The Amino-acid Sequence around the Active Site Cysteine and Histidine Residues of Stem-bromelain

By S. S. HUSAIN and G. LOWE*

(*The Dyson Perrins Laboratory, Oxford University, South Parks Road, Oxford*)

THE proteolytic enzyme stem-bromelain, belongs to a group of plant proteinases which depend for their enzymic activity on the thiol group of a cysteine residue.[1] The amino-acid sequence around the cysteine residue of stem-bromelain alkylated by iodoacetic acid is Gly-Ala-Cys-Trp.[2] The extension of this sequence (at the *N*-terminus) has been reported to be quite different from that found in the closely related enzymes, papain and ficin.[3] This was surprising and we therefore investigated further the amino-acid sequence around the cysteine residue in stem-bromelain.

The reagent 1,3-dibromoacetone irreversibly inhibits stem-bromelain, and cross-links a cysteine

and histidine residue within the same enzyme molecule, the latter through the N-1 of the imidazole group.[4] The amino-acid sequence around these residues has been elucidated with the aid of this reagent.

Stem-bromelain prepared by the method of Ota, Moore, and Stein,[5] was rapidly and completely inhibited at pH 6·0 with just over a molar equivalent of [2-^{14}C]-1,3-dibromoacetone. The thiol contents (determined by the method of Ellman[6]) of the enzyme and inhibited enzyme were 0·85 and 0·11 mol. respectively. The inhibited enzyme in 6M-guanidinium chloride solution was treated with sodium borohydride in order to reduce the ketone and disulphide bonds. The reduced protein was alkylated with iodoacetic acid and separated from excess of reagents on Sephadex G-25. Digestion with trypsin and α-chymotrypsin was followed by chromatography on Sephadex G-25, phosphocellulose, DEAE-Sephadex A-25, and paper, whereby several radioactive peptides were obtained. All of these peptides after hydrolysis showed a single radioactive peak on the amino-acid analyser at the position previously established for Cys·CH_2·CH(OH)·CH_2·His.[7] The structures of the two major peptides (I) and (II) shown in Figure 1 were deduced as follows.

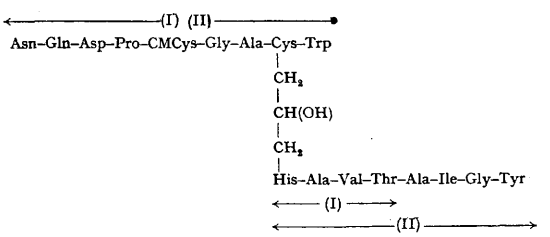

FIGURE 1. *The structures of peptides* (I) *and* (II).

Peptides (I) and (II) after hydrolysis with 6N-HCl (110°, 20 hr., in sealed evacuated tubes) gave respectively the following amino-acid analyses: CMCys (0·67; 0·70), Asp (2·06, 1·94), Thr (0·84, 0·94), Glu (1·00, 1·00), Pro (0·90, 0·95), Gly (1·26, 1·77), Ala (2·04, 2·91), Val (1·00, 1·14), Ile (—, 0·75), Tyr (—, 0·70). Digestion of peptide (I) with carboxypeptidase A for 4 hr. gave Thr (0·8), Val (0·8), Trp (0·8) whereas digestion for 15 min. gave Thr (0·24), Val (0·13), and Trp (0·7). Digestion of peptide (II) with carboxypeptidase A for 4 hr. gave Tyr (0·7) and Trp (1·0). After one cycle of Edman degradation with peptides (I) and (II), only the phenylthiohydantoin of asparagine was extracted. Paper chromatography of the residual peptide fraction after one cycle of Edman degradation with peptide (II), separated a non-radioactive peptide from the radioactive material which after hydrolysis had the following amino-acid analysis: Thr (1·00), Gly (1·19), Ala (1·79), Val (1·00), Ile (0·75), Tyr (0·78). Since the peptide does not contain tryptophan, its *C*-terminal residue must be tyrosine, hence five cycles of "substractive"-Edman degradation sufficed to establish the sequence Ala-Val-Thr-Ala-Ile-Gly-Tyr. Peptide (II) was now subjected to six cycles of "dansyl"-Edman degradation.[8,9] After the first cycle the *N*-terminal residues were, Glx, Ala; after the second cycle, Asx, Val; after the third cycle, Pro, Thr; after the fourth cycle, CMCys, Ala; after the fifth cycle, Gly, Ile; and after the sixth cycle, Ala, Gly. Four cycles of "dansyl"-Edman degradation of peptide (I) gave similar results. Digestion of peptides (I) and (II) with leucine aminopeptidase released some of all the amino-acid residues, which included one residue of Asp, but no Glu; Asn and Gln were present but unresolved on the amino-acid analyser. These data allowed the structures of peptides (I) and (II) to be deduced except that the cross-linked Cys and His residues had not been assigned to their respective peptide sequences. The amino-acid sequence around the active site cysteine however is Gly-Ala-Cys-Trp[2] which enabled this assignment to be made.

