Stability of 11S globulin from Vicia faba seeds

Studies using differential scanning microcalorimetry

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Heat denaturation of 11S globulin, a dodecameric globular protein isolated from Vicia faba seeds was studied using scanning microcalorimetry at pH 7.6 and NaCl concentrations from 0 to 1 M. The specific enthalpy of denaturation was shown to be a linear function of temperature. The ratio of the calorimetric enthalpy to the effective one (Van't-Hoff's) per protomer of 11S globulin was 0.9 ± 0.06. It is concluded that at first approximation 11S globulin protomers denatured independently in conformity with the two-state model. The plotted temperature-dependent specific free energy of 11S globulin denaturation at different NaCl concentrations demonstrated that an increase in the salt content brought about the rise in protein stability. The maximum 11S globulin stability is reached at about 300°K. The molar free energy of denaturation at 300°K in 1 M NaCl is 918 kJ/mol.

Key words: differential scanning microcalorimetry; heat denaturation; 11S globulin

Stability is one of the most important physicochemical characteristics of proteins. It depends on their structure and the interaction with solvents. The change of Gibbs’s free energy at the full protein unfolding (1, 2) is the measure of protein stability. The thermodynamic parameters of the protein denaturation can be determined by various techniques. Calorimetry is the method of choice, since it requires no information on the mechanism of the denaturation process (1). An approach to stability analysis with respect to small globular proteins using differential adiabatic scanning microcalorimetry has been developed by Privalov et al. (1, 3). However, few reports are currently available on application of this method to oligomeric globular proteins (4–6). This paper presents the results of studies on heat denaturation of dodecameric 11S globulin isolated from Vicia faba seeds.

MATERIALS AND METHODS

Materials

11S globulin was isolated from Vicia faba seeds (var. Orlovskie-41, 1982 crop) by selective heat denaturation. It is known that the denaturation temperature of 11S globulin is higher than that of 7S globulin (7). This circumstance makes it possible to use the principle of selective thermal denaturation for isolation of 11S globulin. The thermal denaturation of 7S globulin makes its separation from 11S globulin easier due to the fact that the denatured forms
usually have a more hydrophobic accessible surface than the native form (8, 9). This difference promotes separation of the indicated forms according to their solubility in media with low lipophilicity, for instance in salt solutions.

The isolation of 11S globulin was carried out without preliminary defatting of the meal. The extraction of proteins was performed for 30 min with 0.37 M NaCl solution at pH 5.0 and a meal to water ratio of 1 to 10. Such extraction has been shown to cause the transfer of only proteins into the solution while lipids remain in the residue. The extract was clarified by centrifuging, then the ionic strength of the solution was reduced by half by dilution with water to precipitate globulins. The residue obtained after centrifugation was dissolved in water, and its pH adjusted to 7.0 with 0.1 M NaOH solution. The solution was heated to 80° at a rate of 2 deg/min, then cooled by a two-fold dilution with water. The pH of the solution was adjusted to 5.0 with 0.1 N HCl solution to precipitate globulins. After centrifugation the residue was extracted with a 10-fold volume of 0.5 M NaCl solution. The extract was clarified by centrifugation and the ionic strength of the supernatant then reduced to 0.2 M by dilution with water. The precipitate obtained after centrifugation was dissolved in water, and its pH adjusted to 7.0 with 0.1 N NaOH solution. According to velocity sedimentation analysis, the preparation displayed a single symmetrical peak.

Hen egg white lysozyme (Type A "Biochimreactiv", Olaine, USSR) with the activity of 26000 units was used as a calorimetric standard without additional purification. Salt and other reagents were all of analytical reagent grade.

Stock solutions of 11S globulin (~10%) containing 0.02% sodium azide were kept in darkness at room temperature in hermetically-sealed glass vessels. Under these conditions sedimentation and thermogram patterns of the protein remain unchanged for a month. 11S globulin concentration in the solution was determined by evaporation at 105 ± 5° to a constant weight. The lysozyme concentration was determined by measuring the absorbance at 280 nm and using a value of 26.4 for $E_{1\text{cm}}^{1%}$ (10). 11S globulin solutions for sedimentation and calorimetric studies were prepared by diluting the 10% solution. 0.25% protein solutions in phosphate buffer with pH 7.6 and ionic strength 0.01 were used for calorimetric analysis. The ionic strength of buffer was regulated by adding NaCl.

METHODS

Sedimentation analysis was carried out on the ultracentrifuge MOM 3170B with a double sector cell and a Schlieren optical system at 50 000 rev/min and 20°. The homogeneity of 11S globulin was determined in a phosphate buffer (2.6 mM KH$_2$PO$_4$, 32.5 mM K$_2$HPO$_4$, 0.4 M NaCl) with pH 7.6 and ionic strength 0.5.

Calorimetric measurements were carried out on a differential adiabatic scanning microcalorimeter DASM-1M (Special Design Office of Biological Instrument Making, the USSR Academy of Sciences, Pushchino, USSR) in a temperature range of 20–110°, the scanning rate of 2 deg/min and the excess pressure of 1 atm. In each experiment the heat capacity scale of the microcalorimeter was calibrated by Joule-Lentz effect. Reliability of the electrical calibration was additionally checked by using hen egg white lysozyme as the calorimetric standard. According to Khechinashvili's data (11) the temperature and the specific enthalpy of its denaturation in 0.01 M glycine buffer at pH 2.5 were 338.8°K and 34.8 J/g respectively; the figures were 338.8°K and 33.5 J/g according to our data. The results of both studies are thus in good agreement. Integration of thermograms in the protein's denaturation range was carried out with the planimeter, taking into account a sharp alteration in the heat capacity ($\Delta Q_d$) at the denaturation temperature. Effective (Van't-Hoff's) enthalpy of denaturation was calculated according to eqn. 1 (12).

$$\Delta H^\text{v-H} = 4RT_d^2 \Delta C_d / Q_d$$  (1)

where $T_d$ = denaturation temperature, K; $\Delta C_d$ = peak height, J/K; and $Q_d$ = area under peak J.

