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Nicotinamide Adenine Dinucleotide-specific Glutamate Dehydrogenase of *Neurospora*

V. TRYPTIC PEPTIDES*

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The isolation and sequences of an additional 80 peptides from a tryptic digest of the NAD-specific glutamate dehydrogenase of *Neurospora crassa* are reported. These include an additional peptide containing a lysine residue labeled at the ε-amino group with pyridoxal 5′-phosphate. The sequence of this peptide shows some homology with the reactive lysine residue of other glutamate dehydrogenases.

In an earlier paper of this series (1), several tryptic peptides were described from the NAD-specific glutamate dehydrogenase of *Neurospora crassa*. The protein had been treated with pyridoxal 5′-phosphate to label reactive lysine residues, with *N*-ethyl[1-^{14}C]maleimide to label the single reactive cysteine residue, and with iodoacetate to block other cysteine residues. From a tryptic digest of this material, the isolation and sequences of five PLP[1]-lysine peptides and of the reactive cysteine-containing peptide were described. The present study is devoted to a description of the isolation and sequences of 80 additional tryptic peptides obtained from the same hydrolysate. Many of these peptides have been placed in the COOH-terminal sequence of 669 residues described in the preceding paper (2). However, other isolated peptides belong in the NH_2-terminal part of the molecule and are included here for completeness.

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[1] The abbreviations used are: PLP, pyridoxal 5′-phosphate; BPAW, 1-butanol/pyridine/acetic acid/water as solvent for paper chromatography; PE, paper electrophoresis; PTH, phenylthiohydantoin; in identification of PTH-derivatives: R, regeneration; T, thin layer chromatography; G, gas-liquid chromatography; CN, cyanogen bromide peptide; TM, tryptic peptide from the maleylated protein; AT, aminotripeptidase; Cys(Cm), *S*-carboxymethylcysteine; Met(SO_2), methionine sulfone.

EXPERIMENTAL PROCEDURES

The preparation, chemical modification, and tryptic hydrolysis of NAD-specific glutamate dehydrogenase has been described (1, 3). Trypsin, chymotrypsin, carboxypeptidases A and B, and papain were purchased from Worthington, thermolysin from Calbiochem, and aminopeptidase M from Röhm GMBH Chemische Fabrik. Aminotripeptidase (4) and leucine aminopeptidase (5) were prepared in this laboratory.

Tryptic Hydrolysis and Isolation of Peptides—The modified protein (9.6 μmol) was treated with a total of 34 mg of trypsin for 5 h at 37° and pH 8.0. The initial fractionation of the digest on a column of Sephadex G-50 in 30% acetic acid was described in Fig. 1 of a previous communication (1). Four major fractions, designated as Fractions II to V in the earlier paper, yielded pure peptides on further fractionation.

Fractions II to V were fractionated by combinations of the following methods: chromatography on columns of quaternary aminoethyl (QAE)-Sephadex (A-25) with a KCl gradient in the eluant, Sephadex G-25 with 30% acetic as eluting phase, Dowex 50-X2 with pH gradient of eluant, and Dowex 1-X2 with buffers as described below; preparative descending paper chromatography with 1-butanol/pyridine/glacial acetic acid/water (BPAW, 15:10:3:12 by volume); preparative high voltage paper electrophoresis at pH 1.9 (formic/acetic acid), at pH 3.6 and at pH 6.5 (pyridine/acetic acid) (6). Whatman No. 3MM paper was used for the last four methods. Peptides were located by dipping the paper in ninhydrin-collidine reagent (7). Use of the Rydon-Smith reagents (8) permitted detection of ninhydrin-negative peptides. Peptides containing histidine or tyrosine were detected by use of the Pauly reagent (9). Peptides were eluted from paper with 30% acetic acid and stored at −20°. The chromatography of Fractions III and IV on Dowex 50-X2 was described in an earlier paper (1). Details concerning chromatography on columns of QAE-Sephadex (A-25), Sephadex G-25, and Dowex 50-X8 are included in legends to figures describing these procedures.

Chromatography on Dowex 1-X2—The pH gradient for a column (0.9 × 20 cm) of Dowex 1-X2 was attained with two constant volume (50 ml each) mixing chambers in series and receiving eluant from a third container. The buffer (pH 9.3) used to equilibrate the column and fill the mixing chambers contained 12.5 ml of *N*-ethylmorpholine, 7.5 ml of pyridine, 200 ml of 1-propanol, sufficient acetic acid to attain the desired pH, and water to bring the volume to 1 liter. The gradient was produced by passing into the mixing chambers in succession the following solutions, each of which contained 20% propanol: 25 ml of starting buffer (pH 9.3), 190 ml of 0.1 N acetic acid; 50 ml of 0.5 N acetic acid, 65 ml of 2.0 N acetic acid. At this stage in the elution, the external mixing chamber was removed, and the following solutions containing 20% propanol were passed into the remaining mixing chamber: 150 ml of 2.0 N acetic acid and 50 ml of 5.0 N acetic acid. The column was equilibrated at 40°, and fractions of 1.7 ml were collected at a flow rate of 17 ml/h.

Amino Acid Analysis—Peptide hydrolysis and automated amino acid analysis were performed as previously described (1).

Enzyme Methods—Hydrolyses with chymotrypsin, carboxypepti-

dases A and B, leucine aminopeptidase, aminopeptidase M, thermolysin, papain, and aminotripeptidase were performed as described previously (4, 10).

Edman Degradation—Peptides were degraded by the Edman method, as modified by Peterson *et al.* (11). Details of the method were included in an earlier communication (1). The PTH-derivatives in ethyl acetate were identified by gas-liquid chromatography with a Beckman GC-65 gas chromatograph with glass columns (2 mm × 1.2 m), and the detailed procedure used was as described earlier (1).

PTH-derivatives were also identified by thin layer chromatography (12), using System XP (xylene/isopropyl alcohol 10:20) with Eastman chromatographic sheets (type 6060 with fluorescent indicator). Residues were also identified as the free amino acids after regeneration by hydrolysis in 6 N HCl containing 1% (v/v) mercaptoacetic acid at 150° for 16 h. Identification of these was accomplished by either paper electrophoresis at pH 1.9 or on the amino acid analyzer.

Automated sequential Edman degradation was done with a Beckman sequenator model 890-C with one of the following programs: Dimethylallylamine program 051072 and Quadrol program 050972 (in Ref. 13); with 0.4 M of *N*,*N*-dimethyl-N-allylamine and 0.2 M Quadrol, respectively. Thiazolinone derivatives were converted to phenylthiohydantoins in 0.2 ml of 1 M HCl at 80° for 5 to 10 min.

Partial Acid Hydrolysis—The preferential cleavage of aspartyl peptide bonds with dilute acid was performed by the method of Schultz (14).

RESULTS[2]

Nomenclature and Conventions

Fig. 1, *A*, *B*, *C*, and *D*, shows the sequences of all the peptides obtained in pure form from the tryptic hydrolysate, designated as T peptides. The Roman numeral for the peptide indicates the fraction from which it was isolated. When the sequence was not completely determined, the correct sequence is usually given parenthetically since the remainder of the sequence was ascertained by studies on peptides from other types of hydrolysates; this is mentioned in the text. Peptides containing methionine residues provided overlaps for cyanogen bromide peptides, designated as CN-peptides (16–17). Peptides obtained later from a tryptic digest of the maleylated protein, are designated as TM-peptides (18).

In tables containing the compositions of the tryptic peptides or peptides derived from them by secondary hydrolysis, residues given parenthetically are those based on the known sequence of the peptide. These tables include actual yield figures of the purified peptide and are not corrected for losses in handling, pooling of samples, or aliquots removed for various types of tests. The tables also generally include mobility values on electrophoresis at pH 1.9 (R_{Asp}, 1.9) and chromatography on BPAW (R_{Leu}).

Peptide of Fraction II

Fraction II was applied to a column (2 × 20 cm) of the pyridinium form of SP-Sephadex (C-25) that was equilibrated and eluted with a solvent containing 10% formic acid, 18% acetic acid, 20% 1-propanol, and 0.8% pyridine (pH 2.16). Fractions of 4.4 ml were collected at a flow rate of 13.6 ml/h and analyzed by ninhydrin assay and absorbance at 325 nm. A small amount of material appeared in Fractions 10 to 20, followed by a larger component (IIA) in Fractions 20 to 25. Further elution of the column with solvents at high pH released additional peptide mixtures which on fractionation did not yield pure peptides.

Fraction IIA was desalted on Sephadex G-10 and fractionated on QAE-Sephadex (A-25) (Fig. 2). Peptide II-A1, of 27 residues (Table I), was eluted soon after the KCl gradient was initiated. Fractions eluted after II-A1 did not yield pure peptides.

Peptide II-A1—Edman degradation identified residues 1 and 3, but a difference analysis after Step 4 showed that Asx and 1 residue of serine were lost in addition to the residues directly identified.

Hydrolysis with chymotrypsin yielded three fractions after chromatography on Sephadex G-25 (Fig. 3). From each fraction a single peptide was obtained after purification by electrophoresis at pH 1.9 (Table I). Peptide C1 was resistant to hydrolysis by trypsin, suggesting a Lys—Pro bond, and to hydrolysis by chymotrypsin, suggesting a Tyr—Pro bond. After release of phenylalanine by carboxypeptidase A from Peptide C3, aminotripeptidase yielded tyrosine and a dipeptide that was yellow with ninhydrin; therefore Peptide C3 is Tyr-Gly-Met-Phe.

The sequence (Fig. 1*A*) was completed by studies on Peptides CN-13 and CN-14 (15), for which Peptide II-A1 provided the overlap.

Peptides of Fraction III

The chromatographic separation of Fraction III on Dowex 50-X2 was described previously (Fig. 3(1)) and the elution pattern (Fig. 4) is included here to show the five pooled fractions that yielded pure peptides. These are indicated as Fractions IIIA through IIIE. A ^{14}C-labeled peptide containing 22 residues was eluted between Fractions IIID and IIIE; this peptide was partially sequenced and described earlier (1). Its complete sequence is known from subsequent studies (16, 18). Only Fraction IIIB, which yielded Peptides III-B1 and IV-C, was purified on paper. The remaining peptides (Table II) were purified by chromatography on a column (0.9 × 20 cm) of Dowex 1-X2 (Figs. 5 to 8). The sequences of peptides from Fraction III are shown in Fig. 1*A*.

