A Necessary Modification to the Preparation of Papain from Any High-Quality Latex of *Carica papaya* and Evidence for the Structural Integrity of the Enzyme Produced by Traditional Methods

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A method of preparation of papain (EC 3.4.22.2) from relatively soluble types of latex of *Carica papaya*, including spray-dried latex produced by a controlled and relatively mild process, was devised. Spray-dried latex dissolves easily in water up to 350 mg/ml at 22°C, which corresponds to approx. 230 mg of protein/ml. When the usual method of preparation of crystalline papain contaminated only by its oxidation products, developed by Kimmel & Smith [*J. Biol. Chem.* (1954) **207**, 515–531], is applied to spray-dried latex, the result is a preparation of papain heavily contaminated by chymopapains A and B (EC 3.4.22.6), and to a lesser extent by papaya peptidase A. This applies also to other types of papaya-latex currently commercially available, which, though less soluble than spray-dried latex, are more soluble than the types of latex available when the method of Kimmel & Smith (1954) was developed. This contamination is avoided by adjusting the concentration of the initial latex extract to 65 mg of protein/ml (or less) before salt fractionation. For spray-dried latex this corresponds to 100 mg of latex/ml. Papain isolated from spray-dried latex was characterized by using 2,2'-dipyridyl disulphide and 4-chloro-7-nitrobenzofurazan as thiol-specific reactivity probes and α -N-benzoyl-L-arginine ethyl ester as substrate. Papain isolated from this source appears to have the same catalytic-centre characteristics as papain isolated previously from latex produced by harsher methods. The catalysis of the hydrolysis of α -N-benzoyl-L-arginine ethyl ester by the mixture of thiol proteinases extracted from spray-dried latex by application of the method of Kimmel & Smith (1954) appears to obey Michaelis–Menten kinetics. The presence of the other enzymes results in an increase in the value of K_m and a decrease in the catalytic-centre activity ($k_{cat.}$) relative to the values for the catalysis by papain.

The importance of the latex of the fruit of the papaya plant, *Carica papaya*, as a source of enzymes appears to have been first recognized by G. C. Roy, who published his work in 1873 in the *Calcutta Medical Journal* (see Cayle *et al.*, 1964). One of the enzymes present in papaya latex is papain (EC 3.4.22.2), the most thoroughly characterized of the thiol proteinases [for recent reviews see Lowe (1976) and Polgar (1977)]. Although some of the early studies on papain were carried out on enzyme prepared from fresh latex (see, e.g., Balls & Lineweaver, 1939), most workers have used enzyme isolated from dried latex, the state in which the material is exported from the producing countries, which are mainly in the wet tropics. The production and commercial uses of dried papaya latex have been reviewed by Jones &

Mercier (1974). Papaya latex is obtained by tapping the green unripe fruit and collecting the resulting exudate. Traditionally the latex has then been sun-dried or dried in a kiln or simple wood-burning oven (Becker, 1958; Jones & Mercier, 1974). These methods are much harsher and less carefully controlled than those used to prepare most materials from which enzymes are isolated, and dried papaya latex thus prepared was often a granular and lumpy product with a colour that varied from cream to red-brown and contained substantial quantities (often 50–80% by wt.) of insoluble material. The nature of the latex product and the severity of the methods traditionally used to prepare it suggested the possibility that the well-characterized protein preparation known as papain may be significantly different from the corresponding protein present in the papaya fruit.

It became possible to attempt to examine the question of whether traditionally prepared papain

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might be a 'crippled' enzyme when a new type of papaya latex (here referred to as 'spray dried' papaya latex) produced by a controlled and milder process became available. This paper reports evidence that the main catalytic-centre characteristics of papain prepared from both types of dried latex are after all closely similar. It seems probable, therefore, that the small amount of catalytically active papain that survives the traditional methods of latex production possesses undamaged catalytic-centre structure.

Since the mid-1950s, almost all studies on papain have been carried out on enzyme prepared, at least in the early stages of the procedure, essentially by the method of Kimmel & Smith (1954), which has been described also by Arnon (1970). If this method is used to prepare papain from the improved types of latex currently available commercially, including spray-dried latex, the product contains substantial quantities of other enzymes, notably the chymopapains. The present paper reports a simple modification to the method of Kimmel & Smith (1954) that optimizes the yield of papain from highly soluble papaya latex while avoiding contamination by other enzymes.

