IDENTIFICATION OF THE PEPTIDE SPLIT FROM TRYPSINOGEN DURING AUTOCATALYTIC ACTIVATION*

by

EARL W. DAVIE AND HANS NEURATH

Department of Biochemistry, University of Washington, Seattle, Washington (U.S.A.)

Recent studies of the terminal groups of trypsinogen and DFP-trypsin[1,2] have led to the suggestion that during autocatalytic activation of the zymogen one or more peptides are split off. This suggestion was based on the result that the N-terminal groups of trypsinogen and DFP-trypsin were different, whereas no C-terminal groups reactive toward carboxypeptidase could be detected when these proteins were in the native form. More recent studies have shown, however, that after denaturation by acid[1], DFP-trypsin yields 0.3 equivalents of lysine, and trypsinogen 0.05 equivalents, in addition to lesser amounts of other amino acids[1] which were identical for both protein substrates. The action of carboxypeptidase thus suggests that the carboxyl end of the polypeptide chain may not change during activation of trypsinogen and that the loss of a N-terminal peptide gives rise to a trypsin molecule more susceptible to acid denaturation.

We have succeeded in identifying the single peptide formed during the activation process, as follows: In a typical experiment, 120 mg of twice crystallized trypsinogen[3] was dissolved at 0° in 9 ml of 0.1 *M* borate buffer, pH 8.0, containing 0.05 *M* $CaCl_2$, and activated by the addition of 0.7 mg of trypsin. Aliquots were removed at intervals up to 8 hours and any free amino acids or peptides were adsorbed on Dowex 50 resin (H^+ form, 4 or 12% cross-linked). The reaction products were eluted from the resin and subjected to paper chromatography (butanol-acetic acid-water[4] and methanol-water-pyridine[5]). Control experiments demonstrated that under these conditions adsorption on the resin was quantitative. Simultaneously, the rate of activation was followed by the esterase method using benzoyl-L-arginine ethyl ester as substrate[6]. The zero time control showed the complete absence of ninhydrin-positive products. After 5% activation, a single spot was obtained having an R_F value of 0.10 (butanol-acetic acid-water), 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 6 *N* HCl at 110° for 24 and 48 hours indicated the presence of lysine, valine and aspartic acid in mole ratios of 1:1:5 or 6, and traces of glutamic acid and alanine. The C-terminal position of lysine in the peptide is suggested by the specificity requirements of trypsin, and the N-terminal position of valine by the report that this amino acid occupies a like position in trypsinogen, in contrast to *iso*leucine for DFP-trypsin[2]. Hence, the tentative structure of val-$(asp)_{5 \text{ or } 6}$-lys, with a minimum molecular weight of about 1000, may be assigned to this peptide. The acidic properties of the peptide are in accord with the relative positions of the isoionic points of trypsinogen and trypsin, *i.e.*, 9.3 and 10.1, respectively[7]. Control experiments testing the autolysis of 1% trypsin under the same experimental conditions revealed the presence of a faint spot with an R_F value equal to 0.47 (butanol-acetic acid-water) which, however, failed to increase in intensity with the age of the solution.

REFERENCES

1 E. W. DAVIE AND H. NEURATH, *J. Am. Chem. Soc.*, 74 (1952) 6305.
2 M. ROVERY, C. FABRE AND P. DESNUELLE, *Biochim. Biophys. Acta*, 9 (1952) 702.
3 F. TIETZE, *J. Biol. Chem.*, in press.
4 J. A. GLADNER AND H. NEURATH, *Biochim. Biophys. Acta*, 9 (1952) 335, and manuscript in preparation.
5 R. R. REDFIELD, *Biochim. Biophys. Acta*, 10 (1953) 344.
6 G. W. SCHWERT AND M. A. EISENBERG, *J. Biol. Chem.*, 179 (1949) 665.
7 N. M. GREEN, unpublished results.

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