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Low-temperature heat capacity of biomacromolecules and the entropic cost of bound water in proteins and nucleic acids (DNA)[1]

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Abstract

On the basis of the heat capacity data for proteins and DNA, obtained in the wide temperature range (2–300 K), the amount of the entropic cost of bound water in biomacromolecules is determined. The entropic cost of transferring a single water molecule from the liquid to a site of biopolymers is: 66.9, 58.1, 10.4 and 15.5 J mol^{-1} K^{-1} for fibrous protein (collagen), nucleic acid (double helical DNA), globular protein (Ribonuclease A), desoxynucleotides (d(AMP), d(TMP), d(GMP), d(CMP)) mechanical mixture and DNA polynucleotide chains in the state of statistical coils, respectively. These correspond to transfer-free energy costs as follows (at 298 K): 19.15, 17.5, 3.7 and 4.6 kJ mol^{-1}, respectively. We emphasize that the transfer entropy values determined here are not to be confused with the "entropy of hydration" of polar and nonpolar groups in biopolymers, which are relevant to the thermodynamics of protein folding or DNA double helix winding–unwinding. © 1998 Published by Elsevier Science B.V.

Keywords: Bound water; Entropic cost; Heat capacity; Nucleic acids (DNA); Proteins

1. Introduction

J.D. Dunitz [1], in his interesting and really perspective article about entropic cost that must be associated with the binding of water molecules to proteins or other macromolecules, on the basis of the entropic cost obtained for crystals of inorganic salts, speculates on the amount of the entropic cost: "We have a range of between 0 and 29.3 J mol^{-1} K^{-1} for the entropy cost of transferring a water molecule from the liquid to the protein, corresponding to a free energy cost of between 0 and 8.4 kJ mol^{-1} at 300 K." He correctly observes that "... science has become so specialized that its practitioners in one branch are all too often unaware of what is common knowledge in another" [1]. He states that thermodynamic data from which these entropy changes can be calculated are "nonexisting". However, data for calculating dS/dn, the entropy change occurring when a mole of water is transferred from liquid water to solid protein as a function of (n), the moles of water bound per mole of protein, can be obtained from measurements of sorption isotherms of water vapor on solid proteins at several temperatures [2–6].

We have the possibility to calculate the amount of the entropy cost of bound water in biomacromolecules, more directly, on the basis of heat capacity data for proteins and nucleic acids (DNA) at different levels of hydration (see Refs. [7,10]). This, clearly, is one correct way to obtain the experimental entropies of this systems.

Actually, knowing the temperature dependence of the heat capacity of the condensed systems over a wide temperature range, we not obtain the possibility of

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describing only the specificity of the thermal vibrational spectrum, caused by the peculiarities of the structure of the object under investigation, but also of determining the absolute values of the thermodynamic functions as well, including the entropy S^0_T:

$$S^0_T - S_0 = \int_0^T \frac{C_p}{T} dT - \frac{\Delta H_i}{T_i}$$

where C_p is the heat capacity at constant pressure, ΔH and T the enthalpy and temperature of the ith isothermal phase transition, and $\Delta S_i = \Delta H_i/T_i$ the corresponding entropy. In order to exclude the arbitrary constant of integration, it is obvious, that the measurement of the heat capacity of the object under study should be started from the lowest temperature possible (i.e. from a temperature, close to 0 K) [7,10]. S_0 for a perfectly ordered crystal is conventionally equated to zero (Ice-1 is not perfectly ordered at 0 K and the residual entropy of ice is equal to 34 J mol^{-1} K^{-1} [11], but this does not affect our results, discussed in the following (see, also, Ref. [1]).

2. Experimental

2.1. DNA and protein samples

We used the super-pure samples of Na-DNA of cow spleen (42% GC) and Na-DNA of salmon sperm (ca. 41% GC), the fiberprotein – acid soluble rat skin collagen and globular protein – pancreatic ribonuclease – A (RNase A). The amount of water in native specimens of DNA and proteins was controlled by their exposition at a definite relative humidity as well as by means of slow evaporation of the solvent from polymer gels in calorimetric cells followed by weighing (with accuracy −1 mg). Bidistilled water served as the solvent.

The calorimetric experiments were made for DNA with three water contents:

$n = 0 - 2\ M_{H_2O}/M_{BP}$ (0.050 g_{H_2O}/g_{DNA});
$n = 10 - 12\ M_{H_2O}/M_{BP}$ (0.326 g_{H_2O}/g_{DNA});
$n = 22 - 23\ M_{H_2O}/M_{BP}$ (0.621 g_{H_2O}/g_{DNA}) (see, also, Ref. [8]).

