ISOLATION OF FOUR SOYBEAN TRYPSIN INHIBITORS BY DEAE-CELLULOSE CHROMATOGRAPHY

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A crystalline soybean trypsin inhibitor was first isolated by Kunitz (1946). The preparation of two highly purified soybean trypsin inhibitors, designated SBTIA$_1$ and A$_2$, has been described (Rackis et al., 1962). Since then we have simplified the preparation of SBTIA$_1$ and also isolated two more trypsin inhibitors from raw soybean meal.

MATERIALS AND METHODS

Soybean whey protein, prepared and fractionated according to previously described procedures (Rackis et al., 1959, 1962), was used directly to isolate four trypsin inhibitors. DEAE-cellulose, Selectacel,[1/] reagent-grade, a capacity of 1.05 meq. per gram was purchased from Brown Company, Berlin, New Hampshire. Variable salt gradients were produced with Peterson and Sober's (1959) device. All the chambers contained 30 ml. of 0.01 M potassium phosphate buffer, pH 7.6. In chambers 1-9 consecutively, the following molar concentrations of sodium chloride were also used: 0, 0.08, 0.11, 0.11, 0.16, 0.10, 0.12, 0.20, and 0.20 respectively. Salt concentrations in effluents were determined by conductivity measurements, protein with Lowry's Phenol reagent (1951).

Electrophoretic and ultracentrifugal analyses were made in potassium phosphate buffer, pH 7.6 and 0.1 ionic strength. Electrophoresis was carried

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1/ The mention of firm names or trade products does not imply that they are endorsed or recommended by the Department of Agriculture over other firms or similar products not mentioned.

out at 2° C., with mobilities calculated from the ascending patterns. Sedimentation was done at room temperature in a Spinco ultracentrifuge, Model E. Sedimentation constants were determined according to Svedberg and Pedersen (1940).

Trypsin inhibitor activity, with BAEE as substrate, was determined by the method of Wu and Scheraga (1962). Twice-crystallized trypsin containing 50% magnesium sulfate was obtained from Mann Research Laboratories (Lot No. H 1348). Soybean trypsin inhibitor, crystallized five times, designated SBTI(5X), and purchased from Gallard-Schlesinger Mfg. Corp. (Lot No. A2133), was used to prepare standard curves. However, SBTI(5X) prepared according to Kunitz (1946) was first analyzed for purity and for the presence of denatured trypsin inhibitor by the procedure of Rackis et al. (1962).

RESULTS AND DISCUSSION

Chromatographic Isolation of $SBTIA_1$

In the original procedure of Rackis et al. (1962), $SBTIA_1$ was isolated on DEAE-cellulose by rechromatographing fraction V twice according to the same step-wise procedure shown in Fig. 1. Each time, the protein in tube numbers 32-42 was eluted with 0.18 M sodium chloride, collected, lyophilized, and rechromatographed. The rechromatographic procedure was tedious, and yields of $SBTIA_1$ were quite low.

With the procedure improved by variable salt gradients, fraction V can be simultaneously resolved into three chromatographic peaks (Fig. 2). Protein eluting in effluent volumes, 380-450 ml., peak III, Fig. 2, represents chromatographically pure $SBTIA_1$ having a specific activity value of 1.60, corresponding to the value of highly purified $SBTIA_1$ isolated previously (Rackis et al., 1962). $SBTIA_1$ was also homogeneous in moving boundary electrophoresis, even after back-compensation for 3 hours and in ultracentrifugation. This direct isolation of $SBTIA_1$ from fraction V increased yields at least threefold.

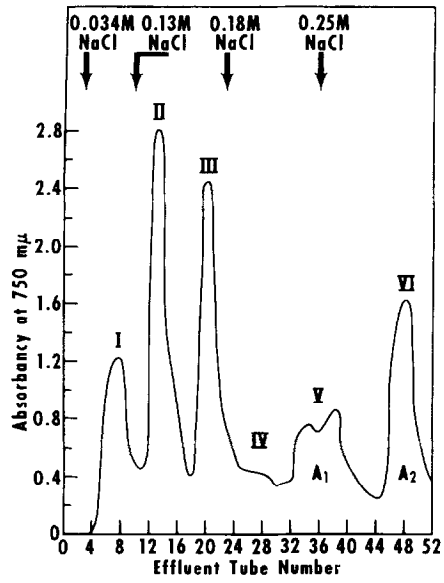

Fig. 1. Stepwise elution diagram of soybean whey proteins: 0.8 to 1.0 g. dialyzed and lyophilized protein in 30 ml. of 0.01 M potassium phosphate buffer, pH 7.6; effluent fraction was 10 ml.; optical density at 750 mμ of a 0.1 ml. aliquot of effluent with Folin reagent (Lowry, et al., 1951). Vertical arrows indicate point of change of sodium chloride concentraction in buffer. Column dimensions of DEAE-cellulose were 2.25 x 39 cm.

