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The Primary Structure of Aspartate Aminotransferase from Pig Heart Muscle. Chymotryptic Peptides

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Atti della Accademia Nazionale dei Lincei. Classe di Scienze Fisiche, Matematiche e Naturali. Rendiconti, Accademia Nazionale dei Lincei, 1974.

Chimica biologica. — *The Primary Structure of Aspartate Aminotransferase from Pig Heart Muscle. Chymotryptic Peptides* (*). Nota di DONATELLA BARRA, FRANCESCO BOSSA, MASSIMO CARLONI, PAOLO FASELLA, FILIPPO MARTINI, RAFFAELE PETRUZZELLI, FRANCESCA RIVA, SHAWN DOONAN, HILARY J. DOONAN, ROBIN HANFORD e JOHN M. WALKER, presentata (**) dal Socio A. ROSSI-FANELLI.

RIASSUNTO. — Sono stati purificati i peptidi prodotti dalla digestione con chimotripsina dell'aspartato aminotransferasi sia carbossimetilata che ossidata e la loro sequenza aminoacidica è stata determinata.

Tutti questi frammenti, che contengono l'86% (353) del numero totale dei residui aminoacidici dell'enzima, possono essere sistemati nella struttura primaria dell'aspartato aminotransferasi e qualcuno è stato di grande importanza per la determinazione della sequenza completa.

In the course of our work on the determination of the primary structure of soluble aspartate aminotransferase from pig heart muscle, we have reported the results obtained by digesting the enzyme with trypsin, pepsin, thermolysin (under various conditions) and elastase [1, 2]. These data plus some fragments obtained with other proteases allowed us to present the complete structure of aspartate aminotransferase [3] which is essentially in agreement with that of Ovchinnikov *et al.* [4]. The present paper reports the full details and results of the chymotryptic digests.

MATERIALS AND METHODS

Details of the following experimental methods are given in the previous papers [1] and Supplementary Publication SUP 50011; [2] and Supplementary Publication SUP 50018: preparation of the enzyme and of the apoenzyme, modification of the enzyme by carboxymethylation and performic acid oxidation; purification of peptides by gel filtration; ion exchange chromatography on SP-Sephadex C-25 and Chromobead P resin; paper chromatography and high-voltage paper electrophoresis at pH 1.9, 3.5 and 6.5; determination of aminoacid compositions of peptides; N-terminal analysis and amino acid sequence determination.

Representative examples of the purification procedures using gel filtration and ion exchange chromatography are shown in figs. 1, 2 and 3.

(*) This research was carried out in the Istituto di Chimica Biologica and Biochimica Applicata and Centro di Biologia Molecolare of Consiglio Nazionale delle Ricerche, Università di Roma, Italy and in the Christopher Ingold Laboratories, Dept. of Chemistry, University College, London.

(**) Nella seduta del 20 aprile 1974.

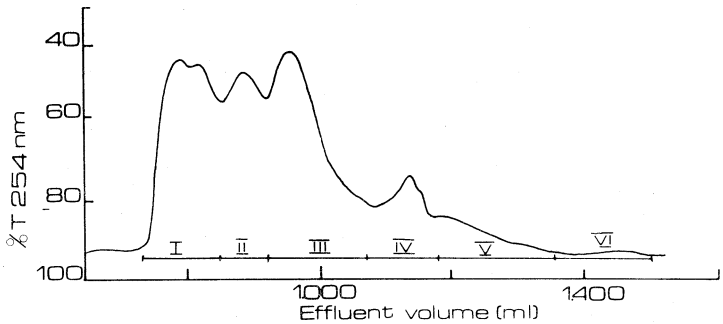


Fig. 1. – Fractionation of the limited chymotryptic digest of performic acid oxidized apo-aspartate aminotransferase on Sephadex G–25 (fine) in 10% acetic acid. Pooled fractions are indicated by bars with roman numbers.