Materials and Methods

Many of the materials and methods have been described previously (see Shipton *et al.*, 1976; Shipton & Brocklehurst, 1978). The spectrophotometric detection of chymopapain contaminants in preparations of papain by titration with 2,2'-dipyridyl disulphide was carried out as described by Baines & Brocklehurst (1978). Buffers were prepared as described by Dawson *et al.* (1969) and by Long (1971). SP-Sephadex (sulphopropyl-Sephadex) 50 was obtained from Pharmacia (G.B.) Ltd., London W.5, U.K., and α -N-benzoyl-DL-arginine *p*-nitroanilide was obtained from Sigma (London) Chemical Co., London S.W.6, U.K.

Spray-dried papaya latex

This latex, known to have been produced by the Boudart (1969) process in Zaire, was generously supplied by Powell and Schofield Ltd., 38 Queensland Street, Liverpool L7 3JG, U.K. It is important to take adequate precautions when handling this material, which is a very fine powder of high proteolytic activity. The respiratory hazards of spray-dried latex in the solid state have been discussed by Flindt (1978). Solid latex should be handled in a good fume cupboard and protective clothing, including face masks, should be worn.

Preparation of papain

This method should provide papain free from contamination by chymopapains A and B (EC 3.4.22.6)

and papaya peptidase A when any commercially available latex of *Carica papaya* is used as starting material.

A solution of the latex containing 65 mg of protein/ml (Lowry *et al.*, 1951) is prepared by dissolving the appropriate amount of latex in 20 mM-cysteine, pH 5.7, containing 1 mM-EDTA. When spray-dried latex is used, this is readily achieved by stirring 25 g of the fine white powder into 250 ml of the cysteine solution at room temperature (approx. 22°C). When the latex dissolves the pH falls to 5.3. Spray-dried latex dissolves rapidly and completely under these conditions. Some other types of papaya latex currently available do not dissolve completely under these conditions, even when the latex is ground with sand in the cysteine solution. If more latex is added to compensate for the insoluble material, care must be taken that the extract does not contain more than 65 mg of protein/ml, or contamination by the chymopapains may result (see the Results and Discussion section).

The pH of the latex solution (250 ml, containing 65 mg of protein/ml) is adjusted from 5.3 to 9.0 by adding 1 M-NaOH (approx. 20 ml) with stirring during 10–15 min at room temperature. The small amount of grey-white precipitate formed is removed by centrifugation (20000g, 4°C, 30 min) and discarded. The supernatant is adjusted to 0.45 saturation by addition of solid $(\text{NH}_4)_2\text{SO}_4$ (0.277 g/ml) with stirring during 20 min. The white precipitate is isolated by centrifugation as above and dissolved in 250 ml of 1 mM-EDTA (disodium salt). This solution is adjusted to 0.40 saturation by addition of solid $(\text{NH}_4)_2\text{SO}_4$ (0.243 g/ml) with stirring during 20 min. Again, the white precipitate is isolated by centrifugation as above and then dissolved in 250 ml of 0.1 M-phosphate buffer ($\text{KH}_2\text{PO}_4/\text{NaOH}$), pH 7.5, containing 20 mM-cysteine and 1 mM-EDTA at room temperature. Solid NaCl (25 g) is then added with stirring during 15 min, and the precipitate is isolated by centrifugation as above. The white precipitate is suspended in 100 ml of 0.1 M-phosphate buffer ($\text{KH}_2\text{PO}_4/\text{NaOH}$), pH 6.5, containing 20 mM-cysteine and 1 mM-EDTA at room temperature. The suspension is left at this temperature for 30 min and then at 4°C for 18 h. The resulting crystals are isolated by centrifugation as above and are stored at 4°C as a suspension in 0.1 M-sodium acetate buffer, pH 5.0, containing 24.3% (w/v) $(\text{NH}_4)_2\text{SO}_4$. The product (usually approx. 1.5 g) when freshly prepared contains approx. 50% active papain, 30% reversibly blocked papain (papain-cysteine mixed disulphide) and 20% inactivatable protein (i.e. based on its protein content the preparation is 50% active when assayed in the absence of a reducing agent and 80% active when assayed in the presence of a reducing agent). On storage for 3 days the proportion of reversibly blocked enzyme increases from 30 to 70% at the

expense of active enzyme, which decreases from 50 to 10%. The 80% activity in the presence of added reducing agent is maintained for at least 1 month.

