PROTEASES OF THE SEEDS OF THE COTTON PLANT

II. SEPARATION AND PURIFICATION

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We separated the fractions with protease activity [1] and purified them by gel filtration on a column of Sephadex G-100 (column 4.5 × 100 cm; rate of elution 60 ml/h) using 0.1 M phosphate buffer with pH 7.4. Two active fractions were obtained: 1 and 2. Fraction 1 was then passed through a column of Sephadex G-200 (column 2 × 75 cm, rate of elution 7 ml/h, 0.1 M phosphate buffer, pH 7.4). The active fractions obtained were called protease I and protease II. Fraction 2 after gel filtration through G-100 was passed through a column of DEAE-cellulose equilibrated with 0.1 M phosphate buffer, pH 7.4 (column 1 × 20 cm, rate of elution 16 ml/h). The protein was eluted by the imposition of a gradient: 0.01 M phosphate buffer with pH 7.4–0.1 M phosphate buffer with pH 7.4 and 0.5 M NaCl. Two active fractions were obtained: one before the imposition of the gradient and the other after it. The fraction eluted after the imposition of the gradient at a concentration of NaCl of 0.1-0.15 M proved to have the maximum specific activity and was called protease III. Experimental information on the separation and purification processes is given in Table 1.

The disk electrophoresis in polyacrylamide gel [2] of proteases I and II showed a single clear band for each and two very weak brightenings while in the case of protease III there was one broad band and five weak brightenings. The molecuar weights of proteases I-III were determined by gel filtration through a column of Sephadex G-200; they were, respectively, 140,000, 70,000, and 30,000. As markers for calibration (column 2 × 75 cm, rate of elution 7 ml/h) we used "Reakhim" catalase (mol. wt. 240,000), "Reanal" lactate dehydrogenase from porcine cardiac muscle (mol. wt. 142,000), "Hungary" bovine serum albumin (mol. wt. 67,000), and "Spofa" trypsin (mol. wt. 24,000). The free volume of the column was determined with respect to dextran blue "Pharmacia."

TABLE 1

Stage of purification	Protein content, g	Proteolytic activity		Yield, %, with respect to		Degree of purification
		specific	total	protein	activity	
First extract in 0.1 M phosphate buffer, pH 7.4	5,2	20	104	—	—	—
Fraction 0-60% saturation $[(NH_4)_2SO_4]$, separation on Sephadex G-100	1,26	70	88,2	24,2	80	3,5
Fraction 1	0,95	40	38	18,6	36,5	2
Fraction 2	0,032	430	13,7	0,6	13,5	21
Separation on Sephadex G-200 of Fraction 1 after G-100	0,84	50	42	16,2	41	2,5
1 (protease I)	0,13	170	22,1	2,4	21,2	8,5
2 (protease II)	0,037	530	19,6	0,7	19,3	26,5
Separation on DEAE-cellulose of Fraction 2 after G-100	0,032	430	13,7	0,6	13,5	21
1	0,016	278	4,98	0,31	5	13
2 (protease III)	0,008	800	6,4	0,11	6,1	40

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Thus, three forms of proteases with dissimilar molecular weights have been found in dormant cotton seeds and a method for their separation has been developed.

LITERATURE CITED

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