The Carboxyl-terminal Sequence of Porcine Pepsin*

T. A. A. Dopheide, Stanford Moore, and William H. Stein
From The Rockefeller University, New York, New York 10021

SUMMARY

The sequence of 27 residues at the carboxyl end of the single polypeptide chain of porcine pepsin has been found to be: -Ile-Leu-Gly-Asp-Val-Phe-Ile-Arg-Gln-Tyr-Tyr-Thr-Val-Phe-Asp-Arg-Ala-Aon-Aon-Lys-Val-Gly-Leu-Ala-Pro-Val-Ala. The peptides from which this sequence has been derived were isolated from tryptic and chymotryptic digests of pepsin and its reduced aminoethylated and trifluoroacetylelated derivatives. All 3 of the strongly basic residues in pepsin (1 lysine and 2 arginine residues) are thus found in this terminal segment of the chain. There is no half-cystine residue in this portion of the molecule, and hence the crosslinking of the enzyme by three -S-S- bonds occurs within a highly acidic sequence of about 300 residues in which the only basic residue is that of the single histidine. The absence of positive charges in this sequence undoubtedly contributes to the acid stability and alkali lability of the enzyme.

The chemical structure of pepsin is currently being explored through several experimental approaches (1, 2).1 The present study is an outgrowth of the work of Rajagopalan, Moore, and Stein (3) on the preparation of purified pepsin from pepsinogen. The enzyme thus prepared from the zymogen is encouragingly homogeneous on the basis of chromatographic behavior, and group analysis, amino acid analysis, and ultracentrifugation (3, 4). With this material as the starting product, tryptic and chymotryptic cleavages of the protein and its derivatives have been investigated on a column (2 X 300 cm) of Sephadex G-75 in 0.1 M phosphate buffer, pH 8.0, at 15 C. The protein concentration was 1.5 to 2%. The process was usually followed by analysis of 50-ml samples of the digest by the ninhydrin reaction. Hydrolysis was complete in 2 to 3 hours. When a buffer-free medium was used for the tryptic digestion and the course of the hydrolysis was monitored with a pH-stat, hydrolysis was nearly complete in 1 hour, and there was less evidence for minor chymotryptic-like cleavages.

Trypsin Hydrolysis—Since pepsin contains only 2 residues of arginine and 1 of lysine, tryptic action can be expected to furnish not more than four peptides in high yield. For most experiments, the inactivated pepsin was digested with 1% of its weight of trypsin in 0.1 M phosphate buffer, pH 8.0, at 37 C. The protein concentration was 1.5 to 2%. The process was usually followed by analysis of 50-ml samples of the digest by the ninhydrin reaction. Hydrolysis was complete in 2 to 3 hours. When a buffer-free medium was used for the tryptic digestion and the course of the hydrolysis was monitored with a pH-stat, hydrolysis was nearly complete in 1 hour, and there was less evidence for minor chymotryptic-like cleavages.

Trypsin Hydrolysis—Since pepsin contains only 2 residues of arginine and 1 of lysine, tryptic action can be expected to furnish not more than four peptides in high yield. For most experiments, the inactivated pepsin was digested with 1% of its weight of trypsin in 0.1 M phosphate buffer, pH 8.0, at 37 C. The protein concentration was 1.5 to 2%. The process was usually followed by analysis of 50-ml samples of the digest by the ninhydrin reaction. Hydrolysis was complete in 2 to 3 hours. When a buffer-free medium was used for the tryptic digestion and the course of the hydrolysis was monitored with a pH-stat, hydrolysis was nearly complete in 1 hour, and there was less evidence for minor chymotryptic-like cleavages.

EXPERIMENTAL PROCEDURE

Materials—Porcine pepsinogen (crystallized), carboxypeptidase A (diisopropyl fluorophosphate-treated), and chymotrypsin were obtained from Worthington Biochemical Corporation. Trypsin (treated with L-(1-tosylamino-2-phenyl)ethyl chloromethyl ketone) was from Calbiochem. Carboxypeptidase B was a gift from Dr. J. E. Folk.

Pepsin was prepared from pepsinogen by the procedure of Rajagopalan et al. (3) scaled up for a starting sample of 500 mg of the zymogen. For structural studies, the enzyme was promptly inactivated by adjusting the eluate from the sulfonethyl-Sephadex column to pH 7.6 by the addition of N-ethylmorpholine (redistilled). The protein solution was desalted on a column (2.5 X 70 cm) of Sephadex G-25 equilibrated with 0.5% N-ethylmorpholine. The lyophilized protein was checked for homogeneity by amino acid analysis (particularly for lysine, histidine, and arginine which should have a ratio of 1:1:2) and for COOH-terminal amino acids with carboxypeptidase A; the release of only alanine by the enzyme is a sensitive criterion for the integrity of the protein (3).

