IDENTIFICATION OF A PEPTIDE RELEASED DURING
AUTOCATALYTIC ACTIVATION OF TRYSINOGEN*

BY EARL W. DAVIE† AND HANS NEURATH

(From the Department of Biochemistry, University of Washington, Seattle, Washington)

(Received for publication, August 19, 1954)

The kinetics of the tryptic activation of trypsinogen have been found by Kunitz (3) to follow the simple chemical equations

\[
\text{Trypsinogen} + \text{trypsin} \rightarrow 2 \text{trypsin} \quad (a)
\]
\[
\text{Trypsinogen} + \text{trypsin} \rightarrow \text{trypsin} + \text{inert protein} \quad (b)
\]

In the presence of calcium ions, at pH 8, reaction (b) is suppressed and the conversion of the zymogen to the active enzyme is essentially complete.

Until recently, little was known of the chemical changes accompanying this conversion. Within the limits of the experimental errors of sedimentation and diffusion measurements, trypsinogen and trypsin have identical molecular weights (4, 5), suggesting that no large fragments arise from the proteolytic activation by trypsin. Rovery, Fabre, and Desnuelle (6) reported valine to be the single N-terminal group of trypsinogen, and isoleucine to occupy the same position in DIP-trypsin.¹ Both proteins were found to be unreactive toward carboxypeptidase (7), suggesting that any C-terminal group which may be present is the same in both proteins. The replacement during activation of the N-terminal valine by isoleucine indicates that 1 or more peptide residue has been removed from the single polypeptide chain of trypsinogen.

Evidence for the appearance of an activation peptide has been obtained in the course of this work and has led to its isolation and chemical characterization, as reported in this communication.

EXPERIMENTAL

Materials

**Trypsinogen**—Two crystalline preparations were employed. One of these was isolated by Dr. F. Tietze in the presence of DFP. The other

* Present in part before the Forty-fifth annual meeting of the American Society of Biological Chemists at Atlantic City, New Jersey, April 12-16, 1954 (1). A preliminary report has been published (2).

† Present address, Biochemical Research Laboratory, Massachusetts General Hospital, Boston, Massachusetts.

¹ The following abbreviations are used: DFP, diisopropyl phosphofluoridate; DIP, diisopropylphosphoryl; DNFB, 2,4-dinitrofluorobenzene; DNP, dinitrophenyl; BAEE, α-benzoyl-L-arginine ethyl ester.

515
TRYPSINOGEN PEPTIDE

was obtained by Dr. N. M. Green, using crystalline soy bean inhibitor instead of DFP to suppress trypsin activity during the process of isolation. The physical properties of the Tietze preparation, containing 0.016 per cent active trypsin, have been published (4). Essentially the same properties were observed for the Green preparation.

DNP derivatives of twenty-two amino acids were prepared by Mr. His-Lung Pan according to published methods (8).

Results

Identification of Valyl Peptide

In order to demonstrate the release of peptides during the tryptic activation of trypsinogen, aliquots were removed at various stages of activation, and any free amino acids and peptides were adsorbed onto and eluted from ion exchange resins and subjected to paper chromatography. Trypsinogen and trypsin were pretreated by exhaustive dialysis against 0.001 N HCl to remove any low molecular weight impurities.

In a typical experiment, 120 mg. of twice crystallized trypsinogen were dissolved at 0° in 9 ml. of 0.1 M borate buffer, pH 8.0, containing 0.05 M CaCl₂, and activated by the addition of 0.7 mg. of trypsin (Worthington, twice crystallized). Aliquots were removed at intervals up to 8 hours, and any free amino acids or peptides were adsorbed on Dowex 50 resin (20 to 50 mesh, hydrogen form, 4 or 12 per cent cross-linked). Simultaneously, the rate and extent of activation were followed by the esterase method, with BAEE as substrate (4). The reaction products were eluted from the resin with NH₄OH (9) and subjected to paper chromatography (butanol-acetic acid-water). As may be seen from the paper chromatogram in Fig. 1, the zero time control showed the complete absence of ninhydrin-positive products. After 10 per cent activation, a single spot was obtained having an \( R_F \) value of about 0.08, the intensity of this spot increasing in proportion to the extent of tryptic activation. Elution of the spot, followed by hydrolysis in a sealed tube in 5.7 N HCl at 110° for 24 and 48 hours, indicated the presence of lysine, valine, and aspartic acid in mole ratios of approximately 1:1:4 or 5 and traces of glutamic acid and alanine.

