THE 8.2 S GLOBULIN OF COTTON SEEDS

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Cotton seeds were defatted with diethyl ether at 3-5°C and the proteins were extracted with 10% NaCl solution. Stepwise dialysis precipitated three fractions: 1) when the concentration of NaCl had been reduced to 5%, 2) to 2.5%, and 3) to 0%.

Fraction 3, consisting of 50% of the combined fractions, was used for the isolation of the 8.2 S globulin. The proteins and the other components (R_f 0.52 and 0.65 in acrylamide gel [1, 2]) soluble in phosphate buffer, pH 7.3, containing NaCl (1.39 g of $Na_2HPO_4 \cdot 12H_2O$, 0.46 g of $NaH_2PO_4 \cdot 2H_2O$ and 10.032 g of NaCl in 1.33 liter) with an ionic strength μ of 0.15 were eliminated by the repeated extraction of fraction 3 (usually three to five times). The residue was dissolved in the same buffer with an ionic strength μ of 0.25, and, after centrifuging (10 min, 5000 rpm), the soluble part was fractionated by chromatography on DEAE-cellulose at room temperature. One hundred ml of the protein solution (10 mg/ml) was deposited on a column (4.0 × 25.0 cm) equilibrated with phosphate buffer having an ionic strength μ of 0.25 and eluted with the same buffer with the creation of a linear NaCl gradient (μ 0.25 – 1.0). The rate of elution was 60 ml/h, and the volume of the fraction 15 ml.

Fractions 4-15 (Fig. 1a) were combined and dialyzed against distilled water at 3-5°C. The precipitate that deposited was separated off by centrifuging (10 min, 5000 rpm) and was freeze-dried. The yield of protein was 35%.

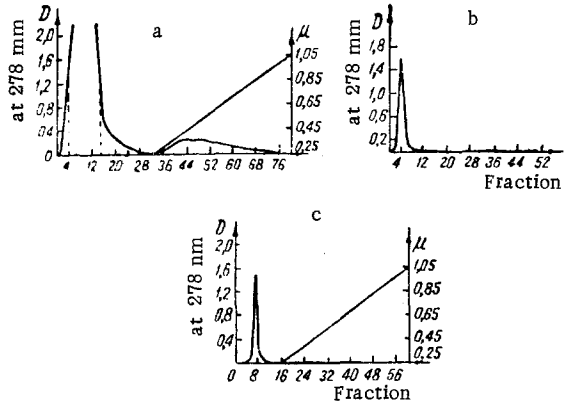

Fig. 1

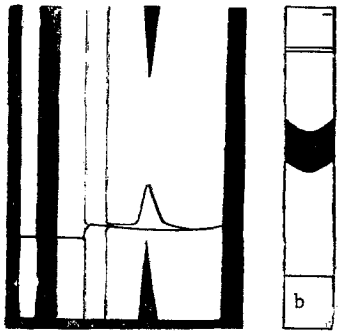

Fig. 2

Fig. 1. Chromatographic separation of the 8.2 S globulin of cotton seeds on DEAE-cellulose (a, b) and Sephadex G-200 (b)

Fig. 2. Sedimentogram (a) and electrophoregram (b) of the 8.2 S component of the globulin of cotton seeds (rate of centrifuging 50,140 rpm, 35 min, θ 20°, T 20°C, concentration of protein 10 mg/ml, medium: phosphate buffer with μ 0.5, pH 8.0.

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Rechromatography on a column (1.0 × 27 cm) of Sephadex G-200 (rate of elution 20 ml/h, fraction volume 5 ml) and on a column (1.3 × 13 cm) of DEAE-cellulose (rate of elution 16 ml/h, fraction volume 4 ml) showed the homogeneity of the protein (Fig. 1b and c, respectively). Its homogeneity was also confirmed by ultracentrifugation (MOM, G-120) (Fig. 2a) and by electrophoresis in acrylamide gel [1] (Fig. 2b).

LITERATURE CITED

1. Yu. Ya. Gofman, Biokhim., 32, 690, 1967.
2. Kh. Mirzarakhmatov and P. Kh. Yuldashev, KhPS [Chemistry of Natural Compounds], 4, 341, 1968.