THE STRUCTURE OF MITOCHRONDRIAL ASPARTATE AMINOTRANSFERASE FROM PIG HEART AND COMPARISON WITH THAT OF THE CYTOPLASMIC ISOZYME

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1. Introduction

We have previously reported that the aspartate aminotransferases (EC 2.6.1.1) from pig heart cytoplasm and mitochondria are homologous proteins [1]. This conclusion was based on limited and fragmentary sequence data but similar conclusions have been reached independently [2,3]. We present here a structure for the mitochondrial aspartate aminotransferase, complete apart from the assignment of some acid and amide side chains, and compare this structure with that of the cytoplasmic isozyme [4,5]. It seems to be of interest to report the structure in its present form because there is still no other case where the structural relationships between a pair of cytoplasmic and mitochondrial isozymes have been established. In addition, work is now in progress on the crystal structure of the mitochondrial aspartate aminotransferase from chicken heart [6]. Published data on the primary structure of the chicken heart enzyme are limited to an N-terminal sequence of 40 residues [7]. In this region, the sequences of the pig and chicken heart enzymes are very similar; indeed, it has been claimed on the basis of immunochemical studies that the sequence identity of the two enzymes may be as high as 80% [8]. Hence it seems likely that the results presented here will be of assistance both in the primary structure analysis and crystallographic analysis of the chicken heart enzyme.

2. Materials and methods

Mitochondrial aspartate aminotransferase was purified by the method previously described [9]. Digestions of chemically modified protein were carried out as follows:

(i) The aminoethylated protein was digested both with trypsin and with the lysine specific protease from *Armillaria mellea.*

(ii) The sodium borohydride-reduced and carboxymethylated protein (iodo-[^{14}C]acetic acid) was digested with chymotrypsin, with thermolysin and with pepsin.

(iii) The protein with lysine residues trifluoroacetylated and cysteine residues aminoethylated was digested with the *A. mellea* protease.

Details of the digestion procedures and the methods used for subsequent isolation of peptides have been described previously [4,10–12]. Sequence analysis was carried out by using the dansyl Edman method as described by Hartley [13]. C-terminal sequences were frequently determined or confirmed by digestion with carboxypeptidases. Some large fragments were subdigested with appropriate proteases and the product peptides isolated and sequenced. Amino acid sequences were usually supported by quantitative amino acid analysis; in later stages of the work sequences of peptides derived from well known regions of the structure could be deduced from amino acid compositions alone. Assignments of side-chain amide groups were made

