Problems of classification of papaya latex proteinases

The proteinases of papaya latex can be divided into three major groups of different basicity: papain (pI 9.55), chymopapain (pI 10.1–10.6), and papaya peptidase A (pI > 11), as they were originally named (for a comprehensive review see Brocklehurst *et al.*, 1981). Due to their considerably different basicities, the three enzymes are readily resolved by ion exchange chromatography.

The major problem of classification of the individual proteinases concerns the large complex band eluted on ion-exchange chromatography between papain and papaya peptidase A. Originally two forms of chymopapain (A and B) were identified in this region, but recently Brocklehurst & Salih (1983) reported the existence of four forms: chymopapains A and B_1–B_3 in order of increasing basicity. The most basic component of the elution band was earlier referred to by Schack (1967) as a new enzyme, distinct from chymopapain, and Lynn (1979) named this component papaya peptidase B as it resembled papaya peptidase A rather than chymopapain. Brocklehurst and his colleagues (Brocklehurst *et al.*, 1980, 1981; Baines & Brocklehurst, 1982; the preceding Letter) proposed that chymopapains A and B can readily be distinguished from each other by the use of the thiol reagent, 2,2′-dipyridyl disulphide. We employed this method (Khan & Polgár, 1983) in order to identify the enzyme in the first peak of the complex elution band (chymopapain A?) which was purified to homogeneity by covalent chromatography and contained exactly 2 thiol groups/mol of enzyme. We also analysed the second peak (chymopapain B_1?) which, however, contained less than 2 thiol groups/mol. The same results were observed with both components: each enzyme resembled partly chymopapain A and partly chymopapain B. As is also described in detail in the preceding Letter (Brocklehurst *et al.*, 1984), our reported measurements related to the biphasic Tsou Chen-Lu plot were indeed performed at pH 9 because the rate constants at this pH were shown to be similar for the reactions of 2,2′-dipyridyl disulphide with both thiol groups of chymopapain A (Brocklehurst *et al.*, 1981), which implied that the activity should linearly decrease to zero with the modification of the two thiol groups. Nonetheless, we performed the measurements over a wider pH range, i.e. between pH 8.2 and 9.0. No significant variation with pH was observed. This was not known to Brocklehurst *et al.* (1984) when they concluded that chymopapain S is chymopapain A.

The following observation might offer an explanation for the different experimental results regarding the first peak of the complex elution band. We have analysed the *N*-terminal amino acid of both the first and the second peak proteins ('chymopapain A/S and B_1/S'). For both enzyme forms we found only tyrosine as *N*-terminus in one particular batch and only glutamic acid in another batch of latex both supplied by Sigma (papain, type I; crude). This is inconsistent with the assumption that chymopapain A has glutamic acid, and chymopapain B tyrosine, as the *N*-terminal residue. Instead, it indicates that some of the chymopapain forms represent artefacts which could be produced during the processing of the latex. Thus, to establish the genuine proteinase pattern of *Carica papaya* L., a study of the fresh rather than the processed latex would be required (Khan & Polgár, 1983). Our experiments were carried out with chymopapains containing glutamic acid as *N*-terminal residue. If Brocklehurst and his colleagues used chymopapain with tyrosine as *N*-terminus, and if the two types of enzymes had different reactivities towards the disulphide compound, then the incongruent results would be explained. Of course, structural differences may also arise at other parts of the protein, not only at the *N*-terminus, which affect the reactivity of the enzyme towards 2,2′-dipyridyl disulphide.

Another uncertainty concerns the most basic component of the complex elution band. It cannot be decided at present whether it represents chymopapain B_3 or papaya peptidase B. Jansen & Balls (1941) pointed out many years ago that chymopapain is very stable at acidic pH. They utilized this unique property of chymopapain to isolate it at pH 2, where both papain and papaya peptidase A were immediately denatured. Until we have sufficient structural and specificity information, I suggest using this criterion to distinguish between chymopapain B_1–B_3 and papaya peptidase B. In fact, we have already used this criterion to classify the most basic re-chromatographed peak displaying the highest enzymic activity (Polgár, 1981) and found that most of the activity disappeared when the enzyme was treated at pH 2. In addition, this enzyme was a monothiol proteinase, which is not a characteristic feature of chymopapain. Of course, the presence or absence of a non-essential thiol group may not be a decisive factor because this may simply be due to a single-site mutation or some kind of oxidation of the second thiol group.

A comparative amino acid sequence analysis of the *N*-terminal portion of the papaya latex enzymes demonstrated unequivocally the presence of at least four different proteins, each originating from an individual gene (Lynn & Yaguchi, 1979). Very extensive homology was observed for the first 17 amino acid residues, which indicated that

papain, papaya peptidase A and B, and chymopapain arose from a common ancestral gene. Thus they may be regarded as isoenzymes.

In summary, at present we have not enough information to classify appropriately the different forms of the complex elution band containing chymopapain. In my opinion, some of the forms are very probably artefacts developed during latex processing. There may be only one genuine chymopapain (A or B), but there may be more. The complex elution band may or may not include a papaya peptidase-type enzyme. The most important question is whether it is justified to classify papain, chymopapains A and B, and papaya peptidases A and B as different enzyme species if there are no marked differences in their specificities. Probably such a consideration led Brocklehurst & Salih (1983) to propose that papain and papaya peptidase A be called papaya peptidase I and papaya peptidase II, respectively. Inspired by their initiative I would agree with a re-evaluation of the nomenclature, which should also include chymopapain, when more extensive structural and specificity information is available. The term 'peptidase', however, may preferably be substituted by 'proteinase' in accordance with the proposals of the Enzyme Commission. Until sufficient information is available, let us adhere to the deep-rooted names, papain and chymopapain, but even now the name of papaya peptidase A could be changed to the more proper papaya proteinase A.

László POLGÁR
Institute of Enzymology,
Biological Research Center,
Hungarian Academy of Sciences, P.O. Box 7,
H-1502 Budapest, Hungary

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