Trypsin Hydrolysis—Since pepsin contains only 2 residues of arginine and 1 of lysine, tryptic action can be expected to furnish not more than four peptides in high yield. For most experiments, the inactivated pepsin was digested with 1% of its weight of trypsin in 0.1 M phosphate buffer, pH 8.0, at 37 C. The protein concentration was 1.5 to 2%. The process was usually followed by analysis of 50-ml samples of the digest by the ninhydrin reaction. Hydrolysis was complete in 2 to 3 hours. When a buffer-free medium was used for the tryptic digestion and the course of the hydrolysis was monitored with a pH-stat, hydrolysis was nearly complete in 1 hour, and there was less evidence for minor chymotryptic-like cleavages.

The tryptic digest obtained with 160 mg of pepsin was fractionated on a column (2 X 300 cm) of Sephadex G-75 in 0.1 M ammonium acetate at pH 10.4. Samples of 100 ml were taken from each effluent fraction (5 ml) for analysis by the ninhydrin method after alkaline hydrolysis (cf. Fruchter and Crestfield (5)). Large peptides, including the cross-linked core of the protein were eluted first; the most retarded peak (600 to 765 ml) contained the smallest peptides. After removal of ammonium acetate by lyophilization and gel filtration on Sephadex G-10, the peptides were submitted to ion exchange chromatography on fine particle size, 8% cross-linked sulfonated polystyrene (cf. Crestfield, Moore, and Stein (6)). Stepwise elution from MS Q15 (Bio-Rad Laboratories) with citrate buffers gave the pattern shown in Fig. 1. Each peptide fraction was desalted by passage over Sephadex G-10 in 25% acetic acid (sample less than 25% of bed volume); the resulting solutions were lyophilized.

Preparation and Tryptic Hydrolysis of (ε-N-Trifluoroacetyllysine)-pepsin—Since pepsin contains only 2 arginine residues, tryptic action when the ε-NH2 group of the lysine residue is
Fig. 1. Chromatography on sulfonated polystyrene of small tryptic peptides obtained from 160 mg of pepsin. The column (15 X 0.9 cm) was operated at 50° and had been equilibrated with 0.2 M citrate, pH 3.25. Elution was performed in a stepwise manner; buffers were changed at 40 ml to 0.2 M citrate, pH 4.25, and at 100 ml to 0.38 M citrate, pH 5.28. The flow rate was 30 ml per hour; the fraction size, 2 ml.

Fig. 2. Chromatography on sulfonated polystyrene of small tryptic peptides obtained from 200 mg of trifluoroacetylated (TFA) pepsin. Conditions were similar to those given for Fig. 1 with buffer changes at 26 ml (to pH 4.25) and at 78 ml (to pH 5.28). The product thus obtained was desalted on Sephadex G-25 in 0.5% N-ethylmorpholine. The lyophilized mixture was fractionated by ion exchange chromatography (Fig. 3).

blocked should cleave the molecule mainly at two points. The derivatization was performed by the procedure of Goldberger and Anfinsen (7). A solution of 200 mg of pepsin in 30 ml of water was adjusted to pH 10 by the addition of 1 M NaOH and 1 ml of S-ethyl trifluoroacetate (Pierce Chemical Company, Rockford, Illinois) was added to the vigorously stirred solution; the pH was maintained between 9.5 and 10.0 by the addition of 1 N NaOH. After about 1 hour, the pH remained constant.

The product thus obtained was desalted on Sephadex G-25 in 0.5% N-ethylmorpholine. The lyophilized derivative was dissolved in 20 ml of 0.1 M phosphate, pH 7.8, and the solution was incubated with 2.0 mg of trypsin for 3 hours at 37° in the presence of a crystal of thymol. Tryptic action was terminated by the addition of 1 ml of glacial acetic acid. The precipitate was removed by centrifugation and washed twice with 50% acetic acid. The soluble peptides in the supernatant solution and the washings were desalted on Sephadex G-10 (in 25% acetic acid), lyophilized, and dissolved in 1.0 M piperidine to remove the trifluoroacetyl group (7).

That part of the resulting peptide mixture, which was soluble in pH 2.2 buffer, was chromatographed on sulfonated polystyrene (Fig. 2).

Preparation of Reduced and Aminoethylated Pepsin—Six further potentially trypsin-sensitive bonds were introduced into the pepsin molecule by reducing and aminoethylating the half-cystine residues. The reducing conditions described by Raftery and Cole (8) had to be made more vigorous in order to obtain complete reduction of the --S--S-- bonds of pepsin. To 100 mg of the enzyme in 3 ml of water were added 3.2 g of urea, 2.5 mg of EDTA, and 0.17 g of 2-methyl-2-amino-1,3-propanediol (Calbiochem); after the final volume had been adjusted to 6 ml, the pH was 10.4. The solution was flushed with pre-purified nitrogen and incubated under a nitrogen barrier (Crestfield (9)) with a 500-fold molar excess (0.60 ml) of mercaptoethanol for 4 hours at 37°. An equal volume of 5 mM 2-methyl-2-amino-1,3-propanediol adjusted to pH 8.5 with HCl was then added. A 2-fold molar quantity (0.90 ml) of ethyleneimine (K and K Laboratories, Brooklyn) was pipetted into the stirred reaction mixture in three equal portions at intervals of 30 min. Disappearance of thiol was monitored with the nitroprusside reaction, which became negative after the last addition of the reagent. Amino acid analysis of the desalted derivative gave 5.97 residues of S-aminoethylcysteine. The color yield of the derivative was experimentally determined to be 91% of that for lysine (cf. Hofmann (10); Raftery and Cole (8)). Only 2.27 residues of aminoethylcysteine (out of the theoretical 6.0) were obtained if the reduction of pepsin was performed at pH 8.5 instead of at pH 10.5.

In order to simplify the isolation of the peptides to be liberated by tryptic hydrolysis at aminoethylcysteine residues, the above derivatization was also applied to pepsin that had already been hydrolyzed by trypsin at its lysine and arginine residues as described above. The unfractionated mixture was lyophilized, aminoethylated, and the product was desalted on Sephadex G-25 in 0.5% N-ethylmorpholine. The lyophilized mixture was fractionated on a column (2 X 300 cm) of Sephadex G-75 in 0.1 M ammonium acetate, pH 10.3, containing 0.25% thioglycol to protect methionine peptides from oxidation (6, 11). The first peak (250 to 370 ml) contained the reduced and aminoethylated core of pepsin separated from the slower moving peptides which had been liberated by the prior tryptic action.

Tryptic Hydrolysis of Reduced and Aminoethylated Pepsin—About 300 mg of the reduced and aminoethylated core were dissolved in 30 ml of 0.1 M phosphate, pH 7.8, and digested with 3 mg of trypsin for 3 hours at 37°. The digest was desalted on a column of Sephadex G-25 (2 X 100 cm) in 0.1 M ammonium acetate, pH 10.2, containing 0.75% thioglycol. The peptide mixture was fractionated by ion exchange chromatography (Fig. 3).

When intact reduced and aminoethylated pepsin (as distinct from the reduced and aminoethylated core) was hydrolyzed by trypsin, the peptides gave a pattern similar to the one shown in Fig. 3, with the presence of two additional peptides, T1 and T2 (cf. Fig. 1). In this instance, the possible presence of an even more strongly adsorbed peptide was sought by changing the...
eluent, after RAE-Ts$^2$ to 1 M acetate, pH 5.50. A major peak was obtained shortly after the breakthrough of the 1 M buffer. This fraction (T4) was further purified by rechromatography on the same column by elution with a gradient (0.19 M citrate, pH 5.28, in a 50-ml mixing chamber into which 1.0 M citrate, pH 5.50, was allowed to flow). Two peaks were obtained, one at 8 ml (T8) and the other at 56 ml (Tn).

Chymotryptic Hydrolysis of Pepsin and Reduced and Aminoethylated Pepsin—With the aim of isolating overlapping peptides so as to establish the order of the peptides of the tryptic series, chymotryptic hydrolysates were examined. Pepsin (64 mg) in 10 ml of water was digested with 1.2 mg of chymotrypsin; the solution was kept at pH 8.0 by the addition of 0.40 M NaOH (pH-stat). After 90 min the reaction was terminated by the addition of glacial acetic acid to bring the solution to pH 3.0. The lyophilized product was extracted with 0.2 M citrate buffer, pH 2.2, and the soluble peptides were chromatographed. After changes in eluent similar to those shown in Fig. 1, a shift at 232 ml to 1 M acetate, pH 5.5, eluted a basic peptide (Chy) at 260 ml.

