Volume 283, number 1, 85–88 FEBS 09694 May 1991
© 1991 Federation of European Biochemical Societies 0014-5793/91/$3.50
ADONIS 001457939100403H

Structural features of stomach aldehyde dehydrogenase distinguish dimeric aldehyde dehydrogenase as a 'variable' enzyme

'Variable' and 'constant' enzymes within the alcohol and aldehyde dehydrogenase families

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Received 8 March 1991

Stomach aldehyde dehydrogenase was structurally evaluated by analysis of peptide fragments of the human enzyme and comparisons with corresponding parts from other characterized aldehyde dehydrogenases. The results establish a large part of the structure, confirming that the stomach enzyme is identical to the inducible or tumor-derived dimeric aldehyde dehydrogenase. In addition, species variations between identical sets of different aldehyde and alcohol dehydrogenases reveal that stomach aldehyde dehydrogenase exhibits a fairly rapid rate of evolutionary changes, similar to that for the likewise 'variable' classical alcohol dehydrogenase, sorbitol dehydrogenase, and cytosolic aldehyde dehydrogenase but in contrast to the 'constant' class III alcohol dehydrogenase and mitochondrial aldehyde dehydrogenase. This establishes that rates of divergence in the aldehyde and alcohol dehydrogenases are unrelated to subunit size or quaternary structure, highlights the unique nature of class III alcohol dehydrogenase, and positions the stomach aldehyde dehydrogenase in a group with more ordinary features.

Dehydrogenase; Enzyme variability; Divergence

1. INTRODUCTION

Alcohol and aldehyde dehydrogenases both exhibit considerable multiplicity with mammalian structures known for four [1] and three [2] 'classes', respectively, and with at least three additional mammalian enzymes [3–6] clearly belonging to these two families (sorbitol dehydrogenase and ζ-crystallin in the case of the alcohol dehydrogenase family; glutamic γ-semialdehyde or 1-pyrroline-5-carboxylate dehydrogenase in the case of the aldehyde dehydrogenase family). In both cases, most studies have concerned the liver enzymes, but alcohol and aldehyde dehydrogenases have wide-spread occurrence, and the two activities also occur in stomach, although only limited amounts of the enzymes have been available for study from this source. Recently, however, the stomach alcohol dehydrogenase was established to constitute a new structural class, class IV [1], while the stomach aldehyde dehydrogenase has been concluded [7–10] to be identical to one of the already known aldehyde dehydrogenases, the form that is inducible or tumor-derived in liver [2,11] and also occurs in bladder [12]. Because of this difference between the stomach alcohol and aldehyde dehydrogenases, it is essential to characterize this aldehyde dehydrogenase further. Only a brief report [10] on limited data without further details exists. Furthermore, knowledge on the structural variation of dimeric aldehyde dehydrogenase is of special interest, since other alcohol and aldehyde dehydrogenases differ widely in being either fairly 'constant' (class III alcohol dehydrogenase and mitochondrial aldehyde dehydrogenase [13–15]) or 'variable' (class I alcohol dehydrogenase, sorbitol dehydrogenase and cytosolic aldehyde dehydrogenase [13,16]).

We therefore now characterized human stomach aldehyde dehydrogenase by analysis of an Asp-specific digest of the protein, giving close to half of the protein structure. This allows firm conclusions on the structural relationships, and shows stomach aldehyde dehydrogenase to be in the 'variable' of the two categories of alcohol and aldehyde dehydrogenases.

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2. MATERIALS AND METHODS

Human stomach aldehyde dehydrogenase was prepared as described [7,8], yielding about 3 nmol enzyme from 10 g wet weight tissue. The enzyme was reductively alkylated by [^{14}C]carboxymethylation and submitted to cleavage with Asp-N protease at a substrate/protease ratio of 1:100 in 1 M urea/0.15 M ammonium bicarbonate, pH 8, for 22 h at 37°C. The digest obtained was separated by reverse phase HPLC on Ultropac TSK ODS-120T (5 μm; Pharmacia/LKB, Bromma, Sweden) in 0.1% trifluoroacetic acid with a gradient of acetonitrile [1]. All fractions obtained were analyzed for purity by solid-phase sequencer degradation in a MilliGen Prosequencer 6600

