Composition of Sesame Seed Protein Components and Purification of the Main Globulin

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ABSTRACT

Phosphate buffer (pH 7.66) containing 0.02M 2-mercaptoethanol and 10% sodium chloride was a satisfactory medium for the extraction of proteins from defatted sesame seed meal. The extracted whole proteins contained more than 85% of the 13S globulin components. The globulin fraction was precipitated by dialysis of the extract against water. After purification by gel filtration, the 13S globulin was homogeneous when examined by ultracentrifugation, disc gel electrophoresis, and double gel immunodiffusion. Ultracentrifuge and slab gel electrophoresis analyses in the presence of urea showed that the 13S globulin has a subunit structure composed of acidic and basic subunits.

Sesame seed globulins were studied before the turn of the century (Ritthausen 1880), but modern methods of protein chemistry were not applied in this field until the late 1950s and early 1960s, when electrophoretic and ultracentrifugal results were first reported (Nath and Giri 1957a, 1957b; Nath et al 1957; Sinha and Sen 1962; Ventura and Lima (1963). Ultracentrifugal studies on the extracted proteins of sesame seeds (Sinha and Sen 1962) with 10% sodium chloride solution revealed the presence of four components having approximate $S_{20,w}$ values of 2S, 7S, 13S, and 19S. The major fraction, α-globulin, represents about 70% of the total proteins in sesame seeds and contains the 13S and 19S components, with low concentration of the 19S component (Sinha and Sen 1962). Gel electrophoresis and double gel immunodiffusion are valuable tools for characterizing a number of complex protein systems but have not been applied to sesame seed proteins. Therefore, full information on the physicochemical properties of the 13S globulin have not been obtained.

This article deals with extraction of the proteins from the defatted meal, examination of the composition of the extracted whole protein components by ultracentrifugation, gel electrophoresis and double gel immunodiffusion, and fractionation and purification of the 13S globulin.

MATERIALS AND METHODS

Materials

Representative white varieties of sesame seeds (*Sesamum indicum* L.) cultivated in Ethiopia, obtained from the Institute of Morinaga Seika Co., Kawasaki, Japan, were stored in the cold until used. These were ground coarsely in a coffee mill and then solvent-extracted for 24 hr in the cold, with petroleum ether (bp 40, −60° C) as solvent. The partially defatted material was ground in a coffee mill and passed through a 60-mesh sieve. The meal was treated repeatedly with portions of the solvent until the final oil content was lowered to 5% and was stored separately in stoppered bottles in the cold. The defatted meal contained 6.8% nitrogen, as analyzed by the micro-Kjeldahl method, and 11.9% water.

Preparation of Globulin Fraction

Globulin fraction was prepared by the method described in Fig. 1.

Slab Gel Electrophoresis

A 19 × 21 × 0.3 cm acrylic plastic cell was made from a 3-mm thick acrylic sheet and 10-mm wide acrylic strips. Molds for the sample slots (0.5 × 3 × 10 mm) were cut from a 0.5-mm thick acrylic plastic plate and set on a cover plate with adhesive at 5-mm intervals. For gel preparation, the acrylic plastic cell was rimmed with vaseline and the cover was firmly fixed with steel clips, leaving a clearance of 0.5 mm between the bottom of the slots and that of the cuvette. The block was fixed in a vertical position. The gel solution, containing 7% acrylamide, 3% bis/acrylamide, 0.08% ammonium persulfate, 0.05% TEMED, 0.02M 2-mercaptoethanol, and 0.076M trishydroxyaminomethane, was adjusted to pH 8.70 with citric acid and then poured into the block through a hole at a corner. Some gels also were made containing 8M urea. The gel block was uncovered and the slots were filled with the sample. The surface of the gel was covered with a thin polyvinyl sheet, avoiding air bubbles. The electrode compartments were filled with saturated sodium chloride solution, which connected with the electrode solution (0.025M Tris-glycine buffer, pH 8.30). Electrical contact between the electrode solution and the gel was made by filter papers. Platinum was used for the electrodes, the positive electrode being farthest from the starting slots. Runs were conducted at constant 200 V (about 20 mA) for about 6 hr. After electrophoresis, the gel was stained for 30 min with 1% amido black 10B or 0.5% Coomassie brilliant blue in acetic acid/water/methanol, 7:50:50 (v/v), and was destained in the same solution free of the dye.