The calorimetric experiments were made also for RNase samples with three moisture contents: 0.03; 0.28; 0.67 $g_{H_2O}/g_{protein}$ (see, also, Ref. [10]) and for dehydrated and hydrated acid soluble collagen (0.35 $g_{H_2O}/g_{collagen}$) specimens [7,11].

2.2. Calorimetry

The low-temperature calorimetric device by means of which the measurements have been carried out, is described in Refs. [8,12]. The main specifications are as follows; the operating temperature range was 2, 5–30 K (standard germanium resistance thermometer); 10–400 K (standard platinum resistance thermometer); the working volumes of the calorimetric cells were 0.4 and 2 cm^3; the temperature was measured by means of a double bridge (Automatic System Laboratory, model A7, 8). The measurements were done in series with the "step" along the temperature interval 1–2 K; all readings data were processed on a computer connected to an "on-line" calorimeter. The maximum error of the heat capacity measurements did not exceed 1% (in the interval 2–5 K), 1.5% (in the interval 5–15 K) and 6% (in the interval 20–300 K).

3. Results and discussion

Figs. 1–3 show the temperature dependence of heat capacity for native DNA, fiber (collagen) and globular (ribonuclease A) proteins, at different contents of water. The heat-capacity peculiarities observed in DNA at very low temperatures are given separately (Fig. 1; the character of this low-temperature "transition" is described in detail elsewhere [13]). A comparison of calculated standard entropies for anhydrous and hydrated proteins and Na-DNA (see Table 1) indicates that each bound-water molecule in this biopolymer contributes a different amount to the standard entropy (ca. 2.92–59.20 J mol^{-1} K^{-1}). These results demonstrate that water in the triple helix of collagen (structural protein of connective tissues) and in the double-helical molecule of heredity-DNA is more "ordered" than water in a typical globular protein (RNase A). The entropy contribution for collagen and Na-DNA can hardly be less than 59.2 J mol^{-1} K^{-1}, 53.6 cal mol^{-1} K^{-1}} (see Table 1) and 41.8 J mol^{-1} K^{-1} [1], the values for globular protein, nucleotides and inorganic salts, respectively. We emphasize that most hydrated biopolymers have

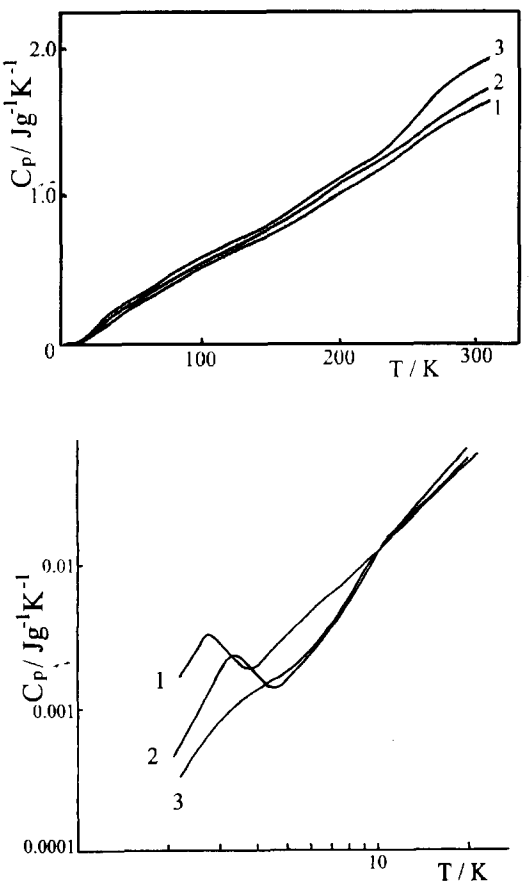

Fig. 1. (A) Temperature dependence of native Na-DNA (of salmon sperm (41% GC)) heat capacity at different water contents: (1) – 0–2; (2) – 8–10; (3) – 23 M H_2O/MBP. (B) Temperature dependence of native Na-DNA heat capacity at low temperatures (2, 5–30 K) (1) – 0–2; (2) – 8-10; (3) – 23 M H_2O/MBP. (see, also, Ref. [13])

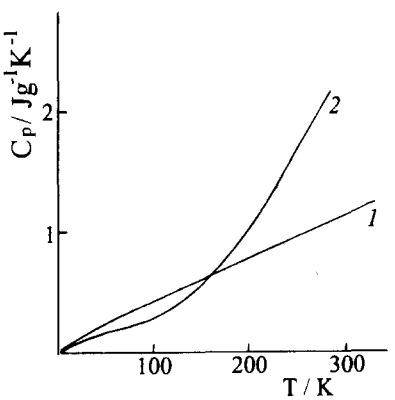

Fig. 2. Temperature dependence of native acid-soluble collagen heat capacity: (1) – dehydrated collagen; (2) – hydrated collagen (5 M H_2O/M tripeptide link).

