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# Class IV alcohol dehydrogenase (the gastric enzyme)

## Structural analysis of human σσ-ADH reveals class IV to be variable and confirms the presence of a fifth mammalian alcohol dehydrogenase class

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Human gastric alcohol dehydrogenase (σσ-ADH) was submitted to peptide analysis at picomole scale. A total of 72 positions were determined in the protein chain, providing information on three aspects of alcohol dehydrogenase structures in general. First, the data establish the presence of a unique class of the enzyme, now confirmed as class IV, expressed in gastric tissue and separate from another novel class, now termed class V. Second, the class IV gastric enzyme has active site relationships compatible with an ethanol-active, zinc-containing alcohol dehydrogenase. Third, this enzyme class is of the variable type, like that for the 'variable', classical liver alcohol dehydrogenase of class I, and in contrast to that for the 'constant' class III enzyme. Known human alcohol dehydrogenase structures now prove the presence of at least seven human genes for the enzyme and nine for the whole protein family.

Gastric alcohol dehydrogenase; Alcohol dehydrogenase classes; Enzyme evolution; Gene duplication; Conserved and variable enzymes

## 1. INTRODUCTION

The known complexity of the mammalian alcohol dehydrogenase system is increasing. Until recently, three classes were well established [1], all of which have been isolated, structurally analyzed, functionally interpreted, to a large extent cloned at the cDNA and genomic levels, and, for a few forms, also analyzed crystallographically (review in [2]; recent additions in [3,4]). However, additional forms have just been established. Thus, a mammalian gastric enzyme has been isolated and proven to be different [5,6]; and a separate liver cDNA for alcohol dehydrogenase has been established (called gene *ADH6* in [7]) and expressed as a protein [8].

The structural details thus far known for the gastric alcohol dehydrogenase concern the rat enzyme that was demonstrated to represent a new alcohol dehydrogenase class (class IV) [5]. The human counterpart ('σ-ADH'[1] in [6]; now σσ-ADH) has similar properties [6,9,10] but also important differences (e.g. $K_m$ for ethanol at pH 7.5, 40 mM vs. 5 M for the rat enzyme [11]) which made it difficult to assign it into the class system. Furthermore, the human protein encoded by *ADH6* has recently been suggested to constitute the gastric enzyme [8], in which case known classes would be four, and the rat and human novel forms [5,7] related as species variants of the same class. Enzyme variability is especially important in relation to class distinctions. The previously distinguished classes I–III differ considerably in variability [12].

We have now resolved the questions on the new enzymes and class(es) by isolation of the human form of gastric alcohol dehydrogenase (σσ-ADH) and structural analysis of that protein. The structural data establish the unique nature of the human gastric enzyme, prove it to be a class IV enzyme, clearly related to the rat gastric alcohol dehydrogenase and different from that encoded by the recently reported human *ADH6* gene. Therefore, a different class (class V) has to be postulated for the enzyme encoded by *ADH6*. Furthermore, species comparisons of the structural data available for class IV indicate that it is a 'variable' enzyme [12], like the classical class I alcohol dehydrogenase, in contrast to class III, which represents a more 'constant' enzyme.

[1]σ to denote σ-subunits, for initial isolation from stomach, but now better named σσ-ADH to emphasize, like in other ADH enzyme and isozyme nomenclature, the dimeric nature of the enzyme and the unique character of the σ-subunit presently established.

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## 2. MATERIALS AND METHODS

Human gastric tissue was obtained from surgical samples (72 g) and stored at −80°C until use. Purification of the gastric enzyme (σσ-ADH) was carried out by chromatography on DEAE-Sepharose and AMP-Sepharose, as described [6]. A modification in the second

chromatography step was the use of 100 mM Tris-HCl, pH 8.0, 0.5 mM dithiothreitol as buffer, instead of 10 mM sodium phosphate, pH 7.4, 0.5 mM dithiothreitol. Under these conditions, the enzyme binds to the column and is specifically eluted by a linear gradient of 0–0.4 mM NADH in the same buffer.