Molar calorimetric enthalpy of denaturation was determined from the correlation

$$\Delta H^\text{cal} = \Delta Q_d \cdot M$$  (2)
11S globulin from Vicia faba seeds

FIGURE 1
Temperature dependences of specific excess heat capacity of 11S globulin solutions (phosphate buffer, pH 7.6, μ = 0.01) at NaCl concentrations of 0 M (1), 0.1 M (2), 0.5 M (3), 1.0 M (4).

where ΔdH = the specific enthalpy of denaturation, J/g; and M = 328 kD, the molecular weight of 11S globulin (13).

The thermodynamic parameters of denaturation were calculated as proposed in ref. 3.

RESULTS

Fig. 1 shows temperature-dependent changes of specific excess heat capacity of 11S globulin solutions at different NaCl concentrations. An increase in the salt level results in the rise of denaturation temperature, but the heat capacity change at the denaturation temperature remains unchanged, ΔdC\_p = 0.3 ± 0.03 J/gK. The dependence on NaCl concentrations of the temperature and the specific enthalpy of 11S globulin denaturation is shown in Figs. 2, 3.

It can be seen that the denaturation enthalpy also rises with increasing NaCl concentrations. The specific enthalpy of 11S globulin denaturation was a linear function of denaturation temperature (Fig. 4). Its slope (according to Kirchhoff's law) corresponds to the heat capacity change, ΔdC\_p = dΔh\_d/dT. The resulting value is ΔdC\_p = 0.33 J/gk, which virtually coincides with the value of denaturation heat capacity change determined direct from the individual calorimetric recordings (Fig. 1).

DISCUSSION

It follows from Fig. 1 that the difference between heat capacity values of denatured and native 11S globulins is independent of NaCl concentrations and equals ΔdC\_p = 0.3 ± 0.03 J/gK. On the other hand, the specific enthalpy of denaturation is a linear function of temperature (Fig. 4); its slope being equal to 0.3 J/gK, which virtually coincides with the value of denaturation heat capacity change determined direct from the individual calorimetric recordings. It is inferred from these data that secondary processes are of no importance; the determined thermodynamic parameters thus obtained can be accounted for in terms of conformational
transition of 11S globulin from native to
denatured state (1).

The two-state model is usually applied to
describe the protein denaturation process (1). There are a number of tests which are used to prove the applicability of this model for describing the protein denaturation process (14). The determination of the ratio of the calorimetric enthalpy $\Delta H^\text{cal}$ to the effective enthalpy (Van't-Hoffs) $\Delta H^\text{v-H}$ is one of them. This ratio being equal to $\sim 1$, the model may be considered applicable to the description of the denaturation process. The ratios of the calorimetric to the effective enthalpies in 11S globulin denaturation are presented in Fig. 5. Obviously, the ratio is independent of the denaturation temperature. It constitutes $10.8 \pm 0.7$ (the significance level is 95%). However, it would constitute $0.9 \pm 0.06$ if we take into account that 11S globulin consists of 12 protomers (13). It is concluded that at the first approximation 11S globulin protomers denatured independently, in conformity with the two-state model.

Values determined experimentally (heat capacity change $-\Delta_d C_p$, specific enthalpy $-\Delta_d h$ and denaturation temperature $-T_d$) were used to calculate the temperature dependent specific enthalpy, entropy and free energy of denaturation according to eqns. 3-5 (15).

$$
\Delta_d h(T) = \Delta_d h + \Delta_d C_p (T - T_d) \tag{3}
$$

$$
\Delta_d S(T) = \Delta_d S + \Delta_d C_p \cdot \ln (T/T_d) \tag{4}
$$

$$
\Delta_d F(T) = \Delta_d h (1 - T/T_d) + \Delta_d C_p [(T_d - T) + T \ln (T/T_d)] \tag{5}
$$

Fig. 6 shows the temperature dependences of specific enthalpy and entropy of 11S globulin denaturation. It is similar to that obtained for "small" globular proteins. Privalov (1) has reported that globular proteins can be divided into two groups with respect to the values of specific enthalpy at 383°K. At this temperature, the specific enthalpy of the proteins in Group A is about 54.5 J/g, but that of the proteins in Group B is < 40 J/g. The proteins in Group A are considered more compact than those in Group B. The specific enthalpy of 11S globulin was 27.7 J/g at 383°K. Thus this protein belongs to Group B. The temperature depen-
Temperature dependences of specific free energy for denaturation of 11S globulin at different NaCl concentrations: 0.1 M (1), 0.3 M (2), 0.5 M (3), 1.0 M (4).

It shows that an increase in the level of salt enhances the specific free energy of 11S globulin denaturation, i.e. its stability. Maximum stability is reached at about 300°K. The specific free energy of 11S globulin denaturation in 1 M NaCl at this temperature is 3.1 J/g or 918 kJ/mol. The latter value is an order of magnitude greater than the average maximum molar free energy of denaturation for "small" globular proteins (1), which is not surprising, since 11S globulin consists of 12 protomers (13).

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