PRIMARY STRUCTURE OF CYTOPLASMIC ASPARTATE AMINOTRANSFERASE FROM CHICKEN HEART AND ITS HOMOLOGY WITH PIG HEART ISOENZYMES

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1. Introduction

Aspartate aminotransferase (L-aspartate:2-oxoglutarate aminotransferase EC 2.6.1.1) is an enzyme of key importance in the conjugated system of amino acid and energy metabolism. AAT in animal cells occurs in two forms, cytoplasmic and mitochondrial, which though homologous differ significantly in structural and functional characteristics [1].

Valuable information available on the catalytic mechanism and the topography of the active center of AAT [2] makes the enzyme an extremely attractive model for studying the interrelationship between structure and function as well as their changes in the course of evolution.

Recently, the primary structures of c- and m-AAT from pig heart have been established [3–6]; the amino acid sequence of chicken m-AAT is being investigated [7]; several X-ray projects are under way [8–10]. Here we report the complete primary structure of c-AAT from chicken heart.

2. Materials and methods

Chicken heart c-AAT was prepared according to [11]. Digestions of carboxymethylated protein were performed in 0.2 M NH_4HCO_3 for trypsin, 0.1 M potassium phosphate (pH 8.0) for staphylococcal protease and in 70% formic acid for cyanogen bromide.

Abbreviations: ATT, aspartate aminotransferase; c-AAT and m-AAT, cytoplasmic and mitochondrial aspartate aminotransferases, respectively.

Hydroxylamine cleavage was performed as in [12]. Peptides were separated according to the following general scheme: preliminary fractionation on a Sephadex column, then, for smaller peptides, chromatography on Chromobeads P cation-exchanger combined with paper chromatography and electrophoresis, the principal technique for separation of larger (20–40 residues) peptides was preparative polyacrylamide gel electrophoresis. The structures of individual fragments were established by the dansyl-Edman method [13] and also by digestions with appropriate exopeptidases. Full experimental detail of this work will be reported elsewhere.

3. Results and discussion

The immunological studies have indicated that the similarity of pig and chicken c-AAT is only of limited character [14,15]. Since the chicken enzyme has no free α-amino group, the sequenator analysis could not be used to estimate the degree of homology of the two proteins. Therefore, the initial step in the structural study of chicken c-AAT consisted in the analysis of a relatively small number of low molecular weight peptides from a limited tryptic hydrolysate of citraconylated c-AAT.

The homology with the pig isoenzyme proved to be high enough to locate unambiguously all 17 arginine-containing peptides in the polypeptide chain. The average sequence identity estimated from the comparison of structures of these peptides with the corresponding regions of pig AAT approximates 80%. At the same time, selective isolation and analysis of

the N-terminal chymotryptic peptide indicated that considerable differences exist between certain loci of the two proteins. It is noteworthy that chicken c-AAT is the only AAT studied which has an acylated N-terminus [3–7,16]. The high level of similarity of chicken and pig c-AAT suggested that the general strategy for the elucidation of complete amino acid sequence of chicken c-AAT could be based on the cleavage techniques leading to the medium-to-small size fragments. According to that notion, tryptic and staphylococcal protease digestions were chosen as basic in our study. Chicken c-AAT was hydrolysed with staphylococcal protease under the conditions providing for the most complete cleavage of all susceptible bonds. 32 individual peptides isolated from that digest accounted for 79% of the total sequence. The analysis of 46 individual peptides recovered from the tryptic digest of chicken c-AAT provided most of the complementary information needed to reconstruct the structure of the protein. However, for two relatively short regions (129–141 and 214–222)* only poor or no evidence could be found in the study of the two hydrolysates. The structure of the latter was determined directly in the study of N-terminal sequence of the cyanogen bromide fragment CB-II (213–287). Cleavage of the 146-residue N-terminal cyanogen bromide peptide CB-I with hydroxylamine followed by isolation and analysis of CB-I–H-1 (125–148) fragment provided the information which permitted completion of the amino acid sequence of c-AAT from chicken heart. Some other cyanogen bromide peptides were also partially analysed in order to establish formal overlaps for the corresponding regions of the molecule. The resulting structure (fig.1) of chicken c-AAT is 410 residues long, i.e., two residues shorter than that of the pig isoenzyme. One residue is missing at the N-terminus and a single deletion is observed at position 120.