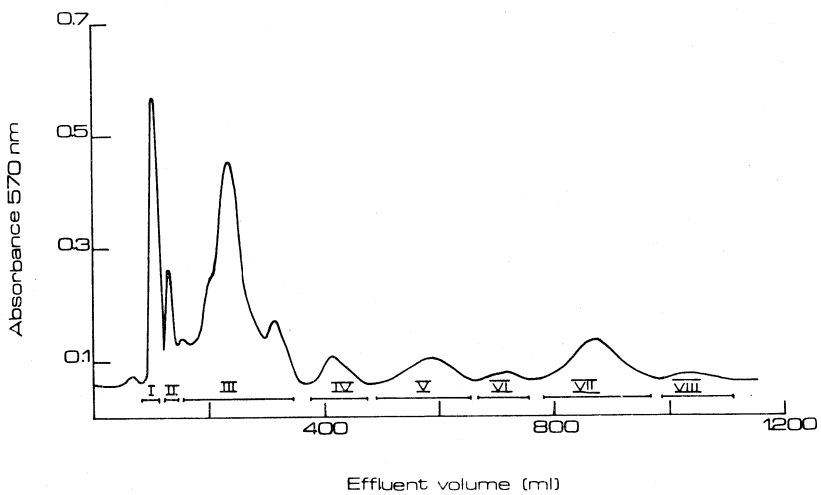


Fig. 2. – Fractionation of fraction IV from Sephadex G–25 (see fig. 1) on SP–Sephadex C–25 under experimental conditions identical to those already indicated [2]. Pooled fractions are indicated by bars with roman numbers.

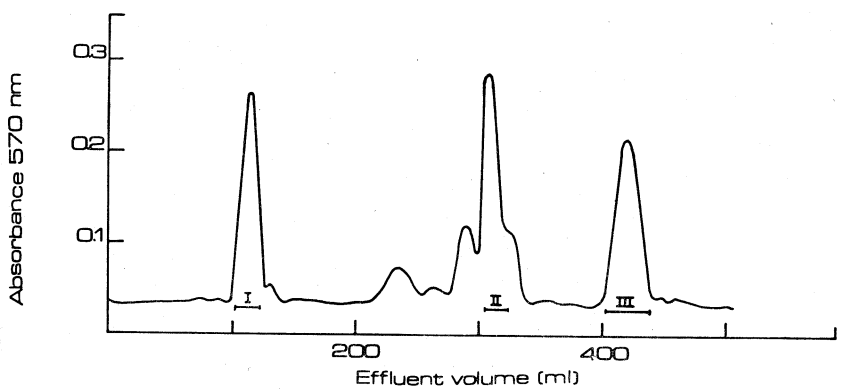


Fig. 3. – Rechromatography of fraction III from SP–Sephadex C–25 (See fig. 2) on Chromobead P (Technicon) resin (0.9 cm × 15 cm.). The buffers used were: (A) pyridine (0.2 M)–formate, pH 2.7; (B) pyridine (0.2 M)–acetate, pH 3.1; (C) and (D) pyridine (2M)–acetate, pH 5.1 and 6.5 respectively. Buffers were mixed in the Autograd according to the following proportions: chambers I, II and III, 100 ml each of A; IV and V, 100 ml each of B; VI, 100 ml of C; VII, 50 ml of C plus 50 ml of D. Flow rate: 1 ml/minute. Peak I contained a peptide identical to CH–1; peak II and III contained CHO–7 and CHO–5 respectively.

TABLE I

Amino acid sequences of peptides from the chymotryptic digestion of carboxymethylated aspartate aminotransferase.

—— Residues from dansyl–Edman method. Residues not underlined were assigned on the basis of the amino acid analysis and by comparison with known sequences.

