Short Communications

The amino acid sequence around the reactive serine of elastase

The group of hydrolytic enzymes which are characterized by being inhibited by DFP contain an unusually reactive serine residue. In the case of trypsin[1] and chymotrypsin[2,3] this serine is present in the sequence Gly·Asp·Ser·Gly· and in other enzymes of the group[1-6] it appears to be present in this or a very similar sequence. Elastase has recently been shown[7] to have an entirely different specificity from trypsin or chymotrypsin and to be inhibited by DFP. The following experiment shows that it too has a similar sequence around its reactive serine residue.

Elastase was prepared by the method of LEWIS, WILLIAMS AND BRINK[8], and purified on a carboxymethylcellulose column[7]. 40 mg of the purified material were dissolved in 25 ml 0.067 *M* phosphate buffer (pH 8.0) and treated with 1.25 ml 0.02 *M* DFP in isopropanol. (The DFP contained 3.5 mC ^{32}P/mg). After incubation for 3 h at 37° the mixture was dialyzed overnight against 5 l 0.01 *M* acetic acid at 2° and freeze-dried. A sample of the di-isopropyl phosphoryl-elastase was then run on a carboxymethylcellulose column. It emerged as a radioactive peak which moved in the same position as unchanged elastase but had no activity against elastin. From the specific radioactivity of the purified material it was calculated that it was 90% pure assuming a molecular weight[8] of 25,000 and that one mole DFP reacted with one mole elastase.

The di-isopropyl phosphoryl derivatives of trypsin and chymotrypsin were prepared similarly but without chromatographic purification. Samples (0.1 mg) of the derivatives were incubated for 3 days at 37° with 12 *N* HCl. After removal of the HCl *in vacuo*, the hydrolysates were subjected to ionophoresis (40 V/cm, 2 h) on Whatman No. 52 paper in pyridine–acetate buffer, pH 3.5, in the apparatus of MICHL[9]. Each sample was applied over 2 cm of the paper and there was slight overlapping (about 0.2 cm) between the different samples. A similar hydrolysate of ^{32}P-labelled ovalbumin was applied for comparison.

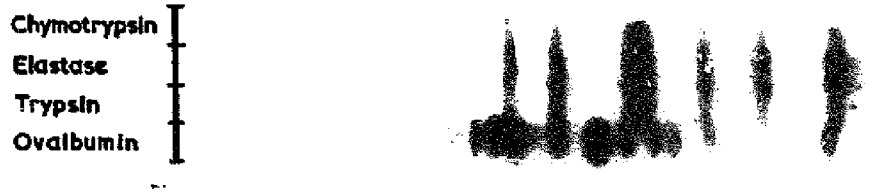

Fig. 1. Radioautograph of ionogram of partial acid hydrolysates of di-isopropyl [^{32}P]phosphoryl derivations of chymotrypsin, elastase, trypsin and of ^{32}P-labelled ovalbumin.

Abbreviation: DFP, di-isopropyl phosphorofluoridate.

Fig. 1 is a radioautograph of the ionogram. The free phosphate band has been allowed to run off the anode end of the paper. The exact correspondence of the radioactive bands from the three di-isopropyl phosphoryl-enzymes indicates that the hydrolysates contain identical peptides of phosphoserine which are entirely different from those derived from ovalbumin. The structure of the different peptide bands is not certain at present but is under investigation. From the number present and their identity in the three enzymes it is probably safe to conclude that elastase, like trypsin and chymotrypsin contains the sequence Gly·Asp·Ser·Gly· around its reactive serine residue.

B. S. HARTLEY
M. A. NAUGHTON
F. SANGER*

Department of Biochemistry, University of Cambridge (Great Britain)

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Received November 27th, 1958

* Member of the external staff of the Medical Research Council.

A simplified spectrophotometric determination of ester groups in lipids

Our laboratory's need for an extremely simple yet highly sensitive and accurate assay of lipid ester groupings in large numbers of chromatographic samples prompted the development of the method reported here. It is based on a hydroxylaminolysis in which an ester reacts with alkaline hydroxylamine to form a hydroxamic acid; the latter forms a purple iron-chelate complex in the presence of acid ferric perchlorate. This method is a modification of more laborious procedures[1-3], and its range extends to 4.00 μequiv. ester.

A standard curve was determined on samples of tristearin, tripalmitin, methyl stearate, or methyl palmitate (California Foundation for Biochemical Research). The weighed lipid was dissolved in Fisher reagent-grade chloroform. Aliquots of the standards were pipetted into test tubes (85 × 15 mm), and the solvent removed under infrared lamps. Acetone (about 0.5 ml) was routinely added to all samples at this point to insure complete removal of the chloroform. The acetone was again evaporated under infrared lamps.

Reagents: *Stock Ferric Perchlorate*[1]. 5 g ferric perchlorate (nonyellow) were dissolved in 10 ml 70 % $HClO_4$ and 10 ml water, then diluted to 100 ml with cold absolute ethanol (stored in refrigerator); *Reagent Ferric Perchlorate*. 4 ml stock ferric perchlorate and 3 ml 70 % $HClO_4$ were diluted to 100 ml with cold absolute ethanol; *Alkaline Hydroxylamine*. Equal vols. of a 4 % ethanolic hydroxylamine solution (2.0 g