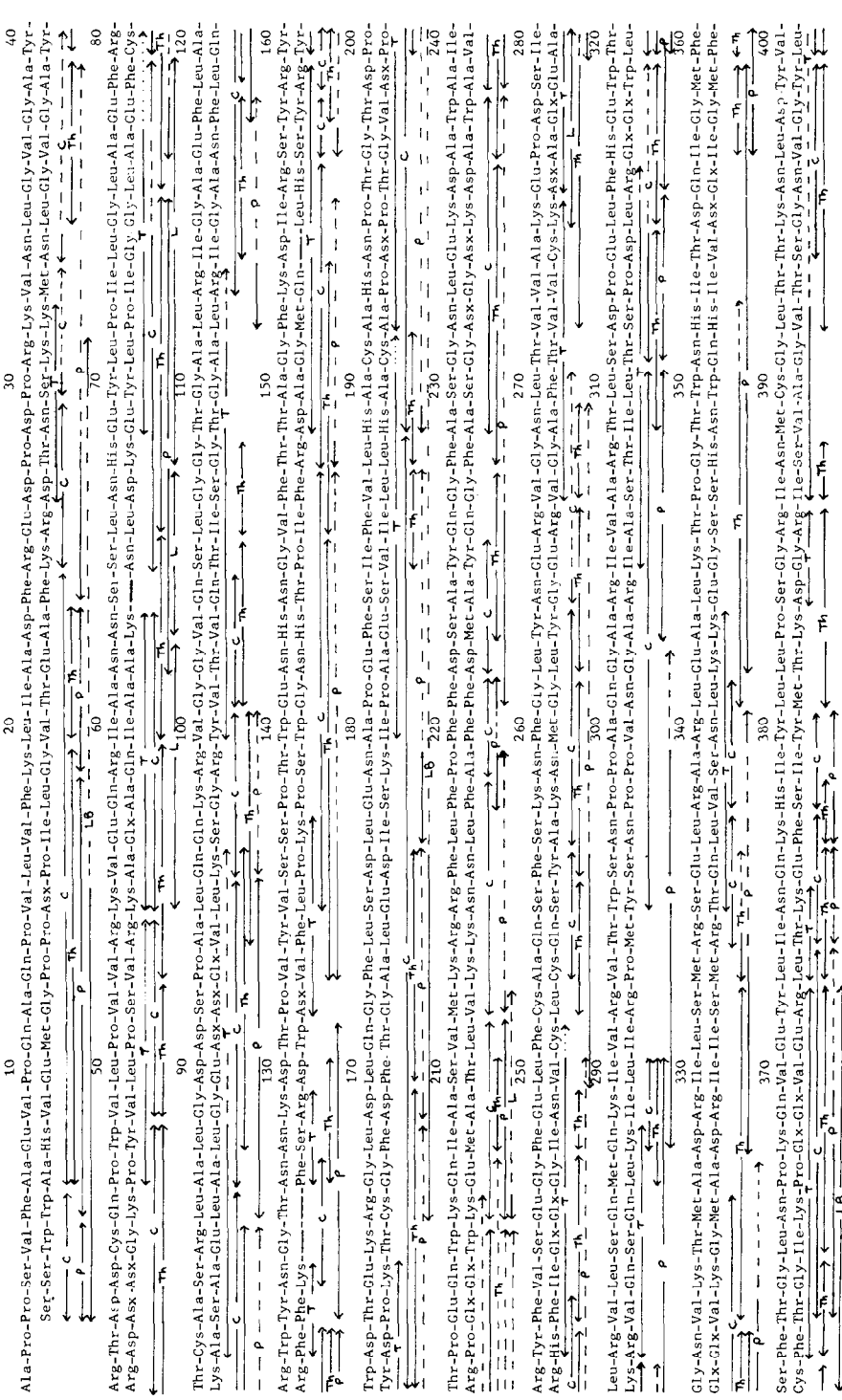

Fig.1. Comparison of the primary structures of cytoplasmic and mitochondrial aspartate aminotransferases. The structure of the mitochondrial isozyme has been aligned with that of the cytoplasmic form so as to provide maximum homology. The peptides used to assemble the structure of the mitochondrial form are indicated by arrows. The symbols T, C, P, Th and L denote peptides derived by digestions of suitably modified protein with trypsin, chymotrypsin, pepsin, thermolysin and *A. mellea* protease respectively; LB denotes peptides from digestion with *A. mellea* protease of the trifluoroacetylated and aminoethylated protein. Full lines denote sequences established directly (see text); dashed lines denote sequences inferred from amino acid compositions and comparison with previously known sequences.

either by amino acid analysis after digestion of peptides with exopeptidases or by measurements of mobility on electrophoresis at pH 6.5 [14].

3. Results and discussion

Figure 1 compares the amino acid sequence of cytoplasmic aspartate aminotransferase from pig heart [4] with that of the mitochondrial isozyme. The major part of the latter structure was determined from peptides obtained by digestion with trypsin, chymotrypsin, thermolysin and pepsin. In some of these digests, particularly that with pepsin, several peptides were found originating from the same regions of the structure due to partial cleavage of susceptible bonds. Generally only one peptide from such regions is shown in fig.1. Recently we have attempted more restricted digestion procedures using the *A. mellea* protease and cyanogen bromide. Results from this work are still incomplete but those that provide essential information have been included here.

There are some points of uncertainty in the structure given and these require comment. In what follows, the numbering system used is that of the cytoplasmic isozyme. The overlap at positions 122–123 cannot by itself be considered sufficient to locate the small tryptic peptide Phe–Phe–Lys with certainty. However, this peptide was also found in a subdigest with trypsin of a cyanogen bromide fragment originating from asparagine-34 to methionine-152; combined with the other evidence in fig.1 this provides strong confirmation of the sequence given. The tryptic peptide at positions 132–137 was assumed to have arisen from a chymotryptic type cleavage after tryptophan but a formal overlap between residues 131 and 132 is lacking; we have no evidence for the existence of a further basic residue in this region.

