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FUNCTIONAL PROPERTIES OF "COTTON PROTEIN."

III. INFLUENCE OF GOSSYPOL AND PHYTATES ON THE SOLUBILITY OF GOSSYPULIN

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The solubility of gossypulin and its derivatives under various conditions has been studied by turbidimetric titration. It was found that the low solubility of the protein under investigation in the neutral range of pH values at a low ionic strength may be due to the presence of phytin and gossypol. The minimum in the solubility of the gossypol-free protein shifted into the more alkaline region (pH 6.0-6.7) as compared with the initial gossypulin (with 0.6% of gossypol). The treatment of gossypulin with dilute solutions of phytin at pH 5.0 led to a shift in the pH of precipitation (pH 3.75), but in the presence of an excess of phytic acid the aggregation capacity of the protein increased and the pH of precipitation changed (pH 4.5-5.0). When Ca^{2+} ions were added, there was a shift of the solubility minimum into the alkaline pH range. In the presence of pectin, the pH of precipitation of all the samples studied shifted into a more acid pH range.

Plant raw material is a potential source of food protein. It can be used for human nutrition provided that it is isolated in the form of preparations with definite functional properties. One of such properties is solubility. The solubility profile as a function of the pH is frequently the first experimentally measured functional property and it can be monitored at each stage of the process of obtaining the protein. A knowledge of the solubility under various conditions (temperature, pH, ionic strength, etc.) enables the optimum conditions for the extraction of the protein to be determined. A number of other functional properties depend on the solubility of the protein, such as its emulsifying and gel- and foam-forming capacities.

We have previously studied the solubility of the native globulin fraction from the seeds of the cotton plant. It was shown that the isoelectric point of the protein is about 6.0. Denaturation of the protein broadens the solubility minimum [1]. We have continued a study of the solubility of gossypulin — quantitatively the main component of the globulin fraction — and have shown how the substances accompanying the protein affect its solubility.

According to Osborne's classification, gossypulin can be assigned to the class of globulins, since it dissolves only in solutions of salts. With a rise in the concentration of NaCl from 1 to 10% at pH 7.3, the solubility of gossypulin increases uniformly from 0.65 to 13.3 mg/ml. A further increase in the concentration of salt (15%) leads to a fall in solubility, i.e., a "salting-out" effect is observed (Fig. 1).

The poor solubility of the protein under investigation in the neutral region at a low ionic strength can be explained by the presence of gossypol and phytin. In the study of solubility we used a dynamic method based on turbidimetry or the nephelometry of the protein solutions. Figure 2 shows the curves of the nephelometric titration of dilute solutions of gossypulin (0.6% of gossypol). As can be seen from Fig. 2, the curves of the nephelometric titration of a gossypulin solution from an alkaline to an acid pH range and from an acid to an alkaline range do not coincide. Apparently, this protein gives different denaturation prod-

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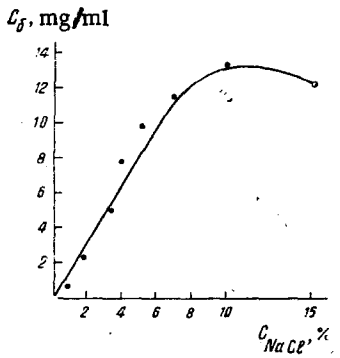

Fig. 1. Solubility of gossypulin in NaCl, pH 7.3.

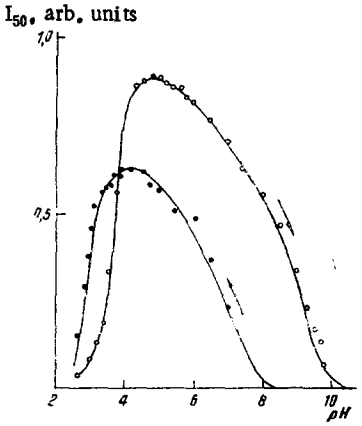

Fig. 2. Curves of the nephelometric titration of gossypulin (with 0.6% of gossypol) by acid and alkali.

