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FUNCTIONAL PROPERTIES OF "COTTONSEED PROTEIN."

V. INFLUENCE OF THE STRUCTURE OF GOSSYPULIN ON COMPLEX-FORMATION WITH PECTIN

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The influence of complex-formation with apple pectin on the solubility of gossypulin has been studied by the turbidimetric titration method. It has been established that apple pectin (amount of $-OCH_3$ groups 5.0%) exerts the same action on the solubility of gossypulin and its derivatives with different degrees of chemical modification and denaturation at a weight ratio of pectin to protein of 1:4. When protein solutions are stabilized in the presence of pectin interactions of ionic, hydrogen, and/or hydrophobic nature take place between the proteins and the pectin.

The interaction of proteins with anionic polysaccharides has been studied by many authors [1-3]. At the present time, this approach is being used to regulate the functional properties of proteins and, in particular to improve their solubility. For the foodstuffs industry it is a matter of interest to obtain water-soluble complexes of proteins with polysaccharides, but their structure and properties have been studied insufficiently completely. There is no unambiguous answer to the question of the nature of the forces stabilizing protein-polysaccharide complexes, either. It has been shown in a series of investigations that the complexes are formed through the forces of electrostatic nature. On the other hand, many authors [4-7] consider that the formation of complexes between proteins and anionic or neutral polysaccharides at pH values above the isoelectric points (IEPs) of the proteins may also take place through forces of nonelectrostatic nature (hydrophobic interactions and hydrogen bonds.)

The properties of the protein-polysaccharide complexes obtained depends directly on the methods by which they have been prepared. It is known that two types of complexes exist [8]. If solutions of macroreagents are mixed under conditions of intensive complex-formation (i.e., at a pH below the IEP of the protein), "mixing complexes" are obtained that are sparingly soluble in water. On the slow titration of a solutions, when such conditions of the medium are created that the interaction is gradually intensified, soluble "titration complexes" are formed.

We have investigated the formation of complexes of gossypulin with apple pectin by the turbidimetric titration method.

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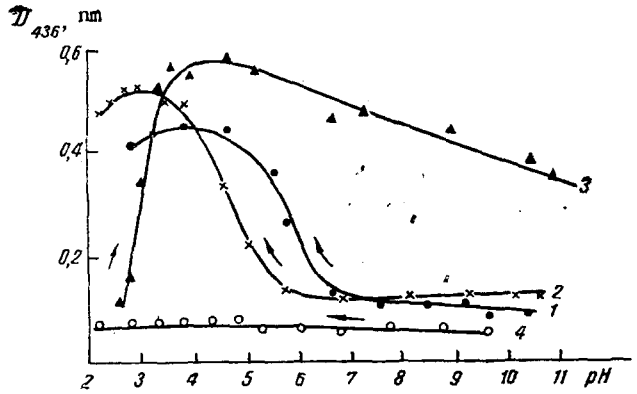

Fig. 1. Turbidimetric titration curves of gossypulin (containing 0.6% of gossypol): 1) gossypulin; 2) gossypulin + pectin (weight ratio 4:1) titrated with acid; 3) gossypulin + pectin (4:1), titrated with alkalis; 4) apple pectin.

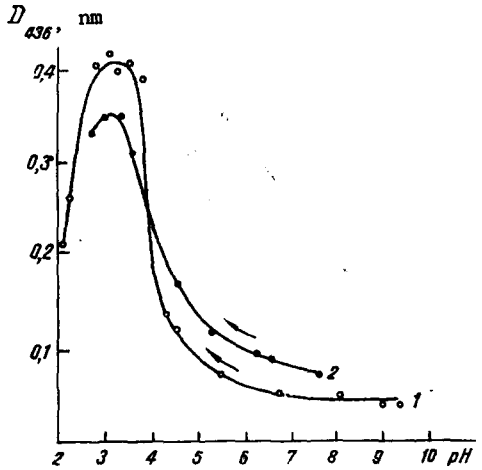

Fig. 2. Curves of the turbidimetric titration of 1) a mixture of gossypulin (0.6% gossypol) + pectin (weight ratio 4:1); 2) the gossypulin/ pectin complex.

In Fig. 1, the difference in the solubility of protein-pectin complexes on titration with acid and with alkali can be clearly seen. Apparently, on the titration of the system from the acid region to the alkaline region, gossypulin forms different denaturation products and begins to precipitate from solution before its interaction with the pectin takes place. Consequently, on titration under these conditions the solubility of the protein becomes worse. On acid titration, the pH of the precipitation maximum of the complex shifts into the more acid region (pH 3.0) as compared with the initial protein (pH 4.0), i.e., an improvement in the solubility of gossypulin in the neutral pH range is observed.

The gossypulin-pectin complex obtained by acid titration is fairly stable in an alkaline medium. If the isolated complex is redissolved under alkaline conditions and then the resulting solution is backtitrated into the acid region, the titration curve will be close to the curve of the initial complex, the composition of the complex remaining constant: N = 11.3% (Fig. 2). This indicates that on the interaction of the protein with pectin it is not a mixture of substances that is formed but a complex the properties of which differ from the properties of the initial macroreagents.