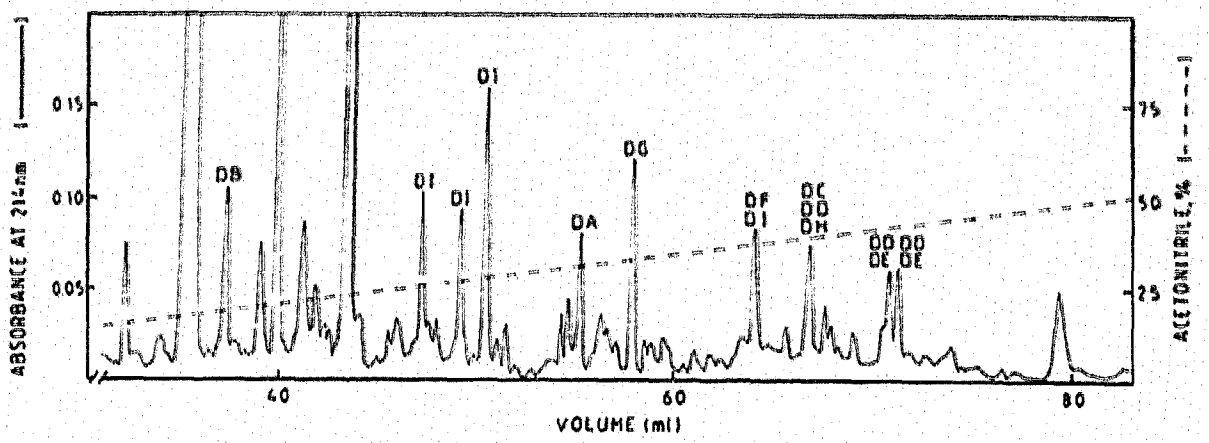

Fig. 1. Reverse phase HPLC of a digest with Asp-N protease of [^{14}C]carboxymethylated human stomach aldehyde dehydrogenase. All major fractions were analyzed for amino acid sequence. Only those labelled DA-DJ gave interpretable results. As shown, a few fractions contained more than one peptide, but the mixtures could be clearly identified by different yields of constituent peptides and by comparisons with adjacent fractions. Several peptides with identical N-terminal structures elute at more than one position, presumably because of separate secondary modifications or different C-terminal cleavages.

utilizing arylamine membranes for C-terminal coupling. Results obtained were correlated with known aldehyde dehydrogenase structures [19] and with the different evolutionary rates for mitochondrial aldehyde dehydrogenase, cytosolic class I and III alcohol dehydrogenases, and sorbitol dehydrogenase, recently established from the structures of those rat/human enzymes (cf. [13-19]).

Table I

Primary structures of ten segments of human stomach aldehyde dehydrogenase now analyzed (Hum D, for dimeric, top line in all 4-set sequences) versus structures known for dimeric rat inducible aldehyde dehydrogenase (Rat D, second line in each set), and the two tetrameric human enzymes from cytosol and mitochondria (Hum-C and Hum-M, bottom two lines in each set)

Positions refer to those known for the entire structure of the tumor-derived rat dehydrogenase [2,11] while peptide designations DA-DJ refer to the Asp-cleavage products purified as shown in Fig. 1. Data for the bottom three lines from [2,11,19]

	1 (positions 50-60)	2 (83-94)	3 (160-180)
	⊢——DA——⊣	⊢——DB——⊣	⊢————DC————⊣
Hum D	DLHKNEWNAYY	DEPVEKTPQTQQ	DLYPVIXGGLPETTELLKER--F
Rat D	DLGKNEWTSYY	DEPVAKTRQTQQ	NLYLVVKGGVPETTELLKER--F
Hum C	NGGKLYSNAYL	KIQGRTIPIDGN	GVVNIVPGYGPTAGAAISSHMDI
Hum M	DNGKPYVISYL	KYHGKTIPIDGD	GVVNIVPGFGPTAGAAIASHEDV