Peptide	Sequence
CH–1	Ala–Pro–Pro–Ser–Val–Phe
CH–2	Ala–Glx–Val–Pro–Glx–Ala–Glx–Pro–Val–Leu–Val–Phe
CH–3	Lys–Leu–Ile–Ala–Asx–Phe
CH–4	Arg–Glx–Asx–Pro–Asx–Pro–Arg–Lys–Val–Asx–Leu– –Gly–Val–Gly–Ala–Tyr
CH–5	Arg–Thr–Asx–Asx–CmCys–Glx–Pro–Trp
CH–6	Val–Leu–Pro–Val–Val–Arg
CH–7	Lys–Val–Glx–Glx–Arg–Ile–Ala–Asx–Asx–Ser–Ser–Leu
CH–8	Ala–Leu–Gly–Asx–Asx–Ser–Pro–Ala–Leu–Glx–Glx– –Lys–Arg–Val–Gly–Gly–Val–Glx
CH–9	Ser–Leu–Gly–Gly–Thr–Gly–Ala–Leu
CH–10	Arg–Ile–Gly–Ala–Glx–Phe
CH–11	Leu–Ala–Arg–Trp
CH–12	Asx–Gly–Thr–Asx–Asx–Lys–Asx–Thr–Pro–Val–Tyr
CH–13	Val–Ser–Ser–Pro–Thr–Trp–Glx–Asx–His–Asx–Gly– –Val–Phe
CH–14	Thr–Thr–Ala–Gly–Phe
CH–15	Lys–Asx–Ile–Arg–Ser–Tyr
CH–16	Arg–Tyr
CH–17	Asx–Thr–Glx–Lys–Arg–Gly–Leu
CH–18	Asx–Leu–Glx–Gly–Phe
CH–19	Leu–Ser–Asx–Leu–Glx–Asx–Ala–Pro–Glx–Phe–Ser– –Ile–Phe
CH–20	Asx–Pro–Thr–Pro–Glx–Glx–Trp
CH–21	Lys–Glx–Ile–Ala–Ser–Val–Met
CH–22	Leu–Phe–Pro–Phe

Continued: TABLE I.

Peptide	Sequence
CH–23	Phe–Asx–Ser–Ala–Tyr
CH–24	Ala–Ser–Gly–Asx–Leu–Glx–Lys–Asx–Ala–Trp
CH–25	Ala–Ile–Arg–Tyr
CH–26	Ser–Phe
CH–27	Ser–Lys–Asx–Phe
CH–28	Gly–Leu–Tyr
CH–29	Asx–Glx–Arg–Val–Gly–Asx–Leu–Thr–Val–Val–Ala–-Lys–Glx–Pro–Asx–Ser–Ile–Leu
CH–30	Arg–Val–Leu
CH–31	Thr–Gly–Asx–Val–Lys–Thr–Met
CH–32	Ala–Asx–Arg–Ile–Leu
CH–33	Arg–Ser–Glx–Leu
CH–34	Arg–Ala–Arg–Leu–Glx–Ala–Leu
CH–34′	Glx–Ala–Leu–Lys–Thr–Pro–Gly–Thr–Trp
CH–35	Leu–Ile–Asx–Glx–Lys–His–Ile–Tyr
CH–36	Leu–Leu–Pro–Ser–Gly–Arg–Ile–Asx–Met
CH–37	Thr–Thr–Lys–Asx–Leu–Asx–Tyr
CH–38	Val–Ala–Thr–Ser–Ile–His–Glx–Ala–Val–Thr–Lys–-Ile–Glx
CH–38′	Val–Ala–Thr–Ser–Ala–Val–Thr–Lys–Ile–Glx

Proteolytic digestion of Aspartate Aminotransferase.

For the chymotryptic digestion, carboxymethylated apo-aspartate aminotransferase (530 mg) was suspended in NH_4HCO_3 (0.1 M; 50 ml; slightly opalescent solution) and incubated at 37° with chymotrypsin (Worthington) (13 mg) for 8 hours; the reaction was stopped by acidification.