TABLE 1. pH Values of the Precipitation of Proteins under Various Conditions

Protein sample	Without additives	Apple pectin	Ca^{2+} ions	Pectin + Ca^{2+} ions
1. Gossypulin (with 0.6% of gossypol)	4,0	3,0	4,0–4,5	2,8–2,9
2. Gossypulin (gossypol-free)	6,0–6,7	4,8–5,2	6,2–7,0	4,5–5,3
3. Gossypulin (1) treated with a 0.01 solution of phytin, pH 5,0	3,75	3,0	3,8–4,0	3,3
4. Gossypulin (1) precipitated with phytic acid	4,5–5,0	2,8–3,0	6,0–6,5	2,7–3,2
5. Gossypulin (1) precipitated with sodium phytate	5,0–5,7	3,1	5,8–7,0	3,1–3,4

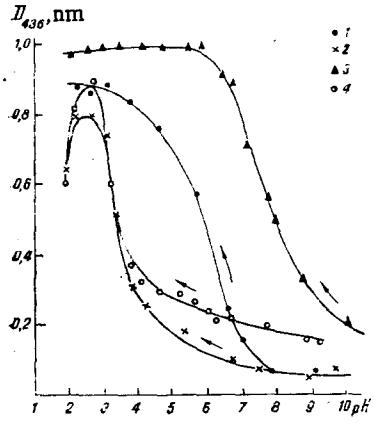

Fig. 3

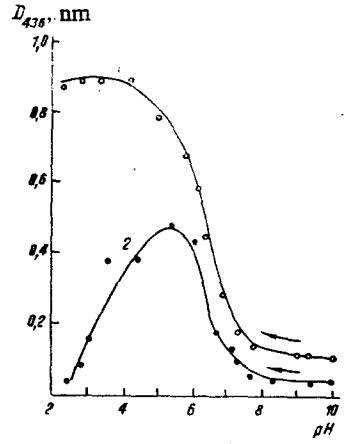

Fig. 4

Fig. 3. Curves of the turbidimetric titration of gossypulin (with 0.6% of gossypol) precipitated by phytic acid: 1) gossypulin; 2) gossypol + pectin (4:1); 3) gossypulin + Ca^{2+} ions 4) gossypulin + pectin + Ca^{2+} ions (0.01% of $CaCl_2$).

Fig. 4. Curves of the turbidimetric titration of gossypol-containing (1) and gossypol-free (2) globulins.

ucts under these conditions, which is responsible for the nature of the solubility curves. In view of this, for all the samples we carried out titration from an alkaline to an acid pH range. The solubility profiles of gossypol-containing (0.6% of gossypol) and gossypol-free globulins differed substantially. The solubility minimum of the gossypol-free protein is shifted into a more alkaline region (pH 6.0-6.7) as compared with the initial gossypulin (with 0.6% of gossypol), and in the same region the precipitation of the gossypol-free glob-

ulin broadens. Table 1 gives the pH values of isoelectric precipitation for gossypulin and its derivatives under various conditions.

The turbidimetric titration of dilute solutions showed that the treatment of gossypulin (with 0.6% of gossypol) with a solution of phytin at pH 5.0 led to a slight shift of the solubility minimum into the acid region (pH 3.75) in comparison with the initial gossypulin (pH 4.0). The shift of the solubility minimum can be explained by the assumption that phytin molecules interact with the surface groups of the protein and thereby lower its isoelectric point and improve its solubility.

It is known that phytic acid is capable of binding strongly to protein at basic amino acid residues (Arg, Lys, His). The greatest binding takes place at pH 2.5, when the protein bears the maximum positive charge. Using gel chromatography, Okubo et al. [2] have shown that soybean glycinin does not bind with phytic acid residues at pH 6.0 and above, i.e., at pH values above the isoelectric point of the protein. Conversely, O'Dell et al. [3] report the formation of a soluble complex of soybean protein with phytates at pH 9.0 and above, while wheat proteins do not form such complexes.