The extent of sequence identity of chicken and pig isoenzymes of c-AAT assessed from our results is 83%, i.e., considerably higher than that estimated in [14,15] and very close to that reported for the mitochondrial pair of isoenzymes from the same sources [7].

* The numbering system used here and below is that of pig c-AAT. The structures of chicken c-AAT and pig m-AAT are aligned with the structure of pig c-AAT as shown in fig.1 and according to [5]

The degree of homology of the two c-AAT varies greatly in different parts of the molecule. The most conspicuous region of high homology is 54 residue long fragment 217–270 which includes only one chemically conservative substitution at position 240. It is noticeable that the functionally important residue Lys^{258} which forms the aldimine bond with the cofactor [2] is located within this region. Similarly, Tyr^{40} and Cys^{390} residues supposed to be near to or in the active center of AAT [2] are also in the regions of high homology (31–43 and 384–412, respectively). Other regions of the most pronounced similarity of the two c-AAT are 46–58, 65–76, 106–116, 188–202 and 346–372.

Further comparison shows that sequence identity of pig c-AAT and its mitochondrial isoenzyme in these regions is also higher than average (48%) though the extent of correlation is quite variable (31–43, 70%; 46–58, 54%; 65–76, 67%; 106–116, 81%; 188–202, 87%; 217–270, 63%). The markedly high similarity of the three evolutionally distant AAT in segments 106–116 and 188–202 is worth further comment. Prediction of the secondary structure of AAT [17] indicates that these segments have a common tendency for β-turn formation, thus conflicting with the general rule that β-turn is the type of secondary structure where the highest percentage of amino acid replacements is observed [18]. Hence, it seems reasonable to suppose that conservation of these sequences is determined by their functional role, rather than being 'architectural'. Also, according to the modern concepts, the active center of AAT in addition to the previously mentioned Lys^{258} contains histidine and arginine residues which are not identified in the sequence [19]. In view of the above speculations, Arg^{112} as well as His^{189} and His^{193} residues may be considered as prime candidates for this role.

The primary structures of both c-AATs contain a region of evident inner homology composed of two segments 285–298 and 300–313 (table 1). No such inner homology is observed in m-AAT; however, considerable similarity between the 285–313 region of c-AAT as a whole and a corresponding region of m-AAT implies their common ancestry, i.e, the relevant duplication of a gene fragment took place before the evolutionary divergence of c- and m-AAT. Thus, it seems possible to deduce that the overall evolutionary pathway of the region in question is

