Class 2 aldehyde dehydrogenase. Characterization of the hamster enzyme, sensitive to daidzin and conserved within the family of multiple forms

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Abstract Mitochondrial (class 2) hamster aldehyde dehydrogenase has been purified and characterized. Its primary structure has been determined and correlated with the tertiary structure recently established for this class from another species. The protein is found to represent a constant class within a complex family of multiple forms. Variable segments that occur in different species correlate with non-functional segments, in the same manner as in the case of the constant class of alcohol dehydrogenases (class III type) of another protein family, but distinct from the pattern of the corresponding variable enzymes. Hence, in both these protein families, overall variability and segment architectures behave similarly, with at least one 'constant' form in each case, class III in the case of alcohol dehydrogenases, and at least class 2 in the case of aldehyde dehydrogenases.

Key words: Aldehyde dehydrogenase; Variability pattern; Evolutionary change; Residue difference; Conserved enzyme

1. Introduction

Aldehyde dehydrogenase has attracted new interest on the basis of four recent findings. One is that the tertiary structure of this group of enzymes has just been determined [1–3], revealing a new family of protein fold. Another is that this enzyme family exhibits great complexity, with at least 10 classes and genes for the human aldehyde dehydrogenase system [4]. This complexity is greater than even that of alcohol dehydrogenase [5] in the same metabolic chain. A third is that these classes differ considerably in evolutionary speed, like the alcohol dehydrogenase classes do, and exhibit at least a 3-fold difference in accumulation of mutational residue exchanges [6]. Finally, aldehyde dehydrogenases are of interest in relation to ethanol consumption and to the action of an antidipsotropic agent [7], especially regarding the mitochondrial [8] and cytosolic enzyme forms.

For all these reasons, the structures of additional forms of aldehyde dehydrogenase are of interest, especially those from hamster, since this animal can exhibit preference for and consume large quantities of ethanol [9,10], which has been used as an animal model of the antidipsotropic agent daidzin. Further, the relative importance of the mitochondrial/cytosolic aldehyde dehydrogenases in hamster appears to differ from that of those in the human [11]. We have therefore now determined the primary structure of hamster liver mitochondrial aldehyde dehydrogenase and correlate it with the enzyme properties.

2. Materials and methods

The protein, purified as described [11], was dissolved in 6 M guanidinium chloride, 0.4 M Tris, pH 8.15, 2 mM EDTA, and carboxymethylated by treatment with neutralized iodo[2-^{14}C]acetate after reduction with dithiothreitol [12]. The carboxymethylated protein was cleaved in separate batches with Lys-C, Glu-C, and Asp-N specific proteases, trypsin, and chymotrypsin at protease:substrate ratios of 1:10–1:150 for 4–20 h at 37°C in 0.1 M ammonium bicarbonate, pH 8.1, with up to 2.2 M urea for solubilization. Peptides obtained were separated by reverse phase HPLC on C_4 or C_{18} columns using linear gradients of acetonitrile in 0.1% aqueous trifluoroacetic acid.

Total compositions were determined by amino acid analysis (Pharmacia Alpha Plus analyzer) after hydrolysis for 24 h at 110°C with 6 M HCl/0.5% phenol. Sequence degradations were performed on Applied Biosystems 470A/477A sequencers or on MilliGen Prosequencers 6600/6625. Peptide molecular masses were determined by MALDI-TOF mass spectrometry using a Lasermat 2000 instrument (Thermo Bioanalysis).

Image construction of the tertiary structure of the bovine enzyme [2] was performed with the program MolScript [13] using the coordinates supplied by Dr. Thomas Hurley, Department of Biochemistry and Molecular Biology, Indiana University, Indianapolis, IN.

