BIOCHEMICAL JOURNAL LETTERS

Fresh non-fruit latex of *Carica papaya* contains papain, multiple forms of chymopapain A and papaya proteinase Ω

The main purpose of the present Letter is to point out (i) that fresh non-fruit latex of *Carica papaya* contains, perhaps unexpectedly, multiple forms of chymopapain A as a major proteinase constituent and (ii) that it is important to determine catalytic site characteristics in order to distinguish between chymopapains A and B. The conclusion reached by Buttle & Barrett (1984), from studies on papaya latex obtained from supermarket fruit, that forms of chymopapain other than chymopapain B are artefacts produced during the commercial preparation of latex, is not supported by our analysis of catalytic site characteristics of the enzymes present in fresh non-fruit latex collected from the growing plant.

An additional purpose is to suggest that the most basic cysteine proteinase present in papaya latex, which has been called variously papaya peptidase A (Schack, 1967), papaya peptidase II (Brocklehurst & Salih, 1983), papaya proteinase A (Polgár, 1984) and papaya proteinase (III) (Buttle & Barrett, 1984) might best be called papaya proteinase Ω.

The latex obtained from the unripe fruit of the pawpaw tree (*Carica papaya*) and dried in the country of origin (see Brocklehurst *et al.*, 1981) has been used extensively as a source of cysteine proteinases. Typically (see Baines & Brocklehurst, 1982; Brocklehurst & Salih, 1983; Brocklehurst *et al.*, 1984) dried latex contains, in order of increasing basicity, the following cysteine proteinases: papain (EC 3.4.22.2), a complex mixture of chymopapains (EC 3.4.22.6) and a very basic cysteine proteinase which has been given the four names listed above.

Papain (the least basic cysteine proteinase component) and the most basic cysteine proteinase appear, respectively, at each end of an ion-exchange elution profile and are unlikely to be confused with any of the chymopapains or with each other. Attempts so far to coin a rational name for the most basic cysteine proteinase have been less than satisfactory. Schack (1967) called this enzyme papaya peptidase A, the suffix 'A' being used presumably because of the possible existence of a papaya peptidase B (see, e.g., Polgár, 1981). In an attempt to preserve a historical link with 'papain peptidase I', the original name used by Bergmann *et al.* (1936) for what was almost certainly the individual enzyme papain (EC 3.4.22.2), Brocklehurst & Salih (1983) suggested that papaya peptidase A might be called papaya peptidase II. This seemed a useful change, particularly as 'papaya peptidase B' appears to be one form of chymopapain. Buttle & Barrett (1984) suggested two further changes. Their point that peptidase should be changed to proteinase, made also by Polgár (1984), is a valid one and should perhaps now outweigh the historical link. Their suffix 'III', however, in our view, is not the optimal suffix because of the multiplicity of cysteine proteinase forms present in the 'chymopapain' elution band of both fresh and dried papaya latex. The number, nature and inter-relationships of these cysteine proteinases are still to be fully determined. We propose, therefore, that 'papaya peptidase A' would most usefully be called papaya proteinase Ω because this enzyme is most certainly the last cysteine proteinase to be eluted in ion-exchange fractionation of papaya latex.

We turn now to the problem of identifying the individual chymopapains. The designation of chymopapains as A or B was made originally by Kunimitsu & Yasunoba (1967) on the basis of basicity, chymopapain A being eluted before chymopapain B in an ion-exchange procedure. The A and B classification is worth preserving even now that improvements in chromatographic techniques have revealed a multiplicity of chymopapain forms. This is because the catalytic site characteristics of chymopapain A are distinctly different from those of the various forms of chymopapain B, and in our experience both A and B forms are always present in commercial dried papaya latex (Brocklehurst *et al.*, 1980; Baines & Brocklehurst, 1982; Brocklehurst *et al.*, 1984). The reaction of the catalytic site thiol group of a chymopapain of the B type with the two-protonic-state reactivity probe (see Brocklehurst, 1982) 2,2′-dipyridyl disulphide is characterized by a plot of

second-order rate constant (k) against pH that has a shape found also for the analogous reactions of many other cysteine proteinases. The main features are a striking rate optimum at pH 3–4, a shallow minimum at pH approx. 6 and a plateau of only slightly higher reactivity in alkaline media approached across a pK_a of approx. 9. The pH–k profile for the reaction of 2,2′-dipyridyl disulphide with the catalytic site thiol group of chymopapain A, however, has a markedly different shape. The bell-shaped component of high reactivity at low pH is missing in the pH–k profile for the chymopapain A reaction and the values of k at high pH are considerably larger (e.g. k = approx. 2×10^4 M$^{-1}\cdot$s^{-1} at pH 8.5, 25°C, I 0.1 M) than those found in the reactions of the B-type chymopapains (e.g. k = approx. 2×10^3–5×10^3 M$^{-1}\cdot$s^{-1} under the same conditions).

It is not the purpose of the present Letter to discuss in detail possible reasons for these marked differences in profile shape. It is sufficient to comment that the bell-shaped component of high reactivity at low pH is considered to arise from reaction of the interactive catalytic site system (containing S^-/ImH^+) with the 2,2′-dipyridyl disulphide monocation and that chymopapain A may contain an additional positive charge in its catalytic site region which is postulated to prevent binding or approach to the thiolate ion of the cationic form of the reactivity probe. Whatever the reason for the differences in profile shape, it is clear that the pH-dependence of k for the reaction of 2,2′-dipyridyl sulphide with the catalytic site thiol group has considerable diagnostic value in distinguishing catalytic sites in A-type chymopapains from those in B-type chymopapains.