The 80%-active enzyme can be used to prepare fully active enzyme, e.g. by covalent chromatography (Brocklehurst *et al.*, 1973, 1974; Stuchbury *et al.*, 1975).

The present method differs from those reported by Kimmel & Smith (1954) and by Arnon (1970) mainly in the smaller quantity of latex and smaller volumes of solutions required to produce 1.5–2 g of papain. Other points of difference are: the extraction medium contains 1 mM-EDTA, and contains 20 mM-cysteine instead of 40 mM-cysteine; two $(\text{NH}_4)_2\text{SO}_4$ fractionation steps (0.45 and 0.4 saturation respectively) replace the one fractionation step (0.4 saturation) and the $(\text{NH}_4)_2\text{SO}_4$ wash; shorter times for the fractionation steps were used. By using the present method it is possible to prepare 80%-active papain contaminated only by inactive and unactivatable protein (probably the papain sulphinic acid; see Drenth *et al.*, 1975) in about 24 h. For most purposes it is not necessary to carry out the crystallization step, which appears to result in little or no improvement in the purity of the product, and omission of this step permits the preparation of 80%-active papain in about 3 h.

Analysis of papain preparations by chromatography on SP-Sephadex

The homogeneity of papain preparations was evaluated by chromatography on SP-Sephadex by using a modification of the method used by Robinson (1975) for the isolation of papaya peptidase A.

A sample of the protein to be evaluated (100–160 mg) was dialysed for 6 h at room temperature against 2×2.5 litres of 0.1 M-sodium acetate buffer, pH 5.0. The sample was then applied to a column (15 cm $\times$ 1.5 cm) of SP-Sephadex 50 gel pre-equilibrated with the same buffer. The column was then washed with 25 ml of the sodium acetate buffer before a linear gradient of 0.1–1.0 M-sodium acetate containing 1 mM-EDTA (total volume 500 ml) was applied and 10 ml fractions were collected. The fractions were analysed by measurement of conductance, A_{280} and catalytic activity towards α -N-benzoyl-DL-arginine *p*-nitroanilide essentially as described by Malthouse & Brocklehurst (1976).

Results and Discussion

Production of spray-dried papaya latex

A significant advance in the production of papaya latex was achieved in Zaire by a Belgian planter, Mr. R. Boudart (see Jones & Mercier, 1974). In the Boudart process (Boudart, 1969) fresh latex is refined immediately after its collection by using only mechanical methods of purification, similar to those

used in a dried-milk plant. The daily collection of fresh latex is transported to the factory in stainless-steel drums. On arrival, the gelled mass is stirred, whereupon it liquifies. Insoluble impurities are removed by filtration, centrifugation, filtration through kieselguhr and finally filtration through a sterile plate filter. The clear filtrate is concentrated under vacuum, spray-dried, sieved, bulked and packaged.

Isolation of papain from spray-dried papaya latex

The Kimmel & Smith (1954) procedure (see also Arnon, 1970, but note that the protein contents in her Table 1 are 1000 times too low as printed) for the isolation of papain from traditionally produced dried papaya latex consists of preparation of an extract of 180 g of latex in 1 litre of 0.04 M-cysteine, pH 5.7, removal of material insoluble at pH 9, $(\text{NH}_4)_2\text{SO}_4$ fractionation, NaCl fractionation and crystallization. This procedure usually yielded about 2 g of crystalline protein, of which about 50% was a mixture of active papain and papain-L-cysteine mixed disulphide, which produces active papain in reducing media (see, e.g., Brocklehurst & Kierstan, 1973). The other 50% was 'irreversibly oxidized papain', at least some of which is the sulphinic acid of papain (see Drenth *et al.*, 1975).