The performic acid-oxidized apoenzyme (570 mg) was dissolved in 4 N–NaOH (30 ml) and the pH of the solution was quickly adjusted to 8.0 with 6 N HCl under vigorous stirring to obtain a fine suspension. The incubation with chymotrypsin (6 mg) was performed at 37° C; the pH was maintained at 8.0 by titration with 0.2 N–NaOH in a pH-stat (Radiometer TTT 1c)

and the reaction was stopped by acidification of the mixture when the equivalent of 20 peptide bonds had been broken as judged by the consumption of NaOH.

RESULTS

Peptides from the chymotryptic digest of carboxymethylated aspartate aminotransferase.

The amino acid sequences of 40 peptides isolated from the chymotryptic digest are shown in Table I. The amino acid compositions, yields and purification steps are reported in Table II; in a few cases the peptides were not subjected to quantitative aminoacid analysis due to lack of material and because the qualitative amino acid analysis by complete dansylation of hydrolysates [1] were consistent with the reported sequences.

TABLE II

Amino acid compositions of peptides from the chymotryptic digestion of carboxymethylated aspartate aminotransferase (*).

	CH–1	CH–2	CH–3	CH–4	CH–5	CH–8	CH–9	CH–10	CH–12	CH–13
CmCys	—	—	—	—	0.6	—	—	—	—	—
Asp	—	—	0.7	2.8	1.9	2.2	—	—	4.0	1.6
Thr	—	—	—	—	0.7	—	0.9	—	2.0	0.8
Ser	0.8	—	—	—	—	0.9	0.6	—	—	1.3
Glu	—	2.6	—	1.0	1.0	3.0	—	1.0	—	1.0
Pro	1.4	1.8	—	1.7	0.7	1.0	—	—	0.5	0.7
Gly	—	—	—	1.6	—	3.0	3.0	1.0	1.0	1.0
Ala	1.0	1.8	1.0	1.0	—	2.2	1.0	1.0	—	—
Val	0.9	2.6	—	1.6	—	1.6	—	—	1.0	1.6
Met	—	—	—	—	—	—	—	—	—	—
Ile	—	—	1.0	—	—	—	—	0.9	—	—
Leu	—	1.0	1.0	0.7	—	1.6	2.1	—	—	—
Tyr	—	—	—	0.8	—	—	—	—	0.8	—
Phe	0.8	0.6	0.7	—	—	—	—	0.8	—	0.6
Lys	—	—	0.8	0.7	—	1.0	—	—	0.9	—
His	—	—	—	—	—	—	—	—	—	0.8
Arg	—	—	—	2.0	0.8	0.8	—	0.9	—	—
Trp (**)	—	—	—	—	+	—	—	—	—	+
Yield (%)	1.5	14	9	7.8	1	5.4	10	21	10	1
Purification stages	GI	GI	GIGE	GIC	GI	GIC	GIC	GICE	GIE	GICE

Purification stages: G, gel filtration; I, ion exchange chromatography on Biorad AG–50 W–X2; C, paper chromatography in solvent BAWP; E, electrophoresis at pH 1.9; E′, electrophoresis at pH 6.5; E″, electrophoresis at pH 3.5.

(*) For the following peptides, not subjected to quantitative amino acid analysis, only the purification stages are given in parentheses: CH–6 (GE′CE); CH–7 (GC); CH–11 (GE″C); CH–24 (GE′C); CG–25 (GE′CE); CH–33 (GE′–GE); CH–34 (GE′CE); CH–37 (GE′CE); CH–38′ (GC).

(**) Presence of tryptophan indicated by reaction with Ehrlich reagent.

Continued: TABLE II.

