The Amino Acid Sequence around the Reactive Thiol Group of Chymopapain B*

JOYCE N. TSUNODA‡ AND KERRY T. YASUNOBU§

From the Department of Biochemistry and Biophysics, University of Hawaii, Honolulu, Hawaii 96822

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SUMMARY

The yellow colored sulfhydryl reagent, N-(4-dimethylamino-3,5-dinitrophenyl)maleimide, was used in the isolation of the peptide containing the reactive sulfhydryl group of chymopapain. Titration of cyanide-activated chymopapain B with p-chloromercuribenzoate at pH 4.6 indicated that there is a maximum of 1.4 moles of sulfhydryl per mole of the enzyme. Alklation of half of the p-chloromercuribenzoate-titratable sulfhydryl groups led to the total inactivation of chymopapain. Pepsin digestion of the N-(4-dimethylamino-3,5-dinitrophenyl)maleimide-treated enzyme and the subsequent isolation of the labeled peptide showed that the label was predominantly in one peptide with the sequence, Lys-Arg-Val-Pro-Asp-Ser-Gly-Glu-Cys-Tyr. This sequence differed from those of the peptides containing the reactive sulfhydryl groups of papain and ficin, although all three enzymes are sulfhydryl proteases.

The detailed structure around the active sulfhydryl group of papain and the position of the "active site" in relation to the entire papain molecule were recently reported (1). A remarkable similarity between the sequence of the peptide containing the reactive sulfhydryl group of papain and that of ficin (2) was found. Chymopapain, like papain, is found in the latex of the papaya, and has been shown to be a sulfhydryl enzyme (3). However, unlike papain, which contains 0.75 p-chloromercuribenzoate-titratable cysteine residues after activation with mercaptans, activated chymopapain B was found to contain a maximum of 1.4 moles of sulfhydryl per mole of the enzyme (3).

This report presents the labeling of a reactive sulfhydryl group of chymopapain. Titration of cyanide-activated chymopapain B with DDPM (Fig. 1) showed that all enzymic activity was lost when chymopapain was treated with 2 eq of DDPM, the presence of residual CMB-reactive sulfhydryl in DDPM-treated chymopapain B are shown in Fig. 3. Although titration of chymopapain B with DDPM (Fig. 1) showed that all enzymic activity was lost when chymopapain was treated with 2 eq of DDPM, the presence of residual CMB-titratable sulfhydryl in DDPM-treated chymopapain indicated that not all of the cysteine residues had reacted with the DDPM added. Therefore, in order to estimate the amount of DDPM incorporated into the protein during the titration of chymopapain with DDPM, the amount of unreacted —SH was calculated as follows. The absorbances of DDPM in buffer (0 to 0.6 ml of DDPM) and of protein plus DDPM (0 to 0.6 ml) were determined. The absorbance differences at 424 nm between the two corresponding solutions were assumed to be a measure of the unreacted —SH. Molar extinction coefficient at 255 nm was 5.5 x 10^4 for mercapto peptide formation in the case of chymopapain.

Titration of chymopapain B with DDPM was carried out as described in the legend for Fig. 2.

Spectral Properties of DDPM and Its Derivatives—The absorption spectra of DDPM, N-(4-dimethylamino-3,5-dinitrophenyl)-succinimido-cysteine (4), DDPS-chymopapain B, and chymopapain B are shown in Fig. 3. Although titration of chymopapain B with DDPM (Fig. 1) showed that all enzymic activity was lost when chymopapain was treated with 2 eq of DDPM, the presence of residual CMB-titratable sulfhydryl in DDPM-treated chymopapain indicated that not all of the cysteine residues had reacted with the DDPM added. Therefore, in order to estimate the amount of DDPM incorporated into the protein during the titration of chymopapain with DDPM, the amount of unreacted —SH was calculated as follows. The absorbances of DDPM in buffer (0 to 0.6 ml of DDPM) and of protein plus DDPM (0 to 0.6 ml) were determined. The absorbance differences at 424 nm between the two corresponding solutions were assumed to be a measure of the unreacted —SH. Molar extinction coefficient at 424 nm for DDPM was calculated

DDPM and 2,4-dinitrofluorobenzene were purchased from M. and used without further purification. Crystalline pepsin, CMB (sodium salt), and casein were obtained from Nutritional Biochemicals. Phenylisothiocyanate was purchased from Eastman Kodak. Nagarse was obtained from Nagase Saigyo Company, Amagasaki, Japan. Disopropyl fluorophosphate-treated carboxypeptidase A and B were obtained from Worthington.