The two other overlaps that are lacking are those between residues 380–381 and 386–387; the positioning of the peptide covering residues 381–386 has, therefore, been done solely on the basis of homology. Given that the rest of the structure is established, this positioning seems entirely reasonable.

Within the limits of the uncertainties discussed above, the polypeptide chain of the mitochondrial isozyme seems to contain 403 amino acid residues, that is, 9 residues less than in the cytoplasmic form. The native enzyme is a dimer of these chains [9]. For maximum homology deletions of two residues at both the N-terminal and the C-terminal ends of the mitochondrial isozyme must be assumed. Other deletions are then at or around positions 64, 125, 126, 154 and 407. The degree of homology between the two isozymes cannot be finally assesed due to incomplete assignment of amide side chains. If the assumption is made that the state of amidation is the same as that in the cytoplasmic isozyme where Asx is coincident with Asp or Asn (and similarly with Glx) then 194 residues occur in identical positions in the two isozymes (i.e. approx. 48% identity). This is clearly, however, an upper limit since 26 acid/amide side chains remain unassigned.

The homology between the two structures varies greatly in different regions. For example, it is very low at the N-terminus (5 identities in the first 30 residues), but this is followed by a region of high similarity (residues 32–41). This latter region contains a tyrosine residue (40) that is thought to be near the active site in the cytoplasmic isozyme if not directly involved in the enzyme action [15]. Similarly, the cofactor binding sites (lysine-258 in both cases [1,3,16]) are in a region of high similarity. It will be of interest to examine the roles of other highly conserved regions when the three dimensional structure of the homologous chicken heart mitochondrial isozyme is available.

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References

[1] Doonan, S., Hughes, G. J., Barra, D., Bossa, F., Martini, F. and Petruzzelli, R. (1974) FEBS Lett. 49, 25–28.
[2] Kagamiyama, H. and Wada, H. (1975) Biochem. Biophys. Res. Commun. 62, 425–430.
[3] Kagamiyama, H., Teranishi, K. and Wada, H. (1975) Biochem. Biophys. Res. Commun. 63, 993–999.

[4] Doonan, S., Doonan, H. J., Hanford, R., Vernon, C. A., Walker, J. M., Airoldi, L. P. da S., Bossa, F., Barra, D., Carloni, M., Fasella, P. and Riva, F. (1975) Biochem. J. 149, 497–506.
[5] Ovchinnikov, Yu. A., Egorov, C. A., Aldanova, N. A., Feigina, M. Yu., Lipkin, V. M., Abdulaev, N. G., Grishin, E. V., Kiselev, A. P., Modyanov, N. N., Braunstein, A. E., Polyanovsky, O. L. and Nosikov, V. V. (1973) FEBS Lett. 29, 31–34.
[6] Gehring, H., Christen, P., Eichele, G., Glor, M., Jansonius, J. N., Reimer, A.-S., Smit, J. D. G. and Thaller, C. (1977) J. Mol. Biol. in press.
[7] Gehring, H., Wilson, K. J. and Christen, P. (1975) Biochem. Biophys. Res. Commun. 67, 73–78.
[8] Sonderegger, P., Gehring, H. and Christen, P. (1977) J. Biol. Chem. 252, 609–612.
[9] Barra, D., Bossa, F., Doonan, S., Fahmy, H. M. A., Martini, F. and Hughes, G. J. (1976) Europ. J. Biochem. 64, 519–526.
[10] Doonan, S., Doonan, H. J., Riva, F., Vernon, C. A., Walker, J. M., Bossa, F., Barra, D., Carloni, M. and Fasella, P. (1972) Biochem. J. 130, 443–452.
[11] Bossa, F., Barra, D., Carloni, M., Fasella, P., Riva, F., Doonan, S., Doonan, H. J., Hanford, R., Vernon, C. A. and Walker, J. M. (1973) Biochem. J. 133, 805–819.
[12] Doonan, S. and Fahmy, H. M. A. (1975) Eur. J. Biochem. 56, 421–426.
[13] Hartley, B. S. (1970) Biochem. J. 119, 805–822.
[14] Offord, R. E. (1966) Nature 211, 591–593.
[15] Polyanovsky, O. L., Demidkina, T. V. and Egorov, C. A. (1972) FEBS Lett. 23, 262–264.
[16] Morino, Y. and Watanabe, T. (1969) Biochemistry 8, 3412–3417.