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RHEOLOGICAL PROPERTIES OF INTERPHASE ADSORPTION LAYERS OF GOSSYPIN AT A LIQUID–LIQUID PHASE SEPARATION BOUNDARY. III. INFLUENCE OF CHEMICAL MODIFICATION

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The rheological properties of interphase absorption layers of gossypin derivatives at liquid phase separation boundaries and the stability of benzene emulsions have been investigated. It has been shown that the acylation of gossypin with acetic and succinic anhydrides lowers the strength properties of the adsorption layers of protein, leading to a change in the stability of benzene emulsions stabilized by gossypin.

We have previously [1, 2] studied the rheological properties of interphase adsorption layers (IALs) of gossypin at liquid-phase separation boundaries as functions of the concentration of protein in the solution and of the ionic strength and the pH of the medium. It was shown that by varying the parameters mentioned it is possible to change the mechanical properties of the layers formed within wide limits. It has been found in a number of studies [3-5] that the chemical modification of cottonseed proteins by acylating agents leads to an improvement in the functional properties (solubility, emulsifying and foam-forming properties) and the extractability of the protein from meal.

In the present paper we give the results of an investigation of the rheological properties of acylated and succinylated cottonseed proteins and the proteins denatured in 8 M urea at a benzene–water phase-separation boundary and of the stability of benzene emulsion stabilized by gossypin derivatives. On the chemical modification of a protein by acylating agents changes take place in the charge of the protein molecules, their capacity for dissociating into subunits, and the conformation of the macromolecules, which, in their turn, may influence the stability of emulsion stabilized by proteins [6].

We have carried out a 100% modification of the lysine residues of gossypin with the aim of changing the structure of the protein [4] and its functional properties to the maximum degree. Figure 1 gives the results of the gel electrophoresis of various specimens of the modified protein in plates of 15% polyacrylamide gel. It can be seen that the chemical modification of the protein with acylating agents leads to a change in the electrophoretic mobilities of the protein bands. At 100% acetylation of the lysine residues in gossypin (sample 2), the bands corresponding to the native subunits disappeared from the electrophoretograms and a diffuse fast-migrating band moving with the marker appeared. Characteristic for succinylated gossypin (sample 3) was the appearance of a band at the start and of protein bands with electrophoretic mobilities lower than those of the initial subunits, the protein bands now being diffuse. On the electrophoresis of the protein denatured in 8 M urea, the pattern of phoresis did not change, apart from the fact that the bands became diffuse.

Rheological Properties of IALs of Gossypin Derivatives

It has been shown previously [4, 6] that the chemical modification of gossypin by acylating agents leads to an improvement in the solubility and in the emulsifying properties of cottonseed proteins. Table 1 gives the results of an

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TABLE 1. Kinetics of the Formation of AILs of Gossypin Derivatives at a Boundary with Benzene at Various pH Values of the Medium (C_p = 0.02%, rate of deformation 0.042 deg/sec)

Sample	Time of forming the layer, h	Rheological properties of the IALs (mN/m) at pH)					
		2.0		7.2		9.3	
		P_{sr}	P_{ss}	P_{sr}	P_{ss}	P_{sr}	P_{ss}
Acetylated gossypin	0.25	3.6	3.3	1.4	1.4	1.2	1.1
	0.5	4.3	4.2	2.0	1.6	1.9	1.7
	1.0	4.8	4.2	2.4	1.9	2.2	1.6
	2.0	5.4	4.2	3.4	2.2	2.2	1.6
	3.0	5.7	4.2	3.5	2.3	2.3	1.6
	14.0	7.2	5.4	3.5	2.5	2.5	1.7
Succinylated gossypin	0.25	0.3	0.2	0.9	0.7	0.036	0.036
	0.5	0.35	0.25	1.2	0.9	0.036	0.036
	1.0	0.4	0.35	1.2	1.0	0.072	0.072
	2.0	0.6	0.4	1.2	1.0	0.108	0.108
	3.0	0.8	0.5	1.2	1.2	0.144	0.144
	14.0	0.9	0.7	2.0	1.4	0.144	0.144
Gossypin denatured in 8 M urea	0.25	5.3	4.0	0.14	0.14	0.28	0.14
	0.5	7.8	6.3	1.2	0.8	0.56	0.35
	1.0	9.3	7.8	2.0	1.4	1.15	1.0
	2.0	10.1	8.0	2.8	1.6	1.8	1.5
	3.0	12.0	8.1	3.8	2.2	2.4	1.8
	14.0	14.1	9.6	4.2	2.3	2.5	2.0
Gossypin	1.0	12.8	5.8	4.3	3.3	11.0	7.5

