THE PRIMARY STRUCTURE OF CYTOPLASMATIC ASPARTATE AMINOTRANSFERASE FROM PIG HEART MUSCLE TRYPTIC HYDROLYSIS PRODUCTS

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Aspartate aminotransferase (L-aspartate: 2-ketoglutarate aminotransferase, EC 2.6.1.1.)* plays an important part in the metabolic activity of the cell, acting as a link between carbohydrate and amino acid metabolism. The mechanism of interaction of the AAT coenzyme (pyridoxal 5′-phosphate) with substrates has been fairly well investigated [1, 2], whereas the role of the apoenzyme in the enzymatic activity is still rather obscure partly because the primary structure of AAT is not yet known. To fill the gap we have investigated the total primary structure of cytoplasmatic AAT from pig heart muscle.

In this paper we report the results of our work on isolation and determination of partial and total sequences of tryptic peptides constituting the greater part of the polypeptide chain of the AAT subunit. To accomplish this we made use of exhaustive tryptic hydrolysis, cleaving the peptide chain at lysine and arginine residues and restricted tryptic hydrolysis affecting only arginine residues, the lysine residues being blocked by treatment with maleic anhydride [3].

It is known that the AAT subunit is a polypeptide chain containing some 400 amino acid residues and devoid of disulfide bridges [4, 5]. The cysteine residues were blocked, prior to tryptic digest (or before treatment with maleic anhydride), by carboxymethylation. Tryptic digests of CM-AAT and of M-CM-AAT in addition to soluble peptides gave some amounts of insoluble peptides. The mixture of soluble peptides was resolved by chromatography on ion-exchange resisns, Aminex 50 X 2, 200–325 mesh (for M-CM-AAT) or Aminex Q-150S (for CM-AAT), into 26 and 27 fractions, respectively. All these fractions were found to be mixtures of several peptides which were further separated by preparative paper chromatography or paper electrophoresis. Thus from the hydrolysis products of M-CM-AAT we obtained more than 20 peptides, the total or partial sequences of which are given in table 1.

Apart from the peptides listed in the table 1 we have isolated a number of peptides which seem to result from partial peptide bond rupture brought about by the rather drastic conditions of the demaleoylation procedure (pH 3, 40°, 40 hr). The bond between aspartic acid and proline residues was especially susceptible to these acidic conditions; thus we have isolated Glu–Asp–Pro–Asp (TA-2-1), Pro–Asp–Pro–Arg (TA-9-3), Pro–Arg (TA-15-1), Pro–Asp (TA-3-1), which obviously are degradation products of peptide TA-6-1 (table 1).

* *Abbreviations:*
AAT : aspartate aminotransferase
CM-AAT : carboxymethylated aspartate aminotransferase
M-CM-AAT: maleoylated and carboxymethylated aspartate aminotransferase

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Table 1
Peptides from the tryptic digest of M-CM-AAT*

TA-6-1	Glu–Asp–Pro–Asp–Pro–Arg
TA-6-2	Thr–Asp–Asp–CMCys–Glx–Pro–Trp–Val–Leu–Pro–Val–Val–Arg
TA-8-1	Thr–CMCys–Ala–Ser–Arg
TA-10-2	Val–Gly–Gly–Val–Gln–Ser–Leu–Gly–(Gly_2, Thr)–Ala–Leu–Arg
TA-11-1	Leu–Ala–Leu–Gly–Asp–Asp–Ser–Pro–Ala–Leu–Gln–Glu–Lys–Arg
TA-12-1	Ser–Glu–Leu–Arg
TA-12-2	Val–Gly–Asn–Leu–Thr–Val–Val–Ala–Lys–Glu–Pro–Asp–Ser–Ile–Leu–Arg
TA-13-1	Ile–Val–Ala–Arg
TA-14-1	Ala–Arg
TA-16-2	Val–Thr–Trp–Ser–Asn–Pro–Pro–Ala–Gln–Gly–Ala–Arg
TA-16-6	Ile–Gly–Ala–Glu–Phe–Leu–Ala–Arg
TA-18-2	Ile–Ala–(His, Asx_3, Ser_2, Glx_2, Pro, Gly, Ala, Ile, Leu_4, Tyr, Phe)–Arg
TA-18-3	Ile–Leu–Ser–Met–Arg
TA-19-1	Lys–Val–Glu–Glu–Arg
TA-20-1	Thr–Leu–Asx–Pro–Glu–Leu–Phe–(Asx, Thr, Ser, Glx, Gly, Val, His, Trp)–Lys–Thr–Met–Ala–Asp–Arg
TA-20-2	Val–Leu–Ser–Gln–Met–Glu–Lys–Ile–Val–Arg
TA-21-1	Ser–Tyr–Arg
TA-23-2	Lys–Leu–Ile–Ala–Asp–Phe–Arg
TA-24-1	Tyr–Trp–Asp–Thr–Glu–Lys–Arg
TA-26-1	Lys–Val–Asn–Leu–Gly–Val–Gly–Ala–Tyr–Arg

