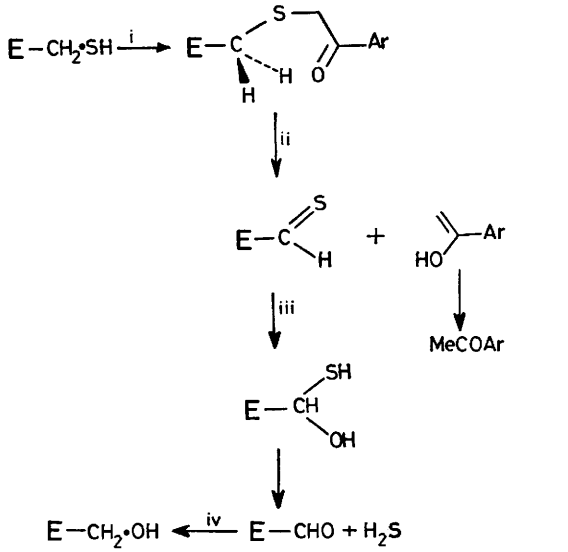
# Chemical Mutations of Papain. The preparation of Ser 25- and Gly 25-Papain

By PETER I. CLARK and GORDON LOWE*
(*The Dyson Perrins Laboratory, Oxford University, South Parks Road, Oxford* OX1 3QY)

*Summary* The active site cysteine residue of the proteolytic enzyme papain has been converted into a serine and a glycine residue by novel chemical methods.

THE conversion of an amino acid residue in a protein molecule into another natural amino acid has rarely been achieved.[1] Such 'chemical mutations'[2] if achieved selectively would provide proteins of great interest in protein chemistry and enzymology. In order to explore more thoroughly the chemistry of the active site of the proteolytic enzyme, papain, and in the hope of generating an enzymically inactive modification of papain with binding properties for peptides similar to those of the native enzyme, a method has been developed for converting the active site thiol group (of Cys 25) into hydroxy-group. The chemical strategy envisaged for this transformation is outlined in Scheme 1. α-Bromoketones selectively alkylate the active site cysteine residue of papain. By using a phenacyl bromide with an absorption band at longer wavelength than that of the enzyme, photolysis of the alkylated enzyme should lead to a Norrish type II cleavage.[3] Steric constraint on the thioaldehyde so generated should prevent oligomerisation, and addition of water followed by spontaneous loss of hydrogen sulphide from the hemithioacetal to give the corresponding aldehyde, seemed the most likely course of events. Reduction of the photolysate with $NaBH_4$ should therefore complete the conversion into Ser 25-papain.

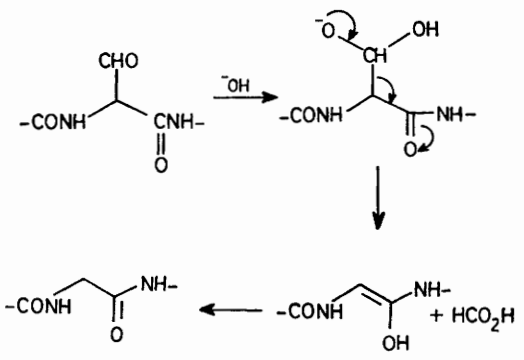



SCHEME 1. Reagents. i, $BrCH_2COAr$; ii, *hν*; iii, $H_2O$; iv, $NaBH_4$.

2-Bromo-2′,4′-dimethoxyacetophenone inhibited papain stoicheiometrically and irreversibly. Photolysis ($\lambda > 320$ nm) of the *S*-(2,4-dimethoxyphenacyl) papain in aqueous solution regenerated 76% of the native enzyme and it was necessary successively to inhibit the photolysate and photolyse again three times in order to achieve a high degree of conversion into the modified protein. The competing mode of cleavage leading to regeneration of native enzyme was probably favoured by steric restriction to the required conformation of the alkylated cysteine residue for the Norrish type II cleavage to be achieved. The final photolysate was treated with the bromoketone (in order to inactivate the small amount of active papain) and applied to an affinity column of sepharose–aminocaproyl-glycyl-L-phenylalanyl-L-arginine.[4] An exponential gradient formed between 20mM-ethylenediaminetetra-acetic acid and water eluted the modified enzyme in almost the same position as the native enzyme. After reduction of the protein with $NaBH_4$ the product was chromatographed on the same affinity column and was eluted at the same position in the chromatogram as the initial photolysis product. The modified forms of the enzyme therefore have retained the specific binding properties of the native enzyme. When the reduction was performed with Sodium boro[$^3$H]hydride and the [$^3$H]-protein hydrolysed, radioactivity appeared only in those fractions from the amino acid analyser containing serine. From the radioactivity in these fractions and the specific activity of the Sodium boro[$^3$H]hydride used, it was calculated that 0·204 moles of serine/mole of protein had been produced, assuming that there is no kinetic isotope effect. Primary kinetic isotope effects of 3·21 to 5·03 are observed for sodium boro[$^3$H]hydride reduction of carbonyl groups with a wide range of reactivity.[5] Thus essentially one mole of serine/mole of protein was formed on reduction with $NaBH_4$.

If reduction of the photolysate with Sodium boro[$^3$H]-hydride is performed immediately after photolysis, the hydrolysed protein contains [$^3$H]-cysteic acid (derived from [$^3$H]-cysteine) and [$^3$H]-serine. This observation confirms that the thioaldehyde is the initial photolysis product which slowly hydrolyses to the corresponding aldehyde.

It seemed possible that dehydro-Ser 25-papain could be converted into Gly 25-papain under mildly basic conditions as outlined in Scheme 2.




SCHEME 2

Dehydro-Ser 25-papain was incubated in [$^3$H]water at pH 9·0 and 20 °C for 3 weeks and the protein then hydrolysed and analysed. Radioactivity was only found in those fractions from the amino acid analyser containing glycine. When a portion of the solution which had been incubated for three weeks was reduced with $NaBH_4$ prior to protein hydrolysis, again only the fractions from the amino acid analyser containing glycine were radioactive. This control experiment excludes the possibility that [$^3$H]-glycine was formed from the dehydroserine residue during hydrolysis of the protein.

Ser 25-papain and dehydro-Ser 25-papain show no enzymic activity towards *N*-benzyloxycarbonyl-glycine *p*-nitrophenyl ester or *N*-benzyloxycarbonyl-L-lysine *p*-nitrophenyl ester which are good substrates for the native enzyme. A Ser 25-papain peptide complex provides therefore an excellent stable analogue of the native enzyme–substrate complex.

We thank the S.R.C. for a research studentship (to P.I.C.). This is a contribution from the Oxford Enzyme Group supported by the S.R.C.

(*Received*, 16*th September* 1977; *Com.* 971.)

[1] L. Polgár and M. L. Bender, *Adv. Enzymol.*, 1970, **33**, 381.
[2] K. E. Neet and D. E. Koshland, *Proc. Nat. Acad. Sci., U.S.A.*, 1966, **56**, 1606.
[3] R. B. Woodward, quoted by K. Heusler in 'Cephalosporins and Penicillins,' ed. E. H. Flynn, Academic Press, New York, 1972, p. 274.
[4] M. R. Bendall, N. Hawcroft, and G. Lowe, unpublished results.
[5] D. J. Pasto and B. Lepeska, *J. Amer. Chem. Soc.*, 1976, **98**, 1091.