In studying the interaction of phytic acid with gossypulin we used the following approach. A protein–phytate complex was obtained at a pH where the greatest binding of phytic acid residues with the protein was observed. The complex did not decompose in the process of subsequent dissolution at alkaline pH values and acid titration. The solubility minimum of such a sample (Table 1, sample 4) had shifted (pH 4.5-5.0) as compared with the solubility minimum of the initial gossypulin (pH 4.0). The treatment of gossypulin in the presence of an excess of phytic acid should lead to a displacement of the isoelectric point of the protein into the acid pH region. Apparently, at high concentrations of phytic acid the aggregation capacity of the protein increases, which leads to a displacement of the pH for the precipitation of gossypulin. Figure 3 shows curves of the turbidimetric titration of a solution of gossypulin precipitate by phytic acid. When a solution of gossypulin (sample 4) was titrated in the presence of Ca^{2+} ions the solubility minimum changed sharply (pH 6.0-6.5). This apparently took place because the Ca^{2+} ions interacted with the phytic acid residues in the phytate–protein complex, which substantially lowered the solubility of the protein. Interesting from our point of view is the influence of pectin on the solubility of the phytate-protein complex. It would appear that pectin should exert no effect on the protein saturated with phytate ions; however, in the presence of pectin the pH for the precipitation of gossypol (sample 4) shifted into the acid region (pH 2.8-3.0). In order to study the competitive capacity of the ions of phytate and of apple pectin on the interaction with the protein, protein–phytate and protein–(phytate + pectin) complexes were obtained and the amounts of nitrogen and phosphorus in them were determined. The results of analysis show that the amount of phosphorus in the sample containing pectin was 0.31% as compared with that in the protein–phytate complex of 0.14%. The amount of nitrogen in the complex with pectin was lowered (13.8%). This indicates that if a solution of the protein contains two types of ions — of phytic acid and of pectin — the protein interacts primarily with the phytate, while the pectin continues to exert a stabilizing action on the protein in solution.

The proteins behave differently on acid titration in the presence of sodium phytate according to the amount of gossypol in the sample. Figure 4 shows curves of the titration of gossypol-containing (0.6%) and gossypol-free globulins in the presence of sodium phytate.

On passing to the study of concentrated solutions of gossypulin (with 0.6% of gossypol), it was found that the preliminary treatment of the protein with a solution of phytin did not give the same effect as in the case of dilute solutions, i.e., an increase in solubility. The pH values of the precipitation of the proteins in concentrated solutions (C_p 0.05-0.5%) shifted into the more alkaline region. Figure 5 shows examples of titration curves for concentrated solutions of gossypulin. The turbidity points of the solutions were determined from the sharp jump in turbidity during titration. Phase diagrams (Fig. 6) were plotted on the basis of the titration curves of solution with given concentrations. It can be seen from the graphs that all the points of pH precipitation are shifted into the alkaline region. This takes place through the aggregation of the protein molecules which, as is well known, intensifies with a rise in the concentration of protein in solution.

Thus, the solubility of gossypulin and its derivatives depends on the presence of accompanying substances (phytates and gossypol). Since the action of these substances changes under different conditions, which may be connected with the denaturation of the protein, in the following publication we shall consider questions of the correlation of the degree of denaturation of the protein with its solubility.

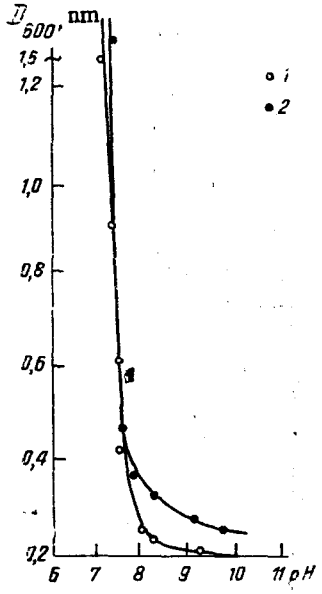

Fig. 5

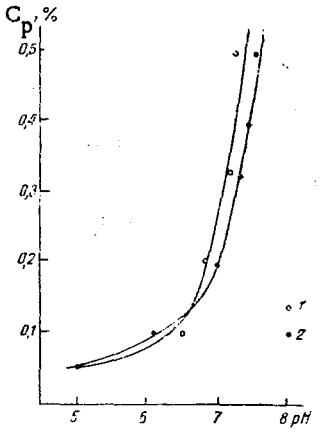

Fig. 6

Fig. 5. Curves of the turbidimetric titration of: 1) gossypulin (with 0.6% of gossypol); 2) gossypulin (1) precipitated with phytic acid, C_p 0.5%.

Fig. 6. Phase diagrams of: 1) gossypulin (with 0.6% of gossypol); 2) gossypulin (1) precipitated with phytic acid.

