SUMMARY

It has been shown that the glycosides D_1, D_2, and D_3 isolated from the starfish *Distolasterias nipon* are, respectively, 5α-cholestane-3β,6α,8β,15β,24ξ-pentaol 3,24-di-O-β-D-xylopyranoside (I), 5α-cholest-22(23)-ene-3β,6α,8β,15β,24ξ-pentaol 24-O-β-D-glucopyranoside 3-O-β-D-xylopyranoside (III).

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CONFORMATIONAL CHANGES OF GOSSYPULIN IN SOLUTIONS AT VARIOUS pH VALUES

INFLUENCE OF SALTS

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UDC 547.962

Work has continued on the study of the conformational transitions of gossypulin from the seeds of the cotton plant under various conditions. The denaturation of gossypulin as a function of the pH of the medium and the influence of salts (sodium chloride and sodium phytate) on the denaturation process have been studied with the aid of circular dichroism. The gossypulin from cotton plant seeds undergoes complex conformational changes in the pH interval from 2 to 13. Sodium phytate stabilizes the protein molecule at pH 2 and 3.

The conformational transitions of reserve proteins, especially the globulins from cotton plant seeds, have prime value in explaining many physicochemical properties. The change in conformation under the action of many factors is responsible for the biological role of these proteins in seeds [1], while since the globulins are one of the main components of food proteins, they also determine the functional properties of food proteins [2]. The conformational changes in the gossypulin of cotton plant seeds are particularly important since it lacks disulfide bridges, which imparts a high conformational mobility to its subunits [3].

In view of this, we have continued investigations of the conformational changes of gossypulin from cotton plant seeds under various conditions. We have studied the denaturation of gossypulin as a function of the pH of the medium, and the influence of salts (sodium chloride and sodium phytate) on the denaturation process. In the present work we used the method of circular dichroism (CD).

Institute of the Chemistry of Plant Substances of the Uzbek SSR Academy of Sciences, Tashkent. Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 255-258, March-April, 1987. Original article submitted October 8, 1986.

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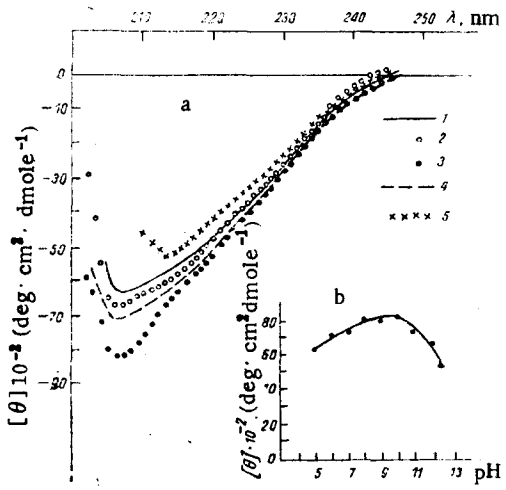

Fig. 1. CD spectra of gossypulin in 0.05 M phosphate buffer containing 10% NaCl at the following pH values: 1) 5; 2) 7.4; 3) 10; 4) 12; 5) 13.

Figure 1a, b shows the CD spectra (in the 200–250 mm region) of gossypulin from cotton plant seeds in 0.05 M phosphate buffer containing 10% of NaCl at pH 5–13. The protein does not dissolve in the presence of 10% of NaCl at lower pH values. It can be seen from the figure that the molecule undergoes considerable changes at pH 10 and 13. While the molar ellipticity at the maximum of the Cotton effect (CE) for the native molecule (pH 7.4), $[\theta]_{max} \approx 6800$ deg·cm^2·dmole^{-1}, at pH 13 the intensity of the EC falls appreciably to ≈ 5000 deg·cm^2 × dmole^{-1}, and at the same time there is a bathochromic shift of the CE to $\lambda = 215$ nm. In the pH interval from 5 to 9, the CD spectrum changes insignificantly, which indicates that the secondary structure of gossypulin is stable in this pH range.

It must be mentioned that the nature of the conformational changes of gossypulin at pH > 10 alters quantitatively (Fig. 1b). While in the pH interval from 6 to 10 the ellipticity rises, and pH > 10 it shows a sharp fall which is apparently connected with the desaturation of the protein. The conformational changes of gossypulin in the absence of NaCl at pH 10.0 and 12.1 have a similar nature (Fig. 2a). However, at pH 13 under these conditions the ellipticity in the CD spectrum increases. This shows a fundamental difference between the products of the denaturation of gossypulin at pH 13 in the absence and in the presence of NaCl (Figs. 1a and 2a). Substantial conformational rearrangements of the molecule are also shown by the CD spectra of the samples in the region of absorption of aromatic amino acids (250–320 nm) (Fig. 2b). In the acid pH range (2.0 and 3.0) gossypulin undergoes more pronounced conformational rearrangements.

