Evidence for the Presence of Histidine-106 in the Active Site of Papain

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THE irreversible inhibition of the proteolytic enzyme papain by iodoacetic acid and by toluene-*p*-sulphonamidomethyl chloromethyl ketone is known to be due to the alkylation of the thiol group of the active site cysteine residue (Cys-25).[1,2] During the hydrolysis of a substrate by papain, an intermediate is formed in which this thiol group is acylated by the substrate.[3]

The suggestion has been made on the basis of kinetic studies that the imidazole group of a histidine residue may also play an essential role in the mechanism of action of this enzyme.[4] In order to provide direct evidence for the propinquity of a histidine residue and cysteine-25 in the tertiary structure of papain, 1,3-dibromoacetone was prepared, which was designed to react first with the active site cysteine residue and then intramolecularly with a second nucleophile.

Twice crystallised papain was rapidly and completely inhibited by one molar equivalent of 1,3-dibromoacetone. The molecular weight of the inhibited enzyme was indistinguishable from papain by gel filtration on Sephadex G-75, showing that cross-linking between enzyme molecules had not occurred. The optical rotatory dispersion curve between 240 and 520 mμ was indistinguishable from that of chloroacetone-inhibited papain. The amino-acid analysis indicated the loss of one histidine residue when compared with that of the native enzyme. The loss of a half-cystine residue was less clear because of the instability of this residue during acid hydrolysis.

Twice crystallised papain was irreversibly inhibited with one equivalent of [^{14}C]-1,3-dibromoacetone and oxidised with performic acid (Baeyer–Villiger). The protein was hydrolysed with acid and the digest analysed on the Technicon amino-acid AutoAnalyzer. The eluate from the ion-exchange column which was not required by the analytical system was used to determine the radiochromatogram. *S*-Carboxymethylcysteine sulphone (and its acid-catalysed degradation products) and 1-carboxymethylhistidine were the only radioactive residues.

In order to identify which of the two histidine residues in papain had been alkylated and to confirm that cysteine-25 was also alkylated, the [^{14}C]-inhibited enzyme was reduced with sodium borohydride and the cysteine residues so generated, alkylated with iodoacetic acid. The protein was separated from excess reagents on Sephadex G-25 and digested with trypsin and α-chymotrypsin. Four radioactive peptides were obtained pure after chromatography of the digest on Sephadex G-25,

phosphocellulose and paper. Peptide (I) (containing 30% of the radioactivity of inhibited papain) and peptide (II) (5%) are shown in the Figure.

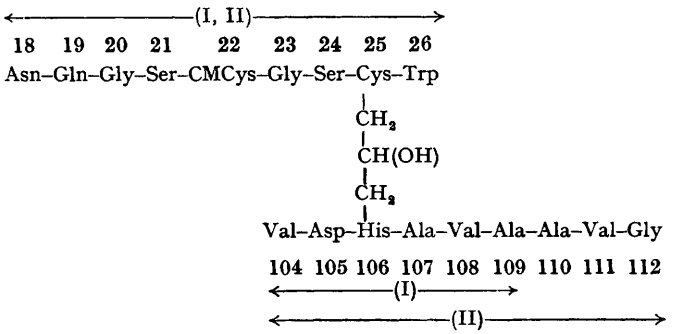

FIGURE. *The structures of peptides* (I) *and* (II).

Peptide (III) (2%) was an artefact arising from rearrangement of the *N*-terminal asparagine residue of peptide (I). Peptide (IV) (2%) contained a lysine residue (this could have arisen from residue 17 or 103 in papain) but was otherwise identical to peptide (I). The structure of the peptides followed from their amino-acid analyses and the amino-acid sequence of the enzyme.[1] The structure of peptide (I) was however confirmed by Edman degradation and leucine aminopeptidase and carboxypeptidase A digestion.

These results provide direct evidence for the presence of the imidazole group of histidine-106 *within* 5 Å of the thiol group of cysteine-25, and support the proposal that a histidine residue plays an essential role in the mechanism of action of papain. The similarity of the amino-acid sequence around cysteine-25 of papain and the active site serine of trypsin has already been noted.[5] It may well be, therefore, that the mechanism of action of the serine and cysteine proteinases are similar. If this is so then the active site thiol and imidazole groups in papain may be hydrogen bonded and act in concert.

Irreversible inhibition of the cysteine proteinases, ficin and stem-bromelain, by 1,3-dibromoacetone have also been shown to involve alkylation of the histidine residue at N(1). The presence of histidine or other nucleophiles close to the active-site cysteine residue of several other enzymes is being investigated with this reagent.

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