Reduced and aminoethylated pepsin was similarly digested with chymotrypsin. Titration indicated that about 35 bonds were hydrolyzed per molecule of protein. The basic peptides were obtained by chromatography on an anodic resin (Fig. 4) with the aim of isolating peptides containing arginine or lysine. Fraction RAE-Chy contained both of these basic amino acids, but analysis indicated that it was not pure. The mixture was submitted to further purification by electrophoresis on paper that had been washed with 1% acetic acid. Elution of the main zone by 0.5% acetic acid yielded a product which gave an analysis for a homogeneous peptide.

Sequential Degradation of Peptides—A modification of the Koningsberg and Hill (12) procedure for the Edman method was used. The whole reaction sequence was performed in a glass-stoppered slightly tapered tube (10 × 1.5 cm). The peptide (0.5 to 1.5 µmole) was dissolved in 1 ml of a pyridine-N-ethylmorpholine-acetic acid buffer, pH 3.5 (pyridine, 150 ml; H2O, 100 ml; N-ethylmorpholine, 29 ml; adjusted to pH 3.50 with glacial acetic acid). Phenyliothiocyanate (50 µl) was added and the tube was thoroughly purged with pre-purified nitrogen. The mixture was maintained at 37° for 1 hour with occasional shaking, extracted five times with 2.5 ml of benzene, and lyophilized. The residue was dissolved in 100 µl of trifluoroacetic acid, the tube was flushed with nitrogen, and the solution was maintained at 37° for 30 min. Trifluoroacetic acid was removed in a nitrogen jet, and the residue was dissolved in 1 ml of 0.4 M acetic acid; this solution was extracted three times with 2 ml of benzene. An aliquot containing 0.05 to 0.1 µmole was taken for hydrolysis and amino acid analysis; the remainder was lyophilized and subjected to another step in the degradation process.

COOH-Terminal Analysis with Carboxypeptidase A and B—Carboxypeptidase A solution was prepared by washing 50 µl of the suspension of the crystalline enzyme twice with water at the centrifuge. The sediment was dissolved in 1.0 ml of 1 M potassium bicarbonate. The enzyme concentration was then adjusted to 1 mg per ml by the addition of the necessary amount of potassium bicarbonate solution, assuming an A280 of 1 mg per ml per cm = 1.94. Carboxypeptidase B was used as a solution in 0.1 M phosphate, pH 7.8.

The abbreviations used are: RAE, reduced and aminoethylated; TFA, trifluoroacetylated; AECys, aminoethylcysteine.

For determination of COOH-terminal residues, peptides were digested with 2 to 4%, by weight, of carboxypeptidase in 500 µl of 0.1 M phosphate, pH 7.8, at 25°. The digestions were terminated by the addition of citrate buffer, pH 2.2, and samples were applied to the amino acid analyzer without further treatment. Whenever a precipitate formed, the samples were filtered through a Millipore filter (0.22 µ in a Swinnex adapter) before analysis.

Net Charge of Peptides—To determine whether the peptides contained aspartic acid or glutamic acid or their respective amides, high voltage paper electrophoresis (Wieland and Pfeiferer (13)) was used (30 volts per cm, pyridine-acetate buffer, pH 6.5). The standards were glutamic acid, glycine, and lysine, with a trace of picric acid as a visible marker. Spots were located by dipping the papers in 0.5% ninhydrin in acetone or, with tyrosine-containing peptides, by spraying with the Pauly reagent.

For determination of COOH-terminal residues, peptides were digested with 2 to 4%, by weight, of carboxypeptidase in 500 µl of 0.1 M phosphate, pH 7.8, at 25°. The digestions were terminated by the addition of citrate buffer, pH 2.2, and samples were applied to the amino acid analyzer without further treatment. Whenever a precipitate formed, the samples were filtered through a Millipore filter (0.22 µ in a Swinnex adapter) before analysis.

Net Charge of Peptides—To determine whether the peptides contained aspartic acid or glutamic acid or their respective amides, high voltage paper electrophoresis (Wieland and Pfeiferer (13)) was used (30 volts per cm, pyridine-acetate buffer, pH 6.5). The standards were glutamic acid, glycine, and lysine, with a trace of picric acid as a visible marker. Spots were located by dipping the papers in 0.5% ninhydrin in acetone or, with tyrosine-containing peptides, by spraying with the Pauly reagent.
RESULTS

The results can be most clearly summarized with reference to Fig. 5. The yields of each peptide have been calculated from the amino acid analyses, performed as previously described (3), without corrections for hydrolytic destruction. The tabular data under the sequence of each peptide refer to the results of amino acid analyses of the intact peptide and of the residue remaining after each step of the Edman degradation; the yield is given for the peptide obtained in the step in question. The amino acid removed is indicated by boldface.