It can be seen from Fig. 2a that the negative CD shifts in the short-wave direction, and at pH 2.0 the CD spectrum is characterized by a negative band with a minimum at $\lambda = 199$ nm and $[\theta]_{199} \approx 16000$ deg·cm^2·dmole^{-1}, while at pH 3.0 there are a band with a minimum at $\lambda = 202$ mm, $[\theta]_{202} \approx 10800$ deg·cm^2·dmole^{-1} and a shoulder at $\lambda = 220$ nm, $[\theta]_{220} \approx 4500$ deg·cm^2·dmole^{-1}. The nature of the change in the conformation of gossypulin in the region of absorption of aromatic amino acids in the acid pH range differs sharply from the changes at pH > 10 (Fig. 2b).

It appeared of interest to study the conformational changes of gossypulin at acid pH values in the presence of sodium phytate, since at these pH values the formation of stable phytate protein complexes takes place. In the presence of sodium phytate (0.015%) in a solution of the protein a less pronounced change in the secondary structure of the gossypulin was observed (Fig. 3a). The value of the molar ellipticity at the maximum of the CE at pH 2.0 was ≈ 11000 deg·cm^2·dmole^{-1}, and at pH 3.0 it was ≈ 9000 deg·cm^2·dmole^{-1}. This is apparently connected with the fact that sodium phytate stabilizes the secondary structure of gossypulin under these conditions. However, in the region of absorption of aromatic amino acids we observed the opposite effect, i.e., the almost complete disappearance of the positive band due to the tertiary structure of the protein (Fig. 3b), which indicates a substantial change in the environment of the aromatic radicals in the protein [4].

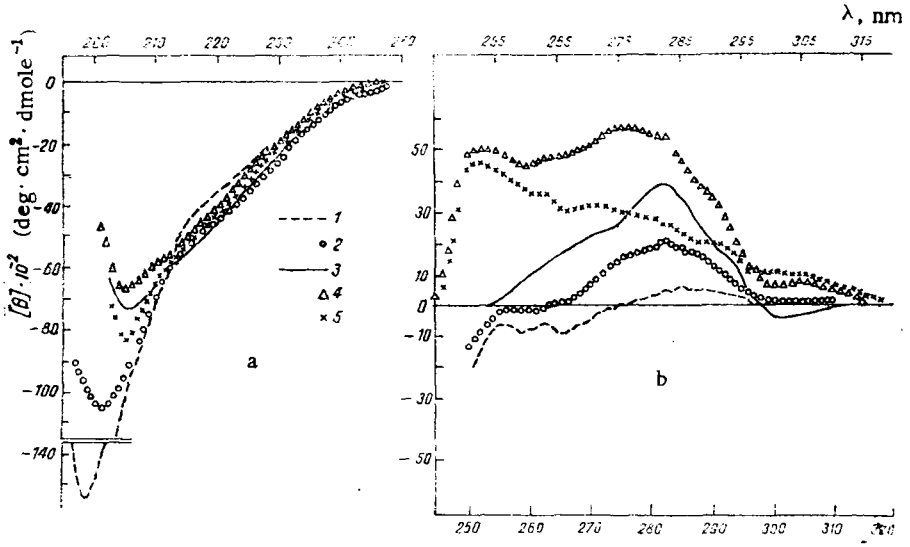

Fig. 2. CD spectra of gossypulin in distilled water at the following pH values: 1) 2; 2) 3; 3) 10; 4) 12; 5) 13.

