CHANGES IN THE CONFORMATION OF CHYMOPAPAIN DURING THE ACTIVATION PROCESS*

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Received January 11, 1966

Plant sulfhydryl proteases are known to require activation by certain types of reagents before enzymic activity becomes manifest (Smith and Kimmel, 1960). The activation process has been viewed as the conversion of masked sulfhydryl groups or cystine residues to free sulfhydryl groups (Smith and Kimmel, 1960; Liener, 1961).

Evidence presented in the present paper indicates that the activation process involves not only the changes mentioned above but also conformational changes which may expose the active center of the enzyme.

Chemical evidence concerning the conformational change was obtained in the following manner. DFP phosphorylates sulfhydryl proteases without inactivating the enzyme (Murachi, 1963; Gould and Liener, 1961). When unactivated chymopapain A and B were reacted with DFP, incorporation of phosphorous was not observed, Table I. After activation of these enzymes with cysteine or cyanide however, approximately one gram atom of phosphorous was incorporated.

The difference spectra of the native and DIP-enzymes have been used by Hess and co-workers (Oppenheimer, et al., 1963; Oppenheimer and Hess, 1963) to demonstrate conformational changes during the reaction of trypsin and chymotrypsin with DFP. When an analogous experiment was run on chymopapain B,

* Supported by Research Grant GB-1363 from the National Science Foundation.

TABLE I

Incorporation of DFP into chymopapain[1]

Enzyme	Unactivated	Cyanide-activated
	g atom P/mole of enzyme[2]	
1. Chymopapain A[3]	0.0	0.88
2. Chymopapain B	0.0	0.89

1 The method for the preparation of DIP-chymopapain has been described previously (Ebata, et al., 1962).
2 DIP-Serine is formed without inactivation of the enzyme.
3 The difference in the chemical properties of chymopapain A and B are described elsewhere (Kunimitsu and Yasunobu, 1965).

TABLE II

Enzyme	Concentration (%)	Solvent	$[\alpha]_{589.3\ m\mu}^{25^\circ}$
Chymopapain	2.75	0.1 M phosphate buffer, pH 7.2	-43.9°
"	2.58	0.1 M phosphate buffer pH 7.2 + 8 M urea, 72 hr.	-57.9°
Chymopapain, cysteine-activated*	1.54	0.1 M phosphate buffer, pH 7.2 + 8 M urea, 72 hr.	-93.8°

* Excess cysteine removed by passing the activation mixture through a mixed bed resin.

the result shown in Figure I was obtained. Non-activated chymopapain did not show a difference spectrum indicating the DFP reacts only with the activated form of chymopapain. This result confirms the fact that the conformation of the native and the activated forms of chymopapain are different.

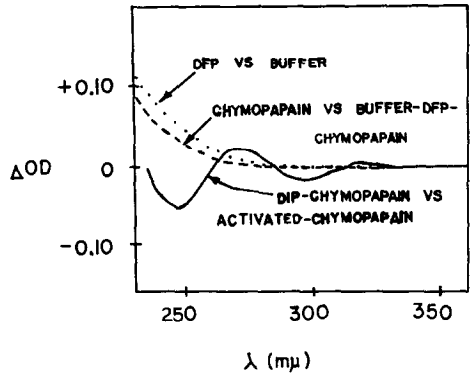

Figure 1. (a) Ultraviolet difference spectra of DFP-treated cyanide-activated-chymopapain versus cyanide-activated chymopapain (_____). 1 ml of NaCN (final cyanide concentration, 0.1 M) was added to 1 ml chymopapain (1.60 mg) in 0.1 M cacodylate buffer, pH 7.2, and the mixture was incubated for 60 minutes at 35°. Then 0.1 ml of DFP in isopropanol was added to the reaction mixture and the final volume was made to 3.0 cc with 0.1 M cacodylate buffer (Aldrich DFP, final concentration, 3.3×10^{-3} M, DFP/enzyme = 188). After further incubation at 35° for 30 minutes difference spectrum was read against the control which consisted of 2 ml of cyanide activated chymopapain and 1 ml of buffer. (b) DFP-treated non-activated chymopapain versus non-activated chymopapain (------). Conditions were identical to (a) except for omission of the cyanide activation step. (c) DFP versus buffer (.....). 2.9 ml of buffer was incubated with 0.1 ml of DFP solution as described above. All readings were made on a Cary Model 14 spectrophotometer.

Optical rotation measurements were made on unactivated and cysteine-activated chymopapain preparations after the removal of the cysteine through a mixed bed, (3 cm of Dowex 1 (OH^- form) first followed by 3 cm of Dowex 50W X8 (H^+ form) and finally 12 cm of Sephadex G-50 at the bottom). The results summarized in Table II demonstrate that very small changes occur upon the addition of 8 M urea to the unactivated enzyme while significant changes occur in the specific rotation upon the addition of 8 M urea to the activated enzyme. Again, this would point to conformational differences between the activated and unactivated chymopapain molecules.

In summary, specific rotation, ultraviolet spectroscopy and phosphorous determinations of unactivated and activated chymopapain clearly demonstrate structural changes during the activation process. A conformational change accompanies the unmasking of the active center of the enzyme. Thus, the activation process is not simply the unmasking of the essential sulfhydryl group. The activation is therefore visualized as more analogous to the activation of other proteases such as trypsin (Neurath, 1964).

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