The gastric enzyme was $^{14}$C-carboxymethylated and submitted to proteolytic cleavage with Lys-C protease as described [5]. Peptides obtained were fractionated by reverse-phase HPLC on a $C_8$ column (Nucleosil 3 C8 100A, 50 × 2 mm) and submitted to sequence analysis by degradation in an ABI 477A instrument fitted with a 120 analyzer. Results obtained were correlated with those known for classes I–III [12], the rat gastric enzyme [5], and the novel human liver cDNA [7] and protein [8].

## 3. RESULTS

Human gastric alcohol dehydrogenase ($\sigma\sigma$-ADH) was isolated by a two-step procedure from surgical specimens. As reported [6], the protein exhibited a considerable ethanol dehydrogenase activity with a $K_m$ of 40 mM and a $k_{cat}$ of 280 min$^{-1}$ at pH 7.5, coenzyme specificity for NAD(H), pH optimum at pH 9.9, and no activity of the class III type (with formaldehyde in the presence of glutathione [13]). Consequently, the human gastric enzyme ($\sigma\sigma$-ADH) behaves enzymatically like a typical mammalian alcohol dehydrogenase. The enzyme was $^{14}$C-carboxymethylated, digested with Lys-C protease (cf. section 2), as described for the rat enzyme [5], and submitted to reverse-phase HPLC (Fig. 1). The peptide pattern obtained showed a profile typical for an alcohol dehydrogenase, revealing only few tryptophan-containing peptides (low $A_{280}/A_{214}$ ratio for most fractions) and many [$^{14}$C]Cys(Cm)-containing fractions.

Major fractions were submitted to sequence analysis. Many gave results difficult to interpret because of impurities or peptide mixtures and small amounts, but the results of fractions sufficiently pure and abundant (initial couplings 15–45 pmol) for clear assignments are summarized in Table I. In total, 72 residues were established. Of these, 23 (32%) differ from those at corresponding positions of the human class I enzyme, and numbers are similar for the differences towards the other classes of the human enzyme (Tables I and IIA), revealing values typical for proteins from different classes of human alcohol dehydrogenase [2]. Consequently, it can be concluded that the gastric enzyme represents a distinct class (class IV) of vertebrate alcohol dehydrogenase, now studied as the human form, and previously analyzed as the rat form [5]. Further-

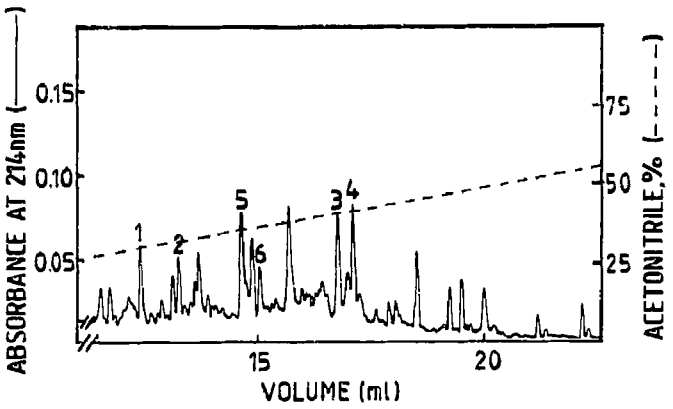


Fig. 1. Reverse-phase HPLC of the Lys-C protease digest (about 200 pmol) of carboxymethylated human gastric alcohol dehydrogenase. Peptides giving interpretable data are shown by numbers, corresponding to the peptides in Table I.