Disc Gel Electrophoresis

Polyacrylamide gel electrophoresis in Tris-glycine buffer was

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performed essentially as described by Davis (1964).

Ultracentrifugation

The ultracentrifugal analysis was done at 20°C with a Model UCA-1 Hitachi ultracentrifuge, and photographs of the schlieren patterns were taken at 6-min intervals. Traces of insoluble sample residue dissolved in phosphate buffer (0.0025M KH_2PO_4, 0.00325M K_2HPO_4, pH 7.66) containing 0.02M 2-mercaptoethanol and 10% sodium chloride were removed by centrifugation (13,400 × g, 20°C, 20 min). The supernatant solution was used for ultracentrifugal analysis after dialyzing against phosphate buffer. This buffer was used extensively during our investigation as an extraction medium for whole proteins from the meal, for ultracentrifugation and gel filtration. Therefore, throughout this article it will be referred to as the standard buffer.

Double Gel Immunodiffusion

Antisera to the protein fraction were prepared by immunization of rabbits as described by Catsimpoolas and Meyer (1968). Double gel immunodiffusion by the micro-Ouchterlony method (Ouchterlony 1949) was done using 1% agar (Difco) in 0.1 or 0.2M phosphate buffer containing 0.001M sodium azide, pH 7.60.

Gel Filtration

A column of Sepharose 6B (2 × 194 cm) was equilibrated with standard buffer. A flow rate of 10 ml/hr was maintained by hydrostatic pressure. The extracted whole protein or globulin fraction, in a volume of not more than 3.0 ml of buffer, was applied to the column and eluted with the same buffer. Each 5-ml aliquot was collected with a fraction collector, and absorbances were measured at 280 nm to detect protein and at 480 nm to detect sugar by the phenol-sulfate method (Dubois et al 1956).

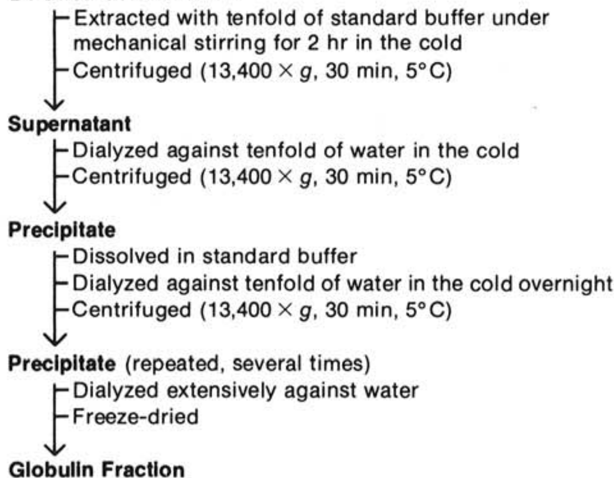

Fig. 1. Preparation method of globulin fraction.