Most of the literature on the cysteine proteinases of *Carica papaya* relates to enzymes isolated from commercially available dried latex (see Brocklehurst *et al.*, 1981 and references therein, for a discussion of latex collection and processing). Recently, however, Buttle & Barrett (1984) reported a study on the enzymes isolated from fresh latex obtained by tapping fruit purchased from a local supermarket. They reported the interesting conclusion that chymopapain is present in fresh latex only as multiple forms of chymopapain B and that forms of chymopapain other than chymopapain B (presumably, notably chymopapain A) are artefacts produced during the commercial preparation of the latex. It seems important now to report our recent finding that fresh papaya latex collected from the stem, leaves and petioles of the growing plant and treated with the reversible thiol blocking agent, 2,2′-dipyridyl disulphide, contains papain, multiple forms of chymopapain A and papaya peptidase Ω. It is clear, therefore, that enzyme with the chymopapain A type of catalytic site is not an artefact produced by commercial processing of the latex but chymopapain B forms might be products resulting from transformation of chymopapain A forms either in the plant, under some circumstances, or in the laboratory after latex collection.

It is important also to note that the ion-exchange elution profile of our fresh non-fruit latex is similar to that reported by Buttle & Barrett (1984) for the supermarket fruit latex. One notable exception is that papain is present in the fresh non-fruit latex, whereas Buttle & Barrett (1984) were unable to detect it in the supermarket fruit latex. Comparison of the ion-exchange elution profiles of the two types of fresh latex with the profile for dried latex would certainly appear to suggest the presence of multiple forms of chymopapain B in fresh latex. The characterization of the catalytic sites of all of the chymopapain components of non-fruit fresh latex as A-type, however, now serves to emphasize that the position of a component in an ion-exchange profile can no longer be regarded as a reliable criterion for identifying a particular type of chymopapain without, in addition, determining the type of catalytic site present. The relationship, if any, between catalytic site type and *N*-terminal amino acid residue (see Baines & Brocklehurst, 1982; Buttle & Barrett, 1984) remains to be established. The possibility must remain, of course, that enzyme forms different from those present in fresh non-fruit latex may be present in fresh fruit latex even though they appear in similar positions in ion-exchange elution profiles. In the absence of information about the types of catalytic site present in the components of fresh fruit latex, it is not possible at present to remove this uncertainty.

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Names and numbers of papaya proteinases

Chymopapain seems to be heterogeneous in at least two respects. Results from many laboratories (cited by Buttle & Barrett, 1984; Brocklehurst *et al.*, 1985) show that there are multiple forms separable by cation-exchange chromatography, and two of the major forms have been termed 'chymopapain A' and 'chymopapain B', respectively. The second form of heterogeneity is that reflected in the reactivity of the catalytic sites with two protonic state probes, reported by Brocklehurst and co-workers (cited by Brocklehurst *et al.*, 1985). The method of identifying forms by use of the probes may not be easy to reproduce in other laboratories (Khan & Polgár, 1983; Polgár, 1984), and the forms distinguished in this way do not necessarily equate with the chromatographically distinguished forms A and B (Brocklehurst *et al.*, 1985). Nevertheless, it has been suggested that chymopapains A and B are two distinct enzymes (sic) (Baines & Brocklehurst, 1982).

We reconsidered the possibility that chymopapain may be best thought of as a single enzyme, despite the existence of multiple forms, and concluded that it was indeed more helpful to 'lump' than to 'split' the chymopapains. One strong reason for this view was that polyclonal antisera raised against chymopapain (a chromatographic 'B' form), reacted with all the peaks of chymopapain from a cation-exchange column in a reaction of complete immunological identity (Buttle & Barrett, 1984). This suggested to us that the multiple forms are products of a single gene, and that differences between them are probably the result of post-translational events. It was our impression that the most important of these post-translational modifications probably occur during commercial processing of the latex, as artefacts, but we agree with Brocklehurst *et al.* (1985) that at least some may occur *in vivo*.

We are disinclined to regard 'chymopapain A' and 'chymopapain B' as distinct proteinases so long as there is no evidence that they differ (*a*) in primary structure, or (*b*) in catalytic specificity. Differences of both kinds would normally be expected between separate enzymes. We would therefore suggest that for the present it is best to think of chymopapain as a single enzyme with multiple chromatographic forms. If, despite the evidence that we have, it transpires that there are multiple genes coding for the multiple forms of chymopapain, and if the forms can be found to differ as proteinases, then it would be necessary to think again.

On the question of the name for the most basic of the proteolytic enzymes of papaya latex, we are pleased that there now seems to be general agreement on the appropriateness of 'papaya proteinase'. Our suggestion of the name 'papaya proteinase III' was on the historical basis that this was the third papaya proteinase to be identified, having been discovered by Schack (1967) after papain (Balls *et al.*, 1937) and chymopapain (Jansen & Balls, 1941). Numbers create less typographical problems than Greek letters, and we feel that this chronological scheme is not only sound, but also establishes a system that can easily accommodate the naming of further enzymes. By contrast, the present controversy over chymopapain serves to illustrate that the ion-exchange chromatographic properties of an enzyme are a poor basis for classification.

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