Table I

Structures of peptides from Lys-C protease cleavages of human gastric alcohol dehydrogenase, compared to corresponding segments for the human/rat variants of classes I–V. Data for the human form of class IV (Hum IV) now analyzed (top line), compared to those for the rat form (Rat IV, quoted in [5]), the same species of the class I–III enzymes [2], and the human class V form [7]. Positional numbers refer to the class I enzyme. Separation of peptides 1–6 now analyzed is shown in Fig. 1. Analyses were performed in an ABI 477A instrument fitted with a 120A analyzer at an average of 20 pmol initial coupling. Established peptide ends are indicated by vertical bars, whereas lack of such a bar indicates additional residue(s) at that end. X and lower case letters denote incomplete residue identifications

| Enzyme | Structure | | | | | |
|---|---|---|---|---|---|---|
| | Peptide 1 | Peptide 2 | Peptide 3 | Peptide 4 | Peptide 5 | Peptide 6 |
| Hum IV | AAVLwEQxRP | ILATGI | FPVIVGHEARGIVESIxExxxxxKPxDKVIPLFLPQCRECNACRNPDGNL | | KPVGRFMnV | CLIGC |
| Rat IV | AAVLWGTNQP | ILATGI | FPVIVGHEAVGIVESVGExVTTVRPGDKVIPLFLPQCRECNPCRNPEGNL | | KPVQHFMnT | CLIGC |
| | 11 20 | 40 45 | 61 88 89 110 | | 135 143 | 170 174 |
| Hum I | AAVLWELKKP | MVAAGI | LPVILGHEAAGIVESVGEGVTTVKPGDKVIPLFTPQCGKCRICKNPESNY | | KPIHHFVGV | CLIGC |
| Rat I | AAVLWEPHKP | MVATGV | LPAVLGHEGAGIVESIGEGVTCVKPGDKVIPLFSPQCGKCRICKHPESNL | | KPIHHFLST | CLIGC |
| Hum II | AAIAWEAGKP | IIATSL | FPVIVGHEAAGIVESIGPGVTNVKPGDKVIPLYAPLCRKCKFCLSPLTNL | | KPVYHFFGT | CLLGC |
| Rat II | | | FPVVLGHECAGIVESVGPGVTNFKPGDKVIPFFAPQCKKCKLCLSPLTNL | | RSIYHFMGV | CLIGC |
| Hum III | AAVAWEAGKP | IIATAV | FPVILGHEGAGIVESVGEGVTKLKAGDTVIPLYIPQCRECKFCLNPKTNL | | KTILHYMGT | CLLGC |
| Rat III | AAVAWEAGKP | IIATAV | FPVILGHEGAGIVESVGEGVTKLKAGDTVIPLYIPQCGECKFCLNPKTNL | | KPILHFMGT | CLLGC |
| Hum V | AAILWKPGAP | VVATGL | YPTILGHEGAGIVESIGEGVSTVKPGDKVITLFLPQCGECTSCLNSEGNF | | KSIYHFGNT | CLISC |

Table II

Summary of structural comparisons of the five known classes of mammalian alcohol dehydrogenase. Values shown without parentheses are those corresponding to the regions established of the class IV enzyme now analyzed (Table I), whereas those within parentheses are the ones established for the whole enzymes characterized [2,7]. Class IV from data now obtained, class I–III, V from [2,5,7]. The similar values for the figures within and without parentheses suggest that the structures now analyzed for class IV are fairly representative of the entire alcohol dehydrogenase structures in general. Species differences are unknown for class V (thus far established only for the human form [7], and therefore empty in (B). As shown, class separations are roughly equidistant in all cases, but highly different 'constant' vs. 'variable' classes are apparent from the species differences, with the presently defined gastric class IV enzyme as a variable alcohol dehydrogenase, like class I, and distinguishable from class III

| A. | Class differences | | | |
|---|---|---|---|---|
| | Human class IV versus human class | | | |
| | I | II | III | V |
| | (residue differences, %) | | | |
| | 32 | 35 | 39 | 38 |

| B | Species differences | | | |
|---|---|---|---|---|
| | Human/rat species variations within class | | | |
| | I | II | III | IV |
| | (residue differences, %) | | | |
| | 18 (18) | 25 | 3 (6) | 15 |

more, by comparison of the two species variants, it can be concluded that class IV is highly variable, exhibiting 15% residue differences in these segments (Table II) between human/rat, i.e. similar to the corresponding differences within class I, and much more than those for class III [12].