The peptide material having larger \( R_F \) values was found to change from one activation experiment to another, some experiments showing nearly complete absence of this material. Upon hydrolysis, these spots usually revealed the presence of lysine, valine, and aspartic acid.

Purification of Peptide on Dowex 50 Column

Preparation and Operation of Columns—In order to isolate the peptide in pure form and good yield, use was made of ion exchange columns in conjunc-
tion with automatic fraction collection, as described by Dowmont and Fruton (10). Dowex 50 (200 to 300 mesh, sodium form, 2 per cent cross-linked) was used in chromatographic tubes 40 cm. long and 0.9 cm. inner diameter. The resin columns (30 cm. high) were prepared by pouring the resin in two or three sections as a slurry in citrate buffer of pH 3.0.

The solvents used for elution were citrate buffers of varying pH and sodium ion concentration. Conditions for optimal separation of peptides were determined empirically. Table I reports the composition of the buffers used in these experiments, as well as the amount of acid or alkali necessary to adjust 1 ml. samples to pH 5, as required for quantitative ninhydrin analysis. All pH values were determined with the glass electrode at 25° by means of a Cambridge model R pH meter and are accurate approximately to 0.02 pH unit.
The columns were operated at room temperature at a flow rate of 2.5 ml. per hour, without external pressure. The peptide mixture was added to the column in 0.5 ml. of citrate buffer of pH 3.0, and effluent fractions of 1.0 or 0.5 ml. were collected and analyzed by the photometric ninhydrin method (11).

Separation of Peptides—The peptides resulting from activation of trypsinogen were adsorbed and eluted from Dowex 50, as described in the section dealing with paper chromatography, and applied to the column. Fig. 2 shows a typical chromatogram of a 63 per cent activated sample. It reveals one major peak (35 to 40 ml. of effluent after addition of buffer of pH 4 to the top of the column) representing the activation peptide (see below) and several minor components. The addition of 0.1 N NaOH to the column following buffer of pH 6.5 failed to produce any further ninhydrin-positive material, indicating that elution was complete.

Since in Fig. 2 the color yield of the peptides was tentatively expressed as leucine equivalents, it was necessary to establish the true color yield of the activation peptide on a molar basis. According to Kjeldahl nitrogen determinations, 22.8 g of peptide nitrogen, corresponding to 0.233 μmole of peptide (molecular weight 705, see below), gave an optical density reading of 1.01 by the usual ninhydrin procedure. This corresponds to a color value of 1.65 on a molar basis relative to leucine (11), a value much higher than the range of 0.70 to 0.90 generally found for peptides (10). It is likely that this high value is due to the additional contribution to the reaction by the ε-amino group of the lysine residue.

---

**Table I**

<table>
<thead>
<tr>
<th>Molarity of citrate</th>
<th>Final Na⁺ concentration</th>
<th>pH</th>
<th>0.10 ml. of NaOH or HCl required to adjust buffer to pH 5.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.192</td>
<td>0.100</td>
<td>3.0</td>
<td>3 N NaOH</td>
</tr>
<tr>
<td>0.132</td>
<td>0.120</td>
<td>3.5</td>
<td>1.5 N NaOH</td>
</tr>
<tr>
<td>0.102</td>
<td>0.130</td>
<td>4.0</td>
<td>0.75 N NaOH</td>
</tr>
<tr>
<td>0.097</td>
<td>0.160</td>
<td>4.5</td>
<td>0.3 N NaOH</td>
</tr>
<tr>
<td>0.105</td>
<td>0.210</td>
<td>5.0</td>
<td></td>
</tr>
<tr>
<td>0.110</td>
<td>0.300</td>
<td>6.3</td>
<td>0.7 N HCl</td>
</tr>
<tr>
<td>0.200†</td>
<td>0.580</td>
<td>6.5</td>
<td>1 N HCl</td>
</tr>
</tbody>
</table>

* All buffers contained 0.5 per cent BRIJ-35 (polyoxyethylene lauryl alcohol).
† 0.1 gm. of disodium versenate and 1.5 ml. of benzyl alcohol per 100 ml. were also added to this buffer.