To facilitate the isolation of pure papain from spray-dried papaya latex and from other relatively soluble grades of latex currently produced commercially, it is necessary to make a small but important change in the procedure described by Kimmel & Smith (1954) and by Arnon (1970). If 180 g of spray-dried latex is added to 1 litre of the cysteine solution (0.04 M, pH 5.5) it dissolves easily and completely; indeed, it is possible to dissolve about 350 g of this type of latex/litre of solution at room temperature (approx. 22°C). This is in marked contrast with the situation that obtains when traditionally produced dried latex is used. It is necessary then to grind the dried latex with sand in the cysteine solution to effect solution, and even with this aid a substantial fraction of the latex fails to dissolve. The higher protein concentration in the initial cysteine solution obtained by applying the Kimmel & Smith (1954) procedure to spray-dried latex results in a final enzyme preparation that contains substantial quantities of two other thiol proteinases, the chymopapains, in addition to papain. This may be demonstrated by chromatography on SP-Sephadex (see Fig. 1a). A papain preparation that is essentially free from the chymopapains can be isolated from spray-dried latex by using an initial solution of latex of the appropriate concentration (see Fig. 1b). It is convenient to work with 250 ml of initial latex solution, and an optimal yield of chymopapain-free papain (approx. 1.5 g of approx. 80%-active enzyme) is obtained by using an initial solution of 25 g of spray-dried latex in 250 ml of the cysteine

Fig. 1. Chromatography on a column (15 cm $\times$ 1.5 cm) of SP-Sephadex-50 of papain prepared from spray-dried latex (a) by the method of Kimmel & Smith (1954) by using an initial solution containing 45 g of latex in 250 ml of solution and (b) by the method described in the Materials and Methods section by using an initial solution containing 25 g of latex in 250 ml of solution Δ , Relative activity towards α -N-benzoyl-DL-arginine *p*-nitroanilide: the highest activity in each elution pattern is taken as 100%; $\blacksquare$, A_{280} ; $\bullet$, concn. of sodium acetate in the elution buffer. For further details, see the text. Fractions: I, papain; II, chymopapain A; III, chymopapain B; IV, papaya peptidase A.

solution. This corresponds to a protein concentration of approx. 65 mg/ml (see Baines *et al.*, 1978). The efficiency of the $(\text{NH}_4)_2\text{SO}_4$ fractionation step, which separates the papain-rich fraction from the chymopapain-rich fraction (Kimmel & Smith, 1954), is very sensitive to protein concentration. Thus when the procedure of Kimmel & Smith (1954) is used, 20–30% of the protein and proteolytic activity is precipitated as the papain-rich fraction when a solution of dried latex is adjusted to 40% saturation with $(\text{NH}_4)_2\text{SO}_4$, whereas 70–80% is precipitated when a solution of spray-dried latex is so treated (Baines *et al.*, 1978). The sensitivity of salt fractionations to protein concentration has been emphasized by Dixon & Webb (1961), and when the initial solution of spray-dried latex is diluted (3–4-fold) the

traditional fractionation pattern observed by Kimmel & Smith (1954) is obtained. A solution containing 25 g of spray-dried latex/250 ml approximates closely to the most concentrated initial solution that reliably provides chymopapain-free papain in the crystallization step. When 30 g/250 ml is used as the initial solution, substantial contamination of the crystalline product by the chymopapains is observed. It is important to point out that when the optimal conditions for the isolation of papain from spray-dried latex (given in the Materials and Methods section), are being used it is necessary to carry out the NaCl fractionation steps in order to remove residual traces of the chymopapains, even though the bulk of these protein contaminants are removed by the $(\text{NH}_4)_2\text{SO}_4$ fractionation.

Routine evaluation of papain preparations for contamination by the chymopapains

Spectral analysis of the reaction of a preparation of papain with the two-protonic-state thiol titrant, 2,2'-dipyridyl disulphide, at two pH values permits the evaluation of papain samples for contamination by the chymopapains.

The theoretical basis of this method, which depends on the difference in reactivity characteristics of the thiol groups of the various enzymes towards the two-protonic-state reagent, has been discussed previously (Baines & Brocklehurst, 1978).