3. Results

The major mitochondrial form of aldehyde dehydrogenase from hamster liver (the class 2 aldehyde dehydrogenase) was purified by a single step affinity chromatography on α-cyano cinnamate-Sepharose 6B after preparation of mitochondria by differential centrifugation [11]. Starting with 59 g liver, 62 mg pure protein, at a specific activity of 4.5 μmol/min/mg enzyme with acetaldehyde as substrate, was obtained after a 160-fold purification. Enzymatic data (K_m 200 nM, k_{cat} 170/min at pH 7.5 and 960/min at pH 9.5) identified the protein as a class 2 liver aldehyde dehydrogenase, and SDS-polyacrylamide gel electrophoresis and gel filtration analysis showed that this enzyme preparation is tetrameric with an apparent molecular weight of ~230 kDa [11].

After enzymatic analysis, the protein was carboxymethylated and digested in separate batches with five different proteolytic enzymes. All digests were fractionated by reverse phase HPLC, and pure peptides were submitted to sequence analysis. In this manner sequence information was obtained from all regions of the subunit by analysis of 111 overlapping fragments. The structure obtained shows a 500-residue subunit (Fig. 1), with a free N-terminus accessible to direct sequence analysis, like the corresponding human enzyme [14]. In

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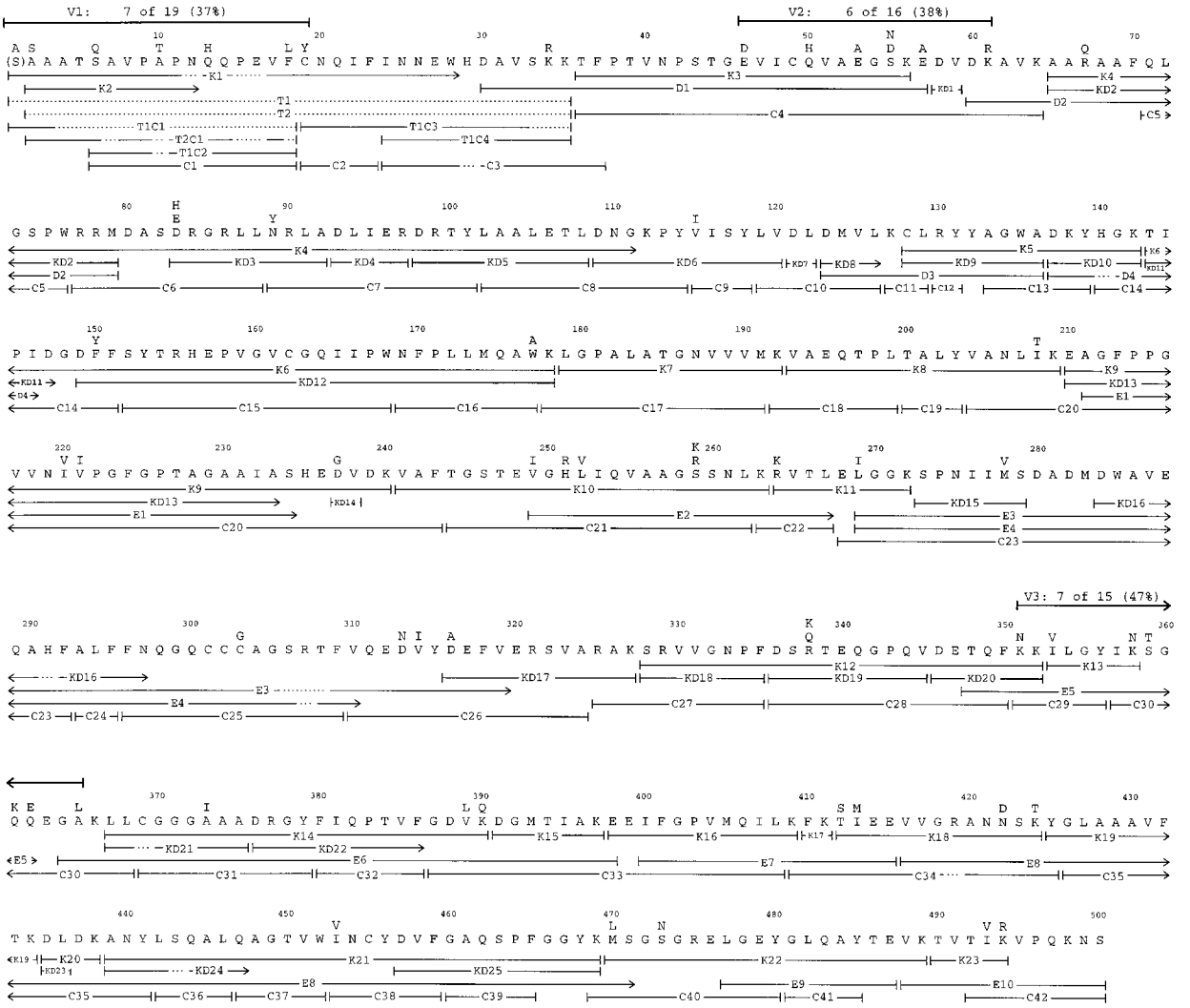

