MODIFICATION OF TRYPTOPHAN RESIDUES IN TRYPSIN, α-CHYMOTRYPSIN AND PEPSINOGEN.

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The structural basis of biological activity in proteins is a problem of great importance and much work has been directed to its solution from many different points of view. In particular, the correlation between enzymatic activity and progressive, selective, chemical modification of certain reactive sites in proteins, has been a fruitful approach.

It is implicit that the chemical reactions used must be specific and that no secondary effects, such as denaturation, occur. These conditions are difficult to realise and only a few useful reagents and procedures have been developed.

In previous reports (1,2) we have shown that reaction with ozone in anhydrous formic acid converts tryptophan residues into N'-formyl-kynurenine, without breakdown of peptide bonds. The reaction occurs with high selectivity and good yield if it is carried out in the presence of certain benzene derivatives (2).

The most important properties of this method are the mild conditions of reaction and the nature of the solvent in which proteins are only reversibly (3,4) inactivated.

In this note we wish to report on the retention of biological activity of trypsin, α-chymotrypsin and pepsinogen

after conversion of tryptophan residues to N'-formyl-kynurenine residues. Treatment of these proteins with ozone results in a selective oxidation of tryptophan residues in these enzymes, as was revealed by the spectral change and amino acid analysis.

In a typical experiment 100 mg of protein and 3.5 mg of resorcinol, dissolved in 100 ml of anhydrous formic acid, were treated at 8°C with a slow current of ozone (4% in oxygen). At suitable intervals, 0.1 ml samples were taken from the reaction mixture and transferred into 2.5 ml aliquots of aqueous 8 M urea-acetic acid (pH 4). The formation of N'-formyl-kynurenine was followed spectrophotometrically at 315 mμ. The increase of adsorbance at 315 mμ of trypsin, α-chymotrypsin and pepsinogen are illustrate in Fig. 1.

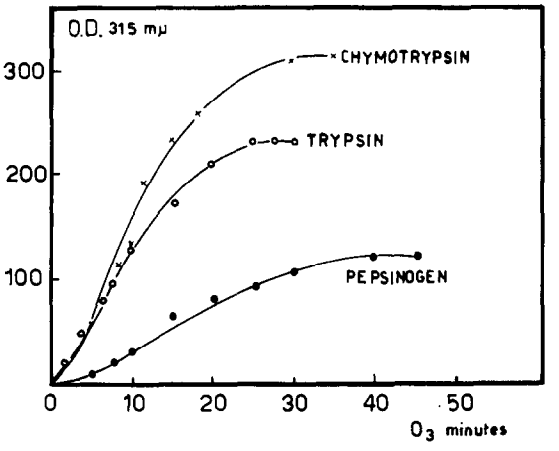

Fig. 1 : Increase of adsorbance at 315 mμ (1 mg in 2.5 ml 8 M urea-acetic acid) versus increasing ozonization.

At appropriate time intervals, parallel samples of 0.1 ml were taken and lyophilized, in preparation for tests of their proteolytic activity. In the case of trypsin and α-chymotrypsin the dry protein residue after lyophilization was dissolved in 1 ml 8 M urea (pH 8) (3) ; the proteolytic activity of 0.1 ml samples was tested using hemoglobin as substrate at pH 8. In the case of pepsinogen, the dry residue was taken up in 1 ml of aque

ous NaOH (pH 8.5) (4) and the extent of proteolytic activity of the modified pepsin tested with hemoglobin as substrate at pH 2.

The decreases of biological activity of trypsin, α-chymotrypsin and pepsinogen as a function of appearance of N'-formyl-kynurenine are plotted in Fig. 2.

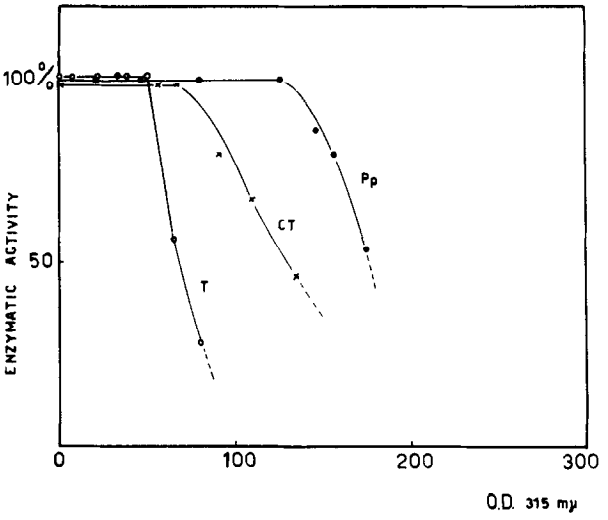

Fig. 2 : Percent proteolytic activity versus 315 mμ adsorbance of 0.1 ml samples.

It is seen that there was no significant decline of enzymatic activity following the oxidation of some of the tryptophan residues in each case.

Protein	O.D. at 315 mμ of 1 mg protein in 2.5 ml 8 M urea-acetic a.	Kynurenine residues per moles of protein
Trypsin	0.130	2
α-chymotrypsin	0.070	1
Pepsinogen	0.052	2

Tab. 1

Amino acid analysis of the three modified proteins with unalterated activity (Tab. 1) has shown that at least two tryptophan residues in trypsin, one in α-chymotrypsin and two in pepsinogen may be converted into N'-formyl-kynurenine without any loss of the original activity.

Further detailed investigations are going in our laboratories.

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ERRATA

Volume 15, No. 6, pp.525-529(1964), in the communication, "Staphylococcal Penicillinase II. Non-Penicillin-Like Cyclic Peptides as Inducers of, and Substrates for, the Enzyme" by Arthur K. Saz and Dolores L. Lowery:

Footnote 1: The name should be Dr. M. Winitz.

Footnote 2: HGS = cyc L-valyl L-arginyl L-leucyl D-phenylalanyl L-prolyl L-valyl L-arginyl L-leucyl D-phenylalnyl L-prolyl.