FUNCTIONAL PROPERTIES OF "COTTON PROTEIN."

I. EMULSIFYING PROPERTIES OF GOSSYPULIN: INFLUENCE ON THEM OF THE STRUCTURE OF THE PROTEIN AND THE PRESENCE OF GOSSYPOL AND OF PHYTATE

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UDC 547.962.5+541.18.051

Samples of gossypulin with different degrees of denaturation of the protein molecule and different levels of gossypol and of phytate have been obtained by chemical modification. It has been established that a change in the conformation of the protein not affecting its secondary structure leads to a slight increase in its emulsifying activity. The presence of gossypol and phytate affects the emulsifying properties and complicates the determination of the contribution of the structure of the protein.

Existing methods of obtaining protein isolates from plant sources amount mainly to extraction in media with alkaline, neutral or acid pH values. The use of particular conditions largely determines the functional properties of the protein product, which is connected with features of the structure of the main protein components and also with the amount of accompanying substances — pigments, phytates, etc. — in it. Protein emulsifying agents differ from nonprotein ones by the complexity of their structures and their high conformational variability. It is just this which opens up broad prospects in the regulation of the emulsifying properties of the proteins.

There is information in the literature on the structure of the main globulin components of cotton seeds [1-3]. However, workers in this field have not yet arrived at a common opinion on a number of questions (subunit structure, nature of the intersubunit interactions). Our systematic investigations of gossypulin — the main protein component of cotton seeds — have been directed to determining the interrelationship between the structure of gossypulin and its functional properties [4, 5].

As we have shown previously [6], the ε-NH_2 group of lysine plays an important role in the stabilization of the quaternary structure of the 11S protein from cotton seeds. Figure 1 shows the results of the gel electrophoresis of succinylated and acetylated gossypulins. The three protein bands correspond to the A, B, and C subunits [7]. At higher degrees of succinylation, dissociation of the protein into subunits is observed even in the absence of NaDDS [6]. The use of proteins with a low degree of modification enabled samples of gossypulin to be obtained with modified quaternary and tertiary structures but no appreciable change in secondary structure (Fig. 2). Table 1 gives the characteristics of the samples of gossypulin used in our work, and also values of the emulsifying activity (EA) and emulsifying capacity (EC).

The gossypol-free protein possessed a low EA. In the presence of gossypol (0.6%) this characteristic increased 7-fold. The succinylated (degree of modification 13%) and acetylated (25%) proteins had higher EAs than the gossypol-free protein but they were lower than that of the gossypol-containing sample (it was the gossypol-containing protein that was subjected to acetylation). A considerable increase in EA was observed for the deaminated proteins. The change in the emulsion stability of the samples studied is shown in Fig. 3. Here the tangent of the angle of slope of the F/C and S/C curves has an inverse relationship as compared with the dependence of the change in the value of the EA for the samples studied.

Thus, the conformational mobility of the protein molecules due above all to the presence of intersubunit and intrachain disulfide bonds in the proteins has fundamental significance for regulating the emulsion properties of the proteins. There are no disulfide bonds in gos-

Institute of the Chemistry of Plant Substances, Academy of Sciences of the Uzbek SSR, Tashkent. Translated from Khimiya Prirodnykh Soedinenii, No. 3, pp. 354-357. May-June, 1986. Original article submitted October 21, 1985.

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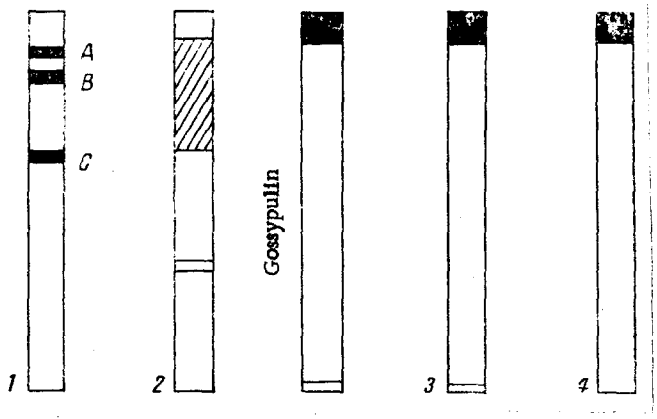

Fig. 1. Gel electrophoresis of gossypulin and its derivatives: 1) the succinylated protein with a degree of modification of 13%; A, B, C) subunits (15% gel, pH 8.9; 0.1% NaDDS); 2) the acetylated protein with a degree of modification of 25% (15% gel, pH 8.9; 0.1% of NaDDS; 3) the succinylated protein (without detergent); 4) the acetylated protein (without detergent).

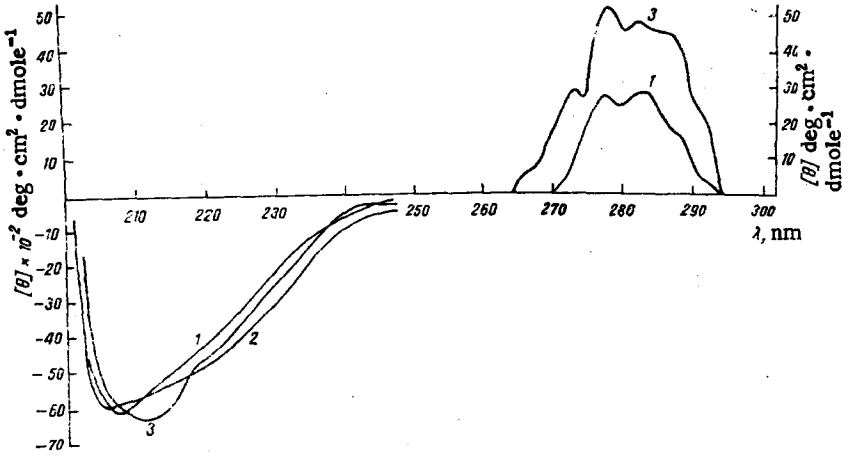

Fig. 2. CD spectra of the modified proteins: 1) succinylated gossypulin; 2) acetylated gossypulin; 3) gossypulin.

sypulin, as has been shown by our results given above and published previously [6]. According to Cherry et al. [3], in the total globulin fraction of cotton seeds there are individual subunits connected by disulfide bonds. We detected nothing similar in gossypulin. It is just the absence of —S—S— bridges in gossypulin that imparts high lability to the molecule and leads to a breakdown of the quaternary structure even at low degrees of chemical modification. Changes at the level of the quaternary and tertiary structures lead to a slight increase in emulsifying activity. On chemical modification, gossypol exhibits a stabilizing effect on the conformational mobility of the polypeptide chain [8]. The denaturation of gossypulin under more severe conditions of deamination greatly increases its emulsifying activity.

The natural accompanying substances of cotton seeds — gossypol and phytate — have a substantial influence on the emulsifying properties of the protein, and this influence of impurities must be taken into account in the study of the functional properties of proteins.

EXPERIMENTAL

Isolation of the Protein Preparations. Gossypulin was obtained from defatted flour (diethyl ether—acetone (1:1)) of the seeds of the cotton plant *Gossypium hirsutum* as described in [9].

Treatment with Phytin and Hydrochloric Acid Solution at pH 5.0. A suspension of 10 g of gossypulin in 50 ml of 0.1% phytin solution at pH 5.0 or in distilled water acidified to

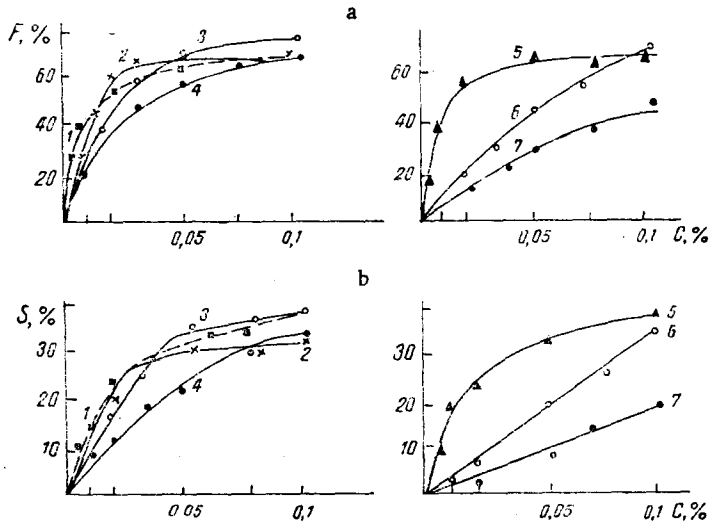

Fig. 3. Curves of the F/C and S/C relationships for gossypulin and its derivatives (C is the concentration of protein solution and S is the area corresponding to the emulsion on emulsion stability diagrams [10]; for F, see Table 2): 1) the succinylated protein; 2) the protein treated with phytate; 3) the protein washed with a HCl solution, pH 5.0; 4) gossypulin; 5) the acetylated protein; 6) the deaminated protein (dried at pH 7.0); 7) the deaminated protein (dried at pH 2.0).

TABLE 1. Emulsifying Activities of the Proteins

Protein	Gossypol, %	Phytin, %	A_1, kg/g	A_1/F_0	C, %	$\frac{F_{max}-F_0}{F_0}$
Gossypulin	0,6	3,1	3,5	0,38	0,1	2,04
Gossypulin (gossypol-free)	—	—	0,5	0,05	0,1	0,4
Acetylated gossypulin	0,1	1,8	2,6	0,3	0,1	2,05
Succinylated gossypulin	0,14	0,3	2,4	0,35	0,1	2,5
Deaminated gossypulin (dried at pH 7.0)	0,4	2,8	5,4	0,5	—	—
Deaminated gossypulin (dried at pH 2.0)	0,4	2,3	5,0	0,85	—	—
Gossypulin washed with HCl solution at pH 5.0	0,4	2,5	2,3	0,23	0,07	1,22
Gossypulin treated with phytin solution at pH 5.0	0,3	2,8	2,1	0,2	0,08	1,12

Note. A_1 is the emulsifying activity of the protein; F_0 is the amount of nonpolar phase passing into the emulsion in a given solvent; F_{max} is the maximum amount of nonpolar phase passing into the emulsion in the presence of the protein; $(F_{max} - F_0)/F_0$ is the emulsifying capacity of the protein; and C is the concentration of protein in solution at which the emulsification limit sets in.

pH 5.0 was kept with stirring for 24 h, the pH of the medium being monitored. Then the sediment was separated off by centrifugation and was twice washed with water. The protein obtained was freeze-dried.

Deamination of Gossypulin. A suspension of 20 g of gossypulin in 100 ml of 0.1% HCl in a 250-ml flask with a reflux condenser was kept in the water bath at 60°C for 8 h. Then the reaction mixture was centrifuged at 6000 rpm for 10 min and the precipitate was separated off. The protein was precipitated from the supernatant solution by two methods: dialysis against distilled water for 24 h, or neutralization of the solution with 0.1 N NaOH to pH 6.0. The

precipitate so obtained was separated off and freeze-dried. The degree of deamination was 50%.

Chemical Modification of Gossypulin. A weighed sample of gossypulin was suspended in borate-alkali buffer, pH 8.8. With stirring, acetic (or succinic) anhydride was added to the protein suspension cooled to 0°C, in a 5- to 300-fold molar excess in relation to the lysine residues. The reaction was performed for 1 h, the pH being maintained by the addition of NaOH solution. After the end of the reaction, the excess of the reagent was eliminated by dialysis against water.

The emulsifying properties of the protein solutions were studied by a method proposed by A. N. Gurov et al. [10].

SUMMARY

1. It has been established that a considerable increase in the emulsifying activity of gossypulin takes place on denaturation affecting the secondary structure of the protein.

2. It has been shown that gossypol and phytates substantially modify the emulsifying properties of proteins.

LITERATURE CITED

1. S. I. Asatov, T. S. Yunusov, and P. Kh. Yuldashev, Khim. Prir. Soedin., 291 (1977).
2. N. L. Ovchinnikova, M. A. Kuchenkova, and P. Kh. Yuldashev, Khim. Prir. Soedin., 404 (1975).
3. H. F. Marshall, M. A. Shirer, and J. P. Cherry, Cereal Chem., 61, No. 2, 166 (1984).
4. T. S. Yunusov, M. T. Turakhozhaev, E. G. Yadgarov, and T. T. Shakirov, Khim. Prir. Soedin., 473 (1981).
5. T. S. Yunusov and M. T. Turakhozhaev, Khim. Prir. Soedin., 366 (1983).
6. T. Yu. Shadrina, T. S. Yunusov, and P. Kh. Yuldashev, Khim. Prir. Soedin., 554 (1979).
7. P. Kh. Yuldashev, M. A. Kuchenkova, T. S. Yunusov, and Sh. S. Azimova, in: Frontiers of Bioorganic Chemistry and Molecular Biology, Pergamon, Oxford (1980), pp. 111-114.
8. Z. S. Yunusova, A. L. Li, T. S. Yunusov, and G. P. Moniseeva, Khim. Prir. Soedin. 132 (1985).
9. Z. S. Yunusova and T. S. Yunusov, Khim. Prir. Soedin., 770 (1981).
10. A. N. Gurov, M. A. Muchin, and N. Larichev, Colloids and Surfaces, No. 6, 35 (1983).