---

2 Heating period of 20 minutes, 8 ml. volume.
Amino Acid Composition of Activation Peptide

The peptide purified by column chromatography (1.7 mg. taken from the top of the major peak shown in Fig. 2) was hydrolyzed in 10 ml. of 5.7 N HCl for 20 hours at 110° without previous desalting. After evaporation of the sample to dryness, it was washed with several ml. of water, taken to

**Fig. 2.** Chromatographic separation of the peptide mixture of a 63 per cent activated sample of trypsinogen. A Dowex 50 column (200 to 300 mesh, 2 per cent cross-linking, sodium form) at 25° was used. Flow rate, 2.5 ml. per hour; column dimensions, 30 x 0.9 cm. The buffers used were as described in Table I. The molar color yield for leucine was used for all ninhydrin-positive materials (see the text for the true color yield of the activation peptide).

**Fig. 3.** Chromatographic separation of an acid hydrolysate of the peptide purified by column chromatography. The column of Dowex 50, 0.9 x 100 cm., was operated in the sodium form, with buffers of the pH and temperature indicated as eluents. A sample of 0.80 mg. of peptide hydrolysate was placed on the column. The approximate position of amino acids not present in the hydrolysate is also shown.
dryness, and this process repeated three times. The amino acids were then taken up in 4.2 ml. of water containing 0.1 ml. of 2 N HCl, and, after centrifuging a small amount of insoluble material, 2.0 ml. samples were sub-

![chromatogram](http://www.jbc.org/)

Fig. 4. Chromatographic separation of the basic amino acids of an acid hydrolysate of the peptide purified by column chromatography. The column of Dowex 50 (0.9 × 15 cm.) was operated in the sodium form at 25°C with the buffers indicated. A sample of 0.8 mg. of peptide hydrolysate was placed on the column. The large initial peak contains aspartic acid and valine. The approximate position of other amino acids not present in the peptide hydrolysate is also shown.

**Table II**

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Amino acid residues</th>
<th>Amino acid residues</th>
<th>Residues relative to lysine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg.</td>
<td>µmoles</td>
<td>moles</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>0.527</td>
<td>4.58</td>
<td>3.98</td>
</tr>
<tr>
<td>Valine</td>
<td>0.117</td>
<td>1.18</td>
<td>1.03</td>
</tr>
<tr>
<td>Lysine</td>
<td>0.147</td>
<td>1.15</td>
<td>1.00</td>
</tr>
<tr>
<td>NH₃</td>
<td>0.0051</td>
<td>0.299</td>
<td>(0.26)</td>
</tr>
</tbody>
</table>

In Table II the amino acid composition of the peptide is given, corresponding to an exact mole ratio of valine-lysine-aspartic, 1:1:4. Thus, a molecular weight of 705 may be assigned to this peptide, assuming the
absence of amide nitrogen. The presence of fractional equivalents of ammonia in the acid hydrolysate of the peptide is not readily accounted for. The possibility of partial decomposition of aspartic acid is discounted by the integral mole ratio of aspartic acid to lysine found. Similar reasoning renders it unlikely that the ammonia has arisen from hydrolysis of an amide group of asparagine, since no stoichiometrically equivalent quantity of ammonia was found. It seems more likely that the ammonia was introduced as an impurity, particularly if the small actual amount of 5.1 γ is taken into consideration.

Quantitative Relation of Peptides to Activation Process

In order to establish the fact that the appearance of the peptide is actually related to the activation process, the amount of peptide formed was compared to the appearance of tryptic activity, as follows:

<table>
<thead>
<tr>
<th>Peptide</th>
<th>29 per cent activation*</th>
<th>49 per cent activation*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Found, μmoles</td>
<td>1.65</td>
<td>2.89</td>
</tr>
<tr>
<td>Calculated,† μmoles</td>
<td>1.78</td>
<td>3.01</td>
</tr>
<tr>
<td>Recovery, %</td>
<td>92.5</td>
<td>96.0</td>
</tr>
</tbody>
</table>

* As measured by esterase activity.
† Calculated on the basis of 1 mole of peptide liberated per mole of trypsin formed.

A solution of trypsinogen was activated by trypsin as described previously, and the reaction was stopped after predetermined time intervals by the addition of an equal volume of ice-cold 10 per cent trichloroacetic acid to aliquots of the solution. The degree of activation was determined by the esterase method. After centrifugation of the precipitate at 0° and washing with 10 per cent trichloroacetic acid, the supernatant solutions were combined and extracted three times with 2 volumes of ether to remove the trichloroacetic acid. The aqueous solution was then lyophilized. The peptide material was taken up in 5 or 10 ml. of citrate buffer, pH 3.0, and an aliquot applied to the Dowex 50 column after centrifugation of a small amount of insoluble material. The chromatograms obtained were similar to those of Fig. 2 except that the peak corresponding to effluent volumes 150 to 160 ml. was larger. Table III presents a comparison of the experimental yield of activation peptide with the amount expected if 1 mole of Val.(Asp)₄Lys (the proposed composition of the activation peptide) had been liberated for each mole of trypsinogen which has undergone activation. The agreement is sufficiently good to warrant the conclusion that the liber-
The other major peak (about 60 ml of effluent after the addition of buffer of pH 5.0 to the top of the column) did not show any such relation to activation and is probably of secondary origin.

**Autolysis Peptides**

Additional experiments were performed to preclude the possibility that the present peptide originated from autolysis of trypsin rather than from hydrolytic activation of trypsinogen.

To this end, a 1 per cent solution of trypsin was first freed of traces of ninhydrin-positive material by passing through a Dowex 50 column (20 to 50 mesh, 4 per cent cross-linked, hydrogen form) at pH 3.0, 25°. The solution was then allowed to autolyze up to 4 hours under conditions identical to those used for activation of trypsinogen. Aliquots were removed at various time intervals and subjected to paper chromatography as described for the activation peptide. While no loss of enzymatic activity could be observed (esterase method), a slight increase in ninhydrin-positive material was noted on the chromatogram, the major portion migrating with an $R_f$ value of 0.47 (butanol-acetic acid-water), compared to 0.08 for the activation peptide. Since, moreover, the former spot was very faint in comparison to the latter, it is unlikely that its appearance is related to activation.

**Properties of Activation Peptide**

**Reaction with DNFB**—In order to establish $N$-terminal groups, the purified activation peptide (approximately 1 mg.) was dissolved in 0.1 ml. of 1 per cent trimethylamine and allowed to react with 0.01 ml. of DNFB previously dissolved in 0.2 ml. of ethanol (13). After the mixture had stood for 2 hours at room temperature, a few drops of water and trimethylamine solution were added, and the excess reagent was extracted three times with ether. The residue, after evaporation of the aqueous solution to dryness, was taken up in several drops of 5.7 N HCl and hydrolyzed in a sealed tube for 8 hours at 105°. After dilution with water, the DNP-amino acids were extracted into ether. The ether- and water-soluble DNP-amino acids were then chromatographed on paper (14), as shown in Fig. 5.

According to Fig. 5, Chromatogram I, the only ether-soluble amino acid was DNP-valine (or $\alpha_1\epsilon$-di-DNP-lysine, which has the same $R_f$ in this solvent). Chromatogram II shows the presence of $\epsilon$-DNP-lysine in the aqueous extract, while Chromatogram III was obtained after development with ninhydrin, free aspartic acid, $\epsilon$-DNP-lysine, and traces of alanine, glutamic acid, and unchanged lysine.
The absence of valine in the aqueous layer confirms the formation of DNP-valine as shown in Chromatogram I and the presence of only one valine per peptide chain. Thus, the peptide has a structure of Val[(Asp)₄-Lys] and not {Val[(Asp)₃-Lys]}ₙ, where n = 2, 3, etc. The finding of only one N-terminal amino acid also indicates homogeneity of this peptide, assuming the absence of α,ε-di-DNP-lysine in Chromatogram I. The reason for the slightly higher Rₚ value of ε-DNP-lysine hydrochloride as