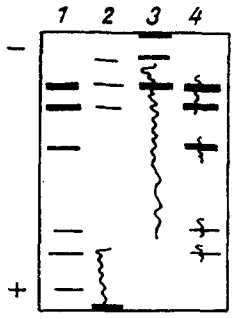

Fig. 1. Electrophoresis in 15% PAAG plates in the presence of 0.1% Na-DDS (tris-glycine buffer, pH 8.3,): 1) gossypin; 2) acetylated (100%) gossypin; 3) succinylated (100%) gossypin; 4) gossypin denatured by 8 M urea.

investigation of the kinetics of the formation of IALs at liquid phase-separation boundaries for various gossypin derivatives at acid, neutral, and alkaline pH values of the medium.

It can be seen that the chemical modification of gossypin by acylating agents leads to a fall in the maximum shear stress of the IALs of the protein (P_{sr}) and the stress of viscous flow of a collapsed layer (P_{ss}) at benzene–aqueous protein solution phase-separation boundary.

The maximum shear stress of the IALs for succinylated gossypin at alkaline pH values of the medium fell by an order in comparison with the initial protein. This is probably connected with a redistribution of the forces in the interphase adsorption layer resulting from a change in the charge of the gossypin macromolecules through the modification of the lysine residues by succinic anhydride. For acetylated gossypin, as well, a change took place in the strength of the IALs at a water–benzene phase-separation boundary, apparently for the same reason. In the case of the protein denatured in 8 M urea, there was a slight change in the strength properties of the IALs at neutral pH values, but at higher pH values the magnitude of P_{sr} was considerably lower than this index for the native globulin, apparently because of the low solubility of the gossypin

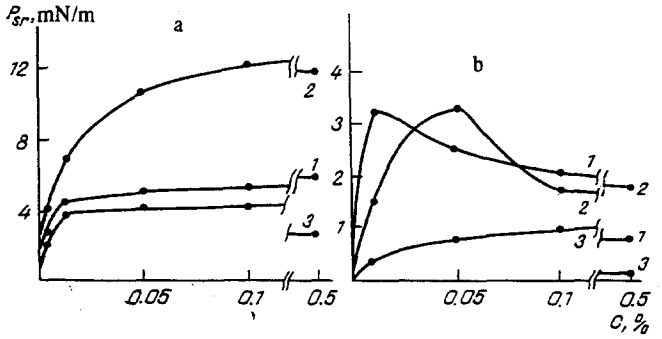

Fig. 2. Influence of the concentration of protein on the rheological properties of IALs of modified gossypin at a boundary with benzene under acid (a, pH 2.0) and neutral (b, pH 7.0) conditions of the medium; 1) acetylated gossypin; 2) gossypin denatured in 8 M urea; 3) succinylated gossypin. Time of forming the layer – 1 h, T = 293 K, $\dot{\varepsilon}$ = 0.002 rad/sec.

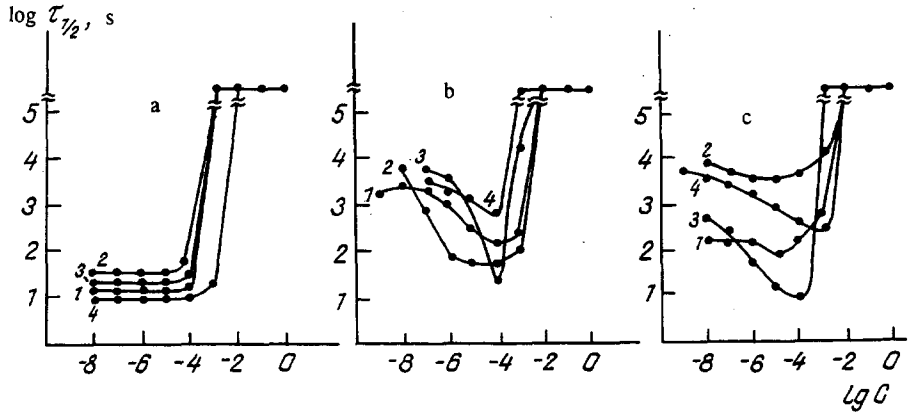

Fig. 3. Dependence of the stability of emulsions of benzene stabilized by gossypin and its derivatives on the concentration of protein for acid (a, pH 2.0), neutral (b, pH 7.2), and alkaline (c, pH 9.3) media: 1) acetylated gossypin; 2) succinylated gossypin; 3) gossypin denatured in 8 M urea; 4) gossypin.