## 4. DISCUSSION

### 4.1. *Overall properties*

The characterized parts of human gastric alcohol dehydrogenase ($\sigma\sigma$-ADH) cover a ligand to the active site zinc atom (His-67, numbering system of class I [2]), and include position 93 which lines the inner part of the substrate pocket [14]. The latter position typically has Phe in class I, but Tyr in human class II and III subunits, in which it contributes to the different substrate pockets [14]. The presence of Phe-93 in class IV (Table II) is compatible with the direct enzyme activity measurements [6]. The structure is clearly different from that of the well-established class I–III human enzymes and is distinguished from each of these to about the same degree (Table II). Combined, these results show that the class IV enzyme constitutes a unique enzyme class, with structure–function relationships interpretable in molecular terms.

### 4.2. *Class IV is a variable structure*

The new structure allows evaluation of species differences regarding the human/rat gastric enzyme. The estimate obtained from the presently known structures of the class IV enzymes shows a considerable variation, similar to that for the classical liver-type class I enzyme (Table II). Consequently, it may be concluded that the gastric enzyme is also a 'variable' enzyme. (This also appears to apply to class II, still incompletely characterized, cf. Table I.) Therefore, the 'constant' class III type [12], is even more distinctly constant within the family than previously realized.

Together, the results thus far suggest that corresponding species variations for the classes differ about 3-fold between the 'constant' class III and the 'variable' classes I and IV. This spread is remarkably constant in relation to that of other dehydrogenase families [12], and suggests that the separate classes of alcohol dehydrogenase fulfil distinct functional roles at their separate sites of expression.

### 4.3. *Physiological significance of class IV*

This new mammalian class is well separated from the other ADH classes regarding structure (Table II), tissue distribution [10,15] and to some extent also regarding kinetic properties [6,11]. Indeed, class IV alcohol dehydrogenase is peculiarly distributed in external (cornea, skin), digestive and respiratory epithelia. This is clearly different from the major, hepatic localization of class I and the ubiquitous distribution of class III. Moreover, class IV has distinct kinetic properties, medium-chain (octanol) and aromatic (*m*-nitrobenzaldehyde) compounds being the best substrates. Taken together, these properties suggest a specific physiological function for class IV. Ethanol is not a good substrate for class IV but at the high ethanol concentrations reached in the digestive tract, it plays a role in the ethanol oxidation and contributes to the first pass ethanol metabolism [15–17].

### 4.4. *Classes of alcohol dehydrogenase*

Definition of the human gastric alcohol dehydrogenase allows direct comparison with the novel liver cDNA (ADH6) recently reported [7]. It is then obvious that the actual protein now analyzed (Table I) is different from the structure deduced from the cDNA of the novel liver-expressed alcohol dehydrogenase [7]. Therefore, the present analysis of the gastric enzyme helps to define that the recent cDNA report [7] concerns yet another enzyme class and is not the human equivalence of the class IV enzyme. Hence, the two recently reported new forms ([5,6], this study) and the enzyme encoded by *ADH6* [7] are distinct entities, in contrast to expectations from studies of the expressed form from the re-

combinant novel cDNA [8]. Inter-class and intra-class differences are summarized in Table II.

At present, five separate classes are known for human alcohol dehydrogenase of the zinc-metalloenzyme type. Suggested nomenclature is alcohol dehydrogenase classes I–III (known before) [1], class IV, the gastric enzyme ($\sigma\sigma$-ADH, structurally reported in [5] and this study), and class V [7,8]. All five classes differ by roughly similar values, around 35% residue exchanges between any pair (Table II). Together with the isozymes within class I [1], this corresponds to minimally seven genes for human alcohol dehydrogenase, and together with the sorbitol dehydrogenase and $\zeta$-crystallins (the latter also present in mammals beyond the initially characterized guinea-pig form (cf. [18]) minimally nine human genes for the protein family. Combined, these data establish a considerable multiplicity and enzyme complexity for the human alcohol dehydrogenase system.

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