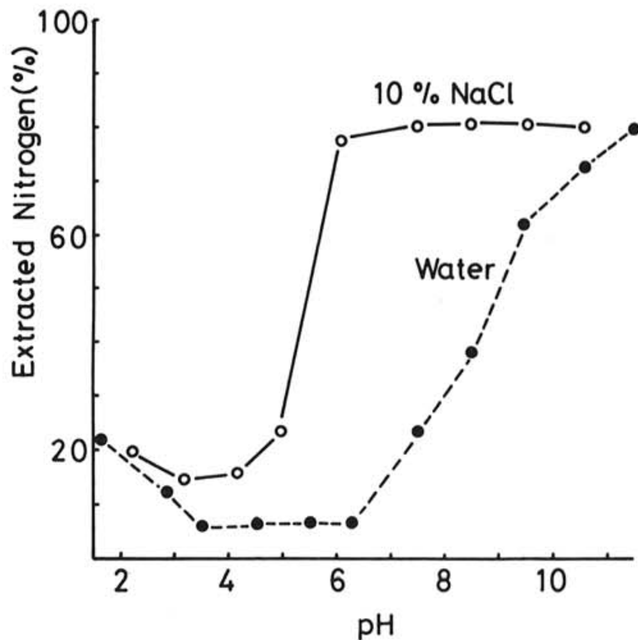

Fig. 2. Effect of pH on extractability of nitrogenous materials from defatted meal with water and 10% sodium chloride solution in the cold (0–5°C).

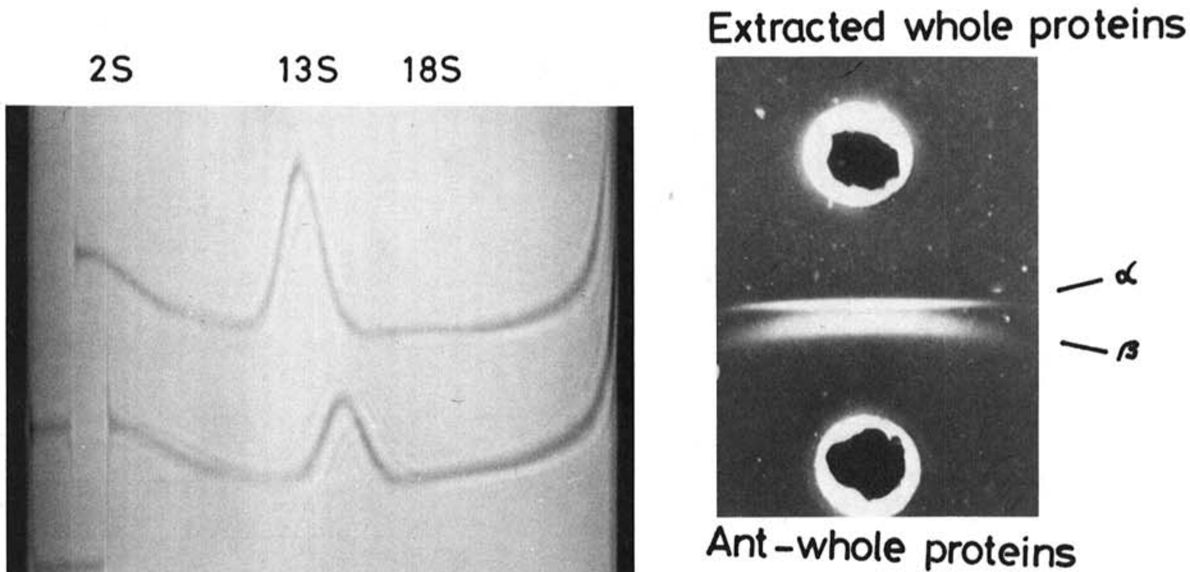

Fig. 3 (**Left**) Ultracentrifugal pattern of extracted whole proteins, using phosphate buffer (0.0026M K_2HPO_4, pH 7.66) containing 10% sodium chloride and 0.01M 2-mercaptoethanol. The protein concentration was 1.0% (upper) and 0.5% (lower). Photograph was taken after 50 min of centrifugation at 55,430 rpm. Direction of sedimentation is from left to right. (**Right**) Double gel immunodiffusion pattern of extracted whole proteins developed with the antiserum.

RESULTS AND DISCUSSION

Extraction of Protein From Defatted Meal

Sesame seeds and the defatted meal contained 2.8 and 6.8% nitrogen, respectively, of which most was proteinaceous nitrogen. Nearly 80% of the nitrogen of the defatted meal could be extracted in the cold with 10% sodium chloride solution at pH 6.0 or at higher pH values (Fig. 2). In the 1.5–11.5 pH range, the extractability was minimum at pH 4.0, above and below which it increased gradually; 10% sodium chloride solution was a better solvent than water over the complete pH range.

