second contained the thiol-dependent activity towards this substrate. The active protein, separated from cysteine by gel filtration, was found to possess a key catalytic site characteristic that is typical of many cysteine proteinases [1, 2, 15]. This step-few kinetic analysis of the reaction of its catalytically essential thiol group with 2-Py-S-S-2-Py showed that the reactivity is a minimum at a pH value of approx. 6 and increases at both lower and higher pH values (approx. 4 and 8), behaviour characteristic of the presence of an −S−/−Im− ion pair [2, 15].

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Investigation of mechanistic consequences of natural structural variation within the cysteine proteinases by knowledge-based modelling and kinetic methods

CHRISTOPHER M. TOPHAM,* JOHN OVERINGTON,* DEVENAND KOWLESSUR,** MARK THOMAS,† EMRY S W. THOMASS§ and KEITH BROCKLEHURST††

*Laboratory of Molecular Biology, Department of Crystallography, Birkbeck College, University of London, Malet Street, London WC1E 7HX.
†Department of Biochemistry, St Bartholomew's Hospital Medical College, University of London, Charterhouse Square, London EC1M 6BQ, and §Department of Biological Sciences, University of Salford, Salford M5 4WT, U.K.

The structural variation within the cysteine proteinases is being used in the study of molecular recognition and catalytic mechanism [1, 2] and work on little-studied members of the family [3–5] continues to reveal behaviour considerably different from that of papain (EC 3.4.22.2). The only high-resolution X-ray structures of cysteine proteinases available are those of papain (1.65 Å) [6] and actinidin (EC 3.4.22.14) (1.70 Å) [7] which differ in active-centre behaviour [1, 2] despite having closely similar crystal structures.

We are extending the availability of variants for mechanistic study by building three-dimensional structures of other members of the family for which complete amino acid sequences are available [8, 9]. In the present work, structures of papaya proteinase Ω [8] and stem bromelain [9] were built (see Fig. 1) by using a knowledge-based approach incorporated in COMPOSER [10–12] and refinement by energy minimization (SYBIL, version 5.2, 1989, Tripos Associates Inc.). The approach is based on simultaneous rigid-body superposition of related known structures, building loops selected from homologous proteins or a wider database, and rule-based building of side-chain geometries. By using the co-ordinate data for papain (9PAP) and actinidin (2ACT) (Brookhaven Data Bank), the two structures were superposed by an iteratively reweighted least squares procedure [11] to define a new set of topologically equivalent residues from an initial set in the catalytic site region of each enzyme (Cys-25, His-159, Asn-175, papain numbering) [13]. The weighted mean of each Ca position comprises the framework for modelling.

The alignment of the papaya proteinase Ω sequence with the consensus sequences (templates) of the framework is trivial in most regions due to the high sequence identity for the core region with papain (72.0%) and actinidin (53.4%) and the result is almost entirely consistent with the sequence alignment reported previously [6]. The alignment of the stem bromelain sequence to the framework differs in three regions from the multiple sequence alignment reported in [9]. The most striking difference occurs around a four residue deletion (168α–d, papain numbering). The sequence alignment in [9] led to the conclusion that the highly conserved Asn-170 and Ser-176 of papain are respectively deleted and mutated to Lys in stem bromelain. That alignment (iii] and (iv)] permits conservation of Tyr-170 in papain, actinidin and stem bromelain but cannot easily be rationalized in structural terms. Thus it would necessitate disruption of the antiparallel β-sheet and hydrogen-bonding pattern common to actinidin and papain.

Abbreviation used: Py, pyridyl.

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Fig. 1. Representations of the Cα backbones of the models of (a) papaya proteinase Ω and (b) stem bromelain.

β-hairpin loop analogous to that in papain. The four residue insertion at position 172 in actinidin [EGGV, see (iii)] is accommodated by extending the β-ladder by forming two additional hydrogen bonds, which permits a class 2:2 I′ β-turn [14]. A similar insertion (Ser-Gly-Gly-Lys) occurs in papaya proteinase Ω. In papain and actinidin Asn-175 is hydrogen-bonded to His-159. Our alignment for stem bromelain places Lys in the position analogous to Asn-175. This would provide an unlikely juxtaposition of positively charged side-chains and suggests the possibility of an error in the sequence reported by Ritonja et al. [9].

We are carrying out kinetic studies on the reactions of stem bromelain and papaya proteinase Ω with a variety of two-hydronic-state thiol-specific reactivity probes R-S-S-2-Py+2-S-S-2-PyCH [15]. Results support the view that residue 175 in stem bromelain is unlikely to be Lys and are revealing substantial differences between the binding site-catalytic site signalling systems [11] of papaya proteinase Ω and papain.

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