Studies on Groundnut Proteins. IV. Effect of Storage or Proteolysis on the Gel Electrophoresis Pattern of Arachin

Changes occur in the gel electrophoresis pattern of arachin on storage. Similar changes are brought about by the action of α-chymotrypsin. The monomer of arachin appears to be degraded more easily than the dimer. A method is described for the preparation of the dimer of arachin in a "pure" form.

Arachin forms the major fraction of the groundnut (*Arachis hypogaea*) proteins (Dechary and Altschul, 1966). Several methods have been described for the preparation of arachin in a homogeneous form (Tombs, 1965; Neucere, 1969; Dawson, 1971; Shetty and Rao, 1974). Purified arachin in gel electrophoresis gives two bands which have been ascribed to the dimer and monomer, respectively (Tombs, 1965). During the course of our work, it was observed that whereas freshly prepared arachin gave two bands of equal intensity in gel electrophoresis, the stored sample gave a different pattern. The difference probably arises out of the action of protease impurities. Also described is a method for obtaining the dimer in a pure form.

EXPERIMENTAL SECTION

Arachin was prepared from defatted groundnuts of the variety *Asiriya mwitunde* by the method of Tombs (1965), Dawson (1971), and Shetty and Rao (1974) and then freeze-dried. The arachin prepared by Tombs' or Dawson's procedure was stored in the cold (4°) for several months. The fresh or the stored sample was dissolved in 0.01 *M* phosphate buffer (pH 7.9), dialyzed against the same buffer, and subjected to gel electrophoresis (150 μg/15 μl) using 7.5% gels in the same buffer at 4 mA/tube for 90 min. The gels were stained for 45 min with 0.5% Amido Black in 7.5% acetic acid solution, destained, and then stored in 7.5% acetic acid.

The freshly prepared arachin was subjected to proteolysis with α-chymotrypsin (Worthington Biochemicals Corporation). Two milliliters of 2% arachin solution in 0.1 *M* borate buffer (pH 7.8) was mixed with 2 ml of borate buffer containing 200 μg/ml of α-chymotrypsin and incubated at 37° for 20 min. The solution was then heated at 50° for 10 min to inactivate the enzyme. The resultant solution was then dialyzed extensively against 0.01 *M* phosphate buffer (pH 7.9). The dialyzed solution was subjected to gel electrophoresis. Sedimentation velocity measurements were made at room temperature (~25°), with a Spinco Model E ultracentrifuge fitted with phase-plate schlieren optics and RTIC unit. Protein solutions (0.5%) in 0.01 *M* phosphate buffer (pH 7.9) were used. The ionic strength of the buffer solution was increased by the addition of NaBr.

RESULTS AND DISCUSSION

The electrophoretic patterns of the fresh, stored, and α-chymotrypsin-treated arachin are given in Figure 1. In each case the fresh sample gave essentially two bands of equal intensity. The faster moving band corresponded to the monomer of arachin whereas the other corresponded to the dimer (Tombs, 1965). The stored arachin prepared by either Tombs' or Dawson's method gave patterns where the intensity of the monomer band had decreased; in addition there were several fast moving bands. This suggested that, on storage, degradation of arachin had occurred. The presence of proteolytic enzyme(s) in groundnut protein has been reported (Irving and Fontaine, 1945; Moseley and Ory, 1973). Probably the enzyme(s) also precipitated with the arachin fraction and thus was an impurity. The degradative action could be due to the presence of the protease.

The freshly prepared arachin samples were tested for proteolytic activity by using hemoglobin as substrate. Proteolytic activity could be detected but could not be assayed quantitatively. This suggested that proteolytic activity was weak; this should be even weaker in the freeze-dried state. That degradation was not caused by freeze drying was shown by the fact that the electrophoretic pattern of arachin immediately after freeze drying was the same as that of fresh arachin. Although the above-described experiment does not specifically indicate that degradation was due to proteases, it is perhaps a reasonable assumption.