Peptide III-A1—The partial sequence was determined by Edman degradation. Step 1: PTH-Leu (G,R); Step 2: PTH-Gly (G,R); Step 3: PTH-Thr (R, aminobutyric acid); Step 4: (not determined); Step 5: PTH-Tyr (G,R); Step 6: PTH-Thr (G); Step 7: (not determined); Step 8: PTH-Leu (G,R); Step 9: PTH-Ile (G,R); Step 10: PTH-Ala (G,T,R); Step 11: PTH-Ala (G,T); Step 12: PTH-Leu (R). The order of Glx and serine in residues 4 and 7 was unknown. The peptide was derived from cleavage of an Asp—Pro bond.

Peptide III-A2—Edman degradation gave the 6 NH_2-terminal residues. Step 1: PTH-Asx (R); Step 2: PTH-Ile (G,R); Step 3: PTH-Pro (G,R); Step 4: PTH-Glx (R); Step 5: PTH-Gly (R, low recovery); Step 6: PTH-Gly (R). The fluorescence of the peptide showed that it contained a Lys(PLP) residue. The analysis (Table II) and the 6 residues identified by Edman degradation showed that this peptide was derived by tryptic hydrolysis between residues 2 and 3 of Peptide IV-01, and by cleavage at an Asp—Pro bond in Peptide IV-F2 to yield Peptide IV-06, Pro—Lys. These relationships were confirmed by isolation of a maleylated tryptic peptide from Peptide CN20 (16). As stated earlier, not all peptides containing PLP-lysine had been isolated from Fraction IV. The large size of this peptide, and its proximity to an acid-labile Asp—Pro bond may have accounted for the difficulty involved in its purification.

[2] Some of the data are presented as a miniprint supplement immediately following this paper. (Figs. 2 through 9, Tables I through XVII are found on pp. 8158–8159.) Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, Md. 20014. Request Document No. 77M-418, cite author(s) and include a check or money order for $4.05 per set of photocopies.

FIG. 1. *A*, amino acid sequences of peptides obtained from Fractions II and III of the tryptic hydrolysate. Peptides containing methionine show the overlaps with cyanogen bromide (CN) peptides described elsewhere (15-17). Residues determined by Edman degradation manually or by sequenator are marked →; by hydrolysis with carboxypeptidases ⟵, ⟵, or ⟵ respectively; by hydrazinolysis ⟵; ⟶, ⟶, or ⟶ for aminopeptidase M, leucine aminopeptidase (*LAP*), or aminotripeptidase, respectively. The following symbols ⟶, or ⟵, or ⟷ indicate identification by two methods. The following symbols are used for peptides derived by secondary hydrolysis: C, chymotrypsin; H, partial acid hydrolysis. Electrophoresis at pH 6.5 was used to determine whether the peptide was neutral, acidic, or basic, when indicated. *B*, sequences of peptides obtained from Fractions IV-C through IV-I of the tryptic hydrolysate (see legend of *A*); *C*, sequences of peptides from Fractions IV-J through IV-K of the tryptic hydrolysate (see legend of *A*); *D*, sequences of peptides from Fractions IV-L through IV-N of the tryptic hydrolysate (see legend of *A*); *E*, sequences of peptides from Fractions IV-O through IV-Q of the tryptic hydrolysate (see legend of *A*); *F*, sequences of peptides from Fraction V (see legend of *A*).

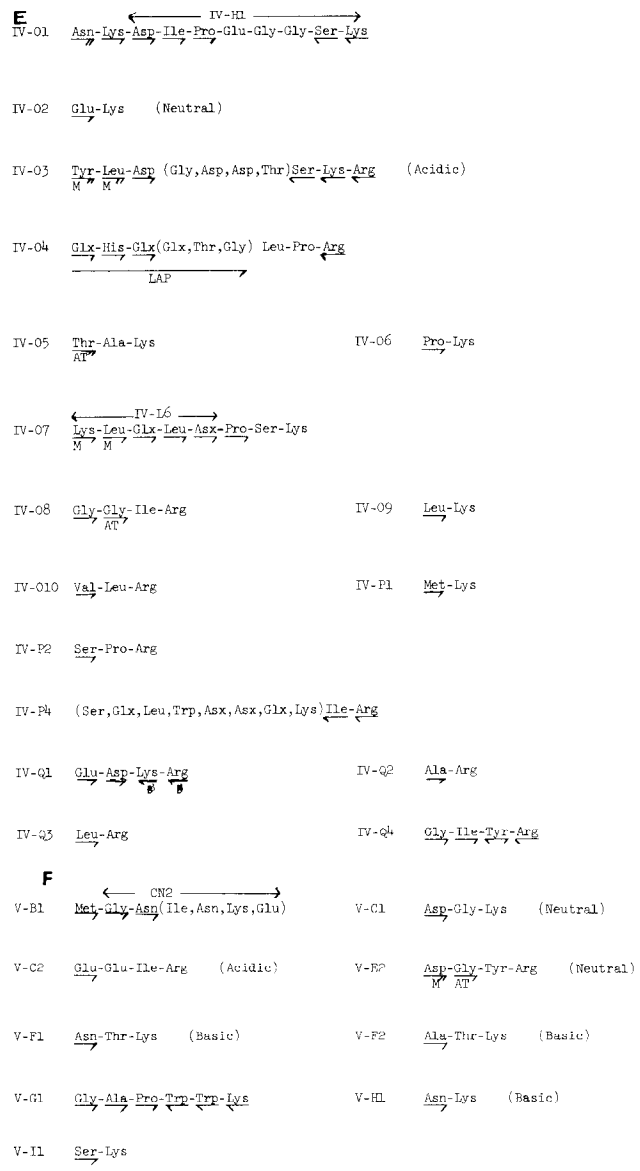

FIG. 1. *Continued.*

Peptide III-B1 – The limited amount of this peptide precluded sequence studies, other than with carboxypeptidases, but its unique composition later served to overlap Peptides CN17 and CN19 (2). Hydrolysis by carboxypeptidases A + B released 5 residues; no arginine was detected on electrophoresis at pH 1.9. Analysis: Ser, 1.8(2); Asn, 0.7(1); Val, 0.8(1); Leu, 1.0(1); Lys, (1) (not analyzed).

Peptide III-D1 – The partial sequence was obtained on the sequenator (Table III), the remainder was determined on Peptide CN30 (17). Leucine aminopeptidase released: Thr, 1.0; Glu, 0.7; Phe, 0.6; Ile, 0.5; Ser, 0.4; Tyr, 0.4; Asp, 0.2; Leu, 0.2. Carboxypeptidases A + B released equivalent amounts of arginine, leucine, and valine.

Peptide III-E – The partial sequence was obtained on the sequenator (Table IV), the remainder by studies on Peptide CN21 (16). Carboxypeptidases A + B released: Arg, 0.9; Leu, 0.9; Gln, 0.9.

Peptides of Fraction IV

The chromatography of Fraction IV on a column of Dowex 50-X2 was described in an earlier communication (Fig. 5(1)). The previous designations of the subfractions (IVA through IVQ) are retained in the present report. The methods for purification on paper and the migration of each peptide relative to a marker amino acid are indicated in the tables giving the compositions of these peptides (Tables V to XV). The sequences of the peptides from Fraction IV are shown in Fig. 1, *B* through *E*.

Peptide IV-C – Hydrolysis by carboxypeptidases A + B relased the five COOH-terminal amino acids: Lys, 1.0(1); Leu, 2.5(2); Ile, 1.0(1); Asn, 0.9(1). Hydrolysis by leucine aminopeptidase released the following: Ser, 1.1(1); Val, 0.8(1); Leu, 1.0(1); Ala, 1.2(1); Asp, 0.3(1). The peptide was neutral at pH 6.5, and hence contained 2 asparaginyl residues. Edman degradation identified the first 10 residues: Step 1: PTH-Ser (G); Step 2: PTH-Val (G,R); Step 3: PTH-Leu (G,R); Step 4: PGH-Ala (G,R); Step 5: PTH-Asx (R); Step 6: PTH-Ala (G,R). Step 7: PTH-Leu (G,R); Step 8: PTH-Pro (G,R); Step 9: PTH-Asn (G); Step 10: PTH-Leu (G,R). The sequence of the COOH-terminal residues was completed by studies on Peptide CN21 (16).

Peptide IV-D1 – Edman degradation was blocked after the first step, which identified the NH_2-terminal residue as PTH-Glx (R). Since the peptide was acidic at pH 6.5 and asparagine was found by hydrolysis with aminopeptidase M, the peptide contained 2 glutamic acid residues. Hydrolysis by carboxypeptidases A + B released Ser, 1.0(1); Leu, 0.6(1); Lys, (1) (not analyzed). Hydrolysis by aminopeptidase M released all the amino acids except proline and 1 glutamic acid residue, suggesting a Glu-Pro sequence: Met(SO_2), 0.6(1); Ser, 1.0(1); Asn, 1.2(1); Glu, 1.0(1); Ile, 1.0(1); Leu, 2.6(3); Tyr, 1.3(1).

Peptide IV-D2 – This ninhydrin-negative peptide was resistant to Edman degradation and to hydrolysis by aminopeptidases. Hydrolysis by carboxypeptidases A + B showed lysine and valine as the only ninhydrin-positive products. Rydon-Smith reagent spray indicated pyrrolidonecarboxylic acid.

Peptide IV-E – The low yield of this peptide precluded sequence studies, but its unique composition provided the important overlap between Peptides CN1 (15) and CN21 (16).

Peptide IV-F1 – Edman degradation identified the NH_2-terminal residues as Ser-Ala. Step 1: residue: Asp, 1.1(1); **Ser, 0.1(0)**; Thr, 1.0(1); Glu, 1.1(1); Pro, 1.0(1); Gly, 2.1(2); Ala, 2.0(2); Val, 2.9(3); Tyr, 0.9(1); Phe, 0.7(1); Lys, (1) (not determined); Step 2: PTH-Ala (R). Hydrolysis by carboxypeptidases A + B released 4 residues. Lys (1) (not determined); Gly, 1.0(1); Glu, 0.9(1); Phe, 1.0(1). Hydrolysis with chymotrypsin gave five peptides that were isolated by electrophoresis at pH 1.9. They were, in order of increasing mobility, Peptides C1 through C5. Peptide C1 was derived from the NH_2 terminus of the intact peptide: Thr, 1.0(1); Ser, 1.0(1); Gly, 1.0(1); Ala, 1.1(1); Val, 1.1(1); Tyr, 1.1(1) (yield, 71%). Edman degradation identified the NH_2-terminal residues as Ser-Ala-Thr: Step 1: PTH-Ser (G); Step 2: PTH-Ala (G); Step 3: PTH-Thr (G). Hydrolysis of the residual peptide with aminotripeptidase released valine and a dipeptide, yellow with ninhydrin, indicating glycine at residue 5.

