THERMAL DENATURATION OF ALBUMIN OF BOVINE BLOOD PLASMA

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INTRODUCTION

Process of thermal denaturation lies in the basis of production of thermotrophic gels of food proteins and also in the basis of method for thermal modification of their functional properties. To optimize regimes of thermotrophic gel-formation and thermal modification of blood proteins, information is needed on parameters of thermal denaturation of these proteins under different conditions.

MATERIALS AND METHODS

Serum albumin was derived from whole blood of beef cattle by method of ethanol fractioning (Mitschmann et al., 1954) with slight modifications. According to data of fast sedimentation, high-performance gel chromatography and gel electrophoresis by method of Davis (Davis, 1964) and Laemmli (Laemmli, 1970), the isolated protein was homogeneous and identical to defatted commercial preparations of beef serum albumin.

For microcalorimetric experiments albumin solutions were used in the corresponding buffer system : 0.01 glycine in acid and alkaline ranges and 0.01 phosphate buffer in the neutral range. Concentration of protein constituted 5.0-10.0 mg/ml.

Microcalorimetric measurements were conducted on differential adiabate scanning microcalorimeter "DASM-4" (scientific & industrial complex "Bioinstrument", Academy of Sciences of USSR, Pushino) in the temperature range 0-110°C and heating rate 2 K/min.

RESULTS AND DISCUSSION

Fig. 1 shows thermogram of albumin denaturation at pH 7.0 (curve 1). It contains 2 denaturation peaks: peak 1 with maximum at $T_{d,1}$=65°C and peak 2 with maximum at $T_{d,2}$= 79°C. Curves 2,3 and 4 present thermograms of albumin solution, pre-heated to 65°C, 74°C and 85°C, accordingly. Change in the form of thermogram after "annealing", points out to irreversible character of the process of albumin denaturation.

From gel chromatography data on the initial albumin solution and on albumin solution heated to 74°C, it is seen, that after "annealing" the system contains 2 components: native protein and component with a higher molecular weight. Relative content of these components constitutes 70 and 30%, correspondingly.

Chromatographic components of albumin after "annealing" were characterized calorimetrically. Component, corresponding to higher molecular weight, does not show cooperative transitions. Consequently, it represents aggregates of denaturated albumin molecules. The other component is characterized by one denaturation peak with maximum temperature, equal to temperature of maximum peak 2 on the thermogram of the initial albumin.

Complex profile of thermogram of albumin denaturation could be explained by at least two reasons: firstly, by availability of two or more domains with different thermodynamics in the rolled up polypeptide albumin chain and secondly, by any heterogeneity of preparation (however, both factors may serve reasons for that).

Reduction of the thermogram profile after "annealiation" of the preparation at temperatures not exceeding temperature of denaturation finish may serve criterion of the domain nature of the complex peak. It was established (Nitschmann et al., 1954) that defatted beef albumin is comprised of the three domains with different stability. As it is seen from fig. 1, initial bimodal thermogram of non-defatted albumin shifts into unimodal one during re-heating of the albumin solutions, initially heated to 74°C, that is to the minimum temperature between peaks 1 and 2. It means, that under these conditions part of molecules of the initial preparation denaturates irreversibly: the complex profile of thermogram of non-defatted albumin depends on heterogeneity of the initial preparation. Let us remember, that according to the data of gel-chromatography and gel-electrophoresis, the non-defatted albumin was homogeneous and identical to defatted commercial preparations of beef serum albumin. Consequently, in case of non-defatted albumin we deal with the system of molecules similar by weight and charge, but different by conformation of native form.

In other words, non-defatted preparation of albumin is a mixture of 2 protein forms: form 1 with denaturation temperature 65°C and form 2 with denaturation temperature 79°C. The content of forms 1 and 2 in preparation equals 70 and 30%, respectively. Thermal denaturation of either of forms was irreversible.

Fig. 2 shows thermograms of albumin denaturation, obtained at different concentrations of sodium decanoate. At small concentrations of sodium decanoate significant stabilization of native conformation of albumin (form 1) is observed.