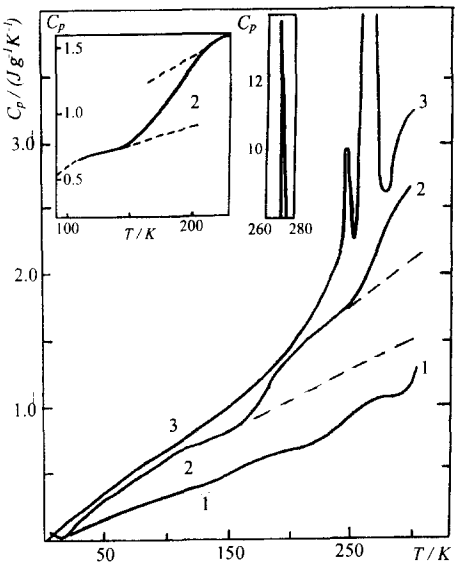

Fig. 3. Temperature dependence of native ribonuclease A heat capacity at different water contents: (1) – 23; (2) – 217; and (3) – 500 M H_2O/M protein).

glass transitions due to freezing of the cooperative motions of biopolymers and bound waters. Hence, the entropies of bound waters, which we obtained, include the residual entropies of biopolymers. For RNase, the calculation of the entropic cost is possible for curves 1 and 2, when all water is in the bound state.

It is well known that in aqueous solution the structure of native collagen triple helix stabilized not only by intermolecular hydrogen bonds but by the "fourth chain" formed by water molecules and tightly built into three polypeptide chains of the protein [7,14,15,25]. These water molecules inserted in the structure of triple helix were deprived not only of translational degrees of freedom, but of rotational ones, as well [12]. This gives the possibility to build the so-called "water-carbonyl helix" model of collagen molecules [15] (see, also, Ref. [25]).

The results of X-ray analysis of hydration of the fragments of a DNA single crystal demonstrates that water molecules also adopt definite equilibrium positions and orientations in the structure of double helix: water molecules near phosphate groups; "water spine" in a narrow groove of double helix in B-DNA; and "water pentagons" in a wide groove in A-DNA [16,17]. According to calorimetric experiments [8], the decrease in the entropy, accompanying

Table 1
Standard entropies (S^0_T) (298 K) of anhydrous biopolymers and their corresponding hydrates. For each hydrate, the number (n) of water molecules per mole of tripeptide links (for collagen), per mole of base pairs (for DNA and nucleotides) and per molecule (for RNase A) is given. Molecular weights of tripeptide link = 270 daltons, of base pairs = 700 daltons and of RNase = 13500 daltons). $\Delta S^0_T/n$ represents the standard entropy difference per bound water molecule

Biopolymers and model compounds	S^0_T/ (kJ mol^{-1} K^{-1})	$\Delta S^0_T/n$/ (J mol^{-1} K^{-1} ± 10%)
Collagen		
0	0.376	—
$n = 5$	0.393	2.92
Na-DNA (41%GC)		
0	1.55	—
$n = 23$	1.82	11.7
Na-DNA (coils)		
$n = 23$	2.80	54.3
Ribonuclease A		
0	15.85	—
$n = 217$	28.70	59.2
Σ (Na-d(AMP), d(TMP), d(GMP), d(CMP)), (41% GC)		
0	20.69	—
$n = 10$	21.30	61.0
$n = 23$	21.91	53.6

the process of organization of the macromolecular structure of DNA, is mainly caused because of the immobilization of water molecules by the structure of the double helix [8]. The heat capacity of bound water in DNA double helix is 3.0–3.5 Jg^{-1} K^{-1} (midway between the heat capacity of ice (4.2 J/gK) and liquid water (2.1 Jg^{-1} K^{-1})). The partial specific heat capacity (at 298 K) of ordered equilibrium form of hydrated DNA (B-DNA), $C_p = 0.75$ Jg^{-1} K^{-1}, is less than the heat capacity of dehydrated, disordered DNA samples: $C_p = 0.96$ Jg^{-1} K^{-1} (see Refs. [8,9,18]).