* The first number in the peptide denomination shows the number of the fraction in ion-exchange chromatography; the second number is that assigned to the peptide in paper chromatography according to increasing R_f-value.

Similarly, in addition to peptide TA-20-1, we have also isolated a peptide TA-23-1 that is shorter than TA-20-1 by three *N*-terminal amino acid residues. Demaleoylation also causes partial conversion of Gln and Asn residues into residues of the corresponding dicarboxylic acids. All this signifies that blocking of lysine residues with maleic anhydride has some drawbacks, as the removal of the protective group requires too drastic a treatment and leads to some artifacts.

Tryptic digestion of CM-AAT gave a great number of peptides identical either to those listed in table 1, or to their fragments. Also some peptides were isolated (table 2) which seem to be fragments of the insoluble peptides from the M-CM-AAT tryptic hydrolysate. Of the peptides from table 2, the more interesting are (1) and (14), which are *C*- and *N*-terminal fragments of the AAT. The structure of the neutral peptide (1) agrees with the AAT *C*-terminal sequence reported by Wada [6]. Determination of the *N*-terminal sequence of AAT using a Model 42–65 Sequenator

Table 2
Amino acid sequence of some tryptic peptides from CM-AAT.

(1)	Ile–Gln
(2)	Glu–Lys
(3)	Leu–Lys
(4)	Ala–Lys
(5)	Asp–Ile–Arg
(6)	Leu–Glu–Ala–Leu–Lys
(7)	Leu–Ile–(Asx, Glx)–Lys
(8)	Thr–Asp–Ala–Ser–Arg
(9)	Asp–Ala–Trp–Ala–Ile–Arg
(10)	Gln–Ile–Ala–Ser–Val–Met–Lys
(11)	Asn–Phe–Gly–Leu–Tyr–Asn–Glu–Arg
(12)	Trp–Tyr–Asn–Gly–(Asn, Thr)–Asn–Lys
(13)	His–Ile–Tyr–Leu–Leu–Pro–Ser–Gly–Arg
(14)	Ala–Pro–Pro–Ser–Val–Phe–Ala–Glu–Val–(Glx_2, Pro)–(Val, Leu)–Lys

(Beckman, GFR) allowed us to establish the following sequence for 9 amino acid residues:

Ala–Pro–Pro–Ser–Val–Phe–Ala–Glu–Val–

(see peptide (14), partial sequence).

For amino acid sequence determination in tryptic peptides a mass spectrometric method was used (for details see [7] and the literature therein) together with Edman degradation as modified by Sjöquist [8], dansylation [9] and carboxypeptidase cleavage. The structures of peptides TA-12-1, TA-12-2 (beginning from the lysine residue), TA-13-1, TA-14-1, TA-19-1, TA-23-2 (table 1); (5), (7), (10), (11) (table 2) were established mass spectrometrically or by combination of mass spectrometry with the Edman procedure and hydrolysis with carboxypeptidases A and B. It is noteworthy that the data we thus obtained were in accord with the results of structural studies carried out in parallel by classical methods.

The enzyme under investigation comprised at least 4 subforms that could be separated by electrophoresis or isoelectric focusing [5, 10]. As no homologous peptides were found in the products of tryptic digestion this seems to confirm the suggestion made earlier [5], that all the AAT subforms have the same amino acid sequence.

We are now completing the structural investigation of the tryptic peptides of AAT and will proceed to study products obtained from AAT by means other than those reported here with the aim of isolating overlapping peptides to allow the elucidation of the total amino acid sequence of the AAT molecule.

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