T1: Val - Gly - Leu - Ala - Pro - Val - Ala (68%)
Composition: 1.8 1.0 1.0 2.0 1.0
Step 1 (80%): 1.0 1.1 1.0 2.0 1.1
Step 2 (76%): 0.9 0.3 1.0 2.0 1.0
Step 3 (100%): 1.0 0.3 0.3 1.9 1.0
Step 4 (100%): 0.9 0.2 0.2 1.2 1.0

Carboxypeptidase A, after 6 hours at pH 7.8, yielded alanine, 0.74, and a trace of valine.

Since T1, obtained in high yield by tryptic hydrolysis, does not contain arginine or lysine, it clearly represents the COOH-terminal peptide liberated by tryptic hydrolysis of pepsin. This conclusion is in agreement with the results obtained when carboxypeptidase A acts on the intact protein (3).

T2: Ala - Asn - Asn - Lys (43%)
Composition: 1.0 2.0(Asp) 0.9
Step 1 (95%): 0.1 2.0(Asp) 0.5
Lysine is assumed to be COOH-terminal from the specificity of trypsin. Electrophoresis showed that the peptide had a net positive charge at pH 6.5; hence, both aspartic acid residues are amidated.

T3: Tyr - Thr - Val - Phe - Asp - Arg (12%)
Composition: 0.7 0.8 1.0 0.8 1.0 0.8
Step 1 (96%): 0.0 0.8 0.9 0.8 1.0 0.9
Step 2 (100%): 0.0 0.1 0.9 0.8 1.0 0.8
Step 3 (100%): 0.0 0.1 0.1 0.9 1.0 0.9
Step 4 (100%): 0.0 0.1 0.1 0.1 0.9 1.0

Electrophoresis at pH 6.5 showed the peptide to be neutral; therefore, the carboxyl group of aspartic acid is charged.

T4: PyrGlu - Tyr - Thr - Val - Phe - Asp - Arg (51%)
Composition: 1.0(Glu) 1.6 1.0 1.0 1.0 1.2 1.0

Rechromatography of T4 yielded two peptides, T4a and T4b, both of which gave essentially the same composition; the former was the purer peptide. Paper electrophoresis at pH 6.5 showed it to be neutral and negative to ninhydrin; it was detected by the Pauly reagent. It is concluded that the more basic of the two, T4a, has a glutamine residue at the NH2-terminal position, and that T4b has formed by cyclization of the glutamine residue during desalting of the peptide in 25% acetic acid to yield a pyroglutamic acid residue.

Pepsin contains only 2 arginine residues, found in Peptides T2 and RAE-T3 (discussed below). From its amino acid composition, T4 must be an extension of T4a with Glu Tyr added at the NH2 end.

TFA - T1: Ala - Asn - Asn - Lys - Val - Gly - Leu - Ala - Pro - Val - Ala (56%)
Comp.: 3.0 2.3(Asp) 1.0 2.0 1.0 1.0 1.1
This peptide was obtained by tryptic hydrolysis when the e-NH2 group of the lysine residue in pepsin had been blocked. Carboxypeptidase A liberated 1.0 alanine in 6 hours; no other residues were found. Tryptic hydrolysis of TFA-T1, followed by chromatography as in Fig. 1 yielded T1 and T2 with the expected amino acid compositions and NH2-terminal sequences as determined by the Edman degradation. Therefore, TFA-T1 establishes the linkage T2-T1 (Fig. 5), and demonstrates that the 1 lysine residue in pepsin is near the COOH end of the molecule.

RAE - Chyl (40%): Asp - Arg - Ala - Asn - Asn - Lys - Val - Gly - Leu - Ala - Pro - Val - Ala
Comp.: 3.0 1.0 3.0 1.0 2.0 1.0 1.0 1.0

From its composition, which includes 1 arginine residue and the single lysine residue of pepsin, RAE-Chyl provides the overlapping peptide joining T2 to T4 (Fig. 5).
Carboxypeptidase B liberated the theoretical amount of arginine in 3 hours, together with 0.66 isoleucine and a trace of phenylalanine. Subsequent hydrolysis with carboxypeptidase A for 30 min released a further amount of isoleucine (0.36) and 0.78 phenylalanine; after 150 min, a trace of valine was also found. These results establish the sequence of the peptide which contains the second arginine residue of pepsin.