Sesame proteins can be extracted with sodium chloride solution from oil free cake (Adolph and Lin 1936, Ritthausen 1880), and the maximum amount (75%) of total proteins is extracted by 6% sodium chloride solution at pH 5.5 (Basu and Sen Cupta 1947). In this study the extractability with 10% sodium chloride solution is almost constant (Fig. 2), being about 80%, at pH 6.0 or at higher pH. The extraction condition using 10% sodium chloride solution in the 6–10 range at low temperature (0–5°C) seemed to be mild; transconformation of the typical globulins in this condition was not observed in several other seeds (Boulter and Derbyshire (1971). Under this condition, almost all protein components could be extracted from the defatted meal and the main components should be acidic proteins with an isoelectric point close to pH 4.0 because of minimum extractability at this pH. Sesame seeds have a higher content of cystein, 2.1%, than do other oil seeds (FAO 1970).

Fig. 4. Schematic diagram of slab gel electrophoresis patterns of extracted whole proteins. Gel concentration was 7.0% acryl amido monomer. Gel was polymerized in 0.076M Tris-citrate buffer, pH 8.7, containing 0.02M 2-mercaptoethanol; electrode buffer was 0.3M boric acid, pH 8.3, adjusted with sodium hydroxide. In the urea system, gel and buffer contained 6M urea.

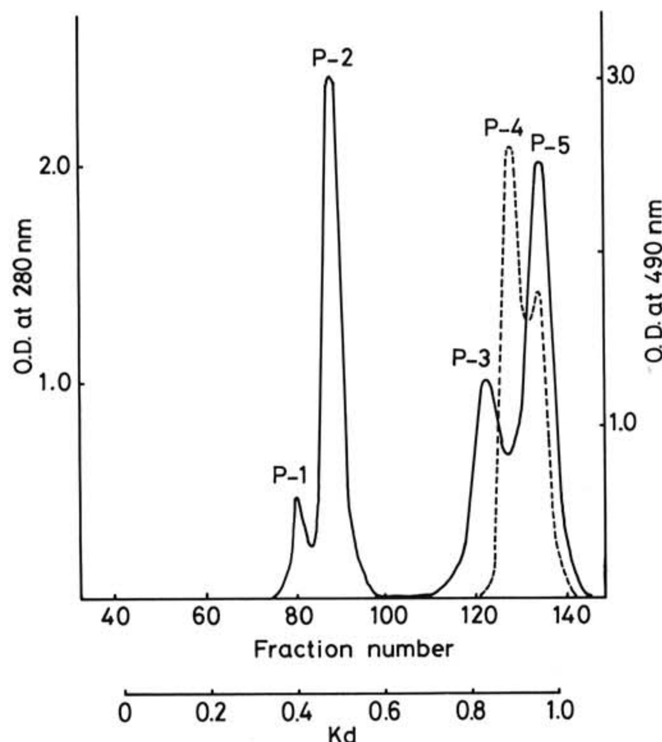

Fig. 5. Sepharose 6B chromatography of sesame seed extract. Elution profile for 1.5 ml of the whole extract after dialysis. Column was eluted with standard phosphate buffer. Flow rate was 10 ml/hr; 5.0-ml fractions were collected.