	4 (181-218)	5 (252-273)
	⊢————————DD————————⊣	⊢————DE————⊣
	DHILYTGSTGVGKIIMTAAAK-HLTPVTLELGGKSPXYV	DPSIQNQIVEK-LKKSLKEFYGE
	DHIMYTGSTAVGKIVMAAAAK-HLTPVTLELGGKSPCYV	DPSIQNQIVEK-LKKSLKDFYGE
	DKVAFTGSTEVGKLIKEAAGKSNLKRVTLELGGKSPCIV	EESIYDEFVRRSVERAKKYILGN
	DKVAFTGSTEIGRVIQVAAGSSNLKRVTLELGGKSPNII	QEDIYDEFVERSVARAKSRVVGN

	6 (280-308)	7 (309-321)	8 (349-369)
	⊢————————DF————————⊣	⊢——DG——⊣	⊢————DH————⊣
	DYGRIISARHFQRVMGLI-----EGQKVA-YGGTG	DAATRYIAPTILT	EAIQFINQREKPLALYMFSSN
	DYGRIINDRHFQRVKGLI-----DNQKVA-HGGTW	DQSSRYIAPTILV	EAIQFINQREKPLALYVFSNN
	TQGPQIDKEQYDKILDLIESGKKEGAKLECGGGPW	GNKGYFVQPTVFS	DVIKRANNTFYGLSAGVFT--
	EQGPQVDETQFKKILGYINTGKQEGAKLLCGGGIA	ADRGYFIQPTVFG	EVVGRANNSTYGLAAAVFT--

	9 (369-418)	10 (435-452)	
	⊢————————DI————————⊣	⊢————DJ————⊣	
	DVIVHITLHSL-PFGGVGNSGMGSYHGKKSF	EEGLKVRYPPSPAKMTQH	Hum D
	DVIVHITVPTL-PFGGVGNSGMGAYHGKKSF	EEAHKARYPPSPAKMPRH	Rat D
	WVNCYGVVSAQCPFGGFKMSGNGRELGEYGF	NS*	Hum C
	WVNCYDVFGAQSPFGGYKMSGSGRELGEYGL	NS*	Hum M

3. RESULTS

Human stomach aldehyde dehydrogenase fragments obtained by digestion of the carboxymethylated enzyme with Asp-N protease were fractionated by reverse phase HPLC. Of 17 fractions obtained (Fig. 1), 10 gave interpretable results upon solid-phase sequencer degradations, thus establishing 215 residues of the protein chain of human stomach aldehyde dehydrogenase, as shown in Table I in relation to corresponding structures for the three types of aldehyde dehydrogenase known before. Several of the cleavages generating the peptides reflect cleavages at Glu residues, which are often found when the Asp-N protease is used in the presence of urea (which was necessary here to solubilize the protein before cleavage).

A comparison of the aldehyde dehydrogenase structures show that the human stomach enzyme is identical in subunit organization at the 215 positions examined to the inducible, or tumor-derived, liver aldehyde dehydrogenase. Thus, both that enzyme and the present one (top two lines in each 4-line set of sequences; Table I) show identical gap positions versus the two types of characterized aldehyde dehydrogenase, the tetrameric cytosolic and mitrochondrial liver enzymes (bottom two lines; Table I). The stomach enzyme and the other dimeric aldehyde dehydrogenase exhibit zero gap differences (Table II), while both have 13 gap differences towards the tetrameric aldehyde dehydrogenases. Similarly, the stomach aldehyde dehydrogenase, now analyzed from human, shows only 38 residue differences towards the tumor-derived aldehyde dehydrogenase previously known from rat [2,11], and this difference (18% in 215 positions) falls exactly in one of the two groups typical of species differences of rat/human dehydrogenases (Table II).

In summary, stomach aldehyde dehydrogenase appears identical to the tumor-derived enzyme and is clearly distinguishable from the common types of normal liver aldehyde dehydrogenase. This result is in agreement with previous expectations [7–10] and considerably extends previous structural knowledge [10]. Parenthetically, it also ascribes a residue for the human stomach enzyme at three positions previously differently ascribed, thus now Glu-298, Thr-321, and Ser-298 (Table I). Finally, the results allow reasonable estimates of the species divergence for the dimeric aldehyde dehydrogenase in relation to divergences for identical species of five other enzymes within the aldehyde and alcohol dehydrogenase families, revealing that the stomach aldehyde dehydrogenase falls into the 'variable' group of alcohol and aldehyde dehydrogenases (Table III).