Peptide C2: Pro, 0.9(1); Ala, 1.1(1); Val, 2.0(2); Phe, 1.0(1) (yield, 85%). Edman degradation identified the NH_2-terminal sequence as Val-Ala. Step 1: PTH-Val (G,R); Step 2: PTH-Ala (G). Hydrolysis of Peptide C2 by carboxypeptidase A released only phenylalanine. Hydrazinolysis of the carboxypeptidase A digest followed by amino acid analysis revealed phenylalanine and valine: Phe, 1.0(1); Val, 0.5(1). This permitted placing proline at residue 9 by difference. Hydrolysis of Peptide C2 by aminopeptidase M gave low recoveries of

alanine and proline, indicating an Ala-Pro sequence: Pro, 0.2(1); Ala, 0.4(1); Val, 2.0(2); Phe, 0.9(1).

Peptides C3 and C5 had an identical composition after hydrolysis, corresponding to the COOH-terminal tripeptide, Gln-Gly-Lys, C3 probably being the deamidation product of C5. Peptide C3: Lys, 1.0(1); Glu, 1.1(1); Gly, 1.2(1) (yield, 38%). Peptide C5: Lys, 1.0(1); Glu, 1.1(1); Gly, 1.2(1) (yield, 43%). Aminotripeptidase released glutamine from Peptide C5. Peptide C4 was an overlap of Peptides C2 and C5; Lys, 1.0(1); Glu, 1.2(1); Pro, 1.2(1); Gly, 1.2(1); Ala, 1.2(1); Val, 1.6(2); Phe, 1.0(1) (yield, 5.5%).

Peptide IV-F2 – Edman degradation identified the NH_2-terminal 4 residues: Step 1: PTH-Gly (R); Step 2: PTH-Val (R); Step 3: PTH-Ile (R); Step 4: PTH-Leu (R). The low yield of aspartic acid and proline after hydrolysis by aminopeptidase M indicated an Asp-Pro sequence: Lys, 1.0(1); Asp, 0.3(1); Pro, 0.3(1); Gly, 1.1(1); Val, 1.3(1); Ile, 1.0(1); Leu, 2.0(2). The sequence was completed by studies on Peptide CN20 (16).

Peptide IV-H1 – Hydrolysis by leucine aminopeptidase released only aspartic acid. Hydrolysis by carboxypeptidases A + B released 3 residues: Lys (1) not analyzed; Ser, 1.0(1); Gly, 0.5(1). Edman degradation completed the sequence: Step 1: PTH-Asp (R); Step 2: PTH-Ile (R); Step 3: PTH-Pro (R); Step 4: PTH-Glx (R); Step 5: PTH-Gly (R); Step 6: PTH-Gly (R).

Peptide IV-H2 – Five steps of Edman degradation gave the NH_2-terminal sequence as Asx-Leu-Phe-Asx-Glx: Step 1: PTH-Asx (R); Step 2: PTH-Leu (R). Step 3: PTH-Phe (R); Step 4: PTH-Asx (R); Step 5: PTH-Glx (R). After hydrolysis with chymotrypsin, two peptides were isolated by paper electrophoresis at pH 1.9. Peptide C1 was the NH_2-terminal peptide: Asp, 3.0(3); Glu, 1.1(1); Leu, 1.0(1); Tyr, 0.9(1); Phe, 0.9(1); yield, 62%. Hydrolysis with carboxypeptidase A released only tyrosine. Partial acid hydrolysis by the method of Schultz (14) released aspartic acid (143%), tyrosine (85%), and glutamic acid (23%). This indicated the presence of the COOH-terminal sequence Asx-Tyr and the sequence Asx-Glx-Asx.

Peptide C2: Arg, 1.0(1); Thr, 1.0(1); Ser, 1.1(1); Glu, 2.1(2); Gly, 1.1(1); Ala, 1.0(1); Leu, 1.1(1); yield, 56%. Hydrolysis with carboxypeptidases A + B released quantitatively, arginine, and 2 glutamine residues, and threonine in lower yield: Arg, 1.0(1); Gln, 2.0(2); Thr, 0.7(1). Edman degradation identified the NH_2-terminal sequence of Peptide C2 as Gly-Leu-Ala-Ser-Thr: Step 1: PTH-Gly (G,R); Step 2: PTH-Leu (G,R); Step 3: PTH-Ala (G,R); Step 4: PTH-Ser (G); Step 5: PTH-Thr (G); residue: Arg, 1.0(1); **Thr, 0.6(0)**; Ser, 0.6(0); Glu, 2.0(2); Gly, 0.3(0); Ala, 0.5(0); Leu, 0.4(0). The data permitted only partial assignments of the amides in Peptide IV-H2.

Hydrolysis of Peptide IV-H2 with aminopeptidase M indicated 1 residue each of aspartic and glutamic acids. Residue 1 was identified as asparagine by virtue of the yellow color with ninhydrin of both Peptides IV-H2 and C1. Residue 5 was identified by difference as the free glutamyl residue, since glutaminyl residues are at residues 13 and 14. The single aspartyl residue could be either at residue 4 or 6. The failure of chymotrypsin to hydrolyze at the phenylalanine residue suggested that residue 4 is aspartyl, since it would constitute, together with the adjacent glutamyl, a region of high negative charge that would inhibit the action of chymotrysin; this was confirmed by studies on Peptide CN20 (15).

Peptide IV-H3 – Edman degradation identified the NH_2 terminus: Step 1: PTH-Leu (R); Step 2: PTH-Glx (R); Step 3: (not determined); Step 4: PTH-Ala (R). Information on the COOH-terminal sequence came from studies on Peptide IV-N7, which possessed Leu-Arg (Peptide IV-Q3) attached to the NH_2-terminal end of Peptide IV-H3. Carboxypeptidases A + B released lysine (not analyzed) and quantitative amounts of tyrosine, valine, and isoleucine, together with lower amounts of other residues: Tyr, 0.9(1); Val, 1.2(1); Ile, 1.0(1); Gly, 0.4(1); Ala, 0.4(1); Gln, 0.5(1); Cys(Cm) 0.1(1). Chymotrypsin released only free lysine, indicating Tyr-Lys as the 2 COOH-terminal residues from which the adjacent pair of residues (Ile, Val) could be derived by difference from the carboxypeptidase data. Hydrolysis for 22 h of both Peptides IV-H3 and IV-N7 gave the low recoveries expected for an Ile—Val bond. The sequence was completed by studies on Peptide CN19 (16).

Peptide IV-H4 – Hydrolysis by aminopeptidase M revealed the presence of 3 residues of isoleucine as compared to 2.1 found after 22-h acid hydrolysis. This indicated the presence of an Ile-Ile sequence. The enzymic hydrolysate also gave free aspartic and glutamic acids: Arg, 0.8(1); Asp, 1.0(1); Thr, 1.1(1); Glu, 1.2(1); Gly, 1.0(1); Ile, 3.0(3); Leu, 1.1(1). The sequence was determined by Edman degradation: Step 1: PTH-Ile (R); Step 2: PTH-Gly (R); Step 3: PTH-Leu (R); Step 4: PTH-Asp (R); Step 5: residue: Ile, 1.1(2) (adjacent isoleucine residues); Gly, 0.3(0); Leu, 0.4(0); Asp, 0.5(0); **Thr,0.6(0)**; Glu, 1.0(1); Arg (1), (not analyzed); Step 6: PTH-Ile (R); Step 7: PTH-Ile (R).

Peptide IV-I – Edman degradation gave the following: Step 1: PTH-Ala (R); Step 2: PTH-Met (R); Step 3: PTH-Ile (R); Step 4: (not identified); Step 5: PTH-Asx (R); Step 6: PTH-Phe (R); Step 7: PTH-Asx (R). Hydrolysis by carboxypeptidases A + B released: Ala, 1.0(1); Ile, 0.9(1); Lys, (1) (not analyzed).

After hydrolysis with chymotrypsin, two peptides were separated by paper electrophoresis at pH 1.9. The composition of the slower migrating neutral Peptide C1 corresponded to the NH_2-terminal sequence of Peptide IV-I: Ala, 1.0(1); Met, 1.0(1); Ile, 1.1(1); Ser, 1.0(1); Asp, 1.1(1); Phe, 1.0(1); yield, 67%. Peptide C2 (neutral) was derived from the COOH terminus: Lys, 1.0(1); Asp, 1.0(1); Ala, 1.1(1); Ile, 1.1(1); yield, 74%. Edman degradation gave: Step 1: PTH-Asp (G,R); Step 2: PTH-Ile (G,R); Step 3: PTH-Ala (G). When the residual tripeptide after one step of Edman degradation of Peptide C2 was hydrolyzed by aminotripeptidase for 16 h, isoleucine was liberated in 18% yield; this confirmed the sequence of Peptide C2 and showed that tripeptides containing a COOH-terminal N^{ϵ}-(phenylthiocarbamyl)lysine residue can serve as a substrate for this enzyme.

Peptide IV-I – This peptide provided an overlap for Peptides CN18 and CN17 (15).

Peptide IV-J1 – Edman degradation gave: Step 1: PTH-Asx (R); Step 2: PTH-Ala (R); Step 3: residue: Asp, 1.0(1); Ala, 1.0(1); **Ser, 0.4(0)**; Lys (1) (not determined); Step 4: PTH-Ala (R). The intact peptide was neutral, indicating the presence of 1 asparaginyl residue; it was positioned from the results obtained by hydrolysis with carboxypeptidases A + B: Lys, 1.0(1); Asn, 1.1(1); Ala, 0.5(1); Ser, 0.2(2).

Peptide IV-J2 – Hydrolysis of this neutral peptide by carboxypeptidases A + B released only arginine.