When the thiol titration with this particular type of reagent is performed on a solution of papain prepared by using an initial protein solution of the appropriate concentration, as described in the Materials and Methods section and shown to be devoid of contaminating chymopapains by chromatography on SP-Sephadex, results exemplified by those given in Fig. 2 are obtained. When a conventional spectrophotometer is used an essentially instantaneous reaction is apparent, which corresponds to the same

thiol content when the reaction is performed at pH 7.6 as when it is performed at pH 4.0 (Fig. 2a). By using the stopped-flow technique a complete progress curve can be recorded and shown to consist of a single phase by a conventional first-order plot (Fig. 2b).

In contrast, when a solution of papain prepared by using the conditions described by Kimmel & Smith (1954) and shown by chromatography on SP-Sephadex to contain chymopapains is tested by titration with 2,2'-dipyridyl disulphide, results exemplified by those given in Fig. 3 are obtained. Conventional spectrophotometry gives a substantially higher thiol content (fast phase) when the reaction is performed at pH 7.6 than when it is performed at pH 4.0 (Fig. 3a) and the stopped-flow method reveals at least two distinct phases in the progress curve which lead to a non-linear first-order plot (Fig. 3b).

The end point of the reaction in Fig. 3(a) corresponds to 1.1 mol of 2-thiopyridone released/mol of enzyme, whereas the end point of the reaction in Fig. 2(a) corresponds to 0.8 mol of 2-thiopyridone

Fig. 2. Progress curves for the reaction of 2,2'-dipyridyl disulphide with papain at 25°C, 10.1 and pH 4.0 and 7.6 (a) Progress curves recorded by using conventional spectrophotometry: papain was prepared from a solution of spray-dried latex (25 g of latex/250 ml) as described in the Materials and Methods section; a sample (4 ml of 70 μ M-protein) was activated by incubation with 20 mM-cysteine at pH 7.8 for 30 min and then separated from low-molecular-weight material by gel filtration on a Sephadex G-25 (fine grade) column (15 cm $\times$ 3 cm); activated, activator-free papain (protein concn. 11.31 μ M, determined by using $\epsilon_{280} = 5.6 \times 10^4 \text{ M}^{-1} \cdot \text{cm}^{-1}$) was allowed to react with 2,2'-dipyridyl disulphide (260 μ M) at pH 4.0 (Δ), and at pH 7.6 ($\bullet$). The points are taken from the continuous trace provided by a Cary 118C spectrophotometer. The recorder was started and the reactants were mixed as the pen crossed a mark on the chart (zero time). The reaction cell was placed in the cell compartment approx. 15 s later. Reactions at both pH values were complete within the response time of the pen. (b) Progress curve and first-order plot for the reaction at pH 7.6: the progress curve was recorded by using a Durrum stopped-flow spectrophotometer and a Tektronix oscilloscope; second-order rate constant = $942 \text{ M}^{-1} \cdot \text{s}^{-1}$.

Fig. 3. Progress curves for the reaction at 25°C, $I = 0.1$ and pH 4.0 and 7.6 of 2,2'-dipyridyl disulphide with the mixture of thiol proteinases prepared by extraction of spray-dried latex by using the procedure of Kimmel & Smith (1954)

(a) Progress curves recorded by using conventional spectrophotometry: the mixture of enzymes was activated and separated from low-molecular-weight material as described in Fig. 2; a sample of the mixture (protein concn. $6.3 \mu\text{M}$, determined by using $\epsilon_{280} = 5.6 \times 10^4 \text{ M}^{-1} \cdot \text{cm}^{-1}$, i.e. the value for papain) was allowed to react with 2,2'-dipyridyl disulphide ($260 \mu\text{M}$), as described in Fig. 2 legend, at pH 4.0 (Δ) and at pH 7.6 ($\bullet$). The reaction at pH 7.6 was complete within the response time of the pen. (b) Progress curve and first-order plot for the reaction at pH 7.6: the progress curve was recorded as described in Fig. 2 legend; the fast phase was isolated from the biphasic logarithmic plot as described by Frost & Pearson (1961). Second-order rate constants: fast phase, $4830 \text{ M}^{-1} \cdot \text{s}^{-1}$; slow phase $630 \text{ M}^{-1} \cdot \text{s}^{-1}$.

released/mol of enzyme. The higher value of Fig. 3(a), which is greater than 1.0 mol of 2-thiopyridone released/mol of enzyme, would be predicted if chymopapains are present as contaminants. This is because the molecular weights and the values of $A_{1\text{cm}}^{1\%}$ at 280 nm of the chymopapains are similar to those of papain (Robinson, 1975) and each contains 2 thiol groups per molecule, whereas papain contains only 1 thiol per molecule (B. S. Baines & K. Brocklehurst, unpublished work).