To determine the nature of the forces responsible for the interaction of the pectin with the protein under these conditions, we used an approach based on determining the proportion of protein (N, %) in complexes of gossypulin and its derivatives with pectin.

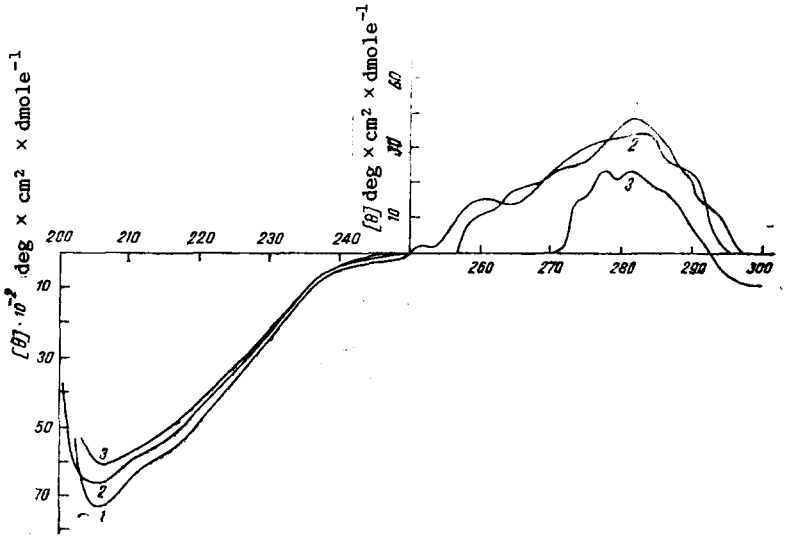

Fig. 3. Circular dichroism (CD) spectra of gossypol-free gossypulin: 1) gossypulin, pH 10.0; 2) gossypulin + pectin (4:1), pH 10.0; 3) gossypulin + pectin, pH 8.7.

Below, we give the results of analyses for nitrogen in complexes of gossypulin and its derivatives with apple pectin (all the complexes were obtained from pectin containing 0.6% of gossypol):

		Nitrogen, %
1.	Initial gossypulin	11.3
2.	Acetylated (degree of modification 25%)	10.9
3.	Succinylated (degree of modification 13%)	11.0
4.	Acetylated (degree of modification 100%)	13.5
5.	Treated with 2% HCl	13.0
6.	Treated with a 0.01% solution of phytic acid at pH 5.3	13.0
7.	Precipitated with phytic acid at pH 2.5	15.3
8.	Deamidated (deprotonated)	12.7
9.	Deamidated (protonated)	13.3

On analyzing these results, the following statements can be made: acetylated (25%) and succinylated (13%) gossypulins with low degrees of modification interact with pectin in the same way as the initial gossypulin (N = 11%). When all the ε-NH_2 groups in the lysine residues of gossypulin were blocked by acetylation (sample 4), the proportion of nitrogen in the complex rose (N = 13.5%), and the proportion of pectin fell correspondingly. This indicates that the interaction of the protein with pectin in the precipitation of the complex takes place through electrostatic forces. In the complex of the protein that had been precipitated by phytic acid (sample 7) the proportion of nitrogen had risen sharply (N = 15.3%) and approximated to the value for the initial gossypulin, although the pectin exerted a stabilizing effect, improving the solubility of the protein in the neutral pH region [9].

A similar phenomenon was also observed for samples 5, 6, 8, and 9, where the proportion of nitrogen increased but the pectin raised the solubility of the protein at neutral pH values. While the increase in the proportion of nitrogen in sample 6 can be ascribed to the blocking of some of the positively-charged groups in the protein by phytic acid, this is not the case for sample 5. Apparently, the increase in the proportion of nitrogen in this complex was connected primarily with a change in the ratio of polar and nonpolar (hydrophilic and hydrophobic) groups on the surface of the protein molecule as a consequence of denaturation. It is probably for this reason that the proportion of nitrogen in the deaminated samples 8 and 9 had increased.

The optimum weight ratio of protein to pectin at which the greatest shift in the pH of the precipitation maximum of the complex was observed was 4:1. An important fact is, in our opinion, that in spite of the different ratios of protein and pectin in the complexes isolated, the pectin had a similar influence on the solubility of the protein, lowering