	CH–14	CH–15	CH–16	CH–17	CH18	CH–19	CH–20	CH–21	CH–22	CH–23
CmCys	—	—	—	—	—	—	—	—	—	—
Asp	—	1.0	—	1.0	0.7	1.8	1.0	—	—	1.0
Thr	1.4	—	—	1.0	—	—	0.8	—	—	—
Ser	—	1.0	—	—	—	1.7	—	1.0	—	0.9
Glu	—	—	—	1.1	1.1	2.2	1.8	1.0	—	—
Pro	—	—	—	—	—	0.6	1.2	—	0.8	—
Gly	1.0	—	—	1.0	1.0	—	—	—	—	—
Ala	1.0	—	—	—	—	1.0	—	1.0	—	1.0
Val	—	—	—	—	—	—	—	1.0	—	—
Met	—	—	—	—	—	—	—	1.0	—	—
Ile	—	0.9	—	—	—	0.7	—	1.0	—	—
Leu	—	—	—	0.9	0.7	1.7	—	—	1.0	—
Tyr	—	0.8	1.0	—	—	—	—	—	—	0.8
Phe	1.0	—	—	—	0.9	1.8	—	—	1.4	1.0
Lys	—	0.8	—	0.8	—	—	—	0.7	—	—
His	—	—	—	—	—	—	—	—	—	—
Arg	—	1.0	1.0	0.9	—	—	—	—	—	—
Trp	—	—	—	—	—	—	+	—	—	—
Yield (%)	10	4	5	0.4	2	2	30	8	4	1.2
Purification stages	GI	GICE	GIC	GIC	GIE	GICE	GI	GIC	GI	GIC

	CH–26	CH–27	CH–28	CH–29	CH–30	CH–31	CH–32	CH–34	CH–35	CH–36	CH–38
CmCys	—	—	—	—	—	—	—	—	—	—	—
Asp	—	1.0	—	2.7	—	1.0	0.6	—	1.0	1.0	—
Thr	—	—	—	0.7	—	1.5	—	—	—	—	1.9
Ser	0.9	0.6	—	0.7	—	—	—	—	—	0.8	0.9
Glu	—	—	—	2.0	—	—	—	1.0	1.2	—	2.2
Pro	—	—	—	0.9	—	—	—	—	—	0.9	—
Gly	—	—	1.0	1.0	—	1.0	—	—	—	1.0	—
Ala	—	—	—	0.9	—	—	1.0	2.0	—	—	1.7
Val	—	—	—	2.4	1.0	1.0	—	—	—	—	2.0
Met	—	—	—	—	—	0.5	—	—	—	0.6	—
Ile	—	—	—	0.7	—	—	0.9	—	1.6	0.8	1.4
Leu	—	—	1.0	1.6	0.8	—	1.1	2.0	0.7	1.6	—
Tyr	—	—	1.0	—	—	—	—	—	0.7	—	—
Phe	1.0	1.0	—	—	—	—	—	—	—	—	—
Lys	—	1.0	—	0.8	—	0.7	—	—	0.9	—	0.8
His	—	—	—	—	—	—	—	—	0.9	—	0.8
Arg	—	—	—	0.8	0.8	—	0.8	1.9	—	0.7	—
Trp	—	—	—	—	—	—	—	—	—	—	—
Yield	1.5	2	23	10	4.5	8	3	1	22	2	1.6
Purification stages	GI	GIE	GI	GIC	GICE	GIC	GICE	GIC	GIC	GIE	GIC

A further number of peptides, originating from more extensive chymotryptic cleavage, were also isolated from this digest but the corresponding sequences are not included in Table I. This phenomenon, which increased the heterogeneity of the digestion mixture so complicating the purification of peptides, is best exemplified by the results obtained from the C–terminal region of the enzyme. In addition to peptide CH–38, the following shorter

peptides were also isolated from the same digest: Ser–Ile–His–Glx–Ala–Val–Thr–Lys–Ile–Gln (18 %); His–Glx–Ala–Val–Thr–Lys–Ile–Glx (2 %); Ala–Val–Thr–Lys–Ile–Glx (0.8 %); Lys–Ile–Glx (3 %) (yields in parentheses).

