HOMOLOGY OF THE PRIMARY STRUCTURES OF CYTOPLASMIC AND MITOCHONDRIAL ASPARTATE AMINOTRANSFERASES FROM PIG HEART

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1. Introduction

Aspartate aminotransferase (AAT) is one of a small group of enzymes which exist in different molecular forms in the cytoplasm and in the mitochondria of the same cell. The isozymes of AAT differ in electrophoretic and immunological properties [1] and in amino acid composition [2]; they are, however, of similar molecular weight [2]. It is a matter of considerable interest whether the two forms of the enzyme are structurally related and arose by divergence from a common ancestral protein or represent a case of convergent evolution of function in originally unrelated proteins. A decision between these two possibilities has not previously been made for any pair of cytoplasmic and mitochondrial isozymes.

We have recently reported the complete primary structure of cytoplasmic AAT from pig heart [3]; similar results were obtained by Ovchinnikov et al. [4]. To establish the structural and evolutionary relationships between the cytoplasmic and mitochondrial isozymes, we are now carrying out studies of the amino acid sequence of the mitochondrial form. Results already obtained show clear homologies between the two structures.

2. Materials and methods

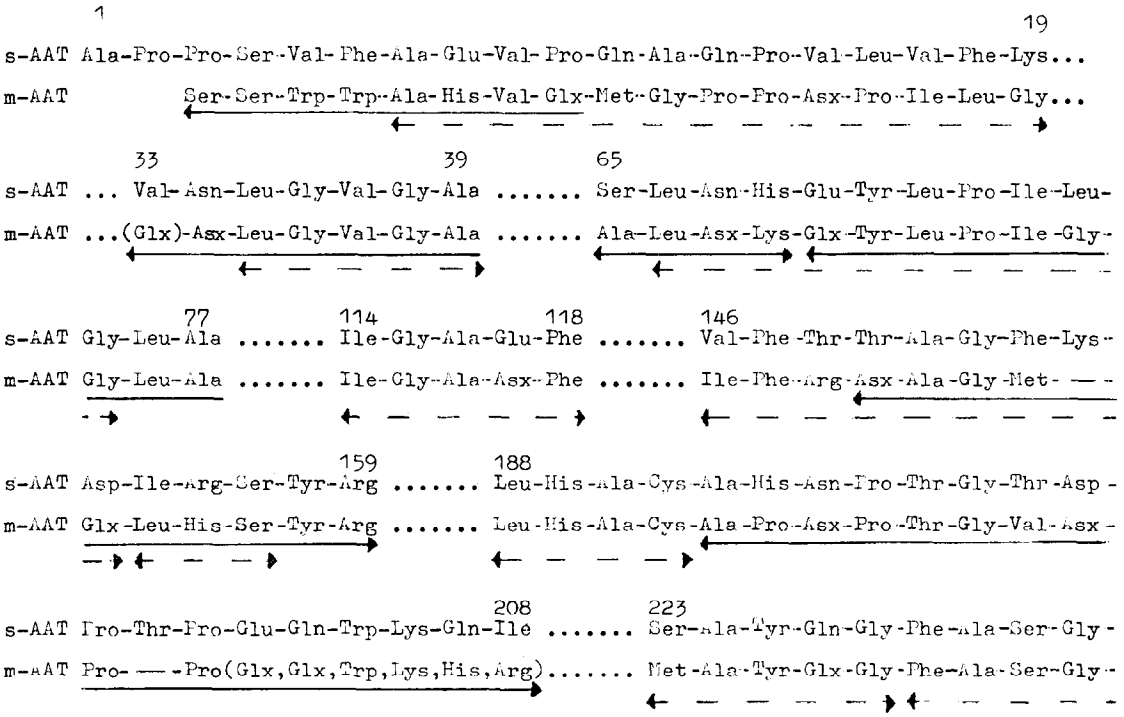
Mitochondrial AAT was purified by a new method which does not involve isolation of intact mitochondria and is, therefore, appropriate for large scale work [5]; the material was homogeneous to starch gel electrophoresis and had an amino acid composition similar to that previously reported [2]. A sample of the protein was amino-ethylated and digested with trypsin and a second sample was reduced with sodium borohydride, carboxymethylated and digested with thermolysin; the methods have been described previously [6,7]. Peptides were mainly isolated by gel filtration through Sephadex followed by chromatography and electrophoresis on paper [6,7]; some of the thermolytic peptides were purified by ion exchange chromatography using SP-Sephadex C-25. Sequences of peptides were determined using the dansyl-Edman method as described by Hartley [8]. In most cases, sequences were confirmed by quantitative amino acid analysis using either a Locarte or a Biocal BC200 automatic analyzer after hydrolysis of the peptide as described by Sanger and Thompson [9]. A sample of maleylated AAT was digested with chymotrypsin and the N-terminal peptide isolated from the digest as described by Bruton and Hartley [10].