denatured by 8 M urea at these pH values. Conversely, in an acid medium at pH 2.0 the maximum shear stress of the IALs for these proteins was at the level of the initial gossypin [2]

We have previously investigated the influence of the concentration of protein on the strength of IALs of gossypin and liquid phase-separation boundaries [1] and have shown that at protein concentrations in the solution of 0.5 and 1.0% a considerable decrease in the strength properties of the protein IALs takes place as compared with concentrations of 0.001 and 0.1%, which is connected with aggregation of the macromolecules in solution. In an investigation of the influence of the concentration of the modified proteins on the rheological properties of IALs at a boundary with benzene in an acid medium (Fig. 2a), it has been shown that in the case of gossypin denatured with 8 M urea a gradual rise in the shear strength of the IALs is observed to 12.0 mN/m at a protein concentration of 0.5%. A smooth increase in the strength of the layers formed is also observed for acyl derivatives of gossypin, the value of P_{sr} being 2-3 times lower than for the initial protein. In a neutral medium (Fig. 2b), the strengths of the IALs for these samples was lower than for the initial protein [2]. For the acetylated gossypin, the maximum shear strength of the IALs rose from 0.144 to 3.24 mN/m with a change in the concentration of the protein from 0.002 to 0.1%. A further rise in the concentration of the protein to 0.5% lowered the strength of the IALs to 0.72 mN/m. In the case of the succinylated gossypin, the shear strength at the same concentrations of protein rose initially from 0.29 to 0.94 mN/m and then fell to 0.10 mN/m at a 0.5% concentration of protein in the solution. In the case of the gossypin denatured 8 M urea, in spite of its low solubility at neutral pH values of the medium, the shear strength rose from 0.036 to 3.24 mN/m with an increase in the protein concentration from 0.002 to 0.05%. A further increase in the concentration of protein to 0.5% lowered the shear strength to 1.76 mN/m.

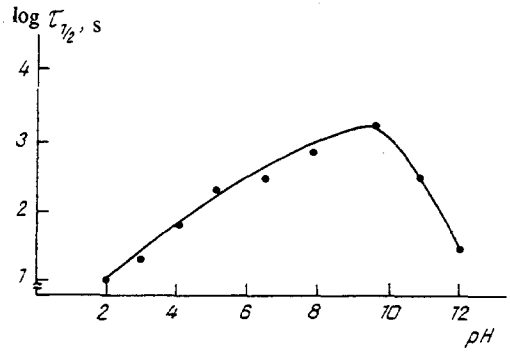

Fig. 4. Dependence of the stability of an emulsion of benzene on the pH of the medium; (volume fraction of benzene 0.2; T = 293 K).

Thus, the chemical modification of the ε-amino groups of the lysine of cottonseed globulins led to a marked fall in the strength of the IALs, although the region of high solubility of the modified protein at different pH values increases [6]. On the other hand, gossypin denatured by 8 M urea lost its solubility to a considerable degree at alkaline pH values, which also lowered the strength of the IALs under these conditions. It must be mentioned that at neutral pH values the characteristic dependence of the strength of the IALs on the concentration of protein in solution was retained for the acylated samples and the urea-denatured samples, as for the native gossypin. The latter is apparently also connected with the high aggregation capacity of gossypin derivatives, as in the case of the native protein [1]. In connection with the results obtained on the strength of protein layers at a phase-separation boundary for a number of gossypin derivatives, its appeared of interest to trace a possible correlation between the strengths of the IALs and the stability of emulsions based on them.

Stability of Benzene Emulsions Stabilized by Gossypin Derivatives

We carried out the investigation of the stability of benzene emulsions stabilized by gossypin and its derivatives by measuring the time of layer separation of half the nonpolar phase ($\tau_{1/2}$) from emulsions obtained at various pH values of the medium as a function of the concentration of protein in the solution [7] (Fig. 3). In an acid medium (pH 2.0), all the modified samples of gossypin formed emulsions with unlimited stability at a concentration of protein in the solution of 0.001% and above (Fig. 3a). For gossypin, stable emulsions of benzene existed only at a protein concentration greater than 0.01%. At neutral pH values of the medium (Fig. 3b), for all the protein samples stable emulsions of benzene were formed at a stabilizer concentration of 0.01% and above. When the concentration of protein in the solution was lowered to 0.01% gossypin still formed stable emulsions, while the protein denatured, while the protein denatured by 8 M urea formed emulsions with $\tau_{i/2}$ = 3.5 h and in the case of the acetylated and succinylated gossypin the stability of the emulsions was $\tau_{1/2}$ = 10 min.

