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DENATURATION AND RENATURATION OF THE 11S GLOBULIN OF COTTON SEEDS BY POLAROGRAPHY

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It has been established by a polarographic analysis of the globulins of cotton seeds that the 7S and 11S golublins possess a two-step polarographic wave with a half-wave potential of −1.42 V. On the basis of the results of a study of the kinetics of thermal denaturation the high lability of the 11S globulin on heating has been shown. The conditions have been determined of the complete denaturation of the 11S globulin in 8 M urea solution and it has been established that the latter is an irreversible process.

The total globulin fraction of cotton seeds consists mainly of two components with sedimentation coefficients of 7S and 11S and molecular weights of 130,000 [1], and 280,000 [2], respectively, which have complex quaternary structures [3, 4]. In a study of the physicochemical properties of the proteins isolated we have established that they possess a two-step polarographic wave and have the same half-wave potential of −1.42 V (Fig. 1).

An investigation of proteins not possessing a quaternary structure has shown that in the process of catalytic reduction of hydrogen ions and the formation of the second step of the wave the main role is played by SH groups and S—S bridges [5]. In the present case, the 11S

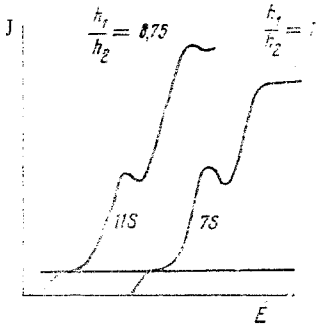

Fig. 1

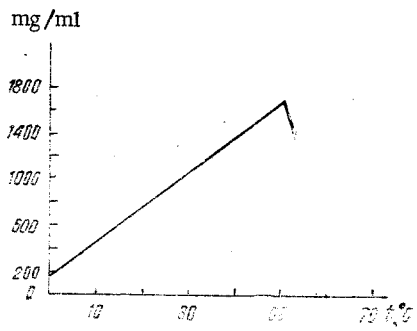

Fig. 2

Fig. 1. Two-step polarographic wave of the native 7S and 11S globulins.

Fig. 2. Temperature dependence of the solubility of the 11S globulin.

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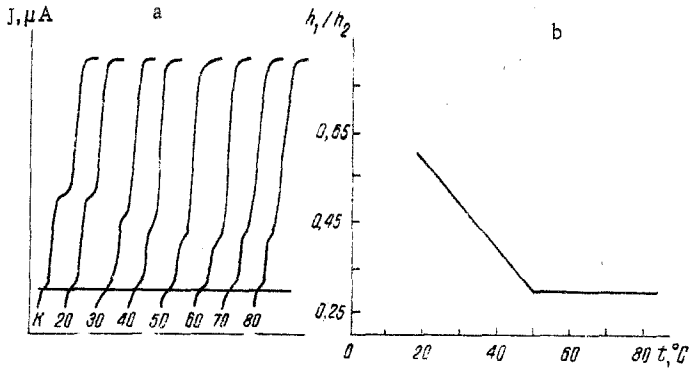

Fig. 3. Influence of the temperature on the heights h_1 and h_2 of the polarographic wave of the 11S globulin and its catalytic activity in 10% sodium chloride solution (a), and a graph of the dependence of the ratio of the first to the second steps of the polarographic wave h_1/h_2, on the temperature (b).

globulin, with a complex quaternary structure, does not contain such groups. However, the 11S globulin does give a two-step wave, which is probably due to the action of various functional groups and bonds present in the protein.

In a study of the solubility of the protein in 10% sodium chloride solution we determined the dependence of the solubility on the temperature (Fig. 2): The solubility of the protein increases with a rise in the temperature to 50°C and then falls sharply. In view of this, the necessity arose for studying the kinetics of its thermal denaturation. We made use of the polarographic method [6], and denaturation was performed with a change in the temperature from 20 to 80°C.

The kinetics of the thermal denaturation was expressed by the ratio of the polarographic activities of the first and second steps of the waves, h_1 and h_2, which for the native protein in 10% sodium chloride solution amounted to 0.60 (Fig. 3). With a rise in the temperature to 50°C, the polarographic activity of the second step of the wave gradually increased, and the ratio h_1/h_2 decreased. A further rise in the temperature did not affect h_1 and h_2, and their ratio remained constant (0.30).

Thus, the marked decrease in the solubility of the 11S globulin at temperatures above 50°C is connected with the complete irreversible denaturation of the protein. A study of the thermal denaturation of the 11S globulin showed a high lability of the molecule: Even at 30°C and 40°C partial denaturation took place. At the same time, as mentioned previously, the 11S globulin has some special characteristics in urea solutions. Thus, on gel filtration through Sephadex G-200 in the presence of 8 M urea the protein was eluted from the column as a single peak, although on disc electrophoresis in PAAG [7] under the same conditions 11 components were revealed.

The denaturation of the protein in 8 M urea took place on heating from 20 to 80°C over 1 h, and at room temperature with an increase in the time of incubation. The denaturation process was followed from the change in the polarographic activities h_1 and h_2. The ratio h_1/h_2 for the protein dissolved in 8 M urea at 20°C was 0.75. When the protein solution in 8 M urea was heated and it was analyzed polarographically, even after incubation at 30°C for 1 h no appreciable changes took place. In this case, the h_1/h_2 ratio was 0.80 (Fig. 4a, b).

A further increase in the temperature led to a rise in the first step of the polarographic wave, and at 50°C h_1/h_2 amounted to 1.32, and then, as can be seen from the polarogram, the same polarographic activities of the first and second steps of the wave were retained at 60, 70, and 80°C. The identity of the polarograms obtained at temperatures of 60°C and above indicates the complete denaturation of the 11S globulin.

As can be seen from the polarogram (Fig. 4c, d), the complete denaturation of the protein in 8 M urea at room temperature sets in only after 6 h, which indicates a high stability of the protein to 8 M urea.

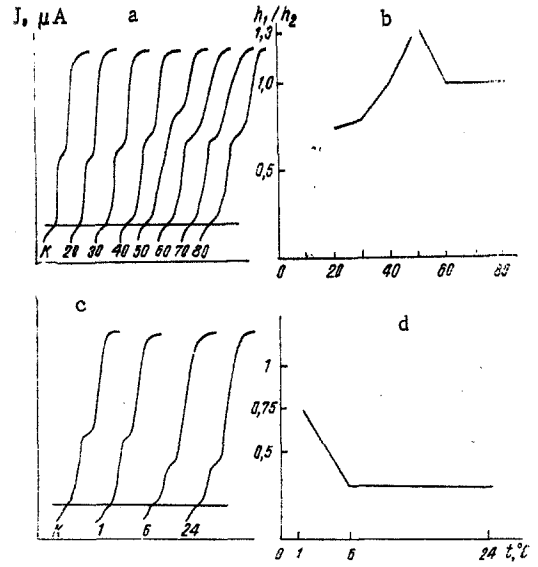

Fig. 4. Graphs of the temperature dependence of h_1 and h_2 of the polarographic wave of the 11S globulin incubated in 8 M urea (a); ratio of the first (h_1) and second (h_2) steps of the polarographic wave as a function of the temperature (b); h_1 and h_2 of the polarographic wave at room temperature as functions of time (c); and ratio of the first (h_1) and second (h_2) steps of the polarographic wave at room temperature as a function of time (d).

The irreversibility of the denaturation of the 11S globulin was established after exhaustive dialysis of the protein solution that had been incubated at 50°C and at room temperature. The h_1/h_2 ratio in the first case was 0.41 and in the second 0.30 which differs greatly from the polarographic wave of the native protein.

The characteristic features of the denaturation of the 11S globulin in 8 M urea solution are apparently due to a number of factors, one of which is the participation of hydrophobic forces in the stabilization of the quaternary structure [8].

It is rather difficult to draw a conclusion concerning the degree of renaturation of the protein in this case, since it possesses a complex quaternary structure, and even on partial denaturation of the molecule the changed quaternary structure may affect the parameters of the polarographic wave. A further investigation of the processes of renaturation on the individual subunits and the use of new methods will permit this question to be answered.

EXPERIMENTAL

Ripe seeds of the cotton plant *Gossypium hirsutum* of variety 108-F were used, and the globulins were extracted from them by a method described previously [4].

Determination of the Solubility of the 11S Globulin. Test tubes were charged with 2-mg portions of the protein and they were each dissolved in 5 ml of 10% sodium chloride solution and kept in the thermostat at various temperatures (from 0 to 70°C) for 30 min. The solutions containing the protein were centrifuged at 18,000 rpm for 10 min. The concentrations of the protein in the solutions were determined by means of the biuret reaction, for which purpose 4 ml of biuret solution was added to 1 ml of protein solution and the mixture was kept in the dark for 30 min. A control experiment was carried out in parallel. After the 30 min, the light-absorption of the protein solution was measured in a photoelectric colorimeter with a green filter.

Purification of the Mercury. To purify the mercury we used a glass cylinder 60 cm high and a funnel with a spiral tip so that on passing through it the mercury disintegrated. To free it from mechanical impurities the mercury (3 kg) was passed through a paper filter in which an aperture had been made with a needle. Then from the funnel with the paper filter placed in it the mercury was passed successively through cylinders containing water and saturated potassium permanganate solution. The excess of potassium permanganate was eliminated by washing the mercury 5-6 times in distilled water. After such treatment, the mercury was passed 3-4 times through a 10% solution of nitric acid and 2-3 times through a 5% solution of sodium nitrite in 5% (by volume) nitric acid. Then distilled water was poured into the cylinder and the mercury was washed several times. To dry the mercury and remove the film from its surface, it was passed through a dry filter and was collected in a bottle with a ground-in stopper.

Determination of the Polarographic Activities of the 7S and 11S Globulins. We used a type LP-7 polarograph with the following parameters: length of the capillary 7 cm; rate of

dropping of the mercury under an applied voltage of 1.6 V 1 drop in 3 sec; area of the surface of a mercury drop 0.91 mm^2; weight of one drop 1.1 mg. To calculate these parameters we used a well-known procedure [9].

For polarographic analysis we took $1 \cdot 10^{-8}$ mole of protein and dissolved it in 2 ml of cobalt–ammonia buffer, pH 9.1. The sample for polarographic analysis was made up in the following way: Into a Novak vessel were poured mercury (as anode), 3 ml of cobalt–ammonia buffer, and 2 ml of the prepared protein solution, to which a voltage of from –1 to –2 V was applied at the rate of 200 mV/min and the analysis was carried out at a sensitivity of the polarograph of S_{100} and a rate of speed of the recorder paper of 200 mV/min. The polarogram was recorded in the form of integral waves and then in the form of differential waves at a sensitivity of 20.

Thermal Denaturation. From a solution of 40 mg of protein in 16 ml of 10% sodium chloride solution 2-ml (5-mg) portions were taken and they were thermostated at 20, 30, 40, 50, 60, 70, and 80°C for 30 min. After the end of this time the samples were cooled to room temperature, treated with 5 ml of cobalt–ammonia buffer, and polarographed.

Denaturation of the 11S Globulin in 8 M Urea. For a solution of 45 mg of the protein in 30 ml of 8 M urea solution 2-ml (3-mg) portions were taken and these were thermostated at 20, 30, 40, 50, 60, 70, and 80°C for 1 h. The denaturation of the protein in 8 M at room temperature for 1 h, 6 h, and a day was performed in parallel.

The renaturation of the protein was effected by dialyzing the incubated tubes against distilled water. Exhaustive dialysis was carried out with constant stirring of the water by means of a magnetic stirrer for two days. The water in the dialyzer was renewed periodically. After the end of the denaturation process and dialysis the samples were placed in a Novak vessel and polarographic analysis was carried out as described above. To compare the results obtained a control experiment was performed. The control sample consisted of the anode mercury, 3 ml of cobalt–ammonia buffer, and 2 ml (3 mg) of protein solution in cobalt–ammonia buffer.

SUMMARY

1. It has been found that the 7S and 11S globulins from cotton seeds possess a two-step polarographic wave with a half-wave potential of –1.42 V.

2. A high lability of the 11S globulin on thermal denaturation has been established.

3. The conditions for the complete denaturation of the 11S globulin in 8 M urea solution has been determined. It has been shown that the latter is an irreversible process.

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