Studies of Enzyme Active Sites: Synthesis and Catalytic Properties of L-Histidyl-glycyl-L-aspartyl-L-seryl-L-phenylalanine

Keyphrases ☐ Enzyme active sites—catalytic peptide ☐ L-Histidyl-glycyl-L-aspartyl-L-seryl-L-phenylalanine—synthesis, catalytic properties ☐ Paper chromatography, electrophoresis—identity

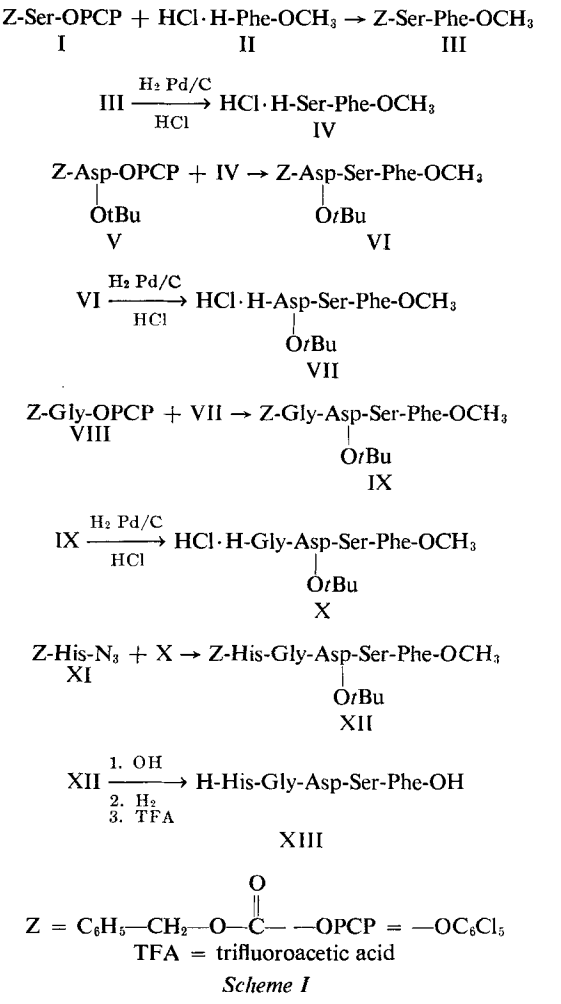
Sir:

Considerable attention has been focused recently on the role played by L-histidine and L-serine in the active site of chymotrypsin and other proteolytic enzymes towards the hydrolysis of various esters such as *p*-nitrophenyl acetate (1–4). One of the approaches for studying the active site of enzymes is the synthesis and evaluation of the catalytic activity of relatively simple peptides embodying as many as possible of the known features of active sites of proteolytic enzymes (5, 6). Katchalski *et al.* reported the synthesis of poly-L-histidine and copolymers of L-histidine and L-serine as model compounds to investigate the catalytic effect on the hydrolysis of *p*-nitrophenyl acetate (7). Sheehan and Cruickshank reported an interesting pentapeptide, L-threonyl-L-alanyl-L-seryl-L-histidyl-L-aspartic acid, possessing considerable catalytic activity (8). In order to provide increased flexibility in the molecule, recently Sheehan *et al.* reported the synthesis of L-seryl-γ-aminobutyryl-L-histidyl-γ-aminobutyryl-L-aspartic acid. This pentapeptide exhibited about 50% more catalytic activity towards the hydrolysis of *p*-nitrophenyl acetate, than that of previously reported synthetic peptides (9). As the separation of serine and histidine in a peptide chain led to relatively more catalytic activity, it was considered worthwhile to investigate the catalytic activity in simple peptides incorporating serine and histidine at different distances in the peptide chains. We now wish to report the preparation of a relatively more potent esterase model, L-histidyl-glycyl-L-aspartyl-L-seryl-L-phenylalanine (XIII).

The catalytic activity of the pentapeptide XIII was determined by the liberation of *p*-nitrophenol from *p*-nitrophenylacetate following the procedure used by Sheehan *et al.* (8, 9). The catalytic coefficient (7) for XIII was 179 l. mole^{-1} min.$^{-1}$ compared with 92 l. mole^{-1} min.$^{-1}$ for L-threonyl-L-alanyl-L-seryl-L-histidyl-L-aspartic acid (8), 147 l. mole^{-1} min.$^{-1}$ for L-seryl-γ-aminobutyryl-L-histidyl-γ-aminobutyryl-L-aspartic acid (9) and 10^4 l. mole^{-1} min.$^{-1}$ for α-chymotrypsin (4).

