CYTOPLASMIC ASPARTATE AMINOTRANSFERASE FROM PIG HEART MUSCLE: PARTIAL SEQUENCE

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1. Introduction

In an earlier paper [1] we reported sequence studies on a number of peptides from the tryptic digest of cytoplasmic aspartate aminotransferase (L-aspartate:2-ketoglutarate aminotransferase, EC 2.6.1.1) from pig heart muscle. Amino acid sequences of a further series of tryptic peptides have been determined, some partial sequences have been completed and data are given on isolation and sequence determination of the products of chymotryptic digestion and cyanogen bromide cleavage of CM-AAT.

2. Methods

The separation and purification techniques for peptides resulting from the hydrolysis of CM-AAT and M-CM-AAT have been described [1]. In several cases we resorted to chromatography on Dowex 1 X 2. Chymotryptic peptides were separated according to a scheme similar to that applied to tryptic peptides. The products of cyanogen bromide cleavage of CM-AAT were separated in the form of citraconyl derivatives by gel filtration on Sephadex G-75. For amino acid sequence determination techniques see [1]. With large tryptic peptides chymotryptic digestion was used for this purpose.

Abbreviations:

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

3. Results and discussion

In addition to peptides described in [1] we also isolated a peptide TA-26-2* from the products of restricted hydrolysis of M-CM-AAT at arginine residues. The total amino acid sequences of peptides TA-10-2, TA-18-2 and TA-20-1 were determined. The sequence of TA-19-1 was defined.

Study of the complete tryptic hydrolysis of CM-AAT led to the following results: we obtained

* For tryptic peptides we use here the same assignments as in [1].

Table 1
Partial sequence of cytoplasmic aspartate aminotransferase from pig heart muscle.
Lys(Pxy) is the N^{ϵ}-pyridoxyllysine residue.

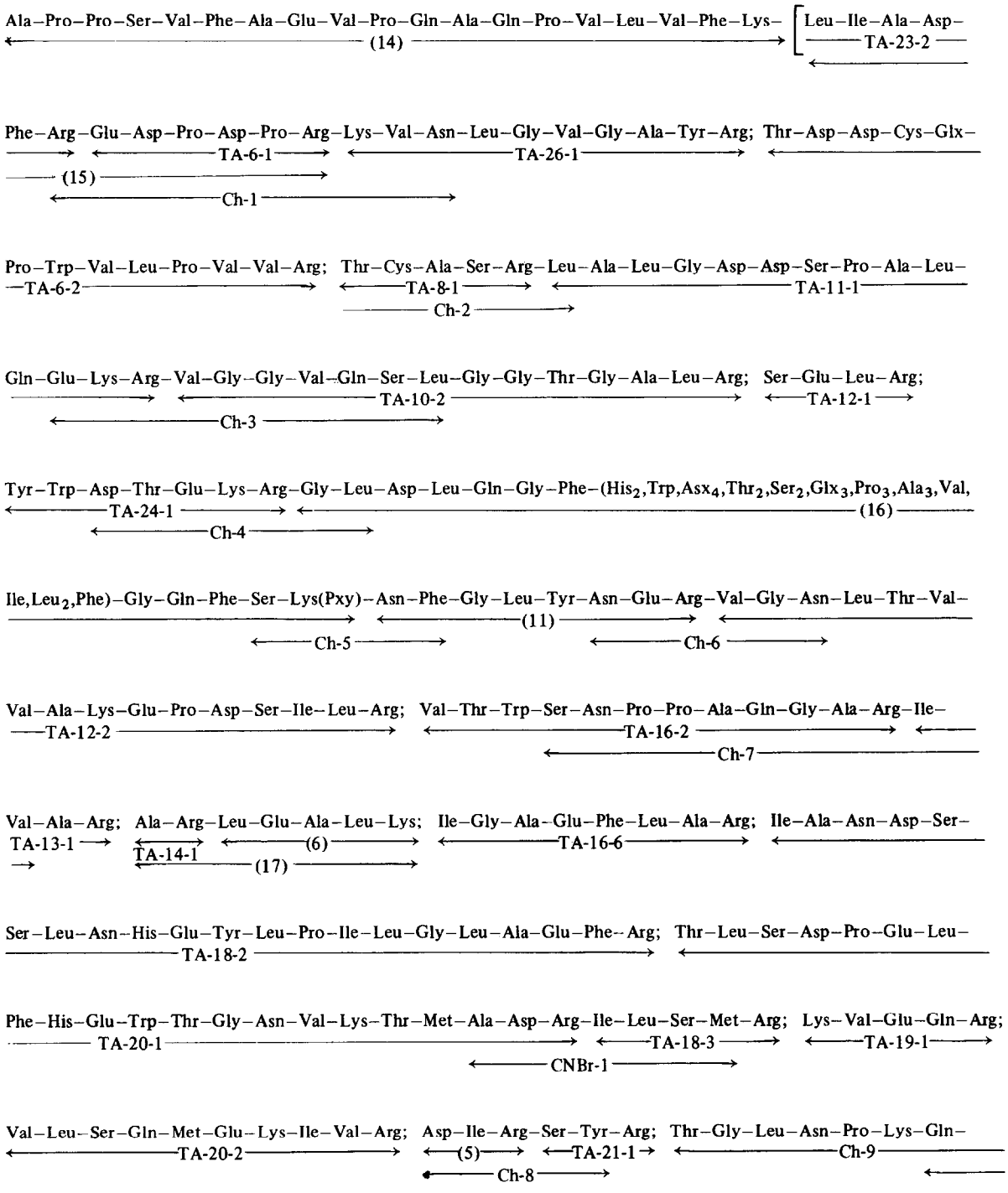
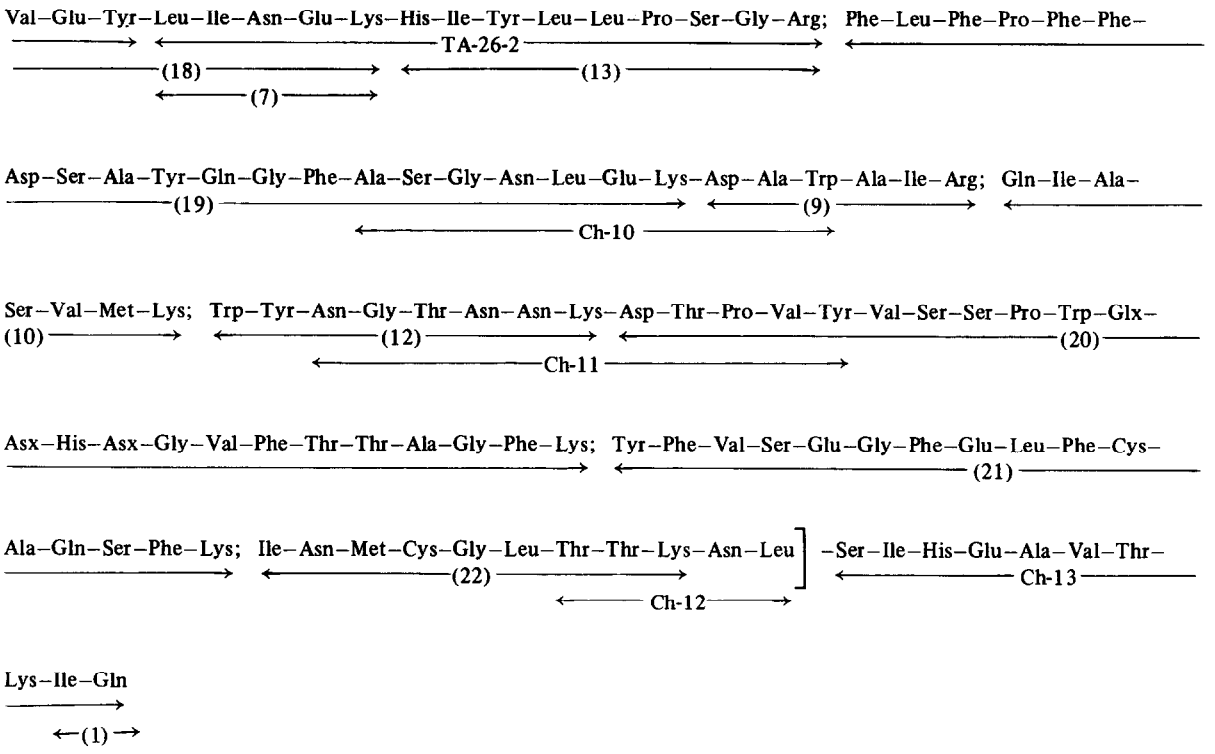