Fig. 1. Primary structure of hamster liver mitochondrial aldehyde dehydrogenase (continuous line), with peptides analyzed to determine the structure (below) and variable positions in other mammalian aldehyde dehydrogenases (above). In the peptides below the continuous structure, the letters denote the type of proteolytic digest from which the peptides originate (C for chymotrypsin, D for Asp-protease, E for Glu-protease, K for Lys-protease, T for trypsin, and more than one letter for successive proteolytic treatments); solid lines show parts analyzed, dotted lines remaining parts. In the designations above the continuous line, all positions which have alternative residues in any of six now known mammalian class 2 aldehyde dehydrogenases (hamster, human, horse, bovine, rat, mouse) are indicated by a letter to show the alternative residue present in at least one other form. Thus, positions without top letters are strictly conserved in all six protein forms of this enzyme. Three regions exhibit the highest variability (V1, V2 and V3), with variable positions of total positions indicated by the numbers and percentage values shown.

addition to the full-size form starting with Ser-1, a truncated form starting with Ala-2 was detectable by direct sequence analysis. The C-terminus was proven to be Ser-500 by identical ends at this residue in peptides from both the digest with Glu-C protease and the one with chymotrypsin. The deduced structure fits excellently with the total composition by hydrolysis.

The obtained structure is of interest for three types of correlation. One is with the tertiary structure thaat has recently become available for the same enzyme from bovine origin [2]. These proteins are closely related (94% residue identities), making functional assignments of residues in the hamster form justifiable and effects of residue exchanges interpretable (below).

The second type of correlation reveals that the hamster form of this protein is about equidistantly related to each of the five mammalian corresponding forms of this enzyme isolated before (94–99% residue identities). Overall, 55 residues (shown in Fig. 1) differ between the hamster and the other forms. Of these, only one residue, a Ser (at position 55), is unique to the hamster protein (all the previously characterized aldehyde dehydrogenase class 2 forms have Asp-55 or Asn-55). This residue constitutes the first residue in the αA helix and occupies a superficial position far from the active site, as indicated in Fig. 2 (constructed from the coordinates of the bovine enzyme [2] using the MolScript program [13]), explaining why the position can vary without functional effects. Of the active site residues, in particular residues 249 and 252 are of interest. They participate in coenzyme binding and differ from the variants of the human protein. However, they represent a set of compensated exchanges and therefore the volume relationship is conserved. Thus,