3. Results and discussion

The results so far obtained by digestion of mitochondrial AAT with trypsin and with thermolysin have allowed identification of 303 individual amino acid residues (approx. 70% of the molecule) in peptides from 3 to 17 residues long. Of these 303 residues, 161 are in peptides which show clear homologies with sequences of the cytoplasmic enzyme. These peptides, and the corresponding regions of the structure of cytoplasmic AAT are shown in fig. 1. Identical amino acids occur in 100 positions if it is assumed that residues provisionally assigned as 'asx' and 'glx' in the mitochondrial enzyme have the same states of amidation as corresponding residues in the cytoplasmic form. Most of the substitutions in the remaining 61 residues are conservative in nature, but changes leading to alterations in charge occur for example at positions, 8, 19, 91, 148, 153, 193, 242 and 383 (the numbering given in fig. 1 refers to the cytoplasmic enzyme but will be taken to apply also to the mitochondrial form). None of the other peptides which we have isolated show sufficient similarities with sequences in the cytoplasmic enzyme to be aligned with confidence at the present time.

Some of the results in fig. 1 require specific comment. The residue at position 33 is shown in brackets since some doubt remains about its identity. The tryptic peptides beginning at residues 3, 33, 69 and 192 could not be sequenced completely; the C-terminal part of the last of these peptides was obtained from its amino acid composition and the first four unsequenced residues have been placed so as to give maximum homology. Gaps have been left in the peptides from mitochondrial AAT at positions 153 and 201; this is to increase homology and does not signify unidentified residues. Similarly, a gap has been left in the sequence of the cytoplasmic enzyme between residues 343 and 344.

The homology of the two sequences from positions 242 to 275 is of particular interest since these sequences contain the active site lysine residues (258) [11]. The active site peptide from the mitochondrial enzyme was previously reported to be Ala–Lys–Asn–Met [11]. In support of this, the thermolytic peptide with the sequence Tyr–Ala–Lys–Asx–Met–Gly ob-