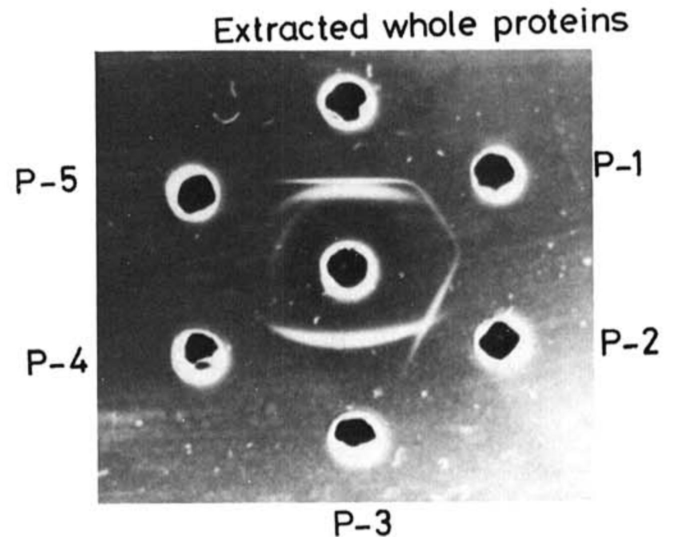

Fig. 6. Double gel immunodiffusion patterns of peaks P-1, P-2, P-3, P-4, and P-5 in Fig. 5 and the extracted whole proteins, developed with antiserum in center well.

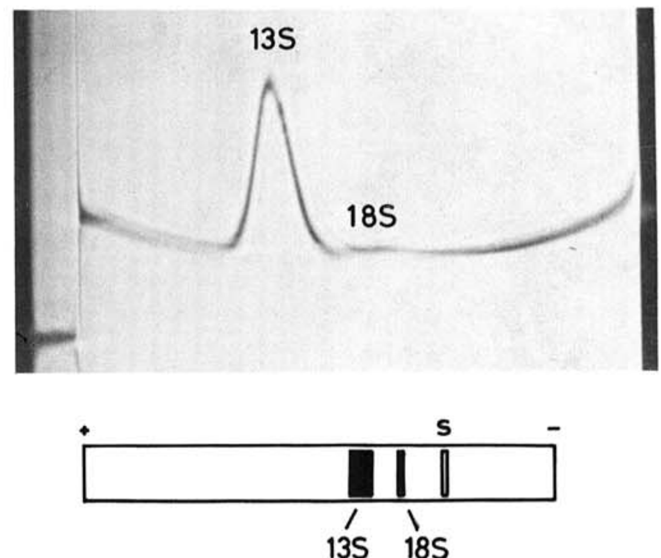

Fig. 7. Ultracentrifugal pattern (above), and schematic diagram (below) of slab gel electrophoretical pattern of the α-globulin under conditions described for Fig. 3 and 4.

Disulfide linkage between protein components affects their extractability and should be disrupted before analyzing protein components. When phosphate buffer (0.0026M KH_2PO_4 and 0.0325M K_2HPO_4, pH 7.66) containing 10% sodium chloride and 0.02M 2-mercaptoethanol was used as extractant of the whole proteins from the defatted meal, 86% of total nitrogen of the defatted meal was extracted.

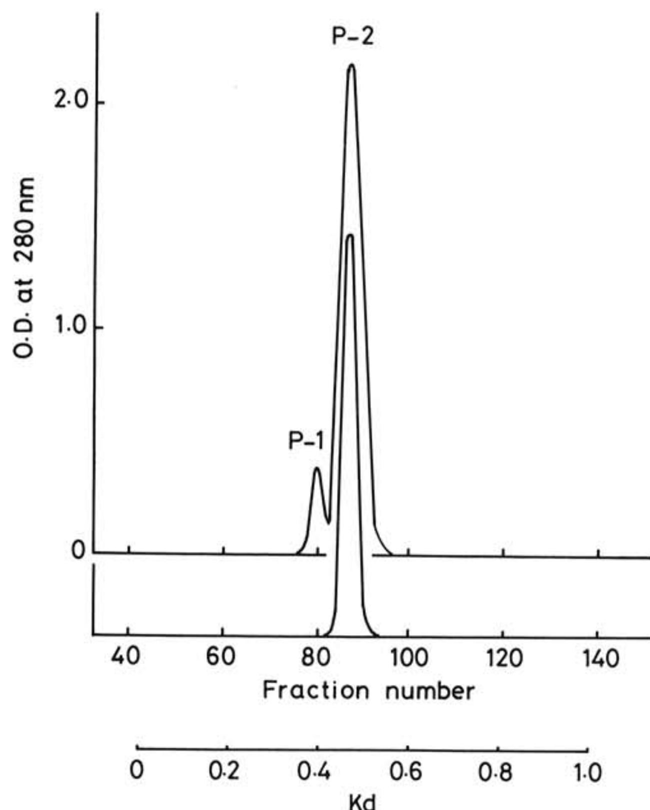

Fig. 8. Fractionation of the α-globulin by gel filtration on Sepharose 6B column (upper) and purification of the 13S globulin by rechromatography of peak P-2 on the same column (lower), under conditions described for Fig. 5.

