

FLUORESCENCE OF GOSSYPIN IN AQUEOUS SOLUTIONS AND EMULSIONS

V. V. Maksimov¹ and T. S. Yunusov²

UDC 541.183.02

Proteins in aqueous solutions fluoresce because aromatic amino acids are excited by UV light [1]. Analysis of emission spectra provides information about the conformation of the protein molecules and its changes.

We investigated the fluorescence of the principal cotton storage protein, gossypin, with the pH changes and decane emulsion stabilized by protein solutions changes in order to explain the effect of the nonpolar phase on the conformation of the protein molecules with formation of interfacial adsorption layers (IAL).

Fluorescence spectra of gossypin were recorded on a Hitachi UV-Vis spectrometer with excitation wavelength 280 nm. Spectra were measured from 315 nm to suppress the effect of scattered light. The optical density of the protein solutions at 280 nm was 0.1. The fluorescence intensity of gossypin at 350 nm and pH 3 was set at 100%. The decane emulsions (0.02%) was obtained by dispersing the nonpolar phase for 1 min in a UZDN-1U4.2 ultrasonic bath at 44 KHz with two-fold dilution of the dispersion by gossypin solution (0.002%). The pH of the solution was monitored with a pH-340 instrument. Figure 1 shows that the fluorescence maximum in the pH range 8.0-10.0 (curves 4, 5, and 6) is practically constant at 340 nm. The quantum yield changes only insignificantly. This indicates that the conformation of the proteins does not change significantly in this pH range. Results from CD spectroscopy of gossypin confirm this [2]. Our data do not agree with results obtained by Indian researchers, who described the fluorescence maximum of gossypin at 325 nm [3]. Apparently this is due to a different array of accompanying substances (gossypol, phytates).

The fluorescence maximum shifts to long wavelength (355 nm) if the pH is made strongly alkaline (up to pH 12.7) (curve 8). This significantly suppresses the fluorescence, indicative of significant conformational changes in the protein structure. The situation is analogous if the pH is decreased to 1.0-2.0 (curves 1 and 2). The only difference is that the fluorescence maximum is observed at 350-355 nm at pH 2.0 and 347-350 nm at pH 1.0. The fluorescence decrease at acidic pH values is less than at alkaline values.

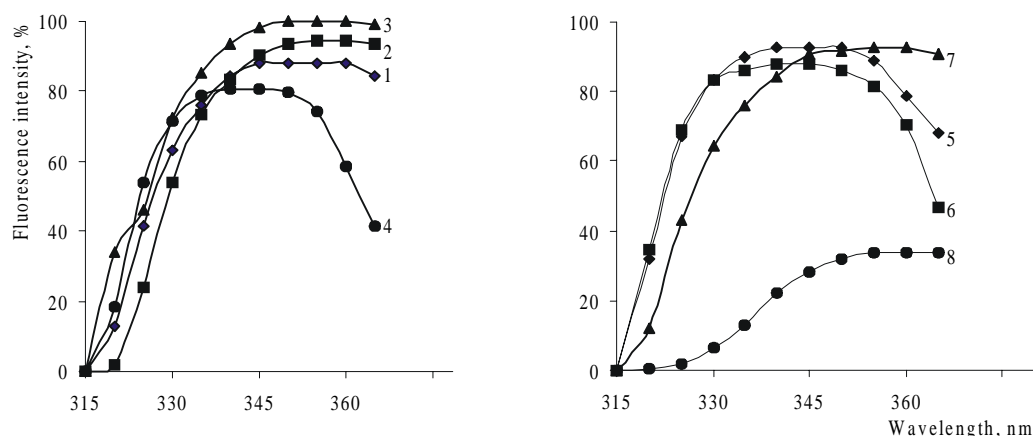


Fig. 1. Fluorescence spectra of gossypin as a function of pH: 1 (1), 2 (2), 3 (3), 8 (4), 9 (5), 10 (6), 12 (7), 12.7 (8).

1) S. Yu. Yunusov Institute of the Chemistry of Plant Substances, Academy of Sciences of the Republic of Uzbekistan, Tashkent, fax (99871) 120 64 75; 2) SP "Mikrel," Tashkent, fax (99871) 191 10 10. Translated from *Khimiya Prirodnikh Soedinenii*, No. 3, pp. 253-254, May-June, 2001. Original article submitted June 12, 2001.

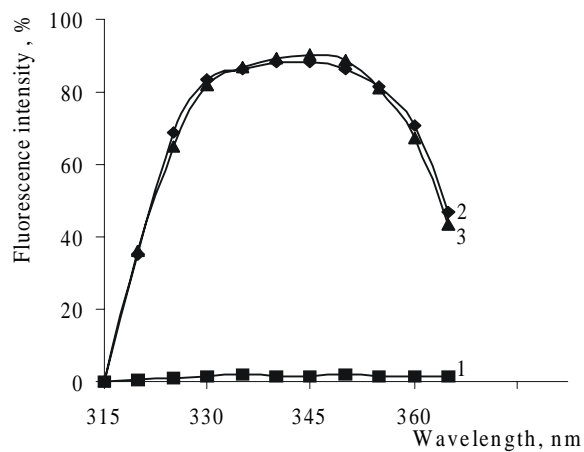


Fig. 2. Fluorescence spectra of decane emulsions stabilized with gossypin at pH 10 (protein:decane ratio 1:10 by mass): decane emulsion at pH 10 (1), gossypin at pH 10 (2), decane emulsion stabilized by gossypin (3).

The study of decane emulsions stabilized by gossypin showed (Fig. 2) that the fluorescence maximum (340 nm) does not change much if the nonpolar phase in the gossypin solution is emulsified at a protein:decane ratio of 1:10 (by mass). The fluorescence quantum yield also does not change, which indicates that the protein undergoes no significant changes under these conditions upon interaction with the nonpolar phase [4].

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