![Fig. 5. Acid hydrolysate of DNP activation peptide. Chromatogram I, tracing of the chromatogram of the ether-extractable compound, and standard DNP-amino acids, resolved in tert-amyl alcohol for 15 hours on paper previously sprayed with phthalate buffer, pH 6.0, and dried at room temperature. The dinitrophenol was bleached by exposure of the paper to HCl vapors. Chromatogram II, tracing of the chromatogram of the water-soluble compound resolved in the same solvent system as Chromatogram I. Chromatogram III, tracing of the chromatogram of the water layer developed in butanol-acetic acid-water and sprayed with ninhydrin, as described in the text.](http://www.jbc.org/)

compared to ε-DNP-lysine on the paper buffered at pH 6.0 (Chromatogram II) is not known.

**Reaction with Carboxypeptidase**—In order to test for the presence of a C-terminal group reactive with carboxypeptidase, 0.40 pmole of the purified activation peptide was incubated at pH 7.8 and at pH 5.5 with carboxypeptidase crystallized six times in substrate-enzyme mole ratios of 2:1. The lower pH range was included in view of the acidic properties of the substrate (15). After 3 hours incubation at 25° no ninhydrin-positive material other than the peptide could be observed by paper chromatography (butanol-acetic acid-water and phenol-water, respectively) with the experimental procedure described previously (9).
Paper Electrophoresis of Activation Peptide—The presence of 4 aspartic acid residues is expected to confer on the peptide acidic properties. Assuming the absence of amide nitrogen, the peptide should have a net charge of -3 at pH 7.8 and would be expected to exhibit anionic properties at that pH. This was demonstrated by electrophoresis with an apparatus similar to that described by Durrum (16). In a phosphate buffer (pH 7.9, ionic strength 0.088), the purified peptide was found to migrate as a single spot toward the positive electrode (duration 180 minutes, initial current 4 ma. per 7.5 cm. width; potential 320 volts).

The isoelectric point of the peptide was determined by the use of citrate buffers ranging from pH 3.20 to 3.80 with a constant ionic strength of 0.05.

Glucose (about 10 γ) and peptide were applied adjacent to each other in the center of a paper strip (7.5 × 35 cm.) and were allowed to migrate for 16 hours at each pH (initial current 1.8 ma. per 7.5 cm. width; potential 200 volts). The paper strip was then cut down the center and one side developed for glucose by use of an aniline trichloroacetate spray (17). The strip containing the peptide was sprayed first with ninhydrin and, after drying, it was sprayed with 3 per cent sodium acetate in 80 per cent ethanol to adjust the pH closer to 5.0.

The distance moved by the peptide in 16 hours relative to the uncharged glucose was plotted against pH, as shown in Fig. 6. The isoelectric point was found to be between 3.4 and 3.5, which is within the range expected for a polyvalent compound of the proposed structure. The peptide appeared to exhibit a small amount of heterogeneity slightly on the basic side of the isoelectric point.
Paper Chromatography of Activation Peptide in Various Solvents—To characterize the activation peptide further, it was of interest to determine its $R_F$ in various solvent systems. These are recorded in Table IV. Since the $R_F$ of a given compound is often very sensitive to small differences in composition of the solvent system used (18), the given values are not to be taken as absolute and should be compared with the three constituent amino acids which are also listed. In some solvent systems, the behavior of the peptide is similar to that of lysine and, in others, to that of free aspartic acid. In all cases the peptide was homogeneous.

Inhibitory Activity of Activation Peptide—In order to test for possible inhibitory activity of the activation peptide toward trypsin and to examine the possibility of an equilibrium of peptide and trypsin, giving rise to limited enzymatic activity, a trypsin solution of known activity was incubated for 5 minutes at pH 7.5 with a 25-fold molar excess of the activation peptide. With BAEE as substrate, no decrease in tryptic activity could be observed. The effect of the peptide on the tryptic hydrolysis of substrates of lower affinity for the enzyme remains to be tested.