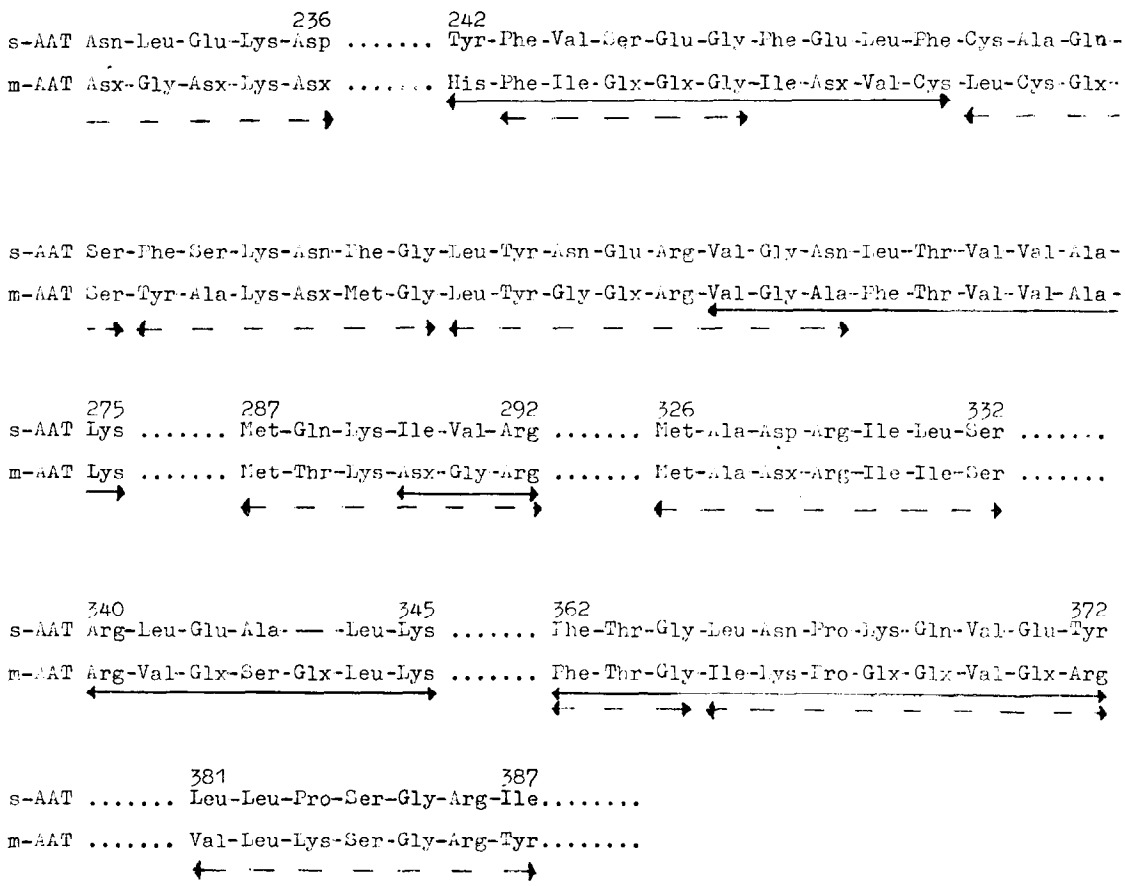


Fig. 1. Comparison of parts of the primary structures of cytoplasmic (s-AAT) and mitochondrial (m-AAT) aspartate aminotransferases. The numbering refers to the primary structure of the cytoplasmic enzyme [3]. Gaps have been left in peptides from m-AAT at positions corresponding to residues 153 and 201 and between residues 343 and 344 of s-AAT in order to increase homology. The notations (⟷) and (← – →) refer to peptides obtained by digestion of m-AAT with trypsin and thermolysin respectively.

tained in the present work was found to contain the pyridoxal phosphate residue covalently bound by reduction with sodium borohydride. The results in fig. 1 extend the known active site sequence to 34 residues.

A peptide was isolated from the N-terminus of the mitochondrial enzyme and shown to have the sequence Ser–Ser–Trp. Overlapping with other peptides extended the N-terminal sequence to that shown in fig. 1. These results confirm the previously reported nine-residue N-terminal sequence [12]. It is of interest that the degree of homology at the N-terminus is much lower than in other regions of the structure; the matching of these parts of the two sequences must be considered tentative. So far, no homologous sequences have been found at the C-terminus; the last pair of sequences given in fig. 1 (positions 381–387) terminate 25 residues from the C-terminus of the cytoplasmic enzyme.

Before the present work, knowledge of the primary structure of mitochondrial AAT was limited to the active site tetrapeptide [11], the N-terminal nonapeptide [12] and a peptide with the sequence Ala–Asp–Arg–Ile–Ile–Ser–Hse which was obtained by cleavage of the enzyme with cyanogen bromide [13]. These data were insufficient to allow any conclusions

to be drawn about the structural relationships between the isozymes. It is now clear from the results in fig. 1 that considerable similarity does exist between cytoplasmic and mitochondrial aspartate aminotransferases, although the full extent of the homology will only become apparent when structural studies are complete. Ultimately, analysis of the homologies between these and other pairs of cytoplasmic and mitochondrial isozymes may yield information on the evolutionary origins of mitochondria.

Acknowledgement

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