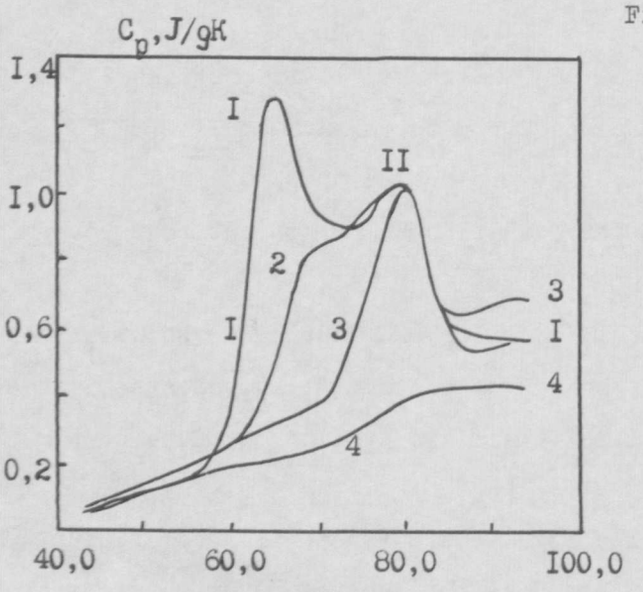


Fig. 1 Thermograms of albumin denaturation before (1)and after annealing at (°C): 65(2), 74(3), 85(4); 0.1 M phosphate; pH 7.0

At some concentrations of decanoate forms 1 and 2 become similar by conformational stability, in other words, initially heterogeneous preparation by its conformational characteristics becomes homogeneous. "Levelling" of stability of the two forms of protein is a sequence of the significant increase in form 1 stability at practically unchanging stability of form 2. It points out to interrelation of decanoate mainly with form 1. The effect of levelling of two forms of albumin stability is also observed at interrelation with sodium miristate. However, at significantly lower concentrations of ligand.

It is known that strengthening of interrelation of lypophilic ligand at the increase of its non-polar radical length is a characteristic feature of hydrophobic nature of this interrelation.

It can be supposed that preparation of non-defatted serum albumin presents a micro-heterogeneous system, comprised of molecules, different in the character of fatty acids binding. Assumption on micro-heterogeneity of non-defatted albumin is in accordance with earlier obtained scientific data.

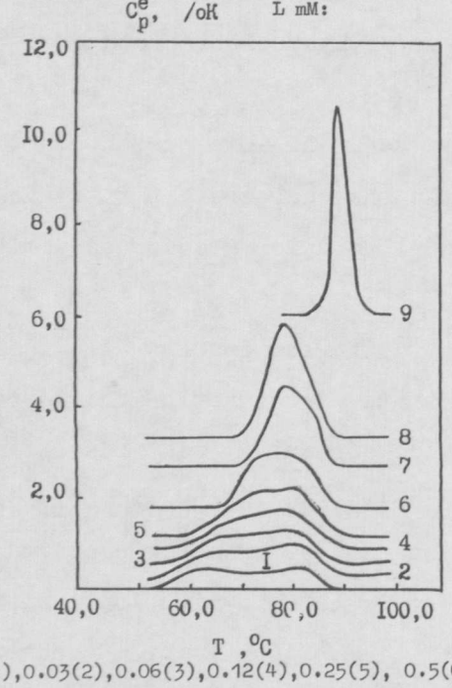
Fig. 3 shows thermograms of albumin denaturation at different concentrations of sodium chloride. In the whole studied range of salt concentrations, denaturation thermograms are bimodal. Increase of ionic strength due to the presence of neutral salt results mainly in the increase of denaturation temperature (and probably of enthalpy) of form 1 of protein. This tendency agrees with the data on salt effect on denaturation temperature of defatted albumin (Gumpen,Hegg, 1979). Obviously, form 1 presents albumin with low content of bound fatty acids, that is with free strong centers of binding. Significant increase of form 1 stability with the increase of salt concentration points to significant contribution of electrostatic interrelation to stabilization of "rolled up" conformation of this form.

Denaturation temperature of form 2 stays constant till concentration of salt achieves 2M. It means that electrostatic interrelations do not contribute much to stabilization of albumin of form 2. Similar result was obtained during study of the effect of sodium chloride on the stability of albumin complex with lauric acid (Gumpen, Hegg, 1979). Thus, form 2 of protein is in fact albumin with higher (as compared to form 1) content of bound fatty acids, that is form with saturated strong centers of binding.

Fig. 4,5 show changes in thermograms of albumin denaturation at acid and alkali pH-ranges. At pH 6.5-6.0 abrupt fall of peak 1 takes place, while peak 2 practically doesn't change. At further lowering of pH, changes in thermograms bear a more monotonous character. After annealing of albumin solution (pH 5.0) to temperatures inside transitional area, profile of the thermogram is restored. It means, that complex profile of thermogram in acid region is explained not only by micro-heterogeneity of albumin, but it also reflects domain character of the rolled up polypeptide chain of a less stable form 1 of protein. Transition to unimodal thermogram at pH 4.0 is connected with "melting out" of a less stable domain of this form. This conformational transition is known in literature as N-F transition (Foster et al, 1965; Foster, 1977; Petersen, Foster,1965).

