PROTEASES OF COTTON SEEDS.

ISOLATION OF PROTEASE A FROM COTTON SEEDS

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The presence of proteolytic enzymes in dormant cotton seeds has been established previously [1-3]. Continuing investigations in this field, we have developed a method for the isolation and purification of protease A which includes the following stages: extraction of the defatted seeds with 0.1 M phosphate buffer, precipitation of the protein with ammonium sulfate at 60% saturation, desalting by dialysis, and ion-exchange chromatography on a column of CM-cellulose (column 5 × 35 cm, rate of elution 60 ml/h, 0.01 M acetate buffer, pH 5.4).

For the further purification of protease A we used rechromatography on a column of CM-cellulose (column 2.5 × 14 cm, rate of elution 30 ml/h, 0.01 M acetate buffer, pH 5.4) and chromatography on a column of DEAE-cellulose (column 1.5 × 8 cm, rate of elution 4 ml/h, 0.01 M phosphate buffer, pH 7.4).

Protease A was eluted with 0.01 M phosphate buffer with the superposition 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. The experimental results on the separation and purification of the protease are given below:

Stage of purification	Volume, ml	Total protein, mg	Activity, units		Yield	
			total	specific	protein, %	activity
Extracted with 0.1 M phosphate buffer, pH 7.4	1500	6000	24000	4	—	—
Precipitation with 60% ammonium sulfate	150	1748	20970	12	100	100
Chromatography on CM-cellulose	100	200	12200	56	12	57
Rechromatography on CM-cellulose	60	52.5	4305	82	5	20
Fractionation on DEAE-cellulose	20	12	1224	102	0,76	5,7

The homogeneity of the enzyme was established by electrophoresis in polyacrylamide gel, by isoelectric focusing in the borate-polyol system (isoelectric point, pI, 4.9), and by a determination of the N-terminal amino acid.

It was established by the GLC method that the protease A contained about 8-10% of carbohydrates. According to the results of gel filtration, the molecular weight of the enzyme was 60,000. The temperature and pH optima of the action of the enzyme have been established (35-40°C and 6.4-7.4, respectively). The protease under investigation was not activated by sulfhydryl agents (cysteine, β-mercaptoethanol) and was not inhibited by p-chloromercuribenzoate, which shows that it does not belong to the thiol proteases.

LITERATURE CITED

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