Composition of Whole Protein Components

An ultracentrifugal pattern (Fig. 3) of the proteins extracted with standard buffer shows three peaks designated as 2S, 13S, and 18S components calculated from the sedimentation coefficient, S_t. Approximate proportions calculated from the peak area on the pattern were 10, 85, and 5%, respectively. The slab gel electrophoretic pattern (Fig. 4) also shows three bands designated as A, B, and C components. Approximate amounts calculated by densitometry were 90, 4, and 6%, respectively. The major component, band A, corresponds to the 13S component on the ultracentrifugal pattern (Fig. 3), and bands B and C to 19S and 2S components, respectively. The relative proportions of the three components of the extracted whole proteins (Figs. 3 and 4) agree with the results reported by Sinha and Sen (1962), except that the 7S component, which amounted to 10.1% of the total proteins, was not detected in this protein fraction. This discrepancy may result from difference in seed varieties or in the calculation method for sedimentation. The 13S component accounted for more than 80% of the extracted whole proteins, as compared with the 11S globulin, legumin, which corresponds to less than 50% of the total proteins in legumes (Boulter and Derbyshire 1971).

Examination of the extracted whole proteins by double gel immunodiffusion (Fig. 3) indicated that a precipitin reaction occurred between the proteins and the antibody and that two immunoprecipitin bands, α and β, were formed. The α-precipitin band may correspond to the major protein component because of the higher density and sharpness of the band. The immunoprecipitin reaction of sesame proteins has not been reported but should become an effective method for protein analysis and identification of sesame globulin.

The slab gel electrophoretic pattern in the presence of urea shows at least 14 bands (Fig. 4), of which nine bands migrated toward the anode and five bands toward the cathode. The electrophoretic results indicate that the main protein components are acidic and have a quarternary structure composed of acidic and basic subunits.

Gel Filtration of Extracted Whole Proteins

As shown in Fig. 5, gel filtration of the extracted whole protein with a column of Sepharose 6B yields five peaks. Peaks P-1, P-2, P-3, and P-5 were proteins, and peaks P-4 and P-5 were carbohydrates. Ultracentrifugal and gel electrophoretic patterns (not shown) indicated that peak P-1 corresponded to 18S component, peak P-2 to 13S component, and peaks P-3 and P-5 to 2S component, respectively. No protein was detected in peak P-4. These results indicate that the 13S and 18S components are not