Enzyme Assay—The proteolytic activity of chymopapain was determined by the method of Kunita (3). Activation of chymopapain was carried out by incubating a suitable volume of enzyme with an equal volume of 0.10 M NaN₃ in phosphate buffer, pH 7.2, at 35°C. Activator-free chymopapain was obtained by passing cyanide-activated chymopapain through a multibed resin consisting of Dowex 50-H⁺, Dowex 1-OH⁻, and Sephadex G-25.

Titration of chymopapain B with DDPM was carried out as described in the legend for Fig. 2.

Spectral Properties of DDPM and Its Derivatives—The absorption spectra of DDPM, N-(4-dimethylamino-3,5-dinitrophenyl)-succinimido-cysteine (4), DDPS-chymopapain B, and chymopapain B are shown in Fig. 3. Although titration of chymopapain B with DDPM (Fig. 1) showed that all enzymic activity was lost when chymopapain was treated with 2 eq of DDPM, the presence of residual CMB-titratable sulfhydryl in DDPM-treated chymopapain indicated that not all of the cysteine residues had reacted with the DDPM added. Therefore, in order to estimate the amount of DDPM incorporated into the protein during the titration of chymopapain with DDPM, the amount of unreacted —SH was calculated as follows. The absorbances of DDPM in buffer (0 to 0.6 ml of DDPM) and of protein plus DDPM (0 to 0.6 ml) were determined. The absorbance differences at 424 nm between the two corresponding solutions were assumed to be a measure of the unreacted —SH. Molar extinction coefficient at 255 nm was 5.5 x 10⁴ for mercapto peptide formation in the case of chymopapain.

Titration of chymopapain B with DDPM was carried out as described in the legend for Fig. 2.

**Experimental Procedure and Results

**Materials**—Chymopapain B was isolated from dried papaya latex (crude standardized papain, Paul Lewis Laboratories) by the method of Kunimitsu and Yasunobu.

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† National Science Foundation Predoctoral Fellow.

§ To whom requests for reprints should be addressed.

1 D. K. Kunimitsu and K. T. Yasunobu, unpublished data.

1 The abbreviations used are: DDPM, N(4-dimethylamino-3,5-dinitrophenyl)succinimido-.
to be $1.35 \times 10^4$. With this value, it was determined that 0.6 eq of DDPM had reacted with the enzyme at the point where the enzyme was completely inactivated.

**Preparation of DDPS-chymopapain**—DDPM in ethanol-acetic acid solvent was slowly added to a solution of cyanide-

**Preparation of DDPX-chymopapain**—DDPM in ethanol-acetic acid solvent was slowly added to a solution of cyanide-

**Fig. 1. Titration of chymopapain with p-chloromercuribenzoate.** The activation of chymopapain by cyanide was carried out under the same conditions as described in the text. One-milliliter aliquots of cyanide-activated chymopapain (1.25 mg) were incubated with 0 to 2.5 ml of CMB in sodium acetate buffer (0.33 M, pH 4.62), for 90 min at room temperature. Changes in absorbance at 255 nm were read against an enzyme blank on the Beckman DU spectrophotometer. Proper corrections were made for contribution of CMB to the absorbance.

