Occurrence of hydroxylysine in trypsin

The reaction of trypsin with diisopropyl phosphofluoridate results in the rapid incorporation into the protein molecule of one diisopropylphosphoryl group which has been shown to be bound to a serine residue[1]. A second diisopropylphosphoryl group is incorporated into the trypsin molecule at a much slower rate than the first[2]. In an investigation of the nature of this second phosphorylation site a phosphorus-containing peptide was obtained from partial hydrolysates of trypsin treated with diisopropyl phosphofluoridate, in which a basic amino acid other than lysine, histidine, or arginine was detected. Because its properties suggested it to be hydroxylysine, an amino acid not previously recognized as a constituent of trypsin, evidence for the presence of hydroxylysine in the trypsin molecule was sought.

Acid hydrolysis of small quantities of trypsin (*e.g.*, 20 mg trypsin in 10 ml of constant-boiling HCl in a sealed tube at 110° for 20 h) gave fair yields of the amino acid in question, as measured by the method of SPACKMAN, STEIN AND MOORE[3]. However, hydrolysis of larger quantities (*e.g.*, 2 g trypsin in 200 ml of constant-boiling HCl in sealed tubes at 110° for 20 h) was incomplete, and more prolonged heating led to severe losses of the amino acid. An enzymic degradation procedure was therefore developed. This procedure involves the use of pepsin at pH 2.0, and subtilisin[4], cotazym[5], and purified aminopeptidase[6]. The total amount of enzyme used for degradation was 1 part per 1000–1500 parts of trypsin. By this procedure it has been possible to obtain the natural isomer of hydroxylysine from trypsin hydrolysates in stoichiometric amounts approximating 1 mole/mole of protein. A control consisting of the hydrolyzing enzymes without trypsin yielded less than 1 % as much hydroxylysine as was obtained from trypsin.

The identity of the amino acid with hydroxylysine has been based on the following observations:

(1) The amino acid appears on a column chromatogram[3] in the same position as does the natural isomer of hydroxylysine.

(2) Short-term heating of the amino acid in acid causes the appearance of twin peaks on column chromatograms which are characteristic of hydroxylysine and allohydroxylysine. When this material is chromatographed on a column[3] together with an authentic sample of a mixture of these stereoisomers of hydroxylysine, the two pairs of peaks super-impose exactly.

(3) A sample of the amino acid separated by the chromatographic procedure of HAMILTON[7] from the remaining amino acids shows the same electrophoretic mobility as an authentic sample of hydroxylysine.

(4) The amino gives the characteristic fluorescence in ultraviolet light when sprayed with a specific reagent for hydroxyamino compounds in which the hydroxyl and amino groups are adjacent[8].

(5) The amino acid behaves as hydroxylysine in two-dimensional paper chromatography[9,10].

The enzymic hydrolysis enables us to obtain hydroxylysine in apparently very good yields. Another interesting feature of this procedure is that it also makes it possible to determine the tryptophan content of the protein. In three different experiments 3.9–4.0 moles of tryptophan and 0.75, 0.90 and 1.05 moles of hydroxylysine/mole of trypsin were found.

Acid hydrolysates of a variety of bovine trypsin preparations have been investigated. Twice-recrystallized trypsin from Worthington Biochemicals and from Mann Chemicals, a five-times recrystallized preparation from Worthington Biochemicals, a three-times recrystallized preparation made in our laboratory and a three-times recrystallized preparation of diisopropylphosphoryl-trypsin were included in these studies. The presence of hydroxylysine, demonstrated in hydrolysates of all of these preparations, suggests that this amino acid is a constituent of the trypsin molecule.

In a previous amino acid analysis of trypsin reported from this laboratory[11] the hydroxylysine was undetected, apparently because the basic amino acids were determined by use of the 15-cm column[3] for basic amino acids instead of the 50-cm column[3] used in this work.

In one analysis of an acid hydrolysate of bovine chymotrypsin a total of 0.6 mole of hydroxylysine and allohydroxylysine per mole of protein was found.

The details of this work will be published elsewhere.

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A nucleotide enzyme complex associated with fowl leukemia virus

The virus particles that can be isolated from blood plasma in the case of fowl myeloblastic leukemia possess a high magnesium-stimulated ATPase activity[1–3]. ADP has been claimed to act as a competitive inhibitor in the hydrolysis of ATP[4].

A closer study of the viral ATPase activity reveals properties, some of which indicate a complex of phosphorylating enzymes. If activated by Mg^{++}, two pH optima can be distinguished (pH 7.0 and 8.5). Besides Mg^{++}, also other metal ions activate

Abbreviations: NTP, ATP, UTP, CTP and GTP for the 5′-triphosphates; NDP, ADP, UDP, CDP and GDP for the 5′-diphosphates; NMP, AMP, UMP, CMP and GMP for the 5′-monophosphates of nucleosides, adenosine, uridine, cytosine and guanosine; P_i, inorganic orthophosphate; Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid.