SOME FEATURES OF COTTONSEED GLOBULINS IN SOLUTIONS

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The main globulin of cotton seeds can exist in various molecular forms with different molecular weights. The existence of such forms is a consequence of features of the structure of the protein, although the possibility of the participation of pigments in it is not excluded. The dimensions and shape of the molecule have been determined by electron microscopy.

Definite advances have been achieved in establishing the chemical structure of cotton globulins [1, 2]. We have studied the properties of the "histidine" globulin from cotton seeds with the aim of considering the questions of its conformation. In our view, the determination of the conformation of globulins supplements the information necessary for understanding the formation of these proteins in the cotton plant and their role and also for solving the complex of problems connected with improving the technology of producing food protein from cotton seeds.

The seeds of the cotton plant of the genus *Gossypium hirsutum* growing in the territory of Uzbekistan contain two globulin components with the N-terminal amino acids histidine and arginine [3, 4]. However, according to the literature, the globulins of cotton seeds can be represented by five components with different molecular weights [5]. The nature of these components has not been established. In the investigation of the conformation of proteins it is necessary to take into account that particular molecular form of the protein which exists in solution, since it may be a consequence of a change in conformation. The histidine globulin (the globulin with histidine as the N-terminal amino acid, or the 11S globulin), like the 11S globulins of the seeds of other oil-bearing plants, is quantitatively the main one [6]. Methods for the isolation and purification of the globulins of cotton seeds have been developed by a number of investigators [7-9]. We have used a method proposed at the beginning of the 1960s [7]. It is based on the different solubilities of the two main n globulins. The solubility of the "arginine" (7S) globulin is higher than that of the histidine (11S) globulin. Moreover, the solubility of the arginine globulin scarcely changed when different cotton seeds (with respect to variety, ripeness, etc.) were used while the solubility of the histidine globulin depended substantially on a number of factors, and, particularly, on the presence of pigments in it. In actual fact, in view of a number of its properties the histidine globulin has a high affinity for pigments (gossypol, etc.), which apparently affects its solubility [10].

We have obtained a histidine globulin with a relatively high solubility. Figure 1 shows the chromatography of the histidine globulin on Ultrogel AcA34. Analysis of the fractions illustrated in the figure showed that they all belonged to a globulin having histidine as the N-terminal amino acid. The molecular weights determined from a calibration graph were as follows: I > 400,000; II ~ 260,000; III ~ 130,000. However, the ultracentrifugation of the histidine globulin obtained by Rossi-Fanelli's method [7] gave one symmetrical peak with a sedimentation coefficient of 11S. Apparently, in gel filtration on a column we observed transitions of different molecular forms of the histidine globulin. Nothing similar was observed in the ultracentrifugation of a relatively concentrated solution of the protein (10 mg/ml). The existence of the histidine globulin in a 7S form has been reported previously [8]. There is information that the histidine globulin in the 11S form can readily dissociate on dilution into a 7S form. This fact has also been reported in a study of the quaternary structure of the histidine globulin [10].

The second globulin component — the arginine globulin — unlike the histidine globulin, on gel filtration gave basically a single peak with a sedimentation coefficient of 7S. Thus,

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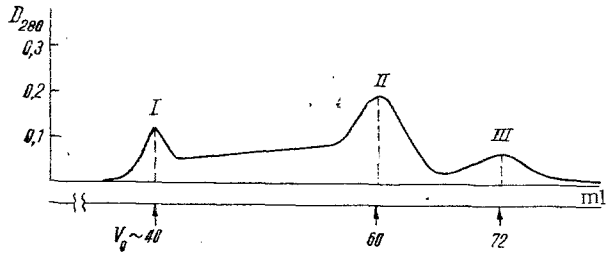

Fig. 1

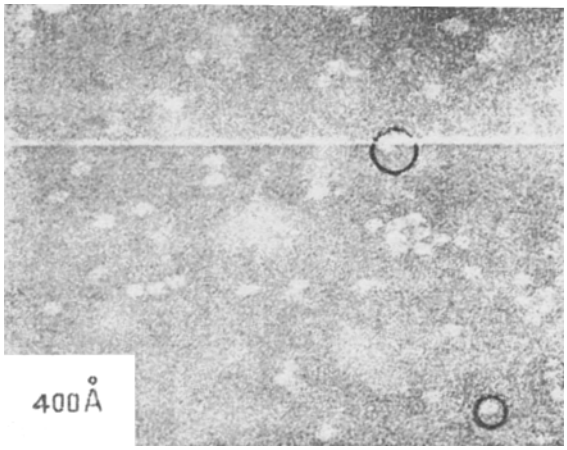

Fig. 2

Fig. 1. Chromatography of the "histidine" globulin in a column of Ultrogel AcA34.