**Fig. 2. Titration of chymopapain with DDPM.** Three milliliters of chymopapain (11.2 mg ml$^{-1}$) were activated with NaCN (final concentration, $1 \times 10^{-3}$ M). The mixture was passed through a multibed resin (Dowex 50 X8, Dowex 1, Sephadex G-25) to remove excess CN$^-$. To test tubes containing 1 ml each of the activated chymopapain solution (protein concentration, 2.8 mg ml$^{-1}$) were added 0.06 to 0.60 ml of DDPM (0.00 to 0.42 mole, DDPM to protein ratio = 0 to 4.5). The reaction mixture was allowed to stand at room temperature for 4 hours. Aliquots of 0.2 ml were then taken from each tube, diluted to 2.0 ml with 0.1 M cacodylate buffer, pH 7.2, and assayed for residual enzymic activity. Remainder of the solution was titrated with excess CMB in 0.33 M acetate buffer, pH 4.6. Concentration of sulfhydryl remaining was calculated from molar extinction coefficients at 255 nm ($5.8 \times 10^3$). Residual activity (---) and residual sulfhydryl content (-----) were plotted against the moles of DDPM added per mole of chymopapain. The arrow denotes the point at which 0.6 eq of DDPM was taken up.

**Fig. 3. Spectral properties.** Absorption spectra of chymopapain B (Δ), DDPS-chymopapain (---), DDPS-cysteine (-----), and DDPM (----). Chymopapain ($1 \times 10^{-4}$ M) was dissolved in 0.1 M cacodylate buffer, pH 7.2 (enzyme concentration, 0.28 mg per ml). DDPS-chymopapain ($1 \times 10^{-4}$ M) was prepared by treating the enzyme with 10-fold molar excess of DDPM, by precipitating with ethanol, and by thoroughly dialyzing as described in the text. DDPS-cysteine ($2 \times 10^{-4}$ M) was prepared according to the method of Witter and Tuppy (4), and dissolved in 50% ethanol-2% HOAc solvent (final concentration, $2 \times 10^{-4}$ M). DDPM ($2 \times 10^{-4}$ M) was dissolved in 50% ethanol-2% acetic acid solvent. All readings were made on the Cary model 14 self-recording spectrophotometer.
Fig. 4. Sephadex G-25 chromatography of first pepsin digestion mixture. Fractions of 15 ml were collected. Represented are the 440-mu absorbance (---) and the 550 mu (ninhydrin) absorbance (——). Cross-hatched bar represents the fraction pooled for second pepsin digestion.

20% of the original ninhydrin-positive material applied to the talc column. The ethanol-acetic acid eluate was concentrated and purified as shown in Fig. 5, A and B. Subsequent amino acid analysis of the two fractions (Table I) showed that Peptide 2 was only 60% pure and differed from Peptide 3 mainly in the low value for tyrosine. Attempts to purify Peptide 2 further led only to the loss of the fraction. Thus, all sequence work was carried out on Peptide 3. Peptide 3 appeared as a single yellow spot at the origin when subjected to high voltage electrophoresis at pH 4.7 (10 ml of pyridine-10 ml of glacial acetic acid-980 ml of water), and no other ninhydrin spots were detected. The ionophoretic behavior of the peptide at pH 4.7 indicated that it was a neutral peptide. In the solvent system, tertiary amyl alcohol-pyridine-water (7:7:5), the labeled peptide moved as a single yellow spot with Rf = 0.5. The yield of this peptide was about 3% of the original starting material.

Amino Acid Analysis—The amino acid composition of the labeled peptide was determined on the Spinco automatic amino acid analyzer (8) after hydrolysis in 6 n HCl for 24-hour and, in some cases, 48-hour periods (Table I). There was no tryptophan present (9), nor was there an intact disulfide bond (10). The possibility of a second half-cystine residue in the peptide was checked by performic acid oxidation (11). As shown in Table II, only 1 cysteic acid residue was present in the oxidized peptide.

NH2-terminal Amino Acid by 2,4-Dinitrofluorobenzene Method
—The amino terminal residue of the isolated peptide was determined by the 2,4-dinitrofluorobenzene method of Sanger (12) as described by Frenkel-Conrat (13). The dinitrophenyl peptide was hydrolyzed in 6 N HCl for 16 to 24 hours and the ether-extractable dinitrophenyl amino acid was subjected to two-dimensional paper chromatography (13). The main yellow spot cochromatographed with authentic bis-dinitrophenyllysine, which indicated that lysine was in the NH₂-terminal position.

\[ \text{NH}_2\text{-terminal Sequence} - \text{The sequential arrangement of the first 4 amino acid residues at the amino terminal end of the peptide was determined by the Edman procedure as described by Konigsberg and Hill (14). The results of the four steps of subtractive Edman procedure are summarized in Table III. From these results, the sequence of the amino acid residues at the amino terminal end of the peptide was Lys-Arg-Val-Pro-} \]