Peptide IV-J4 – Edman degradation identified the NH_2-terminal residues: Step 1: PTH-Asp (G,R); Step 2: PTH-Val (G,R); Step 3: (not identified); analysis after Step 4 showed that residue 3 was serine; Step 4: PTH-Asn (G,R); residue: **Asp, 0.8(0)**; Val, 1.2(1); **Ser, 0.5(0)**; Glu, 3.1(3); Met, 1.8(2); Thr, 1.0(1); Ala, 1.0(1); Phe, 1.0(1); His (1) and Arg (1) (not analyzed). Chymotryptic hydrolysis of the residual peptide from the Edman degradation released only free arginine, thus suggesting the COOH-terminal sequence as Phe-Arg.

Hydrolysis of the intact peptide by carboxypeptidases A +

B released quantitatively 6 COOH-terminal residues: Arg (1) (not analyzed); Phe, 0.9(1); Ala, 1.1(1); Val, 1.0(1); Thr, 1.1(1); Met(SO_2), 1.0(1). The intact peptide was acidic at pH 6.5, and hence contained at least 2 glutamyl residues in addition to the NH_2-terminal aspartyl residue. This peptide provided the overlap for Peptides CN9 and CN14 (15); studies on these peptides completed the sequence of Peptide IV-J4.

Peptide IV-J5 – The sequence was determined by Edman degradation: Step 1: PTH-Pro (G,R); Step 2: PTH-Ala (G,R); Step 3: PTH-Ala (G,R); Step 4: PTH-Ser (G); Step 5: PTH-Ile (G,R); Step 6: PTH-Asn and PTH-Asp (G,R); Step 7: PTH-Gly (G,R). Electrophoresis at pH 6.5 gave two ninhydrin-positive spots of approximately equal intensity, which corresponded to neutral and basic peptides. Analysis of these peptides showed them to be of identical composition. The neutral peptide was therefore a deamidation product of the basic Peptide IV-J5, the latter containing asparaginyl residues. Hydrolysis of Peptide IV-J5 by leucine aminopeptidase released the first 6 residues: Pro, 1.1(1); Ala, 1.8(2); Ser, 0.7(1); Ile, 0.9(1); Asn, 0.9(1); Gly, 0.1(1).

Peptide IV-J6 – Four steps of Edman degradation identified the NH_2-terminal sequence: Step 1: PTH-Ala (R); Step 2: PTH-Glx (R); Step 3: residue: Ala, 0.1(0); Glu, 0.4(0); **Thr, 0.5(0)**; Pro, 0.8(1); Gly, 1.1(1); Ile, 1.0(1); Lys (1) (not analyzed). Step 4: residue: Ala, 0.2(1); Glu, 0.5(0); Thr, 0.5(0); **Pro, 0.5(0)**; Gly, 1.2(1); Ile, 1.0(1); Lys (1) (not analyzed). Hydrolysis by carboxypeptidases A + B released only lysine and isoleucine. Hydrolysis by aminopeptidase M gave low recoveries of threonine and proline, confirming the sequence Thr-Pro: Ala, 1.0(1); Glu, 0.8(1); Thr, 0.05(1); Pro, 1.0(1); Gly, 0.3(1); Ile, 0.2(1); Lys (1) (not analyzed).

Peptide IV-J7 – The sequence was identified by Edman degradation: Step 1: PTH-Gly (R); Step 2: PTH-Asx (R); Step 3: PTH-Ala (R); Step 4: PTH-Pro (R); Step 5: PTH-Glx (R); Step 6: PTH-Phe (G,R); Step 7: PTH-Tyr (G,R); Step 8: PTH-Glx (R); Step 9: PTH-Ala (G); Step 10: PTH-Tyr (G,R); Step 11: PTH-Val (G). Carboxypeptidases A + B released quantitatively the 7 COOH-terminal residues, including glutamine: Gln, 1.0(1); Ala, 1.0(1); Val, 1.1(1); Tyr, 2.1(2); Phe, 0.9(1); Lys (1) (not analyzed). This neutral peptide indicated the presence of a single acidic residue; this was identified as glutamic acid by hydrolysis with aminopeptidase M: Asn + Gln, 2.0(2); Glu, 1.0(1); Gly, 1.0(1); Ala, 0.9(2); Pro, 0.0(1); Val, 0.7(1); Tyr, 2.4(2); Phe, 1.2(1); Lys (1) (not analyzed). The absence of proline and 1 eq of alanine was in accord with the sequence Ala-Pro.

Peptide IV-J7 was also isolated with a blocked NH_2-terminal residue. Hydrolysis with carboxypeptidases A + B released the same seven amino acids as in the study of unblocked peptide: Gln, 1.0(1); Ala, 1.0(1); Val, 0.9(1); Tyr, 1.9(2); Phe, 1.2(1); Lys (1) (not analyzed). However, the residual pentapeptide, which was ninhydrin-negative, was isolated after electrophoresis on paper at pH 1.9; it was located with the Rydon-Smith reagent. It also failed to undergo Edman degradation or hydrolysis with aminopeptidase M. The blocking group was not identified, but could have arisen by cyclization after an α and β shift at the asparagine residue, as described by Jörnvall (19).

Peptide IV-J8 – Edman degradation identified the NH_2-terminal sequence: Step 1: PTH-Leu (R); Step 2: PTH-Glx (R); Step 3: PTH-Val (R); Step 4: PTH-Ile (R). Hydrolysis of the residual peptide with aminotripeptidase released serine. The position of serine was confirmed by Step 5 of Edman degradation which gave the residue: **Ser, 0.5(0)**; Asp, 1.1(1); Arg, 1.0(1).

Peptide IV-K1 – Edman degradation established the NH_2-terminal residue: PTH-Asx (R). Hydrolysis of the residual peptide by aminotripeptidase released isoleucine.

Peptide IV-K2 – Edman degradation established the NH_2-terminal sequence as Ser-Pro-Gly-Val. Step 1: residue: **Ser, 0.1(0)**; Glu, 1.1(1); Pro, 1.0(1); Gly, 2.1(2); Val, 1.0(1); Leu, 1.0(1); Lys (1) (not analyzed); Step 2: PTH-Pro (R); Step 3: PTH-Gly (R); Step 4: PTH-Val (R), residue: Ser, 0.1(0); Glu, 1.0(1); Pro, 0.2(0); Gly, 1.1(1); **Val 0.2(0)**; Leu, 0.9(1); Lys, (1) (not analyzed). Carboxypeptidases A + B released lysine and glutamine after 20 min, and after 6 h allowed identification of the COOH-terminal sequence as Leu-Gly-Gln-Lys: Lys, (1) (not analyzed); Gln, 1.0(1); Gly, 0.4(1); Leu, 0.2(1).

Peptide IV-K3 – Edman degradation gave the NH_2-terminal sequence as Gly-Val-Leu. Step 1: PTH-Gly (R); Step 2: PTH-Val (R); Step 3: PTH-Leu (R). Hydrolysis by carboxypeptidases A + B allowed identification of the COOH-terminal sequence as Leu-Gly-Gln-Lys; Lys, (1) (not analyzed); Gln, 1.0(1); Gly, 0.5(1); Leu, 0.5(1). This peptide was formed presumably by the loss of the NH_2-terminal 2 residues from Peptide IV-K2 (18).

Peptide IV-K4 – The sequence was determined by Edman degradation: Step 1: residue: Asp, 1.0(1); **Thr, 1.8(2)**; Pro, 1.0(1); Phe, 1.8(2); Lys, (1) (not analyzed); Step 2: PTH-Asx (R); Step 3: PTH-Phe (R); Step 4: PTH-Phe (R); Step 5: residue: Asp, 0.2(0); **Thr, 1.2(1)**; Pro, 1.0(1); Phe, 0.3(0); Lys, 0.5(1); Step 6: PTH-Pro (R): residue: Asp, 0.3(0); Thr, 1.0(1); **Pro, 0.5(0)**; Phe, 0.3(0); Lys (1) (not analyzed). Hydrolysis with carboxypeptidases A + B released only lysine. Hydrolysis with aminopeptidase M yielded asparagine.

Peptide IV-K5 – The sequence was determined by Edman degradation: Step 1: PTH-Val (R); Step 2: PTH-Phe (R); Step 3: PTH-Asx (R); Step 4: PTH-Asx (R); Step 5: PTH-Ala (R); Step 6: PTH-Val (R). Hydrolysis by carboxypeptidases A + B gave 2 residues of asparagine: Asn, 2.0(2); Ala, 1.0(1); Val, 2.0(2); Leu, 1.1(1); Phe, 0.9(1); Lys, (1) (not analyzed).

Peptide IV-K6 – Edman degradation gave: Step 1: PTH-Leu (R); Step 2: PTH-Val (R); Step 3: PTH-Val (R); Step 4: PTH-Ala (R); Step 5: PTH-Phe (R).

Peptide IV-L1 – Edman degradation identified the NH_2-terminal sequence as Glu-Val; the degradation was blocked after the second step, probably owing to cyclization of the glutaminyl residue. Step 1: PTH-Glu (G,R); Step 2: PTH-Val (G,R). Hydrolysis by carboxypeptidases A + B released lysine, asparagine, and glutamine: Lys, 1.0(1), Asn + Gln, 2.1(2). Partial acid hydrolysis released lysine (80%) and aspartic acid (67%), allowing identification of the COOH-terminal sequence as Asn-Lys.

Peptide IV-L2 – Edman degradation gave: Step 1: PTH-Ile (R); Step 2: PTH-Glx (R); Step 3: PTH-Asx (R); Step 4: PTH-Asx (R). Hydrolysis by aminopeptidase M released only isoleucine and glutamine: Ile, 1.0(1); Gln, 0.5(1). Hydrolysis by carboxypeptidases A + B liberated arginine, alanine, and asparagine: Arg, 1.0(1); Ala, 1.0(1); Asn, 0.3(1). The neutrality of the peptide at pH 6.5 indicated the presence of aspartic acid at position 3.

Peptide IV-L3 – Edman degradation gave: Step 1: PTH-Ala (R); Step 2: PTH-Glx (R); Step 3: PTH-Glx (R). Hydrolysis with carboxypeptidases A + B at pH 6.5 released all the residues: Lys, 1.0(1); Met (SO_2), 1.0(1); Gln, 0.7(1); Glu, 0.7(1); Ala, 0.7(1). Hydrolysis by aminopeptidase M for 20 min, 60 min, and 22 h gave the ratio of glutamine to glutamic acid as 0.50, 0.68, and 0.78, respectively, allowing identification of the sequence -Glu-Gln-.