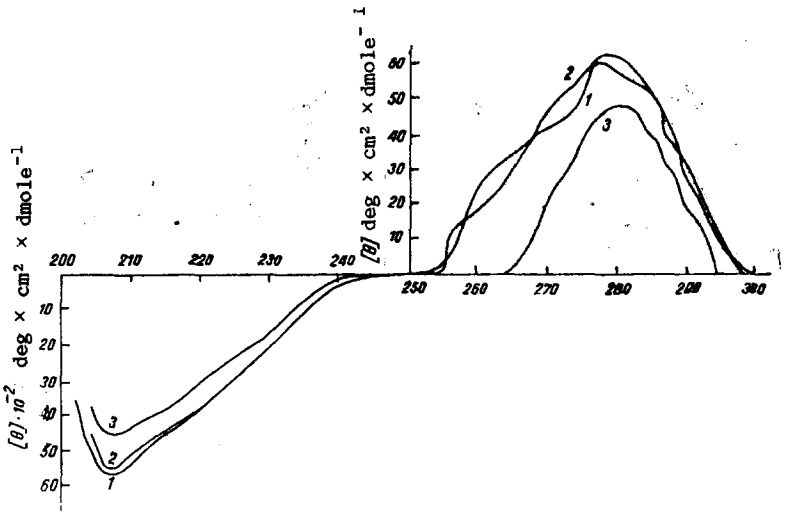

Fig. 4. CD spectra of gossypulin (containing 0.6% of gossypol): 1) gossypulin, pH 7.0; 2) gossypulin + pectin (weight ratio 4:1), pH 10.0; 3) gossypulin + pectin, pH 7.3.

the pH of the precipitation maximum. Apparently, pectin forms a complex with gossypulin also through hydrogen and/or hydrophobic bonds. A confirmation of the fact that the protein-pectin complex is stabilized in solution through noncovalent interaction was obtained in a study of the competing capacity of phytate and pectin ions described in [9].

When proteins interact with polysaccharides (in particular, with pectins), soluble or insoluble complexes are formed (depending on the conditions of their production), the properties of which differ from the properties of the initial macromolecules. The formation of protein-polysaccharide complexes may be considered as the immobilization of the protein on the pectin macromolecules playing the role of a matrix, and this, in its turn, may lead to a change in the structure of the protein. It therefore appeared of interest to study the conformational changes of gossypulin in the presence of pectin.

We studied the circular dichroism (CD) spectra of protein-pectin complexes of gossypulin containing (Fig. 4) and not containing (Fig. 3) gossypol. As can be seen from Fig. 3, the CD spectra of gossypulin itself and of gossypulin mixed with pectin at pH 10.0 differ in the region of the peptide bond (curves 1 and 2), which shows an interaction of the pectin with the protein at these pH values. For samples of gossypulin containing gossypol (Fig. 4), no such influence was observed because of the stabilizing action of the gossypol molecules [11]. In these cases, in the acid titration of the solutions conformational changes of the gossypulins took place, which is apparently connected with a weak interaction of the protein with the polysaccharide matrix. Since such interactions do take palce, they have a substantial effect on the solubility of the protein in the presence of pectin. When the protein is precipitated by pectin, the complex is formed only if ionic interactions between the protein and the pectin predominate.

EXPERIMENTAL

Preparation of the Protein-Pectin Complex. Dry apple pectin [12] was added to an alkaline solution of the protein (pH 10.0-10.5) at a weight ratio of 4:1, and the mixture was stirred until dissolution was complete. Then the solution obtained was titrated with acid (1 N HCl) to a pH at which the deposition of a precipitate set in. The precipitate was separated off, washed with distilled water, and freeze-dried.

The gossypulin derivatives were obtained as described in the literature [9, 10].

The turbidimetric titration of the solutions of the proteins was carried out as in [9].

CD spectra were taken on a JASCO-20 spectropolarimeter. The concentrations of the protein solutions were 0.5-1.0 mg/ml. The cell thicknesses were 0.05 and 1 cm. Nitrogen contents were determined as described in [13].

SUMMARY

1. It has been established that the nature of the action of pectin on the solubility of gossypulin and its derivatives with different degrees of chemical modification and of denaturation is the same at a weight ratio of protein to pectin of 4:1.

2. It is assumed that when protein solutions are stabilized in the presence of pectin, ionic, hydrogen, and/or hydrophobic interactions take place between the proteins and the pectins. When protein is precipitated from solution by acid titration in the presence of pectin, complexes between the protein and the pectin with pronounced ionic interactions are formed.

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SYNTHESIS OF A HEPTAPEPTIDE WITH SEQUENCE 17-23 OF HUMAN CALCITONIN

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Two schemes for the synthesis of a peptide with sequence 17-23 of human calcitonin with the minimum protection of the lateral functions of the amino acids are proposed.

The heptapeptide ZAsnLys(Boc)PheHisThrPheProOH (I) with the natural sequence of human calcitonin [1] is a fragment in the synthesis of the complete calcitonin molecule with the minimum protection of the lateral functional groups of the peptide which permits simpler amino acid derivatives to be used and the yield of the desired product at the end of the synthesis to be raised. In an attempt to synthesize (I) by analogy with [2] without the protection of the hydroxy groups of the threonine residue, a substance was formed that contained two impurities close in chromatographic mobility and other properties to the heptapeptide which could not be separated from the heptapeptide by the usual methods (extraction, recrystallization).

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