containing amounts of phosphoryl choline and glycerophosphoryl choline similar to those in the solutions to be assayed, and appropriate blanks and controls, the technique outlined above offers a means of estimating, chemically, amounts of choline between 0.05–0.5 μmole which could be applied to the study of the enzymic breakdown of choline-containing phospholipids by tissue preparations.

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Preliminary Notes

The action of enterokinase on trypsinogen

Trypsinogen is activated to trypsin either by trypsin itself or by enterokinase[1]. In the former case, a peptide is split off from the amino-end of trypsinogen, the amount of peptide split off being proportional to the tryptic activity developed as suggested by ROVERY, FABRE AND DESNUELLE[2] and recently confirmed by DAVIE AND NEURATH[3]. However, no studies have been made of the chemical changes taking place during the activation by enterokinase which seems to be highly specific for trypsinogen.

In this communication, it is shown that *iso*leucine is the N-terminal amino acid of trypsin developed from trypsinogen by enterokinase activation.

Trypsinogen was prepared from beef pancreas according to the method of NORTHROP AND KUNITZ[4] through crystallization and further purification by trichloracetic acid. Enterokinase of a high degree of purity was prepared from swine duodenal fluid contents according to the author's method[5,6].

The activation was carried out at 0° C and pH 5.6 using 0.1 *M* triethylammonium acetate buffer, 5 mg of trypsinogen and 0.8 mg of enterokinase containing 2000 units per mg being dissolved in 12.5 ml of the activation mixture. Under these conditions, 50% activation could be obtained within 8 minutes. The reaction was essentially of first order kinetics throughout the whole activation without any appreciable signs of autoactivation or autodigestion due to trypsin. The activation was stopped by the addition of 1.0 ml of 1 *N* HCl and aliquots were taken for the measurement of tryptic activity (hemoglobin method) and the rest was submitted to the determination on N-terminal amino acids. EDMAN's method[7] was used for this purpose. Thus, an equal volume of pyridine containing phenyl*iso*thiocyanate in a concentration of 50 μl per ml was added to the activation mixture and the pH was adjusted to 9.0 by the addition of a few drops of triethylamine. The activities of enterokinase and of trypsin were almost instantaneously blocked during this treatment. After the completion of the coupling, the mixture was washed by benzene and the aqueous solution was then lyophilized.

The dried material was hydrolyzed by 1 *N* HCl at 100° C for one hour and the phenylthiohydantoin derivatives of amino acids were extracted by ethyl acetate. The aliquots were

then submitted to paper chromatography using xylol as the developing solvent on Whatman No. 1 paper treated with formamide-acetone (1:3 by volume)[8]. The spots were marked through the ultraviolet absorption with the aid of fluorescence screen. Valine which has been shown to be N-terminal amino acid of trypsinogen both by dinitrofluorobenzene method[2] and Edman's method[9] was detected throughout the whole stage of the activation with practically constant strength. *Iso*leucine which has been shown to be N-terminal amino acid of di*iso*propylphosphofluoridate-treated trypsin (DIP-trypsin)[2] and of trypsin formed by autoactivation[9] was detected with increasing strength accompanying the appearance of tryptic activity. Other spots localized at the original points or of low R_F values were considered to be of minor interest in this connection. The spots corresponding to valine and *iso*leucine were cut off and the phenylthiohydantoin derivatives were eluted by 1 ml of 95% ethanol for one hour at room temperature. The eluates were measured on ultraviolet absorption at 270 mμ. The amounts of *iso*leucine were calculated against those of valine at each run. The agreement between the percentages of the activation and those of *iso*leucine referred to valine is shown in Fig. 1. The elution techniques have been worked out at least for valine, *iso*leucine and alanine[10] and are being applied to other amino acids[11].

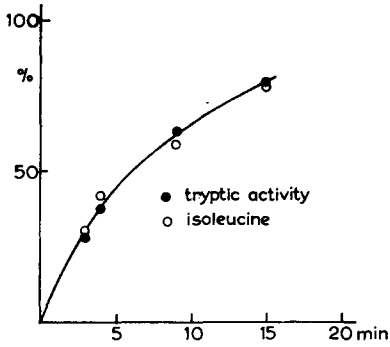

Fig. 1. Relationship between tryptic activity and N-terminal *iso*leucine.

Judging from these experimental results, it is likely that enterokinase acts as a peptidase-splitting valylpeptide[3] from trypsinogen. The details will be published in *Acta Chem. Scand.* in 1956. This work was made possible through a research grant to Professor ERIK JORPES from the Swedish State Medical Research Council. The author's thanks are also due to Dr. PEHR EDMAN for his help during the performance of the work.

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Mitochondrial oxidative phosphorylation in a magnesium-free medium

The assumption, that magnesium is a cofactor in the reactions involved in oxidative phosphorylation[1,2,3], is based on the fact that maximum P/O values for mitochondrial preparations could be obtained only in media containing added Mg^{++}. An assembly of respiratory enzymes, capable of oxidative phosphorylation without added Mg^{++}, has recently been obtained by LEHNINGER'S group[4]. Its endogenous magnesium content is unknown.

With intact rat liver mitochondria, using the rapid platinum electrode technique for simultaneous measurement of respiratory and phosphorylative effects[5], we have obtained respiratory control and maximum P/O values for the oxidation of β-hydroxybutyrate or succinate in a medium containing neither magnesium ions nor other divalent cations. The mitochondria were prepared as in ref. [5]. Table I shows P/O values for β-hydroxybutyrate oxidation in a magnesium-containing (I) and a magnesium-free (II) medium.