Peptide IV-L4 – Edman degradation gave: Step 1: PTH-Leu (R); Step 2: PTH-Glx (R). The presence of a glutamyl residue was established by hydrolysis with aminopeptidase M.

Peptide IV-L5 – Edman degradation allowed identification of the peptide as part of Peptide TIV-L21(1): Step 1: PTH-Glx (R); Step 2: PTH-Ala (R); Step 3: PTH-Tyr (R); Step 4: PTH-Glx (R). Hydrolysis with aminopeptidase M yielded 1 residue each of glutamic acid, glutamine, and asparagine: Asn + Gln, 2.0(2); Glu, 1.0(1); Ala, 2.0(2); Ile, 1.0(1); Tyr, 0.9(1). Since glutamine was previously shown to be among the 6 COOH-terminal residues of Peptide IV-L21 by hydrolysis with carboxypeptidases A + B (1), the NH_2-terminal residue of Peptide IV-L5 and hence residue 3 in Peptide IV-L21 were identified as glutamic acid.

Peptide IV-L6 – The peptide is the product of an Asp-Pro cleavage of Peptide IV-07.

Peptide IV-L7 – Edman degradation gave: Step 1: PTH-Ala (G,R); Step 2: PTH-Ile (G,R); Step 3: PTH-Phe (G,R); Step 4: PTH-Gly (G,R); Step 5: residue: Ala, 1.0(1); Ile, 0.2(0); Phe, 0.3(0); Gly, 0.4(0); **Ser, 1.1(1)**; Tyr, 1.0(1); Leu, 1.0(1); Arg (1) (not analyzed); Step 6: PTH-Tyr (G); Step 7: PTH-Leu (G,R). Hydrolysis of the residual tripeptide with aminotripeptidase released alanine and a basic peptide.

Peptide IV-M1 – Edman degradation yielded: Step 1: PTH-Glx (R); Step 2: residue: Glu, 0.3(1); **Asp, 0.3(0)**; Ser, 1.0(1); Lys (1) (not determined). This neutral peptide gave on hydrolysis with aminopeptidase M: Ser, 1.2(1); Asn, 0.8(1); Glu, 1.0(1); Lys (1) (not analyzed).

Peptide IV-M2 – Hydrolysis by aminopeptidase M released: Leu, 1.0(1); Val, 0.9(1); Asp, 0.5(1); Arg (not determined). Hydrolysis by carboxypeptidases A + B released only arginine. Edman degradation gave: Step 1: PTH-Leu (G); Step 2: PTH-Val (R).

Peptide IV-M3 – Edman degradation gave: Step 1: PTH-Val (G,R); Step 2: PTH-Ala (G,R); Step 3: PTH-Leu (G,R); Step 4: residue: Val, 0(0); Ala, 0.1(0); Leu, 0.05(0); **Ser, 0.3(1)**; Phe, 1.0(1); Arg (1) (not analyzed); Step 5: PTH-Phe (R).

Peptide IV-M4 – Edman degradation gave: Step 1: PTH-Met (G,R); Step 2: PTH-Phe (G,R); Step 3:PTH-Leu (R); Step 4: PTH-Ala (R).

Peptide IV-N1 – Edman degradation identified the NH_2-terminal residue as Glx: PTH-Glx (R).

Peptide IV-N2 – Edman degradation gave: Step 1: PTH-Ala (R); Step 2: PTH-Lys (R); Step 3: PTH-Pro (R). Hydrolysis with carboxypeptidases A + B for 24 h released lysine and serine.

Peptides IV-N3 and IV-N4 – These two tripeptides could not be separated by various methods. The COOH-terminal lysine residues were removed from both peptides by treatment with carboxypeptidase B. The two residual dipeptides, Leu-Ser and Ile-Thr, were separated by paper electrophoresis at pH 3.6 and each was then subjected to Edman degradation.

Peptide IV-N5 – Edman degradation gave: Step 1: PTH-Glx (R); Step 2: PTH-Tyr (R). The neutral peptide must contain a glutamyl residue.

Peptide IV-N6 – Aminotripeptidase released leucine. Edman degradation gave PTH-Leu (R).

Peptide IV-N7 – See Peptide IV-H3.

Peptide IV-N8 – The NH_2-terminal residue by Edman degradation: PTH-Ala (R). Hydrolysis of the residual peptide with aminotripeptidase released alanine.

Peptide IV-N9 – Hydrolysis with aminopeptidase M showed this to be a tripeptide: Lys, 0.9(1), Val, 1.0(1); Ile, 1.0(1). Hydrolysis by aminotripeptidase released isoleucine.

Peptide IV-N10 – The sequence was determined by Edman degradation: Step 1: residue: **Ser, 0.2(0)**; Phe, 1.8(2); Thr, 1.0(1); Gly, 1.1(1); Lys (1) (not determined); Step 2: PTH-Phe (R); Step 3: PTH-Phe (R); Step 4: residue: Ser, 0.2(0); Phe, 0.2(2); **Thr, 0.4(0)**; Gly, 1.0(1); Lys (1) (not determined). Hydrolysis with carboxypeptidases A + B resulted in complete hydrolysis: Thr, 1.0(1); Ser, 1.0(1); Gly, 1.0(1); Phe, 2.2(2); Lys (1) (not analyzed).

Peptide IV-N11 – Hydrolysis with aminotripeptidase released leucine. Edman degradation gave PTH-Leu (R).

Peptide IV-O1 – This peptide overlaps Peptide IV-H1 (Fig. 1*E*). Edman degradation identified the NH_2-terminal sequence: Step 1: PTH-Asx (R); Step 2: PTH-Lys (R); Step 3: PTH-Asx (R); Step 4: not identified but difference analysis after Step 5 indicated residue 4 as isoleucine; Step 5: PTH-Pro (R): residue: Asp, 0.6(0); **Ile, 0.3(0)**; **Pro, 0.4(0)**; Glu, 1.2(1); Gly, 2.0(2); Ser, 1.0(1); Lys (1) (not determined). The presence of Ile-Pro was also indicated by the low recoveries of these amino acids after hydrolysis with aminopeptidase M; Asp, 1.3(1); Ser, 1.3(1); Asn, 0.6(1); Pro, 0.0(1); Glu, 0.9(1); Gly, 2.0(2); Ile, 0.2(1); Lys (2) (not analyzed). By comparison with Peptide IV-H1, the asparagine was assigned to residue 1.

Peptide IV-O2 – The sequence was confirmed by Edman degradation: PTH-Glu (G).

Peptide IV-O3 – This acidic peptide must contain 3 residues of aspartic acid. Edman degradation gave the sequence, Tyr-Leu-Asp: Step 1: PTH-Tyr (R); Step 2: PTH-Leu (R); Step 3: PTH-Asp (R). Hydrolysis of the intact peptide with carboxypeptidases A + B released arginine, lysine, and serine. A time study with carboxypeptidase B gave the COOH-terminal sequence as Ser-Lys-Arg. The sequence was completed by studies of Peptide TM5F (18).

Peptide IV-O4 – Edman degradation identified the NH_2-terminal sequence as Glx-His-Glx; the degradation was blocked after the third step: Step 1: PTH-Glx (R); Step 2: PTH-His (R); Step 3: PTH-Glx (R). Hydrolysis by aminopeptidase M released all the amino acids except proline and a low yield of leucine, suggesting the sequence Leu-Pro; Thr, 0.9(1); Gln, 0.5(1); Glu, 2.4(2); Gly, 1.1(1); Leu, 0.5(1); His (1) and Arg (1) (not analyzed). The COOH-terminal sequence Leu-Pro-Arg was indicated by hydrolysis with leucine aminopeptidase, which released all the amino acids except leucine, proline, and arginine: Thr, 1.0(1); Gln, 1.0(1); Glu, 2.3(2); Gly, 1.1(1); His, 1.0(1). Prolonged hydrolysis with carboxypeptidases A + B yielded only arginine, in agreement with the indicated COOH-terminal sequence. The sequence was completed by studies on Peptide CN21 (16).

Peptide IV-O5 – Hydrolysis with aminotripeptidase released threonine. One step of Edman degradation confirmed the sequence; residue: **Thr, 0.3(0)**; Ala, 1.0(1); Lys, (1) (not determined).

Peptide IV-O6 – The expected sequence was confirmed by Edman degradation: PTH-Pro (R).

Peptide IV-O7 – The sequence was determined by Edman degradation: Step 1: PTH-Lys (R); Step 2: PTH-Leu (R); Step 3: PTH-Glx (R); Step 4: PTH-Leu (R); Step 5: PTH-Asx (R); Step 6: residue: **Pro, 0.4(0)**; Ser, 1.0(1); Lys (1) (not determined).

Peptide IV-O8 – Edman degradation yielded glycine: PTH-Gly (R). Hydrolysis of the residual peptide by aminotripeptidase released glycine.

Peptide IV-O9 – The expected sequence was confirmed by Edman degradation: PTH-Leu (R).

Peptide IV-O10 – Edman degradation: PTH-Val (R).

Peptide IV-P1 – The sequence was verified by Edman degradation: PTH-Met(R).

Peptide IV-P2 – The peptide was not hydrolyzed by aminotripeptidase, suggesting that residue 2 was proline. This was confirmed by Edman degradation: residue: **Ser, 0.2(0)**; Pro, 1.1(1); Arg, 1.0(1).

Peptide IV-P4 – Hydrolysis with carboxypeptidases A + B for 3 h released only arginine and isoleucine. The sequence was elucidated by studies on Peptide CN21 (16).

Peptide IV-Q1 – Edman degradation gave: Step 1: PTH-Glx (R); Step 2: PTH-Asx (R). The COOH-terminal sequence was identifed as Lys-Arg by determining the order of release of lysine and arginine during hydrolysis by carboxypeptidase B. The presence of glutamic and aspartic acid residues was established by prolonged hydrolysis with carboxypeptidases A + B: Glu, 0.5(1); Asp, 0.5(1); Lys, 0.9(1); Arg, 1.0(1).

Peptide IV-Q2 – One step of Edman degradation gave PTH-Ala (R).

Peptide IV-Q3 – Edman degradation gave PTH-Leu (R).

Peptide IV-Q4 – Edman degradation gave: Step 1: PTH-Gly (R); Step 2: PTH-Ile (R); Step 3: PTH-Tyr (R). Hydrolysis by carboxypeptidases A + B gave Arg, 1.0(1); Tyr, 1.1(1).