- COOH-terminal Sequence—The hydrolysis of the peptide by diisopropyl fluorophosphate-treated carboxypeptidase A (substrate-carboxypeptidase, 50:1) was carried out at 36° (13). At various time intervals, aliquots were removed from the reaction mixture and the amino acid released was determined qualitatively on paper and also quantitatively on the amino acid analyzer as tyrosine. Subsequent addition of carboxypeptidase B and incubation for 11 hours led to the release of a yellow substance which cochromatographed with standard DDPS-cysteine in the upper phase of the solvent system, butanol-acetic acid-water (4:1:5). However, quantitative amino acid analysis of the enzymic hydrolysate showed that only 0.25 eq of tyrosine was released by carboxypeptidase A even after 11 hours of incubation. It was not possible to determine quantitatively the amount of DDPS-cysteine released after the addition of carboxypeptidase B since DDPS-cysteine overlapped tyrosine in the chromatogram. The data obtained thus far suggested that, in the isolated peptide, tyrosine was the COOH-terminal amino acid and DDPS-cysteine was the penultimate amino acid. In order to confirm this result, peptide fragments remaining after limited hydrolysis with carboxypeptidase A and B were isolated by paper chromatography in butanol-acetic acid-water (4:1:5). The results are summarized in Table IV, and indicate that the COOH-terminal sequence of the peptide was -(DDPS-Cys)-TyrCOOH. The low yield of tyrosine released from the carboxyl terminal position indicated that the peptide was slowly hydrolyzed by carboxy- peptidase A under the conditions employed. Gold and Segal (15) have observed that carboxypeptidase A was unable to release DDPS-cysteine from the peptide Ala-Ser-(DDPS-Cys)-Thr-Thr-Asn, obtained from the rabbit muscle glyceroldehydrate 3-phosphate dehydrogenase. It is possible that the presence of DDPS-cysteine adjacent to tyrosine prevents the latter from being quantitatively released by the exopeptidase under the conditions used. This has been observed in some cases, most notably in the case of peptides obtained from ribonuclease (16).

**TABLE II**

<table>
<thead>
<tr>
<th>Products</th>
<th>Amino acid residues</th>
<th>Hydrolysis in 6 N HCl</th>
<th>Performic acid oxidation, followed by hydrolysis in 6 N HCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Half cysteine</td>
<td>0.18</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td>CySO₄H</td>
<td>0.04</td>
<td>0.78</td>
<td></td>
</tr>
<tr>
<td>2-Amino-2-carboxethylmercapto-succinic acid</td>
<td>0.43</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Total residues</td>
<td>0.65</td>
<td>0.78</td>
<td></td>
</tr>
</tbody>
</table>

**TABLE III**

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Cycle 1</th>
<th>Cycle 2</th>
<th>Cycle 3</th>
<th>Cycle 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysine</td>
<td>0.36</td>
<td>0.10</td>
<td>0.25</td>
<td>0.17</td>
</tr>
<tr>
<td>Arginine</td>
<td>0.81</td>
<td>0.18</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Valine</td>
<td>0.93</td>
<td>0.10</td>
<td>0.35</td>
<td>0.10</td>
</tr>
<tr>
<td>Proline</td>
<td>0.98</td>
<td>0.85</td>
<td>0.70</td>
<td>0.29</td>
</tr>
<tr>
<td>DDPS-cysteine</td>
<td>1.00</td>
<td>0.92</td>
<td>1.06</td>
<td>1.05</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>0.95</td>
<td>0.93</td>
<td>0.94</td>
<td>0.91</td>
</tr>
<tr>
<td>Serine</td>
<td>1.01</td>
<td>0.97</td>
<td>0.81</td>
<td>0.84</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>1.06</td>
<td>0.96</td>
<td>0.96</td>
<td>0.96</td>
</tr>
<tr>
<td>Glycine</td>
<td>0.91</td>
<td>0.84</td>
<td>0.70</td>
<td>0.64</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>0.55</td>
<td>0.42</td>
<td>0.48</td>
<td>0.54</td>
</tr>
</tbody>
</table>

a Phenylthiohydantoin arginine was separated by high voltage paper electrophoresis at pH 6.5 (pyridine-acetate buffer). The peptide was eluted with 50% acetic acid and used for the subsequent steps.