The high degree of homology between the amino-acid sequence around the active-site cysteine residues in stem-bromelain, papain,[10,11] and ficin[12] is shown in Figure 2. This suggests that these

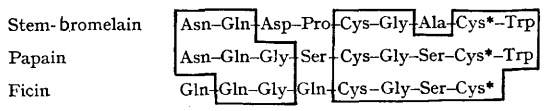

FIGURE 2. *Homologies between the active-site cysteine peptides of stem-bromelain, papain and ficin. The active site cysteine residues are indicated with an asterisk and identical residues in the different enzymes are outlined.*

enzymes have evolved from a common ancestral proteinase and probably adopt a similar catalytic mechanism. It is of interest that in the tertiary structure of papain, Gln-19 is very close to the active site cysteine-25[13] and a glutamine residue is found in each of these enzymes at this position. The even higher degree of homology between the amino-acid sequence around the active site histidine residues in stem-bromelain and papain[14] is shown in Figure 3.

Since the active site histidine residue is at the

N-terminus of the peptide isolated from stem-bromelain after tryptic and α-chymotryptic cleavage, it is reasonable to assume that the adjacent amino-acid (on the *N*-terminus) is not an aspartic or glutamic acid residue. In papain the carboxylic acid group of the aspartic acid residue (105 in the chemical sequence[10] and 157 in the *X*-ray sequence[13]) adjacent to the active site histidine, is about 10 Å from the thiol group of the active site cysteine in the crystal structure.[13] Since this homology does not extend apparently to stem-bromelain, this residue is unlikely to play any significant catalytic role in the mechanism of action of papain.

We thank the Medical Research Council for a grant and the Enzyme Development Corporation, New York, for a generous gift of bromelain-1200 latex.

(*Received, August 26th*, 1968; *Com.*1165).

Stem-bromelain		His*–Ala–Val	–	Thr	–	Ala	– Ile –	Gly–Tyr
Papain	Val–Asp–	His*–Ala–Val	–	Ala	–	Ala	– Val –	Gly–Tyr

FIGURE 3. *Homologies between the active site histidine peptides of stem-bromelain and papain. The active site histidine residues are indicated with an asterisk and identical residues in the enzymes are outlined.*

[1] E. L. Smith and J. R. Kimmel, "The Enzymes", ed. P. D. Boyer, H. Lardy, and K. Myrbäck, Academic Press, New York, 1960, vol. 4, p. 133.
[2] Li-Pen Chao and I. E. Liener, *Biochem. Biophys. Res. Comm.*, 1967, **27**, 100.
[3] T. Murachi, T. Miyake, and M. Mizuno, Seventh International Congress of Biochemistry, Tokyo, 1967, Abstract F-68.
[4] S. S. Husain and G. Lowe, *Chem. Comm.*, 1968, 310; *Biochem. J.*, 1968, **110**, 53.
[5] S. Ota, S. Moore and W. H. Stein, *Biochemistry*, 1964, **3**, 180.
[6] G. L. Ellman, *Arch. Biochem. Biophys.*, 1959, **82**, 70.
[7] S. S. Husain and G. Lowe, *Biochem. J.*, 1968, **108**, 861.
[8] W. R. Gray and B. S. Hartley, *Biochem. J.*, 1963, **89**, 379.
[9] K. R. Woods and Kung-Tsung Wang, *Biochim. Biophys. Acta*, 1967, **133**, 369.
[10] A. Light, R. Frater, J. R. Kimmel, and E. L. Smith, *Proc. Nat. Acad. Sci. U.S.A.*, 1964, **52**, 1276.
[11] S. S. Husain and G. Lowe, *Chem. Comm.*, 1965, 345.
[12] R. C. Wong and I. E. Liener, *Biochem. Biophys. Res. Comm.*, 1964, **17**, 470.
[13] J. Drenth, J. N. Jansonius, R. Koekoek, H. M. Swen, and B. G. Wolthers, *Nature*, 1968, **218**, 929.
[14] S. S. Husain and G. Lowe, *Chem. Comm.*, 1968, 310; *Biochem. J.*, 1968, **108**, 855, 861.