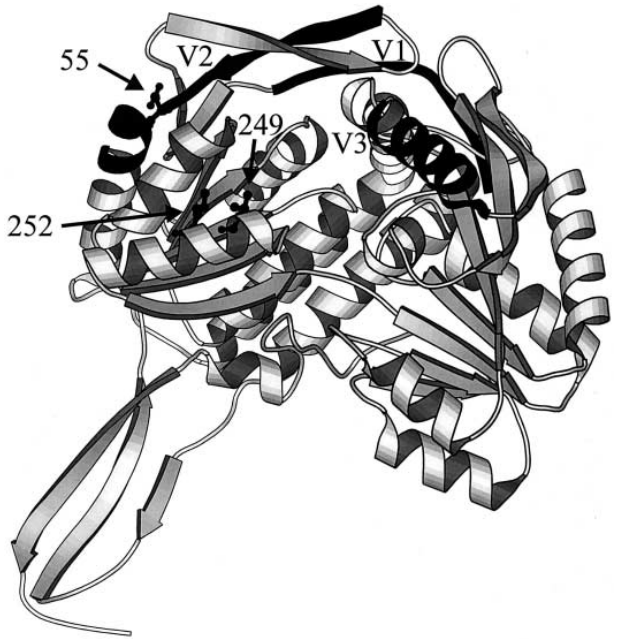

Fig. 2. Spatial positions of the three variable segments in class 2 aldehyde dehydrogenase (black; V1, V2, V3, corresponding to the top indications in Fig. 1), the residue at position 55, thus far unique in this class (arrow, Ser in the present enzyme, Asp in the model pictured), and the compensated active site changes at positions 249 and 252 (arrows), all shown in the schematic representation of the fold of class 2 aldehyde dehydrogenase. Coordinates from crystallographic data for the bovine enzyme in [2] were used for the image construction with the program MolScript [13].

the Ile,Val pair of the human enzyme (at positions 249,252) is found to be Val,Leu in the hamster protein, thus keeping the masses constant in spite of residue differences. The volume conservation in the variable parts of the active site probably explains why the enzymatic properties are largely unaltered, with a k_{cat} of ~1000/min at pH 9.5 in both cases [11].

Finally, the overall architecture of the species variability is of interest. That type of variability in the case of alcohol dehydrogenase, has established distinct functions to the separately variable protein segments [15]. We now find that only three short segments in mammalian class 2 aldehyde dehydrogenases are distinctly variable (Fig. 1): the segments at positions 1–19 (37% positions with non-conserved residues in all six species now known), positions 46–61 (38% non-conserved residues) and 351–365 (47% non-conserved residues). These segments (~40% average non-conservation) are about 5-fold more variable than the remaining structure (8% non-conservation). In the tertiary structure of aldehyde dehydrogenase [2], the three segments (V1–V3 in Figs. 1 and 2) occupy an N-terminal segment (V1) with little definition in the tertiary structure analyzed, one β/α transition (V2, from close to the start of β4 until inside αA), and much of one helix (V3, αJ until close to β14), as shown in Fig. 2. All are superficial and compatible with few functional consequences, in a manner typical of the variable segments of a constant protein as also seen for class III alcohol dehydrogenase with its variable segments in non-functional regions, as opposed to the class I alcohol dehydrogenase, with rapid evolution and variable segments in functional regions [15].

4. Discussion

The present structure characterizes a mitochondrial form of aldehyde dehydrogenase, and shows this protein to be overall strictly conserved in both primary (94–99% residue identities) and tertiary (Fig. 2) structures. Much of the variability that occurs is limited to essentially three regions, highlighted in Fig. 2. They are at non-functional, superficial segments of the subunit, defining the variability pattern to be like that in proteins in general, rather than the variability pattern in functional segments seen in class I alcohol dehydrogenase [15]. Hence, the mitochondrial aldehyde dehydrogenase is concluded to have a well-defined function and to be strictly conserved in mammals.

A few residues at the active site also vary but do so in a manner compensating the effects of the replacements. Similarly, the one unique residue (Ser-55) in the aldehyde dehydrogenase family is also found to occupy a non-functional position in the tertiary structure, explaining the absence of effects on the functional properties.

In conclusion, mitochondrial aldehyde dehydrogenase is a constant enzyme with a variability architecture resembling that of the constant alcohol dehydrogenase of another family (MDR, for medium-chain dehydrogenases/reductases) which is also highly multiple. The overall properties in these two different proteins resemble each other in correlation between the *extent* of overall species variability and the position of variable *sites* in the tertiary structures. Apparently, **little** overall variability is associated with variable segments in **non-functional** parts (for alcohol dehydrogenase class III and aldehyde dehydrogenase class 2). This is the pattern typical of the conserved enzymes that keep the basic function in both families, and is in contrast to the variable enzymes (for alcohol dehydrogenase classes I and II, with **more** overall variations and sites at **functional** segments).

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