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SURFACE PROPERTIES OF THE RESERVE GLOBULINS OF COTTON SEEDS AND THE PRODUCTS OF THEIR PROTEOLYSIS

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The rheological properties of interphase adsorption layers of enzymatic derivatives of the reserve globulins of cotton seeds at liquid phase-separation boundaries have been investigated. It has been established that the modification of the structure of the protein as a result of limited proteolysis by trypsin leads to a change in the surface properties of the layers formed. These layers possess thixotropic properties.

The results of an investigation of the mechanism of surface phenomena at liquid phase-separation boundaries enable us to elucidate the roles of individual functional groups and conformational changes of macromolecules in the occurrence of interphase processes [1]. We have previously [2-4] investigated the rheological properties of interphase adsorption layers (IALs) of the reserve globulins of cotton seeds at liquid phase-separation boundaries as functions of the protein concentration, the ionic strength and pH of the solution, the time of formation of the layer, and chemical modifications of the protein. It was established that a definite role in the manifestation of the functional properties of cottonseed proteins is played by the structural factor [5].

The chemical modification of the main reserve protein of cotton seeds — gossypin — by acylating and denaturing agents led to changes in the structure [6] and the surface properties of the protein. In the neutral pH region, a correlation was observed between the rheological properties (IALs) and the stability of an emulsion of benzene stabilized by acyl derivatives of the protein [4].

The partial hydrolysis of proteins and peptides by proteolytic enzymes is frequently used to improve the nutritional and functional properties of proteins. As a result of hydrolysis, the dimensions of the protein macromolecules decrease and the number of polar groups increases, which usually leads to an improvement in solubility, particularly at the isoelectric point. At the same time, there are changes in other functional properties of the hydrolyzates obtained: the viscosity of solutions, emulsifying activity of the protein and stability of the emulsions, gel-forming activity, and a number of others [8].

We have carried out partial tryptic hydrolysis of the 7S and 11S forms of gossypin and have investigated the rheological properties of the IALs of the modified proteins at liquid phase-separation boundaries (Table 1). On the limited proteolysis of gossypin the electrophoretic mobility of the proteolysis products (Fig. 1) and the strength properties of the IALs of the protein at a boundary with benzene changed considerably. As can be seen from Table 1, for the 7S gossypin the shear strength of the IALs of the proteins (Prs) and the steady-state stress of viscous flow of a disrupted layer fell more than twofold after 3 h. On the other hand, for the 11S form of the protein under the same conditions a 1.5-fold strengthening of the adsorption layers formed was observed.

The differences in properties cannot, apparently, be due to differences in the minor composition of the two forms (Fig. 1). It is known that at neutral pH values of the medium the solubilities of the 7S and 11S forms of gossypin are different [3], which explains the difference in the strength characteristics of IALs of the unhydrolyzed proteins — 8.25 and 2.16 mN/m, respectively. Tryptic hydrolysis of the 11S form of the protein increases its solubility and thereby affects the process of its absorption at a phase-separation boundary, increasing the shear strength of the IALs.

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TABLE 1. Influence of Limited Tryptic Hydrolysis on the Rheological Properties of the IALs of Various Oligomeric Forms of Gossypin (C_p = 0.02%, pH 6.9; t = 20°C; E/S = 1:200; ω = 0.004 rad/sec)

Rheological properties of the IALs, mN/m		Time of proteolysis, min						
		0	5	15	30	60	120	180
7S form	Prs	8.25	4.65	4.41	4.35	4.26	4.20	4.05
	Pss	7.05	4.65	3.87	3.81	3.66	3.57	3.51
11S form	Prs	2.16	2.31	2.46	2.85	2.91	3.21	3.60
	Pss	1.50	1.59	1.67	1.95	2.05	2.22	2.25

TABLE 2. Thixotropic Gel Formation of Interphase Adsorption Layers of Gossypin at Various pH Values of the Medium (C_p = 0.02%; t = 20°C; ω = 0.004 rad/sec)

Rheological properties of the IALs at pH, mN/m		7S	After disruption			11S	After disruption		
		1 h	5 min	15 min	1 h	1 h	5 min	15 min	1 h
2.0	Prs	7.50	6.75	7.20	7.50	7.20	5.85	6.20	6.51
	Pss	7.20	6.45	6.90	7.05	6.60	5.85	6.00	6.20
7.0	Prs	8.16	6.80	7.46	7.85	2.43	2.10	2.40	2.40
	Pss	6.90	6.05	6.67	6.75	2.10	1.92	2.05	2.08
8.8	Prs	8.01	6.45	7.08	7.56	5.58	3.18	3.90	4.60
	Pss	7.20	6.15	6.45	6.96	5.34	2.92	3.36	3.70