The sequences of 6 of these peptides have been included in a previous paper [2]: CH–13 (CH–1); CH–14 (CH–2); CH–15 (CH–5); CH–24 (CH–4); CH–27 (CH–3); CH–37 (CH–6). (The old nomenclature in parentheses). Another peptide (CH–19) has been utilized to overlap fragment C with fragment D to obtain the complete primary structure of the enzyme [3].

TABLE III

Amino acid sequences of peptides from the limited chymotryptic digestion of oxidized aspartate aminotransferase.

—— Residues from dansyl–Edman method. Residues not underlined were assigned on the basis of the amino acid analysis and by comparison with known sequences.

Peptide	Component peptides	Sequence
CHO–1	CH–7	Lys–Val–Glx–Glx–Arg–Ile–Ala–Asx–Asx–
		–Ser–Ser–Leu–Asx–His–Glx–Tyr–Leu–Pro–
		–Ile–Leu–Gly–Leu–Ala–Glx–Phe
CHO–2		Arg–Thr–Cys(O_3H)–Ala–Ser–Arg–Leu
CHO–3	CH–8	Ala–Leu–Gly–Asx–Asx–Ser–Pro–Ala–Leu–
		–Glx–Glx–Lys–Arg–Val–Gly–Gly–Val–Glx–
		–Ser–Leu
CHO–4		Val–Leu
CHO–5	CH–22 CH–23	Leu–Phe–Pro–Phe–Phe–Asx–Ser–Ala–Tyr
CHO–6		Phe–Val–Ser–Glx–Gly–Phe
CHO–6′		Val–Ser–Glx–Gly–Phe–Glx–Leu–Phe–
		–Cys(O_3H)–Ala–Glx
CHO–6″	CH–26	Cys(O_3H)–Ala–Glx–Ser–Phe
CHO–7		Leu–Ser–Asx–Pro–Glx–Leu–Phe
CHO–8	CH–33	Ser–Met(O_2)–Arg–Ser–Glx–Leu
CHO–9		Ser–Phe–Thr–Gly–Leu–Asx–Pro–Lys–Glx–
		–Val–Glx–Tyr

TABLE IV

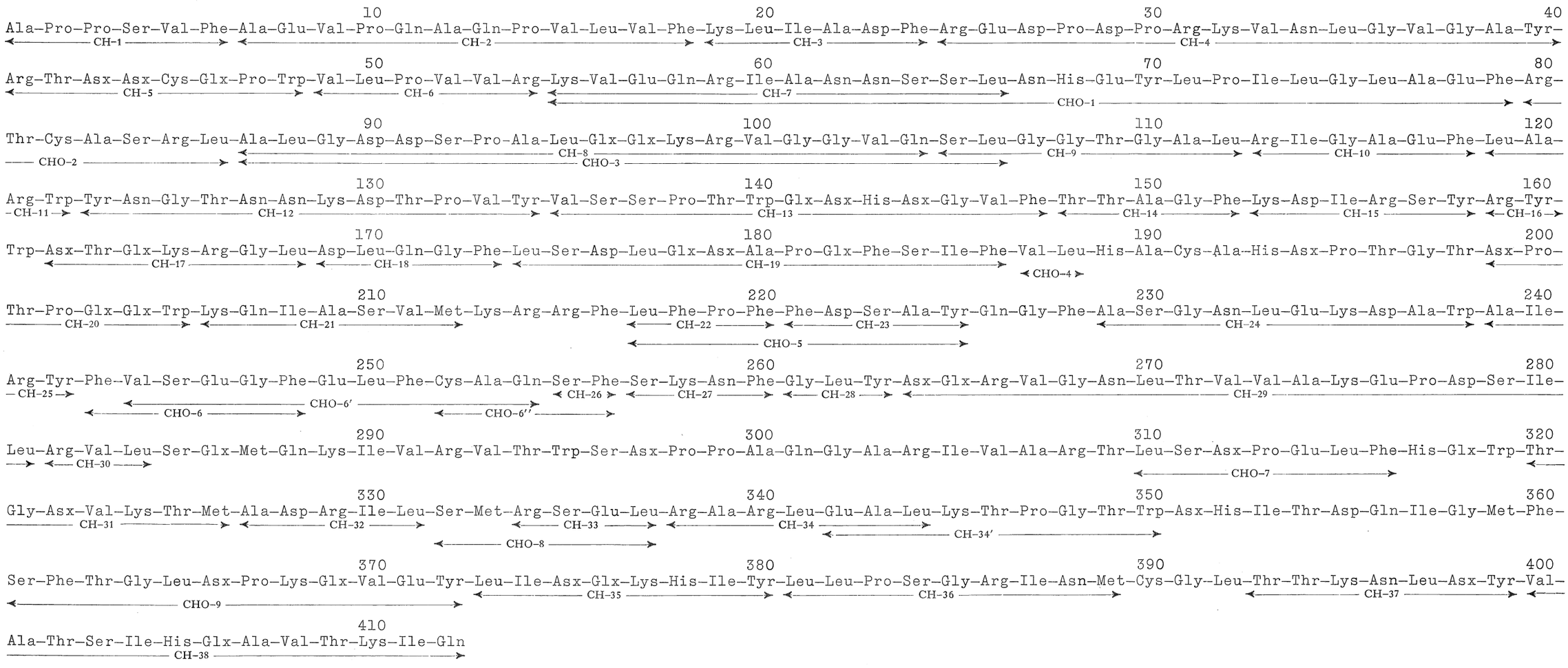
Amino acid compositions of peptides from the limited chymotryptic digestion of oxidized aspartate aminotransferase.