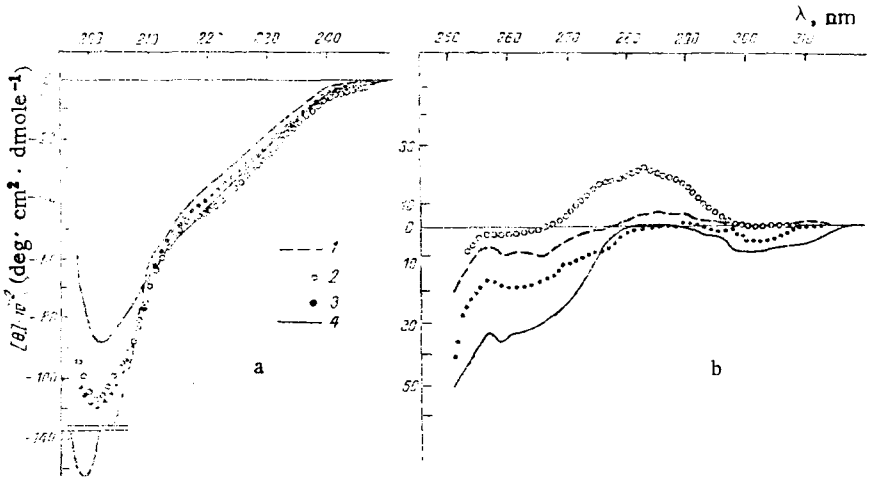

Fig. 3. CD spectra of gossypulin in distilled water in the presence of 0.015% of sodium phytate at the following pH values: 3) 2; 4) 3 (curves 1 and 2 were taken from Fig. 2).

EXPERIMENTAL

Gossypulin from seeds of the cotton plant of variety 108-F was isolated as described in [5].

CD spectra were taken on a Jasco J-20 spectropolarimeter at protein concentrations of 0.5-1.5 mg/ml in cells 0.05 and 1 cm long, the sensitivity of the instrument being 0.002° per 1 cm and the time constant of the instrument 64 sec. The rate of scanning was 2 nm/min. The results obtained were expressed in the form of molar ellipticities calculated to an average amino acid residue [θ]. The mean molecular weight of a residue was calculated from the amino acid composition of the protein as 129.

The proteins were dissolved in 0.05 M phosphate buffer with the addition of 10% of NaCl, and the pH values of the solution were adjusted by 0.1 N solutions of NaOH and HCl. In the absence of NaCl, the protein was suspended in distilled water and the pH of the solution was adjusted with 0.1 N solutions of NaOH and HCl. The final pH values of the protein solutions were checked after they had been stirred for 30 min by magnetic stirrer with the aid of a OP-208/1 precision pH-meter (Hungary). The concentrations of the protein solutions were determined by the biuret method [6].

SUMMARY

It has been established that gossypulin from cotton plant seeds undergoes complex conformational changes in the pH interval from 2 to 13. Sodium phytate stabilizes the protein molecule at pH 2 and 3.

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SEPARATION OF PANTOTHENIC ACID DERIVATIVES AS PRECURSORS FOR THE BIOSYNTHESIS OF THE ACETYLATION COENZYME BY CHROMATOGRAPHY ON DEAE-DELLULOSE

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UDC 577.164.14:615.356

The possibility of the chromatographic separation of pantothenic acid derivatives — 4'-phosphopantothenate, pantethine, 4'-phosphopantetheine, dephospho-CoA and CoA — on a column of DEAE-cellulose (DE-11 or Servacel DEAE 23 SH) has been studied, with their detection by radiometric, enzymatic, or spectrophotometric methods. The affinity of pantothenic acid and that of pantotheine for the ion-exchange resins used are identical. The phosphorylated derivatives of these compounds, which are eluted at higher values of a concentration gradient of lithium chloride, also have identical chromatographic characteristics. The separation of dephospho-CoA and CoA is not achieved on the chromatographic system investigated, but the elution of the fractions to free them from the retained nucleotide-containing precursors of the coenzyme can be used effectively for analytical purposes.

The chemical or enzymatic synthesis of the acetylation coenzyme — pantetheine adenine nucleotide diphosphate (CoA) — is performed in several stages by the successive transformation of its vitaminic precursor — pantothenic acid (I) [1-3]. In the biosynthesis of CoA, intermediate metabolites are 4'-phospho-(I) (II), 4'-phosphopantothenylcysteine, 4'-phosphopantetheine (III) (PPN), and dephospho-CoA (IV). However, among other PAA compounds isolated from biological material must be mentioned pantethine (V), diphosphopantethine (the disulfide derivatives of pantetheine and (III), respectively), and the sulfo derivatives of these two compounds [4]. At least seven derivatives of the vitamin, including the free form of (I) and CoA have been identified in the hepatocytes of higher animals [5], but their accurate differential determination has proved impossible in spite of the simultaneous use of six analytical methods.

Our preceeding communication in this journal [6] related to the quantitative gas-chromatographic determination of pantolactone, which arises on the acid hydrolysis of (I) [7] and compounds containing it [8]. This method is extremely convenient for pharmaceutical chemical purposes [9], although the microbiodetermination of (I) still remains the method of choice [10]. These methods permit analytical problems to be solved with adequate accuracy both in the process of the chemical transformation of compound (I) and also in biochemical investigations.

Institute of Biochemistry, Academy of Sciences of the Belorussian SSR, Grodno. Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 258-262, March-April, 1987. Original article submitted October 1, 1986.

 0009-3130/87/2302-0216$12.50