Peptides of Fraction V

Nine peptides were obtained from Fraction V that were previously unknown (Fig. 1*F*). Twenty other peptides in Fraction V were also found in Fraction IV, and the subfractions of Fraction V containing these are indicated in the appropriate tables. Fraction V was fractionated on Dowex 50 developed with a pyridine/acetate pH gradient (Fig. 9). The pooled fractions were purified by chromatography with BPAW and paper electrophoresis at pH 1.9. The compositions of the unique peptides and the methods of purification are given in Tables XVI and XVII.

Peptide V-B1 – Hydrolysis with aminopeptidase M, followed by electrophoresis at pH 1.9 and 6.5 showed the presence of asparagine, glutamic acid, and no aspartic acid; methionine was present as an oxide. Carboxypeptidases A + B released no free amino acids, suggesting that lysine is not COOH-terminal. Edman degradation identified the NH_2-terminal 3 residues: Step 1: residue: Asp, 2.0(2); Ser, 0.1(0); Glu, 1.0(1); Gly, 1.1(1); Ala, 0.1(0); **Met, 0.2(0)**; Ile, 0.9(1) and Lys (not determined); Step 2: PTH-Gly (R); Step 3: residue: **Asp, 1.6(1)**; Ser, 0.2(0); Glu, 1.0(1); Gly, 0.6(0); Ala, 0.1(0); Met, 0.1(0); Ile, 0.9(1) and Lys (not determined). The sequence and composition are in accord with this peptide being the COOH-terminal portion of the protein, since it contains NH_2-terminal methionine plus the sequence of Peptide CN2 (15).

Peptide V-C1 – This neutral peptide gave PTH-Asp by Edman degradation.

Peptide V-C2 – Edman degradation gave: Step 1: residue: **Glu, 1.3(1)**; Ile, 1.0(1); and Arg (not determined). The residual tripeptide after one step of the Edman degradation was not a substrate for aminotripeptidase, confirming glutamic acid at the NH_2-terminal of this tripeptide, since the enzyme does not release NH_2-terminal acidic residues.

Peptide V-E2 – Aminopeptidase M released aspartic acid and the other residues. Edman degradation gave PTH-Asp (R). Aminotripeptidase released glycine from the residual tripeptide.

Peptide V-F1 – Edman degradation released PTH-Asn (G) from this basic peptide. It is part of Peptide IV-M13 (1).

Peptide V-F2 – Edman degradation gave PTH-Ala (R). It comprises part of Peptide IV-M13 (1).

Peptide V-G1 – Aminopeptidase M released: Trp, 1.9(2); Lys, 1.0(1); Pro, 0.8(1); Gly, 1.1(1); Ala, 0.7(1); Leu, 0.1(0). Carboxypeptidases A + B released only tryptophan and lysine in a ratio of 1.75:1. The peptide remaining after treatment with the carboxypeptidases was yellow with ninhydrin. Three steps of the Edman degradation gave: Step 1: PTH-Gly (R); Step 2: PTH-Ala (R); Step 3: PTH-Pro (R).

Peptide V-H1 – One step of the Edman degradation released PTH-Asn (G). It is part of Peptide IV-O22 (1).

Peptide V-I1 – This is part of Peptide IV-L21 (1).

DISCUSSION

In the present study, 80 additional tryptic peptides are described. Together with the six reported earlier (1), and excluding overlapping peptides, these account for 568 residues of the subunit sequence of the NAD-specific glutamate dehydrogenase. Obviously, this is only about 55% of the estimated 1030 residues in the subunit (3). It should be noted, however, that this was accomplished from only 9.6 μmol of starting material, calculated for the subunit. Indeed, the initial purpose of the study was to obtain the peptides containing the PLP-lysine residues and the [^{14}C]cysteine-containing peptide. The isolation of additional tryptic peptides was undertaken to take advantage of material already in hand. All of these peptides have been useful in diminishing the task of sequencing the larger peptides produced by cyanogen bromide fragmentation (15–17) and by tryptic hydrolysis of the maleylated protein (18). Also, several of the tryptic peptides, particularly those containing methionine, provided important overlaps for the larger peptides isolated after other types of cleavage.

In addition to the five peptides containing lysine residues labeled with pyridoxal 5′-phosphate, another larger labeled peptide was also isolated. In contrast to the peptides that were isolated earlier, this new peptide shows homology with the sequences of the single reactive lysine residue of other glutamate dehydrogenases, as discussed in the preceding paper (2).

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Supplementary Material

to

Nicotinamide Adenine Dinucleotide-specific Glutamate Dehydrogenase of Neurospora V. Tryptic Peptides

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Fig. 2. Chromatography of Fraction IIA on a column (0.8 X 42 cm) of QAE-Sephadex (A-25) with a linear gradient of KCl in 10 mM Tris chloride buffer, pH 9.0, containing 8 M urea. Fractions of 7.5 ml were collected at a flow rate of 37 ml per h and monitored by absorbance at 225 nm; (—) absorbance; (- - -) molarity of KCl. Fraction II-A1 is indicated by a solid bar.

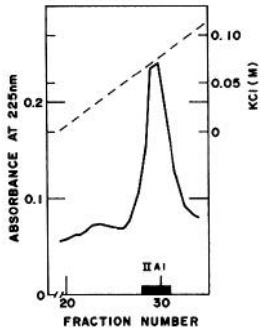

Fig. 3. Chromatography of the chymotrypsin digest of Peptide II-A1 on a column (1 X 158 cm) of Sephadex G-25 (Fine) with 30% acetic acid as the eluting phase. Fractions of 1.3 ml were collected at a flow rate of 9.3 ml per hour and monitored by measuring absorbance at 280 nm. Pooled fractions are indicated by a solid bar.

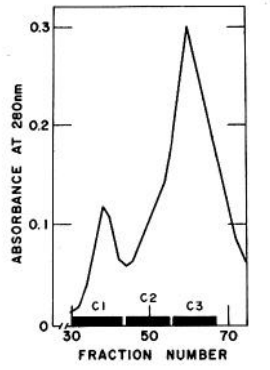

Fig. 4. Chromatography of Fraction III on a column (1 X 50 cm) of Dowex 50-X2 with pH 5.50 to 4.20 gradient buffer. Fractions of 3 ml were collected at a flow rate of 18 ml per hour. (—) ninhydrin assay; (- - -) radioactivity. Pooled fractions are indicated by solid bars.

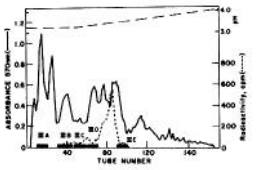

Fig. 5. Chromatography of Fraction IIIA on a column (0.9 X 20 cm) of Dowex 1-X2 maintained at 40° and eluted as described under Methods. Fractions were monitored by ninhydrin assay after alkaline hydrolysis and by measuring pH; (—) ninhydrin assay; (- - -) pH. Pooled fractions are indicated by solid bars.

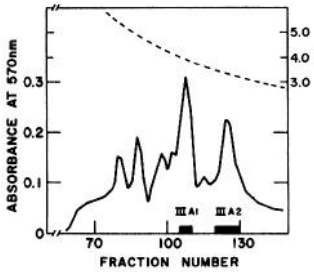

Fig. 6. Chromatography of Fraction IIIC etc. (same as Fig. 5).

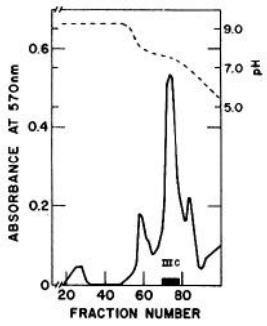

Fig. 7. Chromatography of Fraction IIID etc. (same as Fig. 5).

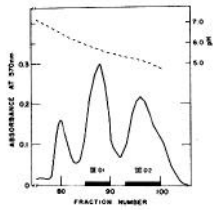

Fig. 8. Chromatography of Fraction IIIE etc. (same as Fig. 5).

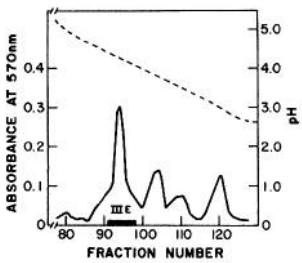

Fig. 9. Chromatography of Fraction V on a column (0.9 X 60 cm) of Dowex 50-X8, 200-400 mesh (pyridinium form) that was eluted initially with 60 ml of 0.2M pyridine-acetate, pH 3.5. The elution was continued with a linear gradient between 0.2M (pH 3.5), and 2.0M (pH 4.9) pyridine-acetate (540 ml); followed by 2.0M pyridine-acetate, pH 4.9 (60 ml) and 1% pyridine. All eluting solvents were made up in 20% 1-propanol. The column was maintained at 40° and fractions of 3 ml were collected at a flow rate of 18 ml per hour. Fractions were monitored by ninhydrin assay after alkaline hydrolysis and by measuring pH; (—) ninhydrin assay; (- - -) pH. Pooled fractions are indicated by solid bars.

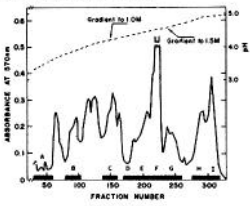

Table I

Compositions of Peptide II-A1 and its chymotryptic peptides

Amino acid	II-A1	II-A1-C1	II-A1-C2	II-A1-C3
Lysine	1.1(1)	0.9(1)		
Arginine	1.0(1)		1.1(1)	
Aspartic acid	1.4(1)	1.0(1)		
Threonine	0.9(1)		0.8(1)	
Serine	3.0(5)	1.0(1)	2.0(2)	
Glutamic acid	3.1(5)	1.8(2)	1.1(1)	
Proline	4.0(4)	4.2(4)		
Glycine	1.7(1)[W]	0.5(0)		1.5(1)
Alanine	0.7(0)[W]			
Valine	1.8(2)	0.8(1)	0.7(1)	
Methionine	1.1(1)			1.0(1)
Isoleucine	1.0(1)		0.8(1)	
Leucine	4.0(4)	2.4(3)	0.9(1)	
Tyrosine	1.9(2)	1.0(1)		0.7(1)
Phenylalanine	2.1(2)	1.0(1)		1.1(1)
Total Residues	27	15	8	4
R_{Asp} (1.9)		0.86	1.23	0.75
Yield (%)	4.5	1.7	1.0	0.4

[W] Contains an impurity as based on later work.

