDIAVAKIDAAAPLDKVCLIGCGFSTGYGSAV
Hum Iγ    DENAVAKIDAASPLEKVCLIGCGFSTGYGSAV
Rat II    SEANLARVDDEANLERVCLIGCGFTSGYGAAI
Hum II    SDINLAKIDDDANLERVCLLGCGFSTGYGAAI
Rat III   ADISVAKIDPSAPLDKVCLLGCGISTGYGAAV
Hum III   ADISVAKIDPLAPLDKVCLLGCGISTGYGAAV
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4 (positions 213-226)

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          ⊢——KE————⊣
Rat IV    AAGASXIIGIDINK
Rat I     TAGAAKIIAVDINK
Hum Iγ    AAGAARIIAVDINK
Rat II    IAGASRIIAIDINS
Hum II    AAGASRIIGIDINS
Rat III   VAGASRIIGIDINK
Hum III   VAGASRIIGVDINK
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5 (positions 232-275)

```
          ⊢——KF————→
                    ⊢——DD——→ ⊢——DE——→  ⊢——DF——→
Rat IV    ALDVGATECXNPRDFTKPISEVLXDLTGXGVLXXXDVIGXLETM
Rat I     AKELGATDCINPQDYTKPIQEVLQEMTDGGVDFSFEVIGRLDTM
Hum Iγ    AKELGATECINPQDYKKPIQEVLKEMTDGGVDFSFEVIGRLDTM
Rat II    AKALGATDCLNPRDLDKPVQDVITELTGGGVDFSLDCAGTAQTL
Hum II    AKALGATDCLNPRDLHKPIQEVIIELTKGGVDFALDCAGGSETM
Rat III   AKEFGATECINPQDFSKSIQEVLIEMTDGGVDFSFECIGNVKVM
Hum III   AKEFGATECINPQDFSKPIQEVLIEMTDGGVDYSFECIGNVKVM
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6 (positions 324-363)

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          ⊢—KG—⊣⊢—KH—⊣⊢————KI————————→
Rat IV    SFDDXPKLVTEFLEKKFDLGQLITHNLPFHNIXEGFELLY
Rat I     SKDAVPKLVADFMAKKFPLEPLITHVLPFEKINEAFDLLR
Hum Iγ    SKESVPKLVADFMAKKFSLDALITNILPFEKINEGFDLLR
Rat II    SVDSVPNLVTDYKNKKFDLDLLVTHALPFDKINDAIDLMN
Hum II    SVDSIPKLVTDYKNKKFNLDALVTHTLPFDKISEAFDLMN
Rat III   SVESVPKLVSEYMSKKIKVDEFVTGNLSFDQINKAFDLMH
Hum III   SVESVPKLVSEYMSKKIKVDEFVTHNLSFDEINKAFELMH
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Table I

Overall residue differences for the 165 positions now known in class IV rat alcohol dehydrogenase, in comparison with the corresponding segments of the class I–III enzymes from rat and human

Class IV enzyme (rat) compared to		Differences at 6 segments covering 165 positions (Fig. 2)	
		Differing residues	Percent differences
Class I	rat	52	32
	human (γ chain)	45	27
Class II	rat	63	40
	human	51	31
Class III	rat	61	37
	human	58	35

Data for the comparisons are as given in Fig. 2. As stated there, 'rat class II' is surprisingly dissimilar and suggests extensive variability within class II or the presence of yet another class.

tent as the other three. This is somewhat surprising since overall enzymatic and physicochemical properties of the stomach enzyme have suggested some resemblance with the class II type in particular [9–12]. However, from the structural data, relationships with class II are not closer than those with the other classes. A functional interest in the structure of human stomach alcohol dehydrogenase follows from a recent suggestion that this enzyme is responsible for the gastric first-pass metabolism of ethanol and correlates with sex-related differences in ethanol oxidation [17].

Residue patterns of the actual replacements (Fig. 2) also differ. Nevertheless, the structure is a 'typical' long chain zinc-containing alcohol dehydrogenase and the homology is sufficient for clear assignments, as shown in Fig. 2. In particular, the segment around position 67, corresponding to the second ligand to the active site zinc atom, is highly conserved and the Gly-His-Glu segment at positions 66–68 is present also in the new class IV form (Fig. 2). This segment is the only three-residue segment common to alcohol dehydrogenases [18] and its presence also in the stomach enzyme reveals that the class IV type is a typical family member, differing in similar manner as the other three already known.

In summary, peptide data confirm the presence of a class IV mammalian alcohol dehydrogenase, and reveal its properties to be distinct from those of the other three classes but to follow the general patterns within the complex family. The existence of a fourth mammalian alcohol dehydrogenase class with special expression in the stomach proves the existence of one additional gene and of further regulations at the genetic level.

Acknowledgements: We are grateful to Dr A. Yoshida for discussions about his new human alcohol dehydrogenase clone. This work was supported by grants from the Swedish Medical Research Council (03X-3532 and 8639), the Swedish Alcohol Research Fund, and the Spanish Dirección General de Investigacion Cientifica y Técnica (PB 86-0156).

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