Some characteristics of papain prepared from spray-dried papaya latex

Most of the characteristics of the papain active centre have been deduced from experiments on papain isolated from latex manufactured by harsh traditional methods. Important examples include:

(i) the interaction of the active-centre thiol (cysteine-25) and imidazole (histidine-159) groups that provide a nucleophilic state of the sulphur atom in neutral media additional to the uncomplicated thiolate ion that predominates above pH 9;

(ii) positive co-operativity of protonic dissociation

from the carboxy group of aspartic acid-158 and from the interactive cysteine-25-histidine-159 pair;

(iii) the possibility of additional modulation of the cysteine-histidine interaction by the state of ionization of a group characterized by a molecular pK_a of 5-6;

(iv) a difference between the active-centre regions of papain and the supposedly analogous thiol proteinase, ficin (EC 3.4.22.3). These characteristics were demonstrated by using pyridyl disulphides and 4-chloro-7-nitrobenzofurazan as thiol-specific reactivity probes (Malthouse & Brocklehurst, 1976, 1978; Shipton *et al.*, 1976; Shipton & Brocklehurst, 1978), and we here report that results closely similar to those obtained previously with papain from traditionally prepared latex are obtained also when papain from spray-dried latex is used.

Thus with both types of preparation: (1) the pH-dependence of the second-order rate constant (k) for the reaction of the papain thiol group with 2,2'-dipyridyl disulphide is described by a profile closely similar to that reported by Shipton & Brocklehurst (1978) (Fig. 5) [the data in the pH range 2.5-9.5

Fig. 4. Apparent adherence to Michaelis-Menten kinetics of the hydrolysis of α -N-benzoyl-L-arginine ethyl ester at 25°C, $I = 0.1$, catalysed by the mixture of enzymes isolated by application of the procedure of Kimmel & Smith (1954) to spray-dried latex

s , 5–80 mM; protein concn. 29.7 μ M (determined by using $\epsilon_{280} = 5.6 \times 10^4 \text{ M}^{-1} \cdot \text{cm}^{-1}$; [SH] reacting instantaneously at pH 4.0 = 17.08 μ M); the linear regression line corresponds to $K_m = 20 \text{ mM}$ and $k_{\text{cat.}} = V/[SH] = 2.2 \text{ s}^{-1}$.

correspond closely to eqn. (1) except in the pH range 4.8–5.2, where values of k are larger than predicted by eqn. (1) and suggest that k depends on an additional pK_a value approx. 5]; (2) the pH-dependence of k for the reaction of the papain thiol group with 4-chloro-7-nitrobenzofurazan is described by a profile closely similar to that reported by Shipton *et al.* (1976) (Fig. 2) [the data in the pH range 2.6–4.4 correspond closely to eqn. (2)].

$$k = \frac{\bar{k}_{\text{XH}_2}}{\left(1 + \frac{[\text{H}^+]}{K_{\text{eI}}} + \frac{K_{\text{eII}}}{[\text{H}^+]}\right)} + \frac{\bar{k}_{\text{XH}}}{\left(1 + \frac{[\text{H}^+]^2}{K_{\text{eI}}K_{\text{eII}}} + \frac{[\text{H}^+]}{K_{\text{eII}}} + \frac{K_{\text{eIII}}}{[\text{H}^+]}\right)} + \frac{k_{\text{X}}}{\left(1 + \frac{[\text{H}^+]}{K_{\text{eIII}}}\right)} \quad (1)$$

$$k = \frac{\bar{k}}{1 + \frac{[\text{H}^+]}{K_{\text{I}}} + \frac{K_{\text{II}}}{[\text{H}^+]}} \quad (2)$$

The values of the parameters (pH-independent rate constants $\bar{k}_{\text{XH}_2}$, $\bar{k}_{\text{XH}}$, k_{X} and $\bar{k}$ and macroscopic acid dissociation constants K_{eI} , K_{eII} , K_{eIII} , K_{I} and K_{II}) that characterize eqns. (1) and (2) are given below for the reactions of papain prepared from spray-dried latex together with the corresponding values in parentheses for the reactions of papain prepared from traditionally produced latex.