4. DISCUSSION

The results confirm that stomach aldehyde dehydrogenase is identical to other dimeric aldehyde dehydrogenases known, the inducible or tumor-derived enzyme, obtained from liver, bladder and other sources [2,7–11]. This is concluded from analysis of close to half the entire structure, and this type of aldehyde dehydrogenase is therefore now possible to compare for large parts of the structures from two different species, rat and human, revealing a species divergence at 18% of all positions. This divergence can be directly compared to that for other dehydrogenases of the same metabolic pathways, mitochondrial aldehyde dehydrogenase, cytosolic aldehyde dehydrogenase classical alcohol dehydrogenase, class III alcohol dehydrogenase, and polyol dehydrogenase, all also known from human and rat (cf. [13–16]).

Unexpectedly, the rate of divergence of these dehydrogenases falls into two distinct ranges (Table III). One is fairly variable, containing classical alcohol dehydrogenase, sorbitol dehydrogenase and cytosolic aldehyde dehydrogenase from before, the other is fairly constant containing class III alcohol dehydrogenase and mitochondrial aldehyde dehydrogenase. The two types differ by a factor of 3 or more. We now find that stomach aldehyde dehydrogenase falls into exactly one of these two types of divergence, i.e. the variant one (Table III), surprisingly with identical values in all cases. This suggests that the evolutionary rate is independent of substrate type (alcohols– aldehydes) quaternary structure (dimers–tetramers), and total size of the subunit (~350 to ~500 residues). The latter point is further illustrated by inclusion of glucose-6-phosphate dehydrogenase, also known from rat and human (cf. [20]), which, like aldehyde dehydrogenase has a large subunit in contrast to the alcohol and polyol dehydrogenases. The lack of correlation with size for the five aldehyde and alcohol dehydrogenases in Table III and their distinction into two classes of variation, is

Table II

Differences among aldehyde dehydrogenases (AldDH), emphasizing the presently analyzed stomach form versus the three types previously characterized [2,11,17]. Data from Table I

Human stomach AldDH versus	Differences			Total positions compared
	Gaps	Residues	Sum	
Rat tumor-derived AldDH	0	38	38	215
Human liver cytosolic AldDH	13	137	150	210
Human liver mitochondrial AldDH	13	142	155	210

Table III

Differences between identical species sets (human/rat) of dimeric aldehyde dehydrogenase, versus other aldehyde dehydrogenases and three different representatives of the alcohol dehydrogenase family (classical class I enzyme, class III enzyme, and sorbitol dehydrogenase)
AldDH, aldehyde dehydrogenase; ADH, alcohol dehydrogenase. For comparison, the identical set of glucose-6-phosphate dehydrogenase, with subunits similarly sized as those for aldehyde dehydrogenase, is also shown. Data for dimeric AldDH from Table II, for previously known structures from [2,11,13-20]

Rat/human enzyme pair	Positions with differences of total positions compared	
	'Constant' enzymes	'Variable' enzymes
Dimeric AldDH		38 of 215 (18%)
Mitochondrial AldDH	18 of 500 (4%)	
Cytosolic AldDH		86 of 500 (17%)
Class I ADH		66 of 373 (18%)
Class III ADH	21 of 374 (6%)	
Sorbitol dehydrogenase		63 of 356 (18%)
Glucose-6-phosphate dehydrogenase	33 of 513 (6%)	

a deviation from the general trend of a correlation between size and evolutionary rate [21]. This deviation establishes that structural and functional requirements influence even closely related and metabolically similar enzymes to different extents. In particular, the class III alcohol dehydrogenase is especially constant within the group (Table III) in relation to its small size, suggesting the possibility that it is influenced by strict requirements on important properties, whereas the stomach aldehyde dehydrogenase is the most variable of those with large subunits perhaps suggesting more variable functions within this metabolic pathway.

Acknowledgements: This work was supported by grants from the Swedish Medical Research Council (Project 03X-3532), the Knut and Alice Wallenberg Foundation, the National Science Council of the Republic of China, and by a visiting scientist fellowship to S.-J.Y. from Karolinska Institutet.

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