A NOTE CONCERNING THE THERMAL UNFOLDING-REFOLDING PROCESS OF PROTEINS

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abstract: A thermodynamic study of bovine serum albumin thermal denaturation reveals significant differences between slow and fast refolding processes. The values of the thermodynamic parameters Gibbs energy, enthalpy and entropy calculated under these conditions suggest that the molecular species resulting form a very slow refolding procedure are closer to the native state of the molecule, unlike the ones obtained after a much faster cooling step during refolding.

Introduction

The processes of protein thermal denaturation consist of irreversible modifications of their secondary structure and of a number of their physical, chemical and biological properties [1]. The denaturation process can be represented by modification of the ratio between tow micro-states: the unfolded one, which is strongly influenced by the solvent, and the (re)folded one, characterised by a high order degree of the molecular structure [2].

Studies on bovine serum albumin (BSA) show that this molecule is able to interact with other proteins through both hydrophobic and hydrophilic interactions. In the native (folded) state, BSA adopts a conformation in which the polar residues are oriented towards the outer surface of the molecule, while the non-polar residues are generally directed toward the inner core of the molecule [3].

The main effect of heat on the protein conformation consists of the redirection of the aminoacid side-chains, which triggers a change in the hydrophobic-hydrophilic equilibrium [4-5].

The present note brings quantitative evidence for the effects of slow and rapid refolding of denaturated BSA molecules, using a thermodynamic approach.

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Experimental procedure

Aqueous solution of BSA (Sigma, fraction V) used without previous protein purification at a concentration of 5 mg ml^{-1}, were analysed for thermal denaturation followed by renaturation using two cooling conditions: very slow cooling of 1.5ºC min^{-1} (**sample B**), and rapid cooling at -20ºC (**sample C**).

Sample A representing the initial thermal unfolding of BSA molecule was considered as reference.

Using a differential dilatometric method, initial volumes of about 12 ml BSA solutions were determined at various temperatures up to 70ºC.

At each temperature, the denaturation time was 15 minutes.

The volumetric resolution scale was of 0.5 µl. The initial densities of the BSA solution (sol) and of the solvent (solv) were determined, and the corresponding values at each temperature were computed.

The partial specific volume ($\overline{V}^t_{BSA}$) calculated at various concentrations (c) and temperatures (t), using the equation:

$$\overline{V}^t_{BSA} = \frac{1}{d^t_{solv}}\left(1 - \frac{d^t_{sol} - d^t_{solv}}{c}\right) \quad (1)$$

For each temperature, the equilibrium constant K_D of the one-step equilibrium considered in the thermal denaturation process

$$(BSA)_N \leftrightarrow (BSA)_D \quad (2)$$

was obtained using the relation:

$$K_D = \left(\overline{V}^t_{BSA} - \overline{V}^N_{BSA}\right)\left(\overline{V}^D_{BSA} - \overline{V}^t_{BSA}\right)^{-1} \quad (3)$$

where N and D stand for native and denaturated molecule, respectively.

Results and Discussion

The values of the partial specific volume $\overline{V}^t_{BSA}$ and Gibbs enthalpy ΔG^o (where $\Delta G^o = -RT\ln K$) were calculated for the samples A, B and C, after two independent parallel measurements for each sample, and using 5 points of linear correlation. The results are shown in Table 1.

From the linear regression of ΔG^o as a function of temperature, the values of ΔH^o and ΔS^o were obtained (Table 2), where N is the number of ΔG^o (T) considered, and R is the correlation coefficient.

Table 1. $\overline{V}^{t}_{BSA}$ **and ΔGº values**

Sp	T(K)	298	303	308	313	318	323	328	333	338	343
A	$\overline{V}$	0.761	0.687	0.614	0.541	0.458	0.400	0.382	0.363	0.345	0.326
	ΔGº (KJ/mol)		4052	1750	-54	-1930	-4274	-5270	-6614	-8795	
B	$\overline{V}$	0.643	0.601	0.559	0.517	0.476	0.446	0.421	0.395	0.370	0.345
	ΔGº (KJ/mol)		4588	2411	816	-649	-1808	-2947	-4466	-6769	
C	$\overline{V}$	0.508	0.431	0.354	0.277	0.200	0.239	0.191	0.143	0.094	0.046
	ΔGº (KJ/mol)		4086	1787	-2.63	-1846	-900	-2147	-3696	-6099	

Table 2. ΔHº and ΔSº values

Sample	ΔSº (J/Kmol)	ΔHº (KJ/mol)	N	R
A	357	112	8	0.996
B	301	95	8	0.995
C	252	80	7	0.977

The differences of Gibbs energy ΔGº, of enthalpy ΔHº and of entropy ΔSº between the three conditions of the studied denaturation processes suggest that the very slow refolding procedure allows the refolding of some molecular structures nearer the original N state of the molecule, while a cooling shock led to much perturbed molecular states of the refolded molecule.

These differences in the thermodynamic parameters between the slow and fast refolding processes are relevant to support such a hypothetical mechanism.

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