$$\begin{aligned} \bar{k}_{\text{XH}_2} &= 5.3 \times 10^4 \text{ M}^{-1} \cdot \text{s}^{-1} (4.2 \times 10^4 \text{ M}^{-1} \cdot \text{s}^{-1}); \\ \bar{k}_{\text{XH}} &= 751 \text{ M}^{-1} \cdot \text{s}^{-1} (850 \text{ M}^{-1} \cdot \text{s}^{-1}); \\ \bar{k}_{\text{X}} &= 1.65 \times 10^3 \text{ M}^{-1} \cdot \text{s}^{-1} (1.7 \times 10^3 \text{ M}^{-1} \cdot \text{s}^{-1}); \\ \text{p}K_{\text{eI}} &= 3.82 (3.85); \text{p}K_{\text{eII}} = 3.89 (3.90); \\ \text{p}K_{\text{eIII}} &= 8.8 (8.8); \bar{k} = 108 \text{ M}^{-1} \cdot \text{s}^{-1} (86 \text{ M}^{-1} \cdot \text{s}^{-1}) \\ \text{p}K_{\text{I}} &= 3.19 (3.24); \text{p}K_{\text{II}} = 3.42 (3.45) \end{aligned}$$

Although a study of the substrate specificity of papain prepared from spray-dried latex was not undertaken, it was ascertained that the Michaelis parameters that characterize the catalysis by this enzyme of the hydrolysis of α -N-benzoyl-L-arginine ethyl ester are similar to those reported previously for enzyme from other latex types (see Brocklehurst *et al.*, 1973). In the present work the values obtained at pH 6.0, 25°C, $I = 0.1$, $K_m = 13 \pm 2 \text{ mM}$ and $k_{\text{cat.}} = 20 \pm 2 \text{ s}^{-1}$, which provides that $k_{\text{cat.}}/K_m \approx 154 \text{ M}^{-1} \cdot \text{s}^{-1}$. These values are the means $\pm$ S.D. of four determinations. The present value of K_m is a little lower than most values reported previously (approx. 18 mM; see Brocklehurst *et al.*, 1973), but is similar to one of the values reported by Whitaker & Bender (1965), who used the twice-crystallized, partially active product of Worthington Biochemicals Inc.

An enzyme preparation prepared from spray-dried latex by the method of Kimmel & Smith (1954) that contained not only papain but also chymopapain-A, chymopapain-B and probably papaya peptidase A and provided the elution pattern shown in Fig. 1(a) was also used to catalyse the hydrolysis of α -N-benzoyl-L-arginine ethyl ester. That the catalysis by this mixture of enzymes appeared to obey Michaelis-Menten kinetics is demonstrated by the linearity of the plot of s/v versus s (Fig. 4). This plot is particularly suitable for the detection of non-linearity (Wharton *et al.*, 1974). The value of K_m obtained (20 mM) is higher than that obtained with papain that had been freed from the other enzymes, and the value of $k_{\text{cat.}}$ for the mixture (2.2 s^{-1} , calculated from V and the rapid phase of the spectrophotometric titration with excess of 2,2'-dipyridyl disulphide at pH 4.0) is very much smaller than the value of $k_{\text{cat.}}$ obtained for papain (20 s^{-1}).

The value of $k_{\text{cat.}}$ obtained in the present work for the papain catalysis is somewhat smaller than some (but not all) of the other values of this parameter reported by other workers [e.g. Sluyterman & Wijderes (1970) gave $k_{\text{cat.}} = 26 \text{ s}^{-1}$ and Blumberg *et al.* (1970) gave $k_{\text{cat.}} = 28.5 \text{ s}^{-1}$]. It seems unlikely that the value of $k_{\text{cat.}} = 20 \pm 2 \text{ s}^{-1}$ here reported results from contamination by other enzymes less effective towards α -N-benzoyl-L-arginine ethyl ester, because these are not revealed either by titration with 2,2'-dipyridyl disulphide (Baines & Brocklehurst, 1978) or by chromatography on SP-Sephadex.

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