Table II

Tryptic peptides from fraction III

Amino acid	III-A1	III-A2	III-B1	III-C	III-D1	III-D2	III-E
Tryptophan				0.4(1)			
Lysine		0.6(1)[W]	1.0(1)	0.8(1)		0.5(0)	0.9(1)
Histidine					0.9(1)	0.9(1)	
Arginine			1.1(1)	0.8(1)	1.0(1)	[W](1)	1.0(1)
Aspartic acid	1.8(1)	1.9(2)	5.1(5)	1.4(1)	1.2(1)	1.2(1)	3.5(3)
Threonine	1.8(2)		1.0(1)	1.0(1)	2.6(3)	2.1(2)	1.1(1)
Serine	1.0(1)	1.3(1)	3.0(3)	1.9(2)	2.2(2)	2.8(3)	3.9(4)
Glutamic acid	1.2(1)	1.3(1)	1.2(1)	2.4(2)	3.3(3)	3.4(3)	2.3(2)
Proline		0.9(1)	2.4(2)	2.7(3)	2.7(2)	2.3(2)	
Glycine	1.1(1)	3.1(3)	3.4(3)	1.0(1)		0.4(0)	0.4(0)
Alanine	1.9(2)					0.5(0)	1.2(1)
Valine		0.9(1)[W]	2.0(2)	1.2(1)	0.8(1)	0.9(1)	0.9(1)
Methionine			1.0(1)			0.3(0)	
Isoleucine	0.9(1)	1.4(2)[W]	2.1(2)	1.2(1)	2.3(3)[W]	1.7(2)	1.2(1)
Leucine	2.7(3)	1.6(2)	1.1(1)	3.4(3)	2.2(2)	2.0(2)	4.9(5)
Tyrosine	0.8(1)			1.3(1)	1.0(1)	1.1(1)	
Phenylalanine			1.0(1)	1.1(1)	1.4(1)	2.1(2)	
Cys (CM)			0.6(1)				
Total residues	13	14	25	20	21	21	20
R_{Asp} (1.9)	1.56	0.72	0.86	0.79	0.66	0.64	
R_{Leu} (BPAW)			0.45				
Yield (%)	1.7	1.0	5.1	5.1	5.1	5.6	5.3
Purified, PE, 1.9	+	+	+	+	-	-	-
Purified, BPAW	-	-	+	+	-	-	-

[W] As PLP-lysine.

[W] Sample lost.

[W] Low analyses because of acid resistant Val-Ile or Ile-Ile bonds.

Table III

Automated Edman degradation of Peptide III-D1

Step	Residue	Yield (%)	Method
1	Thr		G
2	Glu	20	G,T,R
3	(Thr)[W]		
4	Phe	14	G,T,R
5	(Thr)		
6	(Pro)		
7	Asp		R
8	Tyr	6	G,T,R
9	Ile	4	R
10	Leu	3	R

[W] Residues in parentheses were not identified

Table IV

Automated Edman degradation of Peptide III-E

Step	Residue	Yield (%)	Method
1	Ser		G,T
2	Val	32	G
3	Leu	26	G,R
4	(Ser)[W]		
5	Asp	20	G,T,R
6	Lys		T,R
7	Leu	22	G,R
8	(Ser)		
9	Leu	18	G,R
10	Ala	7	T,R
11	Ile	16	G,T,R
12	(Thr)		
13	(Ser)		
14	Leu	5	G,R
15	Asp		T,R
16	Gln		T,R
17	Asx		R
18	Leu	2	G,R

[W] Residues in parentheses were not identified

Table V

Tryptic peptides from Fractions IV-C, D, E and F

Amino acid	IV-C	IV-D1	IV-D2	IV-E	IV-F1	IV-F2
Found also in fraction	III-B				IV-G	
Lysine	0.8(1)	0.9(1)	0.9(1)	0.9(1)	1.0(1)	1.0(1)
Aspartic acid	3.1(3)	1.2(1)		1.5(1)		1.0(1)
Threonine					1.0(1)	
Serine	0.7(1)	1.0(1)		4.3(4)	1.0(1)	
Glutamic acid		2.0(2)	1.1(1)	2.1(3)	1.1(1)	
Proline	1.2(1)	1.1(1)		1.4(1)	1.0(1)	1.0(1)
Glycine				1.5(1)	2.0(2)	1.1(1)
Alanine	2.0(2)			1.1(1)	2.1(2)	
Valine	1.0(1)		1.0(1)	1.1(1)	3.1(3)	0.7(1)[W]
Methionine		1.1(1)		1.0(1)		
Isoleucine	1.0(1)	1.0(1)				0.6(1)[W]
Leucine	4.0(4)	2.9(3)		2.1(2)	1.0(1)	2.0(2)
Tyrosine		0.9(1)		4.0(4)	1.0(1)	
Phenylalanine						
Total residues	14	12	3	20	14	8
R_{Asp} (1.9)	0.70	0.86	1.0	0.0	1.18	1.35
R_{Leu} (BPAW)	0.93	0.53	0.53	1.06	0.60	1.0
Yield (%)	9.1	9.4	10.4	3.4	18.9	10
Purified, PE, 1.9	-	+	+	+	-	-
Purified, BPAW	+	+	+	+	+	+

[W] Acid-resistant bond.

Table VI

Tryptic peptides from Fraction IV-H, and J

Amino acid	IV-H1	IV-H2	IV-H3	IV-H4	IV-I	IV-J1	IV-J2
Found also in fraction	V-A IV-G					V-B	
Lysine	1.0(1)		0.9(1)		1.0(1)	1.0(1)	
Arginine		1.0(1)		0.8(1)[W]			1.0(1)
Aspartic acid	1.0(1)	3.1(3)		1.0(1)	2.2(2)	2.1(2)	0.9(1)
Threonine		1.0(1)		0.9(1)			0.9(1)
Serine	0.9(1)	1.0(1)			1.0(1)	1.0(1)	
Glutamic acid	1.0(1)	3.1(3)	2.1(2)	1.0(1)			1.0(1)
Proline	1.0(1)						1.4(1)
Glycine	2.0(2)	1.1(1)	1.1(1)	0.9(1)			
Alanine		1.0(1)	1.0(1)		1.9(2)	2.2(2)	
Cys (CM)			0.3(1)				
Valine			0.6(1)[W]				
Methionine				0.9(1)			
Isoleucine	1.0(1)		0.5(1)[W]	2.1(2)[W]	1.9(2)		
Leucine		2.1(2)	1.0(1)	1.0(1)			
Tyrosine		0.9(1)	1.0(1)				
Phenylalanine		0.9(1)			1.0(1)		
Total residues	8	15	10	9	10	6	5
R_{Asp} (1.9)	1.20	0.77	1.0	1.12	1.12	1.71	1.84
R_{Leu} (BPAW)	0.19	0.52	0.52	1.03	0.85	0.17	0.25
Yield (%)	23.4	18.1	4.2	9.9	44.5	7.7	1.4
Purified, PE, 1.9	-	+	+	-	+	-	+
Purified, BPAW	+	+	+	+	+	+	+

[W] Acid-resistant bonds.

Table VII

Tryptic peptides from Fraction IV-J (continued)

Amino acid	IV-J3	IV-J4	IV-J5	IV-J6	IV-J7	IV-J8
Lysine	1.0(1)		1.0(1)	1.0(1)	0.9(1)	
Histidine		1.0(1)				
Arginine		1.0(1)				1.0(1)
Aspartic acid	1.6(2)	2.1(2)	2.1(2)		1.1(1)	1.0(1)
Threonine		1.0(1)		1.1(1)		
Serine	0.9 1)	1.0(1)	0.7(1)			1.0(1)
Glutamic ac.d	0.8(1)	3.4 3)		1.1(1)	2.2(2)	1.1(1)
Proline			1.0(1)	1.2(1)	1.1(1)	
Glycine	0.7(1)		1.1(1)	1.2(1)	0.8(1)	
Alanine	1.0(1)	1.1(1)	2.0(2)	1.0(1)	2.1(2)	
Valine		2.0(2)			1.0(1)	0.8(1)V
Methionine		2.0(2)				
Isoleucine			1.0(1)	1.0(1)		0.7(1)V
Leucine						1.0(1)
Tyrosine					1.9(2)	
Phenylalanine		1.0(1)			1.0(1)	
Total residues	7	15	9	7	12	7
R_{Asp}(1.9)	1.18	1.31	1.64	1.70	0.88	1.4
R_{Leu}(BPAH)	0.35	0.44	0.44	0.60	0.74	0.74
Yield (%)	1.4	4.5	3.1	7.4	39.4	18.0
Purified, PE, 1.9	-	+	+	+	+	+
Purified,BPAH	+	+	+	+	+	+

V Acid-resistant bonds.

Table VIII

Tryptic peptides from Fraction IV-K

Amino acid	IV-K1	IV-K2	IV-K3	IV-K4	IV-K5	IV-K6
Found also in fraction					V-A	
Lysine		1.0(1)	1.0(1)	1.0(1)	1.0(1)	
Arginine	1.0(1)					1.0(1)
Aspartic acid	0.9(1)			1.1(1)	2.1(2)	
Threonine				3.1(3)		
Serine		1.0(1)				
Glutamic acid		1.1(1)	1.1(1)			
Proline		0.9(1)		1.0(1)		
Glycine		2.0(2)	2.0(2)			
Alanine	1.2(1)				1.0(1)	1.1(1)
Valine		1.0(1)	0.9(1)		2.0(2)	1.6(2)V
Isoleucine	1.1(1)					
Leucine		0.9(1)	1.0(1)		1.0(1)	1.0(1)
Phenylalanine					0.9(1)	1.0(1)
Total residues	4	8	6	8	8	6
R_{Asp}(1.9)	2.5	1.4	1.6	1.4	1.4	1.5
R_{Leu}(BPAH)	0.48	0.63	0.63	0.72	0.85	1.13
Yield (%)	4.5	11.5	9.6	36.0	30.7	17.0
Purified, PE, 1.9	+	+	+	-	+	-
Purified,BPAH	+	+	+	-	+	+

V Acid-resistant bonds.