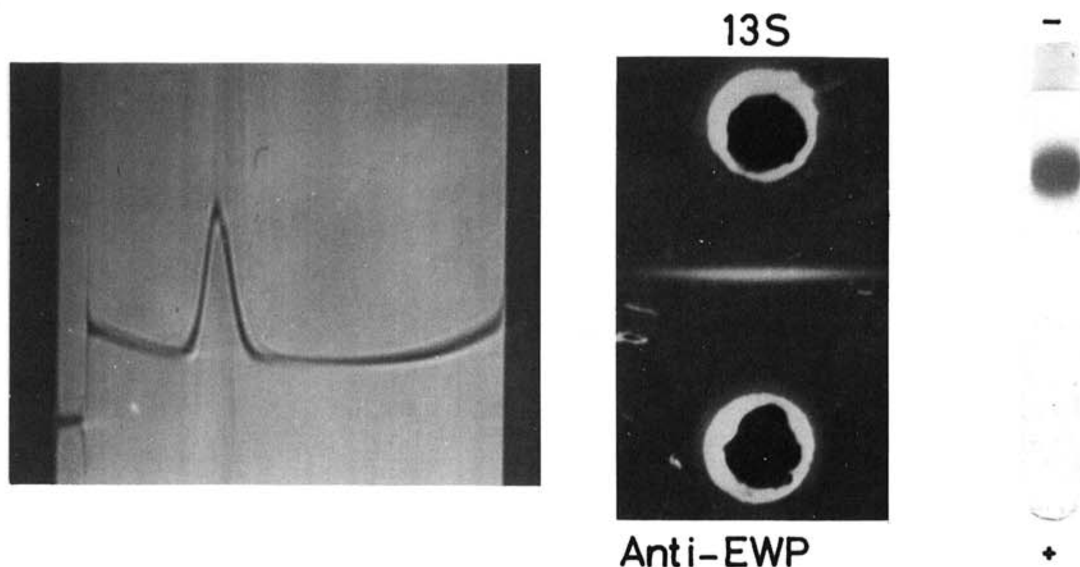

Fig. 9. Ultracentrifugation (left), double gel immunodiffusion (center), and disc electrophoresis patterns of purified 13S globulin under conditions described for Fig. 3 and 4 except that disc gel electrophoresis was performed as described by Davis (1964).

glycoproteins, and can be resolved by gel filtration, ultracentrifugation, and gel electrophoresis. On the double gel immunodiffusion patterns, however, (Fig. 6), cross-reactivity between peaks P-1 and P-2 was detected. Gel electrophoresis of peaks P-1 and P-2 in the presence of urea showed similar patterns, each pattern having eight bands. These results indicate that components 13S and 18S may be composed of the same subunits.

Fractionation and Purification of Main Globulin

Preliminary experiments indicated that 84 or 89% of the whole proteins extracted with standard buffer could be precipitated by salting out with 40% saturated ammonium sulfate or by dialyzing the 10% sodium chloride extract against 10 volumes of water. Using the above procedures, a globulin fraction was prepared as described in Fig. 1. Ultracentrifugal and gel electrophoresis patterns (Fig. 7) showed that the globulin fraction contained 95% of 13S component (main globulin) and 5% of 18S component (minor globulin) but did not contain other proteins corresponding to 2S component. This globulin fraction is therefore regarded as the α-globulin of Jones and Cersdroff (1927), because the α-globulin was identified by Nath and Giri (1957a) as the fraction-I that was obtained by dialysis of one volume of total extract against 10 volumes of water.

Ultracentrifugal studies on the α-globulin by Ventura and Lima (1963) and by Sinha and Sen (1962), indicated that the 13S globulin has a molecular weight of approximately 450,000 and frictional ratio of 1.50 and undergoes denaturation below pH 4.5. To obtain more information on the physicochemical properties of the 13S globulin, however, the 18S component present as a contaminant must be removed from the globulin preparation.

As shown in Fig. 8, gel filtration with Sepharose 6B yields two peaks that correspond to peaks P-1 and P-2 of the extracted whole proteins (Fig. 5). Ultracentrifugation experiments showed that 13S and 18S components were present in peaks P-1 and P-2. The main globulin, 13S, was purified by rechromatography of peak P-2 on the same column (Fig. 8). After purification, the 13S globulin was homogeneous as shown by ultracentrifugal, disc gel electrophoretic, and double gel immunodiffusion experiments (Fig. 9). This isolated 13S globulin is being used currently to determine physicochemical properties.

Because more bands were observed on gel electrophoresis in the presence of urea than in its absence, it is expected that 13S globulin has subunit structure and that the structure can be disrupted by denaturants (urea, SDS, guanidine-HCl, etc.). To examine the subunit structure, the globulin was analyzed in the presence of urea by ultracentrifugation (not shown) and gel electrophoresis (Fig. 10). The approximate sedimentation coefficient of the globulin decreased from 13S to 1.5S and eight bands were observed on gel electrophoresis. The results indicate that the 13S globulin has a subunit structure composed of acidic and basic subunits.

Fig. 10. Slab gel electrophoresis pattern of 13S globulin in the urea system under conditions described for Fig. 4.

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