THE EFFECT OF SOLUBILIZATION ON THE DENATURATION OF EGG ALBUMIN

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Abstract

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THE EFFECT OF SOLUBILIZATION ON THE DENATURATION OF EGG ALBUMIN

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Solubilization of hydrocarbons by proteins has a substantial effect on the physicochemical and biological properties of protein molecules ($^{1-5}$). This is explained by the connection between the solubilization effect and the conformation of protein molecules. Just as changes in the conformation of protein molecules under the action of various factors (^{3}) cause a change in the solubilization effect, solubilization can also influence the conformation of protein molecules. The aim of the present work was to elucidate the effect of benzene solubilization on the conformational stability of a typical globular protein—egg albumin—toward thermal and acid–base denaturation.

Crystalline egg albumin was isolated from hen eggs by the standard method of Sørensen and Høyrup (^{6}) and was recrystallized four times. The albumin was then purified by dialysis and electrodialysis. The purity of the protein was checked by paper electrophoresis. The electropherograms obtained did not differ from those described in the literature (^{7}).

As the solubilized substance we used benzene purified by the generally accepted procedure (^{8}). Twice-distilled water was used for preparing the egg-albumin solutions.

To characterize the denaturation process we used the method of optical rotation. Rotation of the plane of polarization is extremely sensitive to changes in the conformation of protein molecules. It is true that there is no simple and explicit dependence between optical activity and protein structure, but the values of optical activity as a characteristic of the degree of denaturation are generally accepted and play an important role in the study of denaturation processes.

First of all, we investigated the conformational stability of egg-albumin globules to heating. To measure optical rotation we used a thermostatted cuvette, with a constant rate of heating in all cases.

Figure 1 presents the dependence of the specific optical rotation on temperature for a pure solution of egg albumin and for a solution that had solubilized benzene. It is seen from the figure that the sharp rise of curve 1 begins at 60°, whereas that of curve 2 begins at 70°. Hence one may draw the unequivocal conclusion that egg-albumin globules that have solubilized benzene become more stable with respect to thermal denaturation.

Data on the effect of the pH of the medium on the specific optical rotation of egg-albumin solutions are given in Fig. 2. The decrease in the magnitude of the specific optical rotation of egg-albumin solutions that have solubilized benzene (see curves 2 in comparison with curves 1) indicates that the globules become more symmetrical. On the curves, two regions with constant values of optical rotation are observed, expressed to a greater or lesser extent depending on the concentration: 1) at pH $4.7 \div 6.5$ and 2) at pH $8 \div 10.5$; evidently, in these interva···

···pH values the conformation of egg albumin molecules does not change. However, the globule conformations corresponding to the position of these two pH regions are evidently not identical, although in the literature [9] it has until now been considered that the globules of egg albumin are unchanged in the pH interval 4.5–10.5. It was of interest to determine what conformational changes are associated with the appearance of the second plateau at pH 8–10.5. For this purpose the reversibility of changes in optical rotation at pH 9 and 12 was studied. The solutions were acidified to the same pH value, 6.5. As the results of these experiments showed, the changes in optical rotation are reversible for egg-albumin solutions on going from pH 9 to pH 6.5; thus, in a 0.6% solution the specific optical rotation changed from -31.6° to -30.8°.

Fig. 1. Dependence of the specific optical rotation of an egg-albumin solution (1) and of a solution of egg albumin that had solubilized benzene (2) on temperature. $C = 0.55\%$, pH 7.2.

On changing the pH from 12 to 6.5 in solutions of the same concentration, the specific optical rotation changed from 45.2° to 31.8°, i.e., complete reversal was not observed. The reversibility of the change in the specific optical rotation of egg-albumin solutions at initial pH 9, and also the small magnitude of this change, indicate that the appearance of the second region with a constant value of the specific rotation is caused not by a change in the degree of helicity, but rather by deformation changes in the tertiary structure of the protein molecules [11]. The appearance of the second plateau may be regarded as predenaturation changes preceding the irreversible disruption of the conformation of the macromolecules at pH > 11, associated with disruption of the native structure of the globule.

Fig. 2. Dependence of the specific optical rotation of egg-albumin solutions (1) and of a solution of egg albumin that had solubilized benzene (2) on the pH of the medium.
a–$C = 0.7\%$; b–$C = 1.27\%$; c–$C = 2.1\%$.

Comparison of curves 1 and 2 in Fig. 2 shows that sharp rises in the curves (an increase in the negative specific rotation) for solutions of pure egg albumin are observed at lower pH values of the medium (in the alkaline region) and at higher pH values of the medium (in the acid region) than for solutions of egg albumin that had solubilized benzene; moreover, the effect is more pronounced in the dilute solution (Fig. 2). These results indicate an increase in the stability of albumin toward acid-alkali denaturation after solubilization of benzene.

The increase in the stability of egg-albumin globules toward thermal and acid-alkali denaturation is probably caused by interaction of benzene with hydrocarbon portions of the chain and by strengthening of intramolecular van der Waals cohesive forces.

On the basis of the results of experiments on thermal and acid-alkali denaturation of pure egg albumin and of egg albumin that had solubilized benzene, one can explain the phenomenon, hitherto remaining unclear, that less pure extracts or mixtures of protein preparations are more resistant to thermal denaturation than maximally purified ones [10].

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Note: Figure translations are in progress. See original paper for figures.