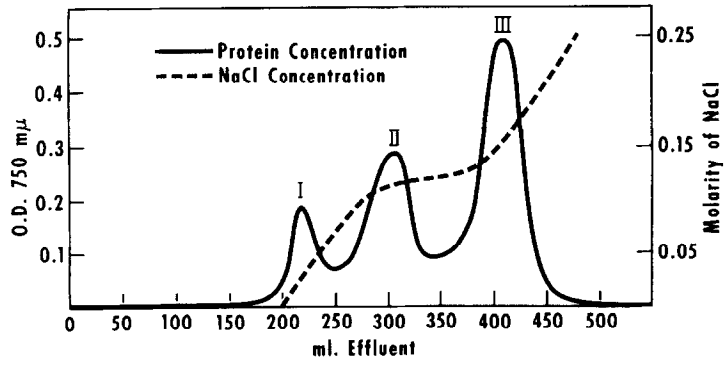

Fig. 2. Resolution of soybean trypsin inhibitors in Fraction V on DEAE-cellulose: 100 mg. dialyzed and lyophilized protein, in 30 ml. of 0.01 M potassium phosphate buffer, pH 7.6; effluent fraction was 5 ml.; broken line and scale at right represent salt concentration in effluent. Other conditions given in Fig. 1 and in text.

Isolation of Additional Trypsin Inhibitors

Peaks I and II in Fig. 2, designated SBTIB_1 and B_2, respectively, represent additional proteins having high-trypsin-inhibitory activity. Cuts taken from the centers of each peak were nearly homogeneous by electrophoresis, ultracentrifugation, and chromatography. Chromatographically pure SBTIA_2 was obtained from fraction VI by discarding the protein in the leading edge of the peak.

Properties of Four Soybean Trypsin Inhibitors

Data on the physical-chemical properties and antitryptic activity of SBTIB_1, B_2, A_1, and A_2 are summarized in Table I. Widely different concentrations of sodium chloride are required to elute the soybean trypsin inhibitors from DEAE-cellulose. The order of elution correlates directly with their respective electrophoretic mobility values. Also, sedimentation constants and specific activity values of the four trypsin inhibitors differ widely. SBTIA_2 and SBTI(5X) have similar physical, chemical, and chromatographic properties.

The soybean trypsin inhibitors account for approximately 6% of the protein in dehulled, defatted soybean meal. The biological function of the trypsin inhibitors is unknown since these proteins do not inhibit soybean protease activity (Ofelt et al., 1955).

Significance of Several Active Trypsin Inhibitors

SBTIA_1, A_2, B_1, and B_2, rechromatographed under identical conditions used in their initial isolation, gave completely reproducible results. This behavior indicates that a true fractionation was obtained. These four trypsin inhibitors are most likely distinct proteins existing as such in the mature seed since no apparent artifacts were formed during the chromatographic process and no extremes in pH and salt concentration were used in the isolation.

Soybean meal must be properly processed with moist heat to inactivate constituents that inhibit growth and enlarge the pancreas and thyroid

Table I. Properties of Soybean Trypsin Inhibitors (SBTI)

Property	B_1	B_2	A_1	A_2	(5X)[a]
Salt conc. for elution, M	0.05	0.11	0.16	0.21	0.21
Electrophoretic mobility, sq. cm./v./sec.	-4.6×10^{-5}	-5.3×10^{-5}	-7.4×10^{-5}	-8.0×10^{-5}	-8.0×10^{-5}
Sedimentation constant, $S_{20}W$	4.07	4.62	1.80	2.29	2.30
Partial specific volume, ml./g.	--	--	0.736	0.735	0.745
Molecular weight, g. $mole^{-1}$	--	--	14,300	21,600	22,700
Specific activity, µg. trypsin inhibited/µg. inhibitor	2.0	1.8	1.6	1.0	1.0
N-Terminal amino acid	--	--	Aspartic	Aspartic	Aspartic

[a] Commercial soybean trypsin inhibitor, crystallized five times and prepared according to Kunitz (1946).

(Liener, 1958). The significance of the presence of SBTIB_1 and B_2 on the nutritional value of soybean meal requires further study; however, it has already been established that SBTIA_1 and A_2 cause exaggerated secretion of pancreatic juice enzymes (Lyman et al., 1962).

SUMMARY

Soybean whey proteins have been resolved by DEAE-cellulose chromatography into four highly purified soybean trypsin inhibitors designated SBTIA_1, A_2, B_1, and B_2. The inhibitors differ in respect to chromatographic behavior, physical-chemical properties, and antitrypsin activity.

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