	CHO–1	CHO–2	CHO–3	CHO–4	CHO–5	CHO–6	CHO–6′	CHO–6″	CHO–7	CHO–8	CHO–9
Cys(O_3H)	—	1.0	—	—	—	—	0.9	1.0	—	—	—
Asp	3.1	—	1.9	—	1.0	—	—	—	1.0	—	1.0
Thr	—	1.0	—	—	—	—	—	—	—	—	1.0
Ser	1.6	1.0	1.6	—	0.9	1.0	1.0	0.8	0.8	1.5	0.9
Glu	3.8	—	3.0	—	—	1.0	2.8	1.0	1.1	1.0	1.9
Pro	0.8	—	1.1	—	0.8	—	—	—	0.5	—	0.9
Gly	1.0	—	3.1	—	—	0.8	1.0	—	—	—	1.0
Ala	1.9	1.0	2.0	—	1.0	—	1.0	1.0	—	—	—
Val	1.0	—	2.0	1.0	—	0.7	0.8	—	—	—	0.9
Met(O_2)	—	—	—	—	—	—	—	—	—	0.9	—
Ile	1.5	—	—	—	—	—	—	—	—	—	—
Leu	4.1	1.2	3.0	1.0	1.0	—	1.0	—	1.8	0.8	0.9
Tyr	0.8	—	—	—	1.0	—	—	—	—	—	0.8
Phe	1.1	—	—	—	2.7	1.7	1.6	1.0	0.7	—	1.0
Lys	0.7	—	0.9	—	—	—	—	—	—	—	0.7
His	0.8	—	—	—	—	—	—	—	—	—	—
Arg	0.7	2.0	1.0	—	—	—	—	—	—	1.1	—
Trp	—	—	—	—	—	—	—	—	—	—	—
Yield (%)	0.8	1.0	1	10	10	2	2	16	10	1.0	10
Purification stages	GICE	GIC	GIC	GIC	GIP	GIE	GIE	GIE	GIP	GICE	GI

Purification stages: G, gel filtration; I, ion exchange chromatography on SP–Sephadex C–25; P, ion exchange chromatography on Chromobead P; C, paper chromatography in solvent BAWP; E, electrophoresis at pH 1.9.