Table 1 (continued)



the total sequences of peptides (7), (12) and that of the *N*-terminal fragment of the enzyme (14); in addition to the tryptic peptides of CM-AAT described earlier [1] we isolated peptides (16), (19), (20), (21), (22) and also a small amount of peptides probably resulting from incomplete cleavage of the enzyme polypeptide chain. These were (15) and (17); they showed the peptide TA-23-2 to be joined to TA-6-1 and TA-14-1 to peptide (6). We also isolated peptide (18), later found to be peptide (7) elongated from the *N*-terminus. Apparently the presence of the latter in the tryptic digest was caused by Tyr–Leu bond rupture which could be due to some residual chymotryptic activity in the trypsin preparation (cf. [2]). Probably the dipeptides (2), (3) and (4) are of similar origin. As to peptide (8), more careful investigation proved it to be identical to TA-8-1.

Chymotryptic digestion of CM-AAT afforded a large number of peptides which in fact were fragments of those sequences we obtained from tryptic digestion of CM-AAT and M-CM-AAT. From this batch of hydrolysis products we also isolated peptides that overlapped sequences of tryptic peptides obtained from CM-AAT and/or M-CM-AAT. These peptides Ch-1–Ch-8, Ch-10, Ch-11 showed that in the AAT polypeptide chain peptide TA-6-1 is joined to TA-26-1; TA-8-1 to TA-11-1; TA-11-1 to TA-10-2; TA-24-1 to (16); (16) to (11); (11) to TA-12-2; TA-16-2 to TA-13-1; (5) to TA-21-1; (19) to (9); (12) to (20). It follows from the sequence of peptide Ch-5 (compare [3]), that it is a portion of the AAT active site carrying the pyridoxyllysine residue. The chymotryptic digest also contained peptides Ch-9, Ch-12, Ch-13 which permitted us to extend the sequences of (18), (22) and that of the *C*-terminal fragment of AAT.

Finally the products of CM-AAT cleavage with cyanogen bromide afforded a peptide CNBr-1 which showed the peptide TA-20-1 to be joined to TA-18-3.

These data allow us to suggest the partial sequence for AAT given in table 1.

The amino acid composition [4, 5] and molecular weight [6] of AAT seem to show the enzyme subunit to contain some 400–430 amino acid residues. The above partial sequence comprises 383 residues, thus accounting for 90% of the constituent amino acid residues.

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