All these results demonstrate that there is a variation in the values of entropy with n (see, also, Refs. [2–6]). Calorimetric investigations suggest that the binding of more tightly held water to a collagen and DNA can cause a decrease in biopolymer entropy as well as a decrease in water entropy [8,12]. But, it is clear that the intramolecular conformational change dramatically influences the mechanism of interaction of water molecules with biopolymers (compare the data for DNA, DNA in the state of statistical coils and nucleotides mixture, at equal levels of hydration $n = 23$). The denaturation increment of the entropy change $\delta S^0/n \equiv \Delta S_{T,\ coil}/n - \Delta S_{T,\ helix}/n = 42.6$ J mol^{-1} K^{-1}, caused by the destruction of ordered water clusters inside the structure of double helix, and of additional layers of "liquid-like" water, should be accompanied by an increase of heat capacity [19] and entropy.

Simultaneously, there is evidence (see Table 1) that immobilized water in globular protein structure has characteristics similar to those of loosely bound water in the channels of zeolites and related minerals, for which the entropy contribution is larger (ca. 58.5 to 62.3 J mol^{-1} K^{-1} [1]). This conclusion also fits well with the X-ray analysis of hydrated protein crystals; according to X-ray data, most water molecules occur on the polar surface of proteins and organize the H-bounded water network including the water present in the "hydrophobic channels" inside the globule [20]. The protein surface water, will behave more like liquid water, similar to the loosely bound nonstructured water, but individual water molecules at the protein surface may significantly contribute to the thermodynamics of folding [9,20]. Highly attractive may be the hypothesis that the native structure of protein is the result of a symbiosis of two fractal objects – the folded

globule and the fractals of bound water forming the hydrate layers of protein surface [21].

As in case of the results obtained for nucleotides, it is necessary to take note of the fact that, in this mechanical mixture, water is associated with the negatively charged phosphate groups and polar groups of nucleotides through strong ion–dipole and charge–dipole interactions [16,17] and, hence, to adopt some definite positions and orientations in this disordered – "chaotic" – structure; in this case, the absolute values of dS/dn, in a mechanical mixture of nucleotides and in polynucleotide chains in the state of statistical coils, coincide [8].

As the standard entropy (298 K) of liquid water is 69.8 J mol^{-1} K^{-1} [1,11], the entropic cost of transferring a single water molecule to a site in the structure of biopolymers is given by:

66.9 ± 8 J mol^{-1} K^{-1} – for fibrous protein – collagen; 58.1 ± 6.0 J mol^{-1} K^{-1} – for Na-DNA; 10.4 ± 1.0 J mol^{-1} K^{-1} – for globular protein – RNase A; and 15.5 ± 2.0 J mol^{-1} K^{-1} – for nucleotides and DNA "coils".

This corresponds to a transfer of free energy cost (at 298 K) as follows: 19.5 ± 2.0 kJ mol^{-1} – for fibrous protein – collagen; 17.5 ± 2.0 kJ mol^{-1} – for Na-DNA; 3.7 ± 0.4 kJ mol^{-1} – for globular protein – RNase A; and 4.6 ± 0.5 kJ mol^{-1} – for nucleotides and DNA "coils".

In this way, we attempt to answer the nontrivial questions related on thermodynamic of hydration of biopolymers. We obtain the precise quantitative data about how large really is the entropic cost of bound water in the main biomolecules, namely proteins and nucleic acids (DNA). We emphasize (see, also, Ref. [1]) that the transfer entropies determined here are not to be confused with the "entropies of hydration" of polar and nonpolar groups in biopolymers, which are relevant to the thermodynamics of protein folding [22,24]) or DNA double helix winding-unwinding [8]).

Now, we can ask a question: "Is the scandal, that so little is known about the interactions of macromolecules and their aqueous environment, about to be removed?" [23]. The point is that biopolymer functions, including enzyme catalysis, processes of biosynthesis, winding–unwinding and folding–unfolding processes in biomolecules, are complex, and water may play several roles that are interdependent, "possibly to such an extent that it may be inappropriate to try to separate them" ([20], p. 146); particularly, in terms of energy and entropy units.

It is true that conformation of macromolecules is determined by the primary structure of proteins and nucleic acids and by hydration mechanisms, and the latter determine the conformational dynamics of biopolymers [24]. As our understanding in protein folding and DNA winding–unwinding processes develops, the prediction of biomolecule conformational dynamic will become more accurate. Simultaneously, many diseases are themselves the result of errors in protein and DNA dynamics caused by mutations [24]. Hence, the interplay of physics of biopolymers, solvation chemistry theory and experiments and art of site-directed mutagenesis is likely to be major themes for future work.

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