ISOLATION OF A MONODISPERSE PROTEIN FRACTION FROM COTTONSEEDS*

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Received January 6, 1964

Little is still known about the chemical, physicochemical and biological properties of individual seed proteins (Brohult and Sandegren, 1954; Altschul, 1963).

To a great extent, this is due to the fact that these are extracted from the seeds in complex mixtures and often what was considered a pure protein fraction is found to be heterogeneous by modern fractionation techniques (Altschul, 1963).

As far as cottonseed proteins are concerned, fractionations have been carried out in the past, but all the preparations were found to be heterogeneous in the centrifuge or in electrophoresis (Altschul, Lyman, and Thurber, 1958).

We wish to describe here the isolation and some properties of a monodisperse protein component which accounts

* Supported by a grant FG-It-125 from the U.S. Department of Agriculture.

for a large fraction of the proteins in cottonseeds. In analogy with similar proteins from other seeds, we have called this protein "Acalin A."

EXPERIMENTAL AND RESULTS

Acala glandless cottonseeds were kindly supplied by the Southern Regional Laboratory, U.S. Department of Agriculture.

The seeds were dehulled, flaked, extracted with 5 volumes (W/V) of acetone cooled at -20°, and dried by suction on a filter.

The acetone powder was then stored in the cold. Most of the protein present in the acetone powder of cottonseeds can be extracted with 2 M NaCl at room temperature. After the removal of the insoluble material, the pale yellow solution shows, in the ultracentrifuge, the presence of several components. Most of the proteins which are present in this extract can be reversibly precipitated by lowering the ionic strength of the solution. In preliminary fractionation experiments, it was noted that the temperature had an unusually large effect, at a given ionic strength and pH, on the solubility of a protein fraction. A procedure was therefore devised to fractionate the extract making use of the effect of different salt concentrations and temperatures on the solubility of the various proteins. A number of fractions have so been obtained, which show, in the ultracentrifuge, different amounts of individual components. The procedure,

which will be described below, leads to the isolation of the protein component, the solubility of which is particularly affected by temperature (Acalin A).

The extract in 2 M NaCl, buffered at pH 7, is dialyzed in the cold against water for 24 hours. During the dialysis a heavy precipitate appears in the dialysis sack. The water soluble proteins are removed by centrifugation and the precipitate is extracted with NaCl 0.05 M in the cold. The supernatant is discarded and the precipitate is then suspended in 0.3 M NaCl, at pH 7, and extracted at a temperature near 30° C. The material is then centrifuged at the same temperature. The precipitate is extracted again with smaller portions of 0.3 M NaCl at 30° C, and the clear supernatants obtained from the extractions cooled at 0-2° C. On cooling, a white precipitate appears and is collected by centrifugation in the cold. The bulk of the precipitate is dissolved in a minimum amount of 0.3 M NaCl at 30° C and the insoluble material removed by centrifugation. The solution is then cooled again at 0-2° C. The precipitate which forms is collected and dissolved in 0.3 M NaCl at 20° C.

At room temperature and neutral pH the protein so isolated is insoluble in water; it dissolves in salt solutions at $\mu > 0.2$; it is precipitated by ammonium sulfate at a saturation over 70%. The solubility in ammonium sulfate, just as in dilute salt solutions, is markedly affected by temperature.

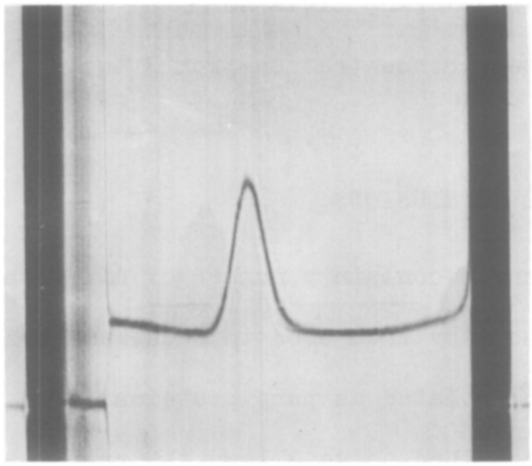

Fig. 1 Sedimentation pattern of "Acalin A." Protein concentration: 7 mg/ml. Solvent: 0.5 M NaCl, pH 7.

The protein appears homogeneous in the ultracentrifuge, as shown in Figure 1. It shows a single band in zone electrophoresis on cellulose acetate (phosphate buffer, μ=0.3, pH 7); it is eluted as a single peak from absorption on DEAE-sephadex columns, with step wise gradient of NaCl at pH 7.

The protein contains 18% nitrogen. Its ultraviolet light absorption spectrum has a maximum at 278 mμ. At this wavelength the extinction coefficient $E^{1\%}_{1cm}$ is 7.00.

The sedimentation coefficient in 0.3 M NaCl, pH 7.0 ($S^{0}_{20,w}$) is 9.2 S. The sedimentation constant shows little dependence on concentration.

The weight average molecular weight was determined by light scattering measurements in a Brice Phoenix Photometer at λ=546 mμ and with the procedure routinely used in our laboratory (Rossi-Fanelli, Antonini and Caputo, 1959). The weight average molecular weight in 0.3 M NaCl, pH 7 is about 180,000 on the basis of a value of dn/dc=0.192 ml/g.

The slope of the light scattering plots Hc/τ is essentially zero over a protein concentration from 7 to 0.3 mg/ml.

DISCUSSION AND CONCLUSIONS

Aqueous extracts from seeds contain a number of different protein components which also vary from seed to seed, and relatively few of them have been isolated as pure proteins.

The simple procedure described above allows the preparation from cottonseeds of a protein fraction, which behaves as a homogeneous monodisperse material. This protein, for which we propose the name Acalin A, appears to be one of the major protein components of cottonseeds. It shows a marked dependence of solubility on temperature, and this property forms the basis of the isolation procedure. In this respect and in its other molecular properties, it is similar to other "cold precipitable" globulins of different seeds (Brohult and Sandegren, 1954; Altschul, 1963).

Further characterization of this protein and comparison of its properties with those of other seed proteins are now in progress in our laboratory.

REFERENCES

Altschul, A.M. (1963) in Symposium on Food Proteins and Their Reactions. Corvallis, Oregon. September 4-6.

Altschul, A.M., Lyman, C.M. and Thurber, F.H. (1958) in "Processed Plant Protein Foodstuffs." A.M. Altschul, editor. Academic Press, New York.

Brohult, S. and Sandegren, E. (1954) in "The Proteins." H. Neurath and R. Bailey, editors. Academic Press, New York.

Rossi-Fanelli, A., Antonini, E. and Caputo, A. (1959) in J.Biol.Chem., 234, 2906.