TABLE V

Localization of the chymotryptic peptides in the complete primary structure of aspartate aminotransferase [3].



Peptides from the limited chymotryptic digest of oxidized aspartate aminotransferase.

A further 11 peptides that were isolated after limited digestion of the oxidized enzyme but were not found in the prolonged chymotryptic digest, are shown in Table III. The corresponding amino acid compositions, yields and purification steps are reported in Table IV. Where these peptides were composed, in part, of one or more peptides obtained from the other chymotryptic digest, the numbers of the corresponding shorter peptides are given in parentheses.

DISCUSSION

The amino acid sequences of the peptides obtained by digestion of aspartate aminotransferase with chymotrypsin under two different sets of conditions have been positioned in the complete primary structure of the enzyme presented in Table V. The combined chymotryptic digestions allowed the identification of 353, i.e. 86%, of the amino acid residues in the protein. The figures for the other digests were: trypsin, 78 %, pepsin, 73%; thermolysin, 93%; elastase, 40%. The chymotryptic cleavage followed the reported specificity [5]. All but 3 of the peptides presented here have tyrosine, phenylalanine, tryptophan, leucine or methionine at their C–termini; the exceptions have glutamine (2) or arginine (1) (frequency in parentheses). It must be remembered that the reported yields of the peptides are only very approximately correlated to the extent of chymotryptic cleavage at the C–termini, since recovery of a peptide depends also on the specificity of the cleavage at the other side (as exemplified above with peptide CH–38) and on the difficulties in the purification procedures.

The limited digestion of oxidized aspartate aminotransferase with chymotrypsin was performed to obtain larger peptides and to facilitate isolation of peptides containing cysteine and methionine residues. This double aim was only in part attained probably because it was not pursued in separate digests (as was the case for thermolysin [2]) or/and because of the relatively small amount of aspartate aminotransferase submitted to proteolysis. However, peptides like CHO–1, which contains 4 peptide bounds potentially susceptible to chymotryptic attack, have been isolated, although in poor yield.

As far as the problem of overlapping fragments of aspartate aminotransferase was concerned, the chymotryptic peptides were of minor usefulness compared with peptides obtained with other proteolytic enzymes of comparably restricted specificity (e.g. thermolysin) essentially for the following reasons. The chymotryptic digestions were performed after the peptic and the thermolytic ones, which had already given most of the information provided by chymotrypsin. In addition, in the sequence of aspartate aminotransferase 13 tryptic peptides have C–terminal peptide bonds susceptible to chymotryptic attack (18–19; 24–25; 40–41; 79–80; 112–113; 153–154; 158–159; 205–

206; 212–213; 281–282; 333–334; 337–338; 344–345). In these cases a greater usefulness of the thermolytic peptides compared with the corresponding chymotryptic ones is easily predictable on the basis of the specificity of the two proteolytic enzymes.

Peptide CH–38′ is worthy of special attention. Despite the fact that lack of material did not allow a quantitative aminoacid analysis, the sequence analysis was unambiguous and gave evidence of a deletion of 3 residues in positions 404–406 of the polypeptide chain.

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References

[1] 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*, 442–452.

[2] Bossa F., Barra D., Carloni M., Fasella P., Riva F., Doonan S., Doonan H. J., Hanford E., Vernon C. A. and Walker J. M. (1973) – « Biochem. J. », *133*, 805–819.

[3] Doonan S., Doonan H. J., Hanford R., Vernon C. A., Walker J. M., Bossa F., Barra D., Carloni M., Fasella P., Riva F. and Walton P. L. (1974) – « FEBS Lett. », *38*, 229–233.

[4] Ovchinnikov Yu. A., Egorov Ts. 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., Polvanovsky O. L. and Nosikov V. V. (1973) – « FEBS Lett. », *29*, 31–34.

[5] Smyth D. G. (1967) – In *Methods in Enzymology*, (Hirs, C. H. W. ed.), *11*, pp. 214–230, Academic Press, New York.