**TABLE IV**

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>C-1 (RF = 0.12)</th>
<th>C-2 (RF = 0.28)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tyrosine</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>DDPS-cysteine</td>
<td>0.0</td>
<td>0.7</td>
</tr>
<tr>
<td>Glycine</td>
<td>0.7</td>
<td>1.1</td>
</tr>
<tr>
<td>Serine</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Valine</td>
<td>1.0</td>
<td>0.7</td>
</tr>
<tr>
<td>Proline</td>
<td>1.0</td>
<td>0.7</td>
</tr>
<tr>
<td>Lysine</td>
<td>0.8</td>
<td>0.7</td>
</tr>
<tr>
<td>Arginine</td>
<td>0.8</td>
<td>0.7</td>
</tr>
</tbody>
</table>

Yield: 10% 14%

**Effect of performic acid oxidation on DDPS-peptide**

Peptide 3 (0.00 μmole) was treated with 2.0 ml of cold performic acid reagent (9.0 ml of 88% formic acid + 1.0 ml of 30% H₂O₂). The mixture was allowed to stand at -4° for 4 hours, dried under nitrogen, and hydrolyzed in 6 N HCl for 24 hours. The amino acid composition was determined in the amino acid analyzer.

**Overlapping Fragments**—In order to complete the sequence determination, the peptide was hydrolyzed with Nagarse. Peptide 3 (1 μmole) was dissolved in deionized water and the solution was adjusted to pH 7.7 to 7.8 with 1% NaHCO₃. To this peptide solution was added 0.1 ml of 0.1% solution of Nagarse in 0.1 M phosphate buffer, pH 7.7, and the mixture was incubated for 8 hours at 37°. The reaction was terminated by the addition
of 2 to 3 drops of glacial acetic acid. The digestion mixture was then dried under nitrogen and the residue was taken up in a minimal amount of deionized water. A small amount of white precipitate which formed at this stage was centrifuged off. The evidence which led to the reconstruction of the sequence of the peptide containing the reactive sulphydryl group of chymopapain B and from papain and ficin was surprising and unexpected. Although it appears that a part of the cysteine residues in the chymopapain B was preferentially labeled by DDPM with concomitant loss of enzyme activity, the essential nature of the remaining cysteine residue has not been ruled out by the present study.

In spite of the similarities in the over-all properties of chymopapain B and papain, there are several definite differences which cannot be overlooked. The ability of papain to hydrolyze every substrate tested at a much faster rate and the greater milk-clotting activity of chymopapain B reflect some differences either in the basic mechanism or in the substrate-binding ability of the two enzymes (3). These differences in turn could be due to the difference in the amino acid sequence of the active center peptide. Jolles (17) has shown that enzymes with similar properties have different sequences in the active center in the case of hen egg white and dog spleen lysozymes.

Finally, the most striking difference between the sequence of the labeled peptide from chymopapain B and from ficin and papain is the fact that another half-cystine residue which is located 2 amino acid residues (Ser-Gly-) away from the labeled peptide from chymopapain B and those from ficin and papain B and papain, there are several definite differences which cannot be overlooked. The ability of papain to hydrolyze every substrate tested at a much faster rate and the greater milk-clotting activity of chymopapain B reflect some differences either in the basic mechanism or in the substrate-binding ability of the two enzymes (3). These differences in turn could be due to the difference in the amino acid sequence of the active center peptide. Jolles (17) has shown that enzymes with similar properties have different sequences in the active center in the case of hen egg white and dog spleen lysozymes.