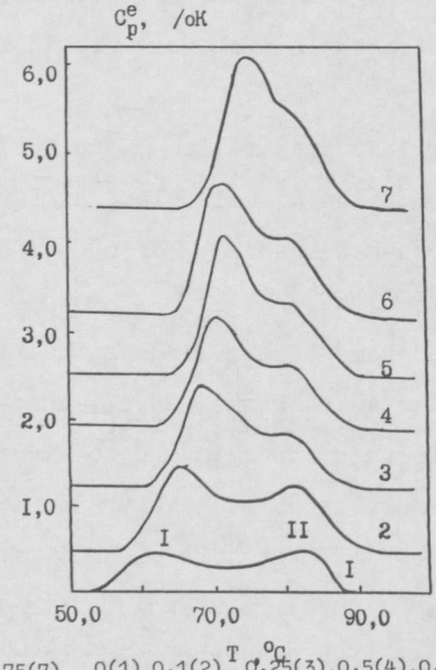
Fig. 5 shows thermograms of albumin denaturation at alkali pH. One can see a common tendency to widening of conformational transition with pH rise. In pH range from 6.5 to 9.5 denaturation temperature of albumin of form 1 lowers significantly (fig.5). Denaturation temperature of albumin of form 2 in this range changes slightly. This fact again confirms conclusion about secondary role of electrostatic factors in stabilization of albumin form, saturated with fatty acids.

Fig.2 Thermograms of albumin denaturation at different concentrations of sodium decanoate



0(1),0.03(2),0.06(3),0.12(4),0.25(5), 0.5(6),0.75(7) 1.0(8),10(9). 0.01 phosphate buffer; pH 7.0

Fig. 3 Thermograms of albumin denaturation at different concentrations of NaCl, M:



0(1),0.1(2), 0.25(3),0.5(4),0.75(5), 1.0(6), 2.0(7). 0.01 buffer, pH 7.0

Fig.4 Thermograms of albumin denaturation at different pH-values:7.0(1),6.5(2),6.0(3), 5.5(4),5.0(5), 4.7(6),4.5(7), 4.2(8),4.0(9).

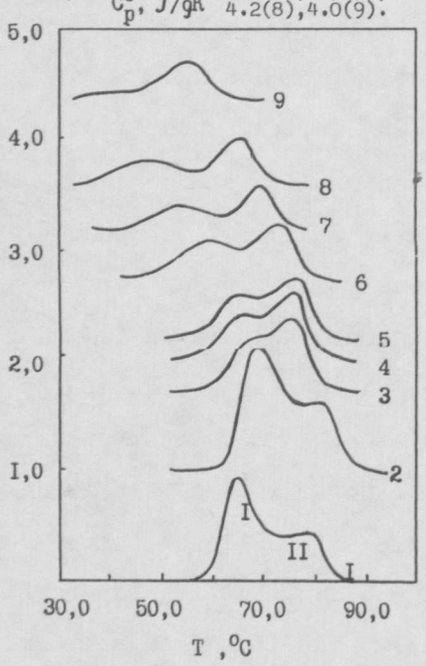
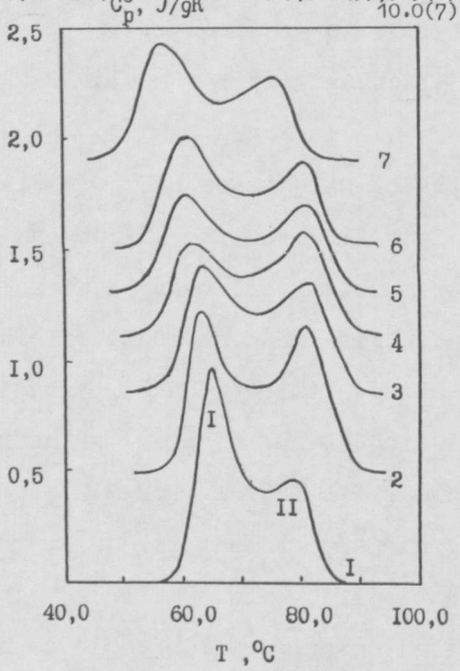


Fig.5 Thermograms of albumin denaturation at different pH-values (continuation): 7.0(1), 7.6(2),8.0(3), 8.6(4),9.0(5),9.5(6), 10.0(7).



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