Fig. 2. Electron-microscope photograph of the "histidine" globulin from cotton seeds. Highly diluted solution of the protein (50-200 μg/ml) in 1 M ammonium acetate buffer, pH 6.85, were used to obtain the films. They were contrasted with tungstophosphoric acid, pH 7.0. 1 cm — 400 Å (photograph by V. Ya. Stelmashuk, Institute of Crystallography, Academy of Sciences of the USSR).

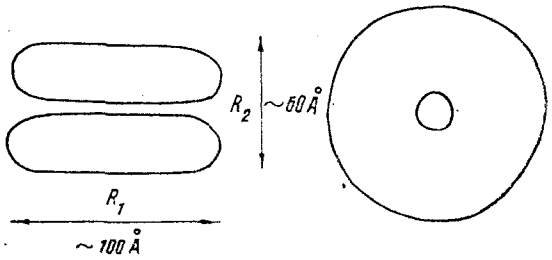

Fig. 3. Schematic illustration of the "histidine" globulin from cotton seeds.

the histidine globulin is the cause of that multiplicity of molecular forms that appears in the study of the total globulin fraction by gel filtration. The multiplicity is apparently a consequence of intermolecular interactions [11] leading to a change in the forms of existence of the molecule under different conditions [12]. However, a definite role may also be played by the low-molecular-weight substances (such as pigments) that are present in large amount in cotton seeds.

We have studied the protein by electron microscopy. Figure 2 is a photograph of the histidine globulin at a magnification of 250,000. At pH 5.0 the protein precipitates completely, which did not permit the use of a number of contrasting agents in the acid pH range. Analysis of photographs of the histidine globulin makes it possible to present a schematic illustration of the protein (Fig. 3).

Electron microscopy showed the identity of the different fractions obtained in the gel chromatography of the histidine globulin (see Fig. 1). In a study of the quaternary structure of the 11S globulins from grape seeds [13] and sunflower seeds [14] it was found that the molecules of the 11S globulins with molecular weights of about 300,000 have larger dimensions. Thus, the largest dimension of the molecule of the 11S globulin from grape is abou 120 Å, and as the authors have shown, its form is close to spherical. The molecule of the 11S globulin of the sunflower is also close to spherical and has dimensions of 104 × 104 × 88 Å. According to the results of electron microscopy, the histidine globulin apparently exists in a form with a molecular weight of about 130,000, since films are obtained from highly dilute solutions. It must be mentioned that the relative ease of dissociation of the 11S globulin of cotton seeds distinguishes it from the analogous seed proteins of other plants.

Thus, it may be concluded that the protein obtained is homogeneous and that the method of purification used gives it in the native state. As is well known, the conformation of proteins is investigated by the CD method in relatively dilute solutions, and at such concentrations (less than 0.5 mg/ml) the histidine globulin will be present in a form with a molecular weight of ~130,000.

EXPERIMENTAL

The globulins were isolated as described by Rossi-Fanelli, with some modifications. The isolation of the two main globulin components, based on their different solubilities, was carried out at room temperature (not above 25°C). The sparingly soluble globulin fraction was washed three times with a 0.3 M solution of NaCl. Then 10-mg portions of each globulin fraction, dissolved in 1 ml of 10% NaCl, pH 7.4, were deposited on a column of Ultrogel AcA34 (LKB, Sweden). The rate of elution was 8-10 ml/h, 2- to 2.5-ml fractions being collected. The eluates were analyzed at a wavelength of 280 nm in a flow-through cell. The column was calibrated with known proteins: catalase (mol. wt. 240,000), glucose oxidase (mol. wt. 150,000), hemoglobin (mol. wt. 67,000). The free volume (V_0) was determined with respect to dextran blue (mol. wt. 2,000,000). The chromatographic conditions have been described above.

Ultracentrifugation was carried out on MOM 3170 ultracentrifuge at a rotor speed of 50,000 rpm, the concentration of protein being 10 mg/ml in 10% NaCl, pH 7.4.

SUMMARY

1. It has been established that the histidine globulin from cotton seeds can exist in several molecular forms.

2. The approximate dimensions of the molecule of the histidine globulin has been determined.

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