Table IX

Tryptic peptides from Fraction IV-L

Amino acid	IV-L1	IV-L2	IV-L3	IV-L4	IV-L5	IV-L6
Found also in fraction				V-C	V-B	
Lysine	1.0(1)		1.0(1)	1.0(1)	0.9(1)	0.9(1)
Arginine		0.9(1)			1.1(1)	1.0(1)
Aspartic acid	1.1(1)	2.1(2)				
Serine				1.0(1)		
Glutamic acid	1.9(2)	1.1(1)	2.1(2)	1.2(1)	2.1(2)	1.0(1)
Alanine		1.0(1)	1.0(1)		2.1(2)	
Valine	1.0(1)					
Methionine			1.0(1)			
Isoleucine		1.0(1)			1.0(1)	
Leucine				0.9(1)		2.0(2)
Tyrosine					1.0(1)	
Total residues	5	6	5	4	8	5
R_{Asp}(1.9)	1.8	1.5	1.85	2.07	1.40	1.50
R_{Leu}(BPAH)	0.15	0.29	0.27	0.31	0.49	0.76
Yield (%)	9.5	14.1	17.7	26.8	4.4	5.8
Purified, PE, 1.9	+	+	+	-	+	+
Purified,BPAH	+	+	+	+	+	+
Purified					PE,3.5	

Table X

Tryptic peptides from Fractions IV-L (continued), M and N

Amino acid	IV-L7	IV-M1	IV-M2	IV-M3	IV-M4	IV-M5
Found also in fraction		V-C			IV-L	V-F
Lysine		0.9(1)			1.0(1)	
Arginine	1.0(1)		1.0(1)	0.9(1)		1.0(1)
Aspartic acid		1.0(1)	1.3(1)			
Threonine						1.1(1)
Serine	2.0(2)	1.1(1)		1.1(1)		
Glutamic acid		1.0(1)				1.0(1)
Glycine	1.0(1)					
Alanine	1.9(2)			1.1(1)	1.1(1)	
Valine			0.9(1)	1.0(1)		
Methionine					1.0(1)	
Isoleucine	1.0(1)					
Leucine	1.1(1)		0.8(1)	1.0(1)	1.0(1)	
Tyrosine	0.9(1)					
Phenylalanine	1.0(1)			1.0(1)	1.0(1)	
Total residues	10	4	4	6	5	3
R_{Asp}(1.9)	1.00	1.90	2.3	1.12	1.26	2.12
R_{Leu}(BPAH)	1.04	0.22	0.52	1.04	1.06	0.22
Yield (%)	18.3	20.6	2.3	15.1	14.6	10.2
Purified, PE, 1.9	+	+	+	-	-	+
Purified,BPAH	+	+	+	+	+	+

Table XI

Tryptic peptides from Fraction IV-N (continued)

Amino acid	IV-N2	IV-N3V	IV-N4V	IV-N5	IV-N6	IV-N7
Found also in fraction		V-E	V-E	V-D	V-E	
Lysine	1.8(2)	V-F	V-F	V-E	V-F	
Arginine		(1)	(1)	1.0(1)	1.1(1)	1.0(1)
Threonine	1.0(1)		1.0(1)			0.9(1)
Serine	1.9(1)	1.2(1)				
Glutamic acid				1.5(1)		2.3(2)
Proline	1.2(1)					
Glycine						1.2(1)
Alanine	0.8(1)				1.0(1)	1.1(1)
Cys (CM)						(1)
Valine				0.9(1)		0.6(1)V
Isoleucine			1.0(1)			0.6(1)V
Leucine		0.8(1)			1.0(1)	2.0(2)
Tyrosine				0.9(1)		0.9(1)
Total residues	6	2(3)	2(3)	4	3	12
R_{Asp}(1.9)	2.43	2.30	2.30	1.64	2.24	1.36
R_{Leu}(BPAH)	0.22	0.43	0.43	0.53	0.53	0.65
Yield (%)	4.2	10.9	0.3	16.8	31.6	3.0
Purified, PE, 1.9	+	-	-	+	+	+
Purified,BPAH	+	-	-	+	+	+

V Isolated as dipeptides from mixture of two tripeptides after hydrolysis with carboxypeptidase B (see text).

V Low yield because of acid resistant bond

Table XII

Tryptic peptides from Fractions IV-N (continued) and O

Amino acid	IV-N8	IV-N9	IV-N10	IV-N11	IV-O1	IV-O2
Found also in fraction		V-E	V-C	V-E		
Lysine		1.0(1)	1.0(1)	1.0(1)	2.1(2)	1.0(1)
Arginine	1.0(1)					
Aspartic acid					2.2(2)	
Threonine			1.0(1)			
Serine			0.9(1)		1.0(1)	
Glutamic acid					1.2(1)	0.9(1)
Proline					0.8(1)	
Glycine			1.1(1)		2.1(2)	
Alanine	2.1(2)					
Valine		0.4(1)V				
Isoleucine		0.3(1)V		1.0(1)	1.0(1)	
Leucine	1.1(1)			1.0(1)		
Phenylalanine			2.0(2)			
Total residues	4	3	6	3	10	2
R_{Asp}(1.9)	2.45	3.0	1.67	2.90	1.83	3.90
R_{Leu}(BPAH)	0.63	0.63	0.89	0.89	0.14	0.24
Yield (%)	10.2	22.4	29.7	12.0	2.5	0.8
Purified, PE, 1.9	+	+	-	+	+	+
Purified,BPAH	+	+	+	+	+	+

V Acid-resistant bonds.

Table XIII

Tryptic peptides from Fraction IV-O (continued)

Amino acid	IV-O3	IV-O4	IV-O5	IV-O6	IV-O7	IV-O8
Found also in fraction			V-C			V-G
Lysine	1.0(1)		1.0(1)	1.0(1)	2.0(2)	
Histidine		1.0(1)				
Arginine	1.0(1)	0.9(1)				0.9(1)
Aspartic acid	3.2(3)				1.1(1)	
Threonine	1.1(1)	1.0(1)	0.9(1)			
Serine	1.0(1)				1.0(1)	
Glutamic acid		2.3(3)			1.0(1)	
Proline		1.1(1)		1.0(1)	0.9(1)	
Glycine	1.2(1)	1.0(1)				2.3(2)
Alanine			1.0(1)			1.0(1)
Isoleucine						
Leucine	1.1(1)	1.1(1)			2.0(2)	
Tyrosine	0.9(1)					
Total residues	10	9	3	2	8	4
R_{Asp}(1.9)	1.57	1.80	2.20	2.70	2.18	2.60
R_{Leu}(BPAH)	0.31	0.31	0.31	0.31	0.48	0.53
Yield (%)	7.8	13.0	24.3	14.6	13.0	28.7
Purified, PE, 1.9	+	+	+	+	+	+
Purified,BPAH	+	+	+	+	+	+

Table XIV

Tryptic peptides from Fractions IV-O (continued), and P

Amino acid	IV-O9	IV-O10	IV-P1	IV-P2	IV-P3	IV-P4
Found also in fraction	IV-P V-H			V-H	V-H	
Tryptophan						(1)
Lysine	1.0(1)		1.0(1)			1.0(1)
Arginine		1.0(1)		1.0(1)	(1)	0.9(1)
Aspartic acid						2.1(2)
Serine				0.9(1)		0.9(1)
Glutamic acid						2.2(2)
Proline				1.2(1)		
Valine		1.0(1)				
Methionine			0.7(1)			
Isoleucine						0.9(1)
Leucine	0.9(1)	1.0(1)				1.0(1)
Total residues	2	3	2	3	1	10
R_{Asp}(1.9)	4.12	3.04	3.51	2.83	3.12	2.11
R_{Leu}(BPAH)	0.57	0.86	0.22	0.38	0.38	0.68
Yield (%)	52.3	16.7	2.1	20.6	78.3	1.4
Purified, PE, 1.9	+	-	+	+	+	+
Purified,BPAH	+	+	+	+	+	+

Table XV

Tryptic peptides from Fraction IV-Q

Amino acid	IV-Q1	IV-Q2	IV-Q3	IV-Q4
Found also in fraction	V-H			V-H
Lysine	1.0(1)			V-I
Arginine	1.0(1)	1.0(1)	1.0(1)	1.0(1)
Aspartic acid	1.0(1)			
Glutamic acid	1.0(1)			
Glycine				1.0(1)
Alanine		0.9(1)		
Isoleucine				1.0(1)
Leucine			1.0(1)	
Tyrosine				1.0(1)
Total residues	4	2	2	4
R_{Asp}(1.9)	3.3	5.5	3.1	1.7
R_{Leu}(BPAH)	0.16	0.37	0.67	0.88
Yield (%)	10.2	9.9	30.2	25.8
Purified, PE, 1.9	+	+	+	+
Purified,BPAH	+	+	+	+

Table XVI

Tryptic peptides in Fraction V-B, C, E and F

Amino acid	V-B1	V-C1	V-C2	V-E2	V-F1
Lysine	1.0(1)	1.0(1)	0.1(0)		1.0(1)
Arginine			0.8(1)	1.0(1)	
Aspartic acid	2.1(2)	1.0(1)		1.0(1)	1.0(1)
Threonine					1.0(1)
Serine			0.1(0)		
Glutamic acid	1.1(1)		2.4(2)		
Glycine	1.1(1)	1.1(1)		1.1(1)	
Methionine	0.8(1)				
Isoleucine	0.9(1)		1.0(1)		
Leucine	0.1(0)				
Tyrosine				1.0(1)	
Total residues	7	3	4	4	3
R_{Asp}(1.9)	1.00	2.23	1.62	1.89	3.29
R_{Leu}(BPAH)	0.44	0.09	0.28	0.39	0.22
Yield (%)	3.6V	11.6	1.8	5.6	15.0
Purified, PE, 1.9	+	-	+	+	-
Purified,BPAH	+	+	+	+	+

V Also purified by electrophoresis at pH 3.6.

Table XVII

Tryptic peptides in Fractions V-F (continued), G, H, and I

Amino acid	V-F2	V-G1V	V-H1	V-I1
Tryptophan		1.0(2)		
Lysine	1.1(1)	1.0(1)	1.0(1)	1.0(1)
Aspartic acid			1.0(1)	
Threonine	1.0(1)			
Serine				0.8(1)
Proline		0.8(1)		
Glycine		1.1(1)		
Alanine	0.9(1)	0.7(1)		
Total residues	3	6	2	2
R_{Asp}(1.9)	2.07	1.47	3.10	3.10
R_{Leu}(BPAH)	0.42	0.75	0.19	0.34
Yield (%)	15.8	13.4	19.2	15.2
Purified, PE, 1.9	+	-	-	-
Purified,BPAH	+	+	+	+

V After complete hydrolysis with aminopeptidase M