**DISCUSSION**

The present experimental data indicate strongly that during the conversion of trypsinogen to trypsin a hexapeptide is split off, having the probable structure Val-(Asp)$_4$-Lys. The N-terminal position of valine has been proved by labeling with DNFB, whereas the C-terminal position of lysine can only be inferred from (a) the specificity requirements of the activating enzyme, trypsin, and (b) the lack of reactivity of the purified peptide toward carboxypeptidase.

The analytical composition of the peptide is also in accord with other known properties of trypsinogen and trypsin (or DIP-trypsin) listed in Table V. Within the limits of experimental error, the molecular weights,
as determined by sedimentation and diffusion, are the same. However, if it is assumed that the two proteins have identical tyrosine and tryptophan contents, the respective extinction coefficients at 280 m\(\mu\) would correspond to a difference in molecular weight of approximately 1000 compared to a calculated peptide molecular weight of 705. N-Terminal analysis, aspartic acid content, and the difference in nitrous acid-reactive amino groups all are in accord with the loss of 1 valine, 4 aspartic acids, and 1 lysine from the trypsinogen molecule.\(^3\) The isoionic points are also in accord with the loss of predominantly acidic groups from the zymogen.

### Table V

**Comparison of Some Properties of Trypsinogen and Trypsin (or DIP-Trypsin)**

<table>
<thead>
<tr>
<th>Property</th>
<th>Author</th>
<th>Trypsinogen</th>
<th>Trypsin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mol. wt.</td>
<td>Cunningham et al. (5)</td>
<td>23,800</td>
<td>23,800</td>
</tr>
<tr>
<td></td>
<td>Rorary et al. (6)</td>
<td>1 valine</td>
<td>1 isoleucine</td>
</tr>
<tr>
<td>N-Terminal (carboxypeptidase)</td>
<td>Davie and Neurath (7) and this work</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>N. M. Green (unpublished)</td>
<td>9.3</td>
<td>10.1</td>
</tr>
<tr>
<td>Isoionic point (mixed bed ion exchange resin)</td>
<td>P. E. Wilcox (unpublished)</td>
<td>15.1 per</td>
<td>13.8 per</td>
</tr>
<tr>
<td></td>
<td>This work and Davie and Neurath (25)</td>
<td>13.9</td>
<td>14.4</td>
</tr>
<tr>
<td>Free amino groups (Van Slyke)</td>
<td></td>
<td>25.0 per</td>
<td>20.9 per</td>
</tr>
<tr>
<td></td>
<td></td>
<td>23,800</td>
<td>23,100</td>
</tr>
<tr>
<td>(E_{280}^{1%}) (column chromatography)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^*\) Values taken from amino acid analysis of 18 hour hydrolysates (5.7 N HCl) of trypsinogen and DIP-trypsin (foot-note 3).

According to the present data, the most significant molecular changes accompanying the tryptic activation of trypsinogen consist in the hydrolytic cleavage of a lysyl-isoleucyl bond of trypsinogen, yielding as reaction products Val-(Asp)<sub>4</sub>-Lys and trypsin.\(^4\) It is likely that during this process the C-terminal portion of the polypeptide chain remains unaffected, since

\(^3\) Complete amino acid analyses of trypsinogen and DIP-trypsin will be reported elsewhere (E. Cohen, E. W. Davie, and H. Neurath, in preparation).

\(^4\) While this work was in progress, Desnuelle and Fabre (19) reported the isolation of DNP peptides from partial hydrolysates of DNP-trypsinogen, the largest fragment containing 1 mole of DNP valine, about 4 moles of aspartic acid, and 1 mole of lysine. These findings lend additional support to the conclusion that the present peptide has arisen from the N-terminal portion of the polypeptide chain of the zymogen.
both trypsinogen and DIP-trypsin remain unreactive toward carboxypeptidase, even after 3 hours preincubation with 6 M urea,5 pH 7.8, at 0°.