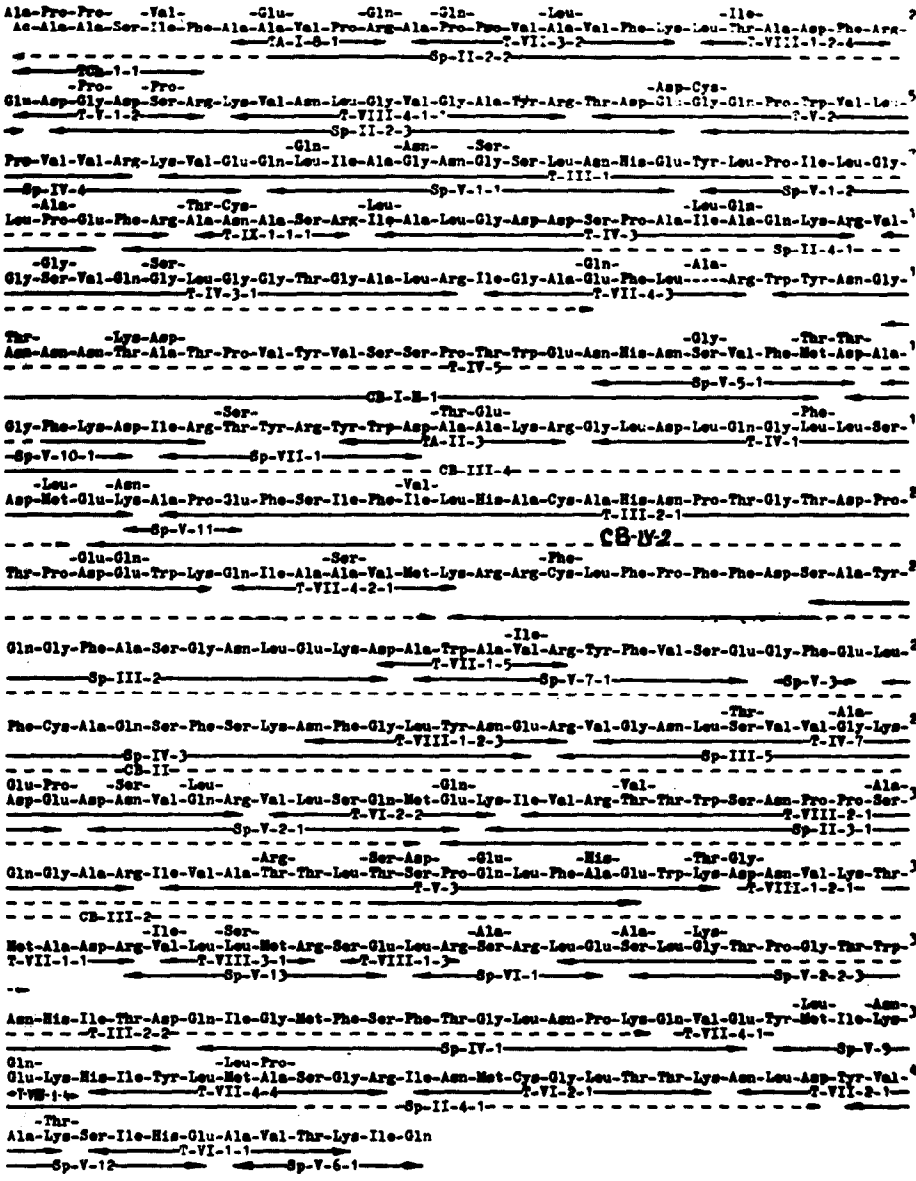

Fig.1. Comparison of the primary structures of c-AAT from chicken and pig heart. The continuous sequence is that of chicken c-AAT. Residues differing in the pig enzyme are shown above the corresponding positions. The residues are numbered according to the structure of pig c-AAT. The peptides used to assemble the structure of chicken c-AAT are indicated by arrows. Symbols T, TA, Sp, CB, H, Ch denote fragments obtained by treatment with, respectively, trypsin, citraconylation plus trypsin, staphylococcal protease, cyanogen bromide, hydroxylamine and chymotrypsin. Full lines denote sequences established directly, dashed lines those inferred from amino acid compositions and comparison with previously known sequences.

Table 1
Regions of inner homology of AAT

Isoenzyme of AAT	Positions	Amino acid sequence
Chicken c-AAT	285–298	-Ser-Gln-Met-Glu-Lys-Ile-Val-Arg-Thr-Thr-Trp-Ser-Asn-Pro-
	300–313	-Ser-Gln-Gly-Ala-Arg-Ile-Val-Ala-Thr-Thr-Leu-Thr-Ser-Pro-
Pig c-AAT	285–298	-Ser-Gln-Met-Gln-Lys-Ile-Val-Arg-Val-Thr-Trp-Ser-Asn-Pro-
	300–313	-Ala-Gln-Gly-Ala-Arg-Ile-Val-Ala-Arg-Thr-Leu-Ser-Asp-Pro-
Pig m-AAT	285–298	-Ser-Gln-Leu-Lys-Ile-Leu-Ile-Arg-Pro-Met-Tyr-Ser-Asn-Pro-
		-Val-Asn-Gly-Ala-Arg-Ile-Ala-Ser-Thr-Ile-Leu-Thr-Ser-Pro-

much longer for the mitochondrial isoenzyme than for the cytoplasmic one.

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