At alkaline pH values (Fig. 3b) all the samples of protein stabilized a benzene emulsion down to concentrations in the solution of 0.01%. When the concentration of protein in the solution was lowered to 0.001%, unstable emulsions were formed only in the case of the 8 M-urea-denatured protein. It must be mentioned that at a concentration of protein in the solution of 10^{-4}% and below an increase in the stability of the disperse system formed was observed. This is connected with the fact that the stability of benzene emulsions without the addition of an emusifying agent (Fig. 4) is two orders greater at pH 9.5 than for acid pH values of the medium, i.e., the observed effect can be ascribed to the properties of benzene itself at alkaline pH values.

On analyzing the rheological investigations of IALs of gossypin derivatives at liquid phase-separation boundaries and the results on the stability of benzene emulsions (Table 1 and Fig. 3) it is possible to observe the existence of a correlation between these properties (the rheology of the IALs and the stability of the emulsions) only at neutral pH values of the medium. When the pH deviated into the acid and alkaline regions, no definite correlation was observed which is apparently connected with a sharp change in the contributions of the different factors (charge, conformation of the protein molecules, etc.) to the mechanism of the stabilization of benzene emulsions.

EXPERIMENTAL

Chemical Modification of Gossypin [4]. A weighed sample of protein was dissolved in 8 M urea in borate buffer at pH 9.0. With constant stirring, acetic or succinic anhydride was added to the solution cooled to 0°C (500-fold excess of the agent per 1 mole of lysine). The reaction was conducted at 0°C for 1 h (pH 8.9-9.5), the pH being maintained by the addition of 5 M NaOH. After the end of the reaction, the excess of the reagent was eliminated by dialysis against distilled water, pH 8.0 and 6°C, and then the protein solution was freeze-dried.

Determination of the Degree of Chemical Modification. The number of free ε-amino groups of lysine was determined by the reaction with trinitrobenzenesulfonic acid [8].

The rheological properties of the IALs of protein at liquid phase-separation boundaries were investigated by the method described in [9]. Benzene purified by a standard procedure and distilled at 80.0°C/740 mm Hg, $n_D^{20} = 1.5013$, was used as the nonpolar phase.

Gel electrophoresis was conducted on plates of 15% polyacrylamide gel in tris-glycine buffer, pH 8.3, with the addition of 0.1% of Na-DDS. The amount of protein deposited was 20 μg, and electrophoresis conducted at 40 mA/gel. Protein bands were fixed with 5% trichloroacetic acid and were stained with Coomassie R-250. The excess of dye was washed out with 7% acetic acid.

The stability of the emulsions was investigated by the method of [7]. Benzene was emulsified in protein solutions with various concentrations at 3000 rpm for 5 min (volume fraction of benzene – 0.2), after which the emulsions were transferred to calibrated test-tubes and the time of half-separation of a layer of the nonpolar phase from the emulsion was measured.

REFERENCES

1. V. V. Maksimov, T. S. Yunusov, and V. N. Izmailova, Uzb. Khim. Zh., No. 2, 12 (1991).
2. V. V. Maksimov, T. S. Yunusov, and V. N. Izmailova, Uzb. Khim. Zh., No. 2, 29 (1993).
3. Y. R. Choi, E. W. Lusas, and K. C. Rhee, J. Am. Oil Chem. Soc., **58**, No. 12, 1044 (1981).
4. T. S. Yunusov, A. L. Li, A. N. Gurov, and V. B. Tolstoguzov, Khim. Prir. Soedin., 354 (1986)
5. E. H. Rahma and M. S. Narasinga Rao, J. Agric. Food Chem., **31**, 352 (1983).
6. G. A. Piyakina and T. S. Yunusov, Khim. Prir. Soedin., 471 (1986).
7. D. A. Malkova, V. N. Izmailova, and G. P. Yampol'skaya, Vestn. Mosk. Univ., Khimiya, **25**, No. 5, 480 (1985).
8. J. Adler-Nissen, J. Agric. Food Chem., **27**, No. 6, 1256 (1979).
9. V. N. Izmailova, V. N. Yampol'skaya, and B. D. Summ, Surface Phenomena in Protein Systems [in Russian], Khimiya, Moscow (1988), p. 240.