The pentapeptide was synthesized as outlined in Scheme I. In order to limit the degree of racemization, the peptide chain was extended from the *C*-terminal residue phenylalanine and all peptide bonds were formed using the pentachlorophenyl active ester method (10, 11). *N*-Benzyloxycarbonyl-L-serine pentachlorophenyl ester (I) was condensed with L-phenylalanine methyl ester hydrochloride (II) and the resulting dipeptide, *N*-benzyloxycarbonyl-L-seryl-L-phenylalanine methyl ester (III) was hydrogenated in the presence of hydrogen chloride in anhydrous methanol to afford L-seryl-L-phenylalanine methyl ester hydrochloride (IV). Coupling of IV with *N*-benzyloxycarbonyl-β-*t*-butyl-L-aspartic acid pentachlorophenyl ester (V) afforded *N*-benzyloxycarbonyl - β - *t* - butyl - L - aspartyl - L - seryl - L - phenylalanine methyl ester (VI). Hydrogenation of VI by the usual method afforded β-*t*-butyl-L-aspartyl-L-seryl-L-phenylalanine methyl ester hydrochloride (VII). Coupling of VII with *N*-benzyloxycarbonyl glycine pentachlorophenyl ester (VIII) afforded *N*-benzyloxycarbonyl glycyl - β - *t* - butyl - L - aspartyl - L - seryl - L - phenylalanine methyl ester (IX). Glycyl-β-*t*-butyl-L-aspartyl-L-seryl-L-phenylalanine methyl ester hydrochloride (X) was isolated by the hydrogenation of IX. Coupling of *N*-benzyloxycarbonyl-L-histidine azide (XI) with X led to the formation of *N*-benzyloxycarbonyl-L-histidyl-glycyl - β - *t* - butyl - L - aspartyl - L - seryl - L - phenylalanine methyl ester (XII), m.p. 158–159°.

Anal.—Calcd. for $C_{37}H_{47}N_7O_{11}$: C, 58.04; H, 6.14; N, 12.81. Found: C, 58.31; H, 5.97; N, 12.62.



Scheme I

Complete deprotection of the pentapeptide XII was achieved by saponification, hydrogenation and treatment with trifluoroacetic acid affording L-histidyl-glycyl-L-aspartyl-L-seryl-L-phenylalanine (XIII), m.p. 215–218° dec.

Anal.—Calcd. for $C_{24}H_{31}N_7O_9$: C, 51.34; H. 5.53; N, 17.47. Found: C, 51.12; H, 5.68; N, 17.19.

The free pentapeptide XIII was homogeneous to paper chromatography and paper electrophoresis under a variety of conditions.

Further studies on the catalytic activity and the chemistry of the pentapeptide XIII and other peptides incorporating histidine and serine separated by different distances of amino acids are under investigation.

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A. KAPOOR
SOON M. KANG
MARY ANN TRIMBOLI
College of Pharmacy
St. John's University
Jamaica, NY 11432

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Interference of Polycarboxylic Acids in the Determination of Ester Degradation by the Hydroxamic Acid Procedure

Keyphases □ Ester degradation determination—polycarboxylic acid interference □ Hydroxamate assay procedure—polycarboxylic buffer interference □ Colorimetric analysis—spectrophotometer

Sir:

In recent studies dealing with the kinetics of degradation of polyethylene glycol-600-mono-oleate in hydro-

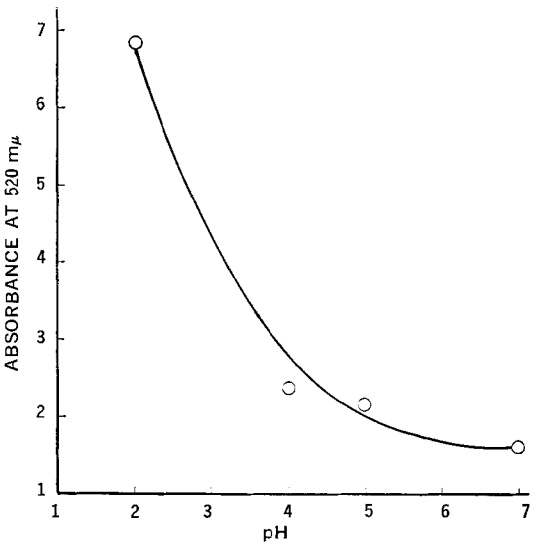

Figure 1—*Absorbance of ferric hydroxamate after heating hydroalcoholic solution buffered to various pH values with succinic acid for 24 hr.*

alcoholic solution at 80°, inconsistent results were observed. The degradation of the ester was determined by the ferric hydroxamate procedure (1), which indicated an apparent increase in ester concentration as a function of time. Investigation of the system leads us to believe that the succinate buffer system employed appears to react with the ethanol present in the system to form esters.

Blank solutions were prepared containing 0.1 *M* succinic acid in 50% ethanol and adjusted to pH 2, 4, 5, and 7 with 0.1 *N* sodium hydroxide and then subjected to refluxing at 80° for 24 hr. Samples were then taken and assayed by the hydroxamate procedure (1) with the results shown in Fig. 1. It is readily apparent that even in the absence of the test compound there is an increase in absorbance at 520 mμ which indicates the formation of an ester species. The reaction probably proceeds *via* an intermediate anhydride formation as reported earlier (2, 3), which would show an identical pH dependency. As expected, similar results were noted in our experiments using other polycarboxylic buffer systems such as citrate and tartrate. The interference was essentially eliminated by the use of phosphate systems.

These findings clearly indicate again the potential side reactions induced by buffers which may give rise to significant interference with analytical procedures. To test the validity of the assumption that the reaction involved esterification of the ethanol, identical solutions were prepared but substituting acetone for ethanol. Acetone was chosen since, if the proposed mechanism were correct, it would not react with the proposed anhydride intermediates. Solutions, after heating under the identical conditions as cited previously, showed no change in absorbance at 520 mμ. These findings would further support the hypothesis that esterification was taking place during the heating process.

The above-cited interference could cause significant errors in the interpretation of high-temperature kinetic