While quantitative data have demonstrated that the liberation of this hexapeptide and the appearance of tryptic activity are related phenomena (see Table III), they do not exclude the possibility that other peptides may have been liberated as well. Since any peptide devoid of a free amino group would fail to be adsorbed initially by the ion exchange resin, use was made of trichloroacetic acid to separate peptides from proteins. Whereas a major second peptide component was actually found, there was no proportional relation of this component to the appearance of tryptic activity, suggesting that it had originated from a non-specific attack of trypsin on the protein components present in the system. The possibility of the appearance of peptides unreactive toward ninhydrin has not been subjected to experimental tests.

It is of interest to note the high degree of selectivity which governs the hydrolytic activation of trypsinogen. Although trypsinogen contains peptide groups contributed by 14 lysine and 2 to 3 arginine residues,3 all of these conforming to the specificity requirements of trypsin, only one of these bonds is hydrolyzed in the process of activation. While the same principle of "limited hydrolysis" applies to the activation of chymotrypsinogen (20, 9) and pepsinogen (21), it is particularly impressive in this instance, since the breaking of a single bond apparently completes the activation process and no intermediary active forms seem to be involved. It is inviting to speculate that electrostatic repulsion between the 4 adjacent aspartic acid residues causes the N-terminal portion of the polypeptide chain of trypsinogen to remain in an extended configuration, thus rendering the lysyl-isoleucyl bond exposed to the attack by trypsin, whereas all other peptide bonds which conform to the specificity requirements of trypsin remain inaccessible within the helical configuration of the remainder of the polypeptide chain. Under conditions favoring "denaturation" of trypsinogen or trypsin (absence of calcium ions, pH lower than 7 to 8) the helical structure might become sufficiently distorted to expose additional peptide bonds to tryptic hydrolysis, yielding "inert protein" (3) or more profoundly degraded products.

As an alternative interpretation of the properties of the activation peptide it might be suggested that in the intact trypsinogen molecule the acidic regions of the peptide mask basic groups of the potential active center. This situation would be somewhat analogous to the postulated interaction between acidic groups of ovomucoid and soy bean trypsin inhibitor, re-

5 The reactivity of carboxypeptidase in the presence of this denaturing agent has been established by Dr. Y. D. Halsey in experiments to be published elsewhere.
respectively, with free amino groups of trypsin (22), except that in the present case, because of its small size, attachment of the hexapeptide to the protein through the lysyl-isoleucyl bond is required for strong interaction. (This qualification is introduced because of lack of inhibitory activity of the free hexapeptide.) Although no experimental evidence for a basic grouping within the active center of trypsin is at hand, such an assumption is entirely compatible with the concept of an esteratic site (23) with an electrophilic component, particularly since trypsin, like acetylcholine esterase, possesses esterase activity (24). The requirement of basic groupings in specific substrates suggests, in further analogy, an additional anionic site within the active center.

It should be recognized that the activation process, as visualized in this discussion, applies strictly to the tryptic activation of trypsinogen and that it remains for future work to establish to what extent these considerations are also applicable to the activation by enterokinase or penicillium kinase (3).

Our thanks are due to Miss Elaine Cohen and Dr. Y. D. Halsey for their assistance and advice in the experiments involving column chromatography.

This work has been carried out, in part, under contract No. Nonr-477-04 between the University of Washington and the Office of Naval Research, Department of the Navy, and was also supported by funds made available by the people of the State of Washington, Initiative 171, and by the United States Public Health Service.

SUMMARY

The tryptic activation of trypsinogen leads to the formation of a peptide having the probable structure Val-(Asp)4-Lys. The peptide has been isolated by chromatographic methods, its structure and properties being in accord with certain known properties of trypsinogen and trypsin. Evidence is provided that the peptide is directly related to the appearance of tryptic activity, and it is suggested that the hydrolysis of a lysyl-isoleucine bond in trypsinogen is the single hydrolytic event leading to the formation of active trypsin.

BIBLIOGRAPHY


IDENTIFICATION OF A PEPTIDE RELEASED DURING AUTOCATALYTIC ACTIVATION OF TRYPsinOGEN

Earl W. Davie and Hans Neurath


Access the most updated version of this article at http://www.jbc.org/content/212/2/515.citation

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/212/2/515.citation.full.html#ref-list-1