Chy:

\[
\text{Ile} - \text{Arg} - \text{Gln} - \text{Tyr} \quad (39\%)
\]

Composition:

\[
1.0 \quad 1.0 \quad 1.2(\text{Glu}) \quad 1.0
\]

Carboxypeptidase A liberated 1.0 tyrosine and 0.44 glutamine. The peptide provides the overlap which ties RAE-T₁ to T₄ (Fig. 5).

DISCUSSION

The unexpected simplicity of the derivation of the sequence shown in Fig. 5 places all 3 of the strongly basic amino acid residues of pepsin in a 27-residue, COOH-terminal segment which is devoid of half-cystine residues. Had the two arginines and the single lysine been more evenly distributed through the chain of 321 residues, the continuous sequence shown in Fig. 5 could not have been derived primarily from the short peptides of the tryptic series.

The present findings are in agreement with the results of Kuznetsov, Kovaleva, and Stepanov (1); the sequence which they have established is the same as the first 9 residues of RAE-Chy₁ (Fig. 5). The conclusion of Vaganova, Levin, and Stepanov (14) that the 3 residues at the COOH terminus are -Pro-Val-Ala is confirmed. The present data are also in agreement with the results of Jones and Perham\(^\text{3}\) and of Koehn and Perlmann\(^\text{4}\) who have isolated peptides from enzymatic hydrolysates of pepsin and pepsinogen.

It is tempting to postulate that the isoleucine residue at the NH₂ terminus of the 27-residue segment shown in Fig. 5 is preceded by a half-cystine residue. This would mean that in the reduced, aminoethylated enzyme, tryptic hydrolysis would occur at an aminoethylcysteyl-isoleucyl bond. However, the possibility of a chymotryptic-like hydrolysis cannot be excluded, in which case the preceding residue would probably be an aromatic amino acid. The yield of RAE-T₁ (Fig. 3) is only 18% and small amounts of chymotryptic-like cleavages have been encountered in this series of experiments. Hydrolysis between -Tyr-Tyr-gives a 12% yield of T₄; in the absence of buffer salts, when the pH-stat is used, the yield of this peptide is much lower. Other chymotryptic-like cleavages are the cause of the formation of the minor components, such as a small quantity of Asp-Arg (10%) eluted just prior to T₂ (Fig. 1). An ancillary result in the present study has been the isolation of two peptides containing aminoethylcysteine (RAE-T and RAE-T₁, Fig. 3). The peptides have the sequences Ser-Ser-Leu-Ala-AECys and Ser-Gly-Gly-AECys. These structures, which have been established by Edman degradation, are compatible with Tang and Hartley's results\(^\text{8}\) on the peptides containing the half-cystine residues which form the -S-S- bonds of pepsin; our findings do not fully agree with those of Keil, Moravek, and Sörm\(^\text{2}\) on the same subject.

The present research emphasizes one unusual feature of the structure of pepsinogen. From the experiments of Herriott (cf. Reference 15) and recent amino acid analyses of the zymogen and the enzyme (Blumenfeld and Perlmann\(^\text{16}\) and Arnon and Perlmann\(^\text{17}\); Rajagopalan et al.\(^\text{10}\)), it is known that about 42 residues are cleaved from the NH₂ terminus of pepsinogen during its conversion to pepsin. The segment removed is very basic; it contains 9 lysine and 2 arginine residues. As shown in Fig. 5, all of the remaining basic amino acids of the pepsinogen molecule are near the COOH terminus. Therefore, between the NH₂- and COOH-terminal portions, there must exist a sequence of about 300 residues that contains exclusively neutral and acidic amino acids with the sole exception of a single histidine residue. The existence in pepsin, beginning at the NH₂ terminus, of this extended region almost devoid of positive charges may be related to the unusual acid stability and exceptional alkali lability of the enzyme.

Acknowledgments—We wish to acknowledge the skilled technical assistance of Miss Mary Fitzgerald and Miss Wanda Jones in the performance of this research.

REFERENCES


\(^{3}\) G. Jones and R. Perham, personal communication.

\(^{4}\) P. Koehn and G. E. Perlmann, personal communication.
The Carboxyl-terminal Sequence of Porcine Pepsin
T. A. A. Dopheide, Stanford Moore and William H. Stein


Access the most updated version of this article at http://www.jbc.org/content/242/8/1833

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/242/8/1833.full.html#ref-list-1