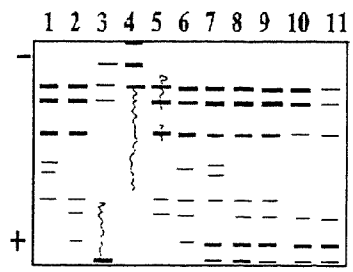

Fig. 1. Gel electrophoresis of gossypin in 15% PAAG plates in the presence of 0.1% Na-DDS at pH 8.3 (*1-5*) and 1% Na-DDS (*6-11*): *1*) 7S form; *2*) 11S form; *3*) acetylated; *4*) succinylated; *5*) denatured in 8 M urea; *6*) hydrolyzed with trypsin for 5 min; *7*) hydrolyzed with trypsin for 15 min; *8*) hydrolyzed with trypsin for 30 min; *9*) hydrolyzed for 1 h; *10*) hydrolyzed for 2 h; *11*) hydrolyzed with trypsin for 3 h.

The strong stabilizing action of high-molecular-mass SAAs is explained by the appearance of a gel-like or liquid-crystal structure of the adsorption layer through the separation of a new phase, differing greatly in its rheological characteristics from the bounding bulk phases [9]. For gel-like systems a process of reversible change of their physicomechanical properties under mechanical action — thixotropy — is known; for various systems this process has been described in detail in a monograph [10]. It has been shown [11] that when the limit of the shear strength of complexes of serum albumin and dextran sulfate is reached, the stationary IALs undergo brittle disruption, after which partial restoration of their structure takes place.

We have shown that adsorption layers of gossypin formed in 1 h at a boundary with benzene and disrupted under mechanical action are capable of a spontaneous restoration of their structure when held under isothermal conditions for 5, 15, and 60 min (Table 2). The thixotropic properties were determined at various pH values of the medium. The results presented show that, under the conditions investigated of the formation of adsorption layers of different oligomeric forms of gossypin

at liquid phase-separation boundaries, their strength is restored to 80% of its initial value 5 min after disruption and to 90—95% of it after 1 h; i.e., IALs of gossypin possess thixotropic properties, which explains the high strength of disperse systems stabilized by these emulsifiers.

EXPERIMENTAL

Tryptic Hydrolysis of Gossypin. With heating in a thermostat to 37°C and stirring, a solution of trypsin (*E/S* = 1:200) was added to 50 mg of gossypin in 50 ml of 0.1 M ammonium hydrogen carbonate. After predetermined intervals of time, 5-ml aliquots of the hydrolyzates were taken, proteolysis was stopped by heating the reaction mixture in the boiling water bath for 5 min, and it was frozen and lyophilized. The dried preparations were stored at 4°C in a desiccator over phosphorus pentoxide. The degree of proteolysis was determined as described in [12].

Electrophoresis of the protein was conducted on 15% PAAG plates in the presence of 0.1 and 1.0% Na-DDS. The protein bands were fixed in 10% TCA and were stained with Coomassie R-250.

The rheological properties of the IALs of the proteins at liquid phase-separation boundaries were determined as described in [2].

REFERENCES

1. V. N. Izmailov, G. P. Yampol'skaya, and B. D. Summ, Surface Phenomena in Protein Systems [in Russian], Khimiya, Moscow (1988), p. 240.
2. V. V. Maksimov, T. S. Yunusov, and V. N. Izmailov, Uzb. Khim. Zh., No. 2, 12 (1991).
3. V. V. Maksimov, T. S. Yunusov, and V. N. Izmailov, Uzb. Khim. Zh., No. 2, 29 (1993).
4. V. V. Maksimov, T. S. Yunusov, and V. N. Izmailov, Khim. Prir. Soedin., 528 (1994).
5. T. S. Yunusov, V. V. Maksimov, R. A. Rafikov, and V. N. Izmailova, "Food proteins: structure and functionality," in: Fourth Symposium on Food Proteins: Structure, Functionality, Relationships (K. D. Shewenke and R. Mothes, eds.), VCH, Weinheim (1993), p. 297.
6. Z. S. Yunusova, A. L. Li, T. S. Yunusov, and G. P. Moiseeva, Khim. Prir. Soedin., 132 (1985).
7. T. S. Yunusov, A. L. Li, A. N. Gurov, and V. B. Tolstoguzov, Khim. Prir. Soedin., 354 (1986).
8. V. V. Maksimov, T. S. Yunusov, A. L. Li, and G. A. Piyakina, Khim. Prir. Soedin., 520 (1990).
9. A. A. Trapeznikov, Koll. Zh., **21**, No. 1, 108 (1959).
10. V. N. Izmailova and P. A. Rebinder, Structure Formation in Protein Systems [in Russian], Nauka, Moscow (1974), p. 268.
11. A. N. Gurov, P. V. Nuss, and G. P. Yampol'skaya, Vestn. Mosk. Un-ta, Ser. 2, Khimiya, **28**, No. 2, 186 (1987).
12. Jens Adler-Nissen, J. Agric. Food Chem., **27**, No. 6, 1256 (1979).