EXPERIMENTAL

Nephelometric titration was carried out on a Specol spectropolarimeter (DGR) with a special attachment for nephelometric titration in which the scattering of the light is recorded at an angle of 90°. Wavelength 436 nm (selected experimentally), cell volume 30 ml. The solutions were stirred with the aid of a magnetic stirrer at a constant rate of rotation. The value of I_{90} is given in arbitrary units of the scale of the recording instrument. Titration was performed with the aid of a OP-204/1 Radelkis pH-meter (Hungary) with a combined electrode.

The turbidimetric titration of the protein solutions was performed on a SF-16 spectrophotometer. Wavelength 436 nm, cell volume 4 ml. The solutions were stirred continuously with the aid of a magnetic stirrer. To determine pH values, a type pH-340 pH-meter was used. Concentrated solutions were used at a wavelength of 600 nm.

The protein–phytate complex was obtained by precipitating the protein from acid solution (pH 2.3-2.5) with sodium phytate, the system being stirred for 30-40 min. The precipitate was filtered off by centrifugation (3000 rpm, 5 min) and was washed with distilled water. The completeness of precipitation of the protein was checked from its amount in the supernatant liquid.

The treatment of the gossypulin was carried out with a 0.01% solution solution of phytin at pH 5.0 for 30-60 min with monitiring of the pH of the medium. Then the precipitate was separated off, washed with water, and freeze-dried.

Gossypol-free gossypulin was obtained by the procedure described in [4].

Protein concentrations in solution were determined by the microburet method described in [5].

SUMMARY

1. The pH values of the precipitation of gossypulin under various conditions have been determined by turbidimetric titration.

2. In the presence of apple pectin, a shift in the pH of precipitation to the more acid pH range took place (for all samples).

3. The presence of gossypol and of phytates substantially changes the solubility of gossypulin.

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LIGNIN OF THE FINE-FIBERED COTTON PLANT OF VARIETY S-6030 AFFECTED BY FUSARIAL WILT. II

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A comparative study of the products of cleavage by sodium in liquid ammonia of the dioxane lignins (DLAs) from healthy and fusarial-wilt-affected stems of the fine-fibered cotton plant of variety S-6030 and a study of the PMR spectra of both lignins has shown that the DLA from the healthy stems is more highly condensed than the DLA of the stems affected by wilt. The main structures of DNA wilt are of the guaiacyl type. In the DLA from the affected stems, the amount of p-coumaryl structures had increased, which confirms the demethylating action of fusarial wilt on cotton-plant lignin.

Continuing a study of the dioxane lignins (DLAs) from ripe stems of the thin-fibered cotton plant of variety S-6030, both healthy and affected by fusarial wilt [1, 2], we have cleaved the DLAs with metallic sodium and liquid ammonia by the procedures adopted for lignins [3]. The total yield of monomeric cleavage products from the DLA of the healthy plant (15.62%) was only half that from the DLA of the wilt-affected cotton plant (30.70%). This, like the yield of the product of nitrobenzene oxidation [2], indicates a greater degree of condensation of the lignin from the healthy stems. The yield of high-molecular-weight cleavage products was 33.49% from the DLA of the healthy stems and 34.97% from the DLA of the wilt-affected stems. The gel chromatography of these combined materials on a column of Sephadex LH-20 (with aqueous methanol as eluent and solvent) showed their similarity. Using the coefficients found previously [4], it was possible to delimit on the gel chromatograms the regions of oligomers (fraction 1), trimers (fraction 2),dimers (fraction 3), and monomers (fraction 4). The bulk consisted of the oligomeric and monomeric fractions.

Analysis of the total monomers on a gas-liquid chromatograph showed the presence of phenols belonging to three types of phenylpropane structural units (PPSUs): p-coumaryl, guaiacyl, and syringyl. The ratio of the p-coumaryl, guaiacyl, and syringyl components in the monomers from the healthy stems was 0.09:1:0.32, and from the affected stems 0.15:1:0.22.

Below we give the yields of monomeric products from the cleavage of lignins with metallic sodium and liquid ammonia (% on the DLA):

Substance	DLA from healthy stems	DLA from affected stems
p-Hydroxyphenylpropane	—	0,40
3-(p-Hydroxyphenyl)propanol	0,65	1,58
Guaiacol	0,26	0,26
Guaiacylethane	0,38	0,56
Guaiacylpropane	6,03	10,42
1-Guaiacylpropanol	0,90	2,25
Syringylpropane	2,92	2,86

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