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The occurrence of both papain and chymopapain in the papaya

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**TABLE V**

Peptide fragments obtained from Nagarse digestion of Peptide 3

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Electrophoretic Mobility</th>
<th>Color with ninhydrin</th>
<th>Amino acids released on 24-hr 6 N HCl hydrolysis, with molar ratios</th>
<th>NH2-terminal residue</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>+20</td>
<td>Faintly purple</td>
<td>Lys (0.6), Arg (0.8), Val (1.0), Pro (0.9), Asp (0.9), Ser (1.0)</td>
<td>Gly (0.7), Glu (1.0), DDPS-Cys (0.5)</td>
<td>Gly</td>
</tr>
<tr>
<td>S2</td>
<td>+5</td>
<td>(Yellow) Brown</td>
<td>Asp (1.0), Ser (0.6), Gly (0.8), Glu (1.0), DDPS-Cys (0.8)</td>
<td>Asp</td>
<td></td>
</tr>
<tr>
<td>S3</td>
<td>-2.5</td>
<td>Dark purple</td>
<td>Lys (0.6), Arg (0.8), Val (1.0), Pro (0.9), Asp (0.9), Ser (1.0)</td>
<td>Lys</td>
<td></td>
</tr>
</tbody>
</table>

**DISCUSSION**

DDPS-chymopapain B was prepared by titrating the enzyme with DDPM until approximately half of the original CMB-titratable sulfhydril groups were substituted and the enzyme lost all of its activity. From this derivative, only one type of labeled peptide was isolated from the peptic digest of DDPS-chymopapain B.

The amino acid sequence of the peptide containing the labeled sulfhydril group of chymopapain B was found to be different from those of papain (1) and of ficin (2) as follows.

Chymopapain B: Lys-Arg-Val-Pro-Asp-Ser-Gly-Glu-Cys*-Tyr-

Papain: Pro-Val-Lys-Asn-Gln-Gly-Ser-Cys-Gly-Cys*-Trp-

Ficin: Pro-Ile-Arg-Gln-Gly-Gln-Cys-Gly-Cys*-Ser-Cys*-

This difference in the amino acid sequence of the labeled peptide from chymopapain B and from papain and ficin is surprising and unexpected. Although it appears that a part of the cysteine residues in the chymopapain B was preferentially labeled by DDPM with concomitant loss of enzyme activity, the essential nature of the remaining cysteine residue has not been ruled out by the present study.

In spite of the similarities in the over-all properties of chymopapain B and papain, there are several definite differences which cannot be overlooked. The ability of papain to hydrolyze every substrate tested at a much faster rate and the greater milk-clotting activity of chymopapain B reflect some differences either in the basic mechanism or in the substrate-binding ability of the two enzymes (3). These differences in turn could be due to the difference in the amino acid sequence of the active center peptide. Jolles (17) has shown that enzymes with similar properties have different sequences in the active center in the case of hen egg white and dog spleen lysozymes.

Finally, the most striking difference between the sequence of the labeled peptide from chymopapain B and those from ficin and papain is the fact that another half-cystine residue which is located 2 amino acid residues (Ser-Gly-) away from the labeled half-cystine is missing in the chymopapain B peptide. The role of this second half-cystine in papain appears to be that of bringing together residues 165 (Asp) and 24 (the essential Cys), which are located at different parts of the enzyme molecule (1). Although the corresponding information for ficin is not available, it is likely that the second half-cystine residue in ficin has a similar function. Also significant is the fact that in both ficin and papain no free carboxyl group was located within the peptide studied. However, in the case of chymopapain B, a free glutamic acid was located adjacent to the labeled cysteine residue. In the absence of independent data, it cannot be concluded that this glutamic acid contributes the kinetically important grouping with the pK of 4.5. However, if this is so, then the amino acid residues kinetically implicated in the hydrolytic mechanism of the plant sulfhydril protease (the sulfhydril group and the carboxyl group) are found within the same peptide fragment in chymopapain B. Therefore, a specially placed disulfide bridge is not necessary to bring the essential amino acids together.

The occurrence of both papain and chymopapain in the papaya
latex raises an interesting question as to their roles in vivo. That papain, the smaller of the two, is a partially hydrolyzed product of chymopapain is ruled out on the basis of all the data (3) available on the differences in physical, chemical, and enzymic properties of the two enzymes, including the difference in the amino acid sequence around the essential sulfhydryl group. Since it seems redundant for two enzymes of similar general properties and mechanism of action to be found in the same source for the purpose of carrying out identical functions, each of the two enzymes may have unique and definite functions in the papaya latex.

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