To test the hypothesis that the different pattern obtained with the stored sample was due to proteolysis the freshly prepared arachin was subjected to the action of α-chymotrypsin. The gel electrophoresis pattern obtained with the α-chymotrypsin-treated, fresh sample showed a less intense monomer band and also several fast moving components. The same results were obtained with all three arachin preparations.

It was interesting that the dimer of arachin was rather resistant to proteolysis. Tombs (1965) has reported that the monomer is more susceptible to dissociation with a reagent such as 10 *M* formamide. Shetty and Rao (1973) have reported that 0.5% sodium dodecyl sulfate dissociates the monomer and does not seem to affect the dimer. Therefore, it might be possible to prepare the dimer in a pure form, by the combined action of α-chymotrypsin and sodium dodecyl sulfate. The following experiment was performed. Arachin prepared by the method of Shetty and Rao (1974) was first treated with α-chymotrypsin and the enzyme was inactivated by heating at 50° for 10 min. The solution was dialyzed extensively against 0.01 *M* phosphate buffer (pH 7.9) and made to 0.5% with respect to sodium dodecyl sulfate. This was followed by dialysis against the same buffer to remove free sodium dodecyl sulfate. The dialyzed solution was then passed through a column of Sephadex G-75 (95 × 2.5 cm) equilibrated with 0.01 *M* phosphate buffer. The gel filtration pattern is given in Figure 2. As could be

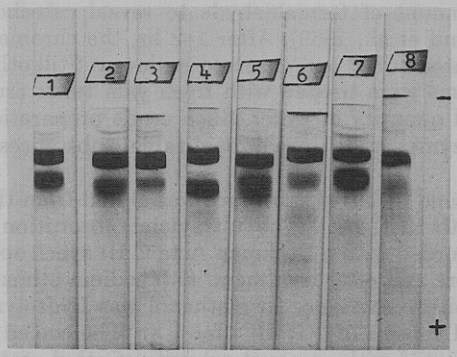

Figure 1. Polyacrylamide gel electrophoresis patterns: (1) arachin by Tombs' method (fresh); (2) arachin by Tombs' method (stored in the cold for several months); (3) arachin by Tombs' method (fresh, after treatment with α-chymotrypsin); (4) arachin by Dawson's method (fresh); (5) arachin by Dawson's method (stored in the cold for several months); (6) arachin by Dawson's method (fresh, after treatment with α-chymotrypsin); (7) arachin by Shetty and Rao's method (fresh); (8) arachin by Shetty and Rao's method (fresh, after treatment with α-chymotrypsin).

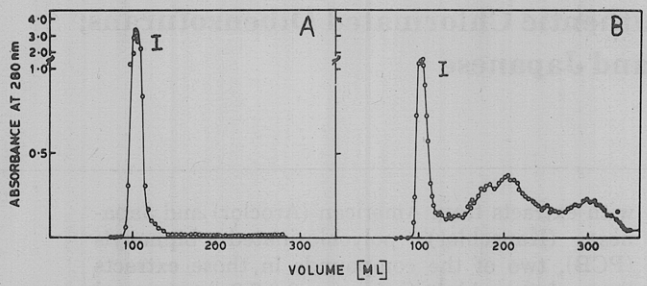

Figure 2. Gel filtration patterns: (A) arachin (by the method of Shetty and Rao); (B) arachin subjected to the action of α-chymotrypsin and sodium dodecyl sulfate.

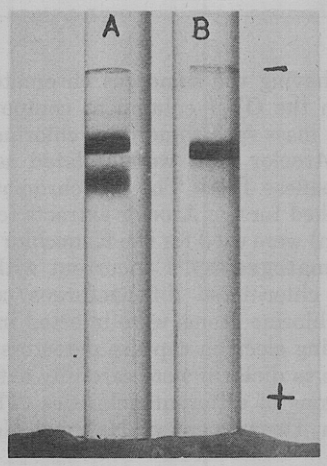

Figure 3. Polyacrylamide gel electrophoresis pattern: (A) arachin; (B) fraction corresponding to peak I in Figure 2B.

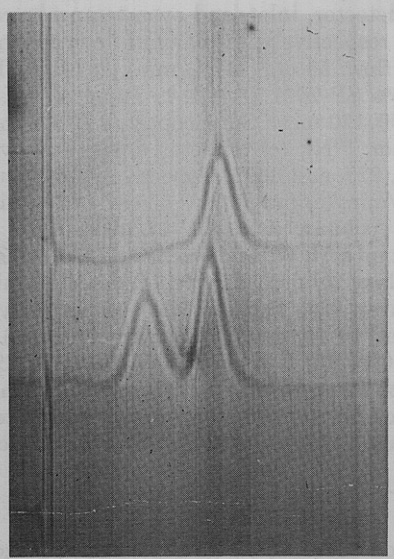

Figure 4. Sedimentation velocity patterns in 0.01 *M* phosphate buffer (pH 7.9): (lower) arachin; (upper) fraction corresponding to peak I in Figure 2B.

expected the enzyme and sodium dodecyl sulfate treated arachin contained many low molecular weight components in addition to the high molecular weight component. The fractions corresponding to peak I were pooled and subjected to gel electrophoresis (Figure 3). It consisted of a single band whose position corresponded to that of the dimer of arachin. This suggested that the high molecular weight fraction in gel filtration was perhaps the dimer of arachin.

Both the untreated arachin and the high molecular weight fraction (after concentration) were subjected to ultracentrifugation in 0.01 *M* phosphate buffer (pH 7.9). The sedimentation velocity patterns are given in Figure 4. The untreated sample gave two peaks with apparent sedimentation coefficients of 14 and 10 S. Johnson and Shooter (1950) have reported that arachin dissociates into halfmers in buffer solution of low ionic strength and the two peaks obtained with the untreated samples were undoubtedly due to the dissociation reaction. The high molecular weight fraction gave only one peak of 14 S, suggesting that this fraction did not undergo dissociation in buffers of even low ionic strength. Johnson and Shooter (1950) have also reported that in buffers of high ionic strength the dissociation of arachin is suppressed. The sedimentation velocity experiments were, therefore, repeated in 0.01 *M* phosphate buffer (pH 7.9) containing 0.5 *M* NaBr (Figure 5). Both the proteins gave single peaks with 14 S. This further suggested that the high molecular weight fraction did not dissociate in buffers of low ionic strength and also did not aggregate to higher molecular weight components in buffers of high ionic strength. Thus, this fraction differed from the arachin fraction and perhaps was the dimer. Further characterization of this fraction is in progress.

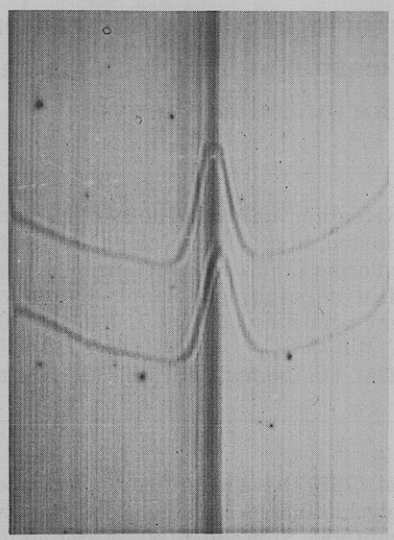

Figure 5. Sedimentation velocity patterns in 0.01 *M* phosphate buffer containing 0.5 *M* NaBr: (lower) arachin; (upper) fraction corresponding to peak I in Figure 2B.

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K. Jayarama Shetty
M. S. Narasinga Rao*

Central Food Technological Research Institute
Mysore 570013, India

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