Journal of Molecular Liquids 264 (2018) 579–584

Contents lists available at ScienceDirect

Journal of Molecular Liquids

journal homepage: www.elsevier.com/locate/molliq

Water and cold denaturation of small globular proteins

Antonio Grimaldi, Giuseppe Graziano *

Dipartimento di Scienze e Tecnologie, Università del Sannio, Via Port'Arsa 11, 82100 Benevento, Italy

ARTICLE INFO

Article history:
Received 20 January 2018
Received in revised form 9 May 2018
Accepted 21 May 2018
Available online 22 May 2018

Keywords:
Cold denaturation
Water density
H-bonds
Solvent-excluded volume effect
Water translational entropy
Chain conformational entropy
Electrostatic repulsion

ABSTRACT

Cold denaturation is an intriguing phenomenon that deserves special attention because its rationalization is surely linked to a deeper understanding of the molecular determinants of the marginal stability of small globular proteins dissolved in water and aqueous solutions. It is here reviewed and discussed a theoretical approach that has offered a reliable explanation for the occurrence of both cold and hot denaturations. A cornerstone of the approach is the recognition of the role played by the density of water and its temperature-dependence that are largely determined by the special energetic and geometric features of H-bonds. In fact, the density of water determines the magnitude of the solvent-excluded volume effect, which is a main ingredient of the hydrophobic effect. The relationship between density and solvent-excluded volume effect establishes a strong connection between the physico-chemical properties of water and the conformational stability of small globular proteins.

1. Introduction

Experimental studies have unequivocally demonstrated that small globular proteins in water and aqueous solutions undergo two reversible conformational transitions [1–3]. The usual hot denaturation happens on increasing the temperature above room temperature, and the unusual, yet universal, cold denaturation happens on decreasing the temperature below room temperature. It is unusual because stable small globular proteins show cold denaturation at very low temperature (i.e., well below 0 °C), and its direct observation needs a partial destabilization of folded structure [1–3]. Nevertheless, cold denaturation is an intriguing phenomenon because it is characterized by a negative enthalpy change (i.e., it is exothermic) and a negative entropy change that is difficult to associate with the attainment of an unfolded structure [1–3]. These evidences imply that water molecules have to play a pivotal role for the conformational stability of globular proteins, and the existence of two denaturation transitions as a function of temperature. Note that cold denaturation has been reported also for amyloid fibrils of α-synuclein [4].

Over the last thirty years several statistical mechanical models have been devised to try to reproduce the occurrence of two reversible conformational transitions [5–14]. The problem of these models is that: (a) the chain is represented as a self-avoiding walk on a regular lattice or something similar; (b) the role of water comes from the outside, inserting preconceived relationships for the temperature dependence of the hydrophobic effect (i.e., the effective interaction of nonpolar side chains with water [15]). The apparent success of these models does not lead to a real explanation of the molecular origin of cold denaturation.

Recently, one of us has devised a theoretical approach that, notwithstanding its simplicity, has provided a clear relationship between the physico-chemical properties of water and the occurrence of two reversible denaturation transitions for small globular proteins [16–18]. A cornerstone of the approach is the recognition of the role played by the density of water and its temperature-dependence that are largely determined by the special energetic and geometric features of H-bonds. Liquid density determines the solvent-excluded volume effect (associated with the need to make space-create a cavity in a liquid in order to host a solute molecule), whose magnitude and temperature dependence in water are different with respect to the other common liquids, due to the presence of the 3D H-bonding network [17]. The decrease in solvent-excluded volume occurring when the polypeptide chain folds leads to a gain in translational entropy of water molecules, favouring the folded structure. The stability of the latter, however, is opposed by the chain conformational entropy which decreases on folding. The balance between the translational entropy of water molecules and the chain conformational entropy rules the thermodynamic stability of small globular proteins [16–18]. This balance depends on temperature because the magnitude of the solvent-excluded volume effect is ruled by the small size of water molecules and by the temperature dependence of water density [19]. It is worth noting that Kinoshita and coworkers have developed a theoretical model, different from the present one, that, however, leads to a similar scenario for the molecular origin of cold denaturation and the conformational stability of small globular proteins [20,21].

* Corresponding author.
E-mail address: graziano@unisannio.it (G. Graziano).

https://doi.org/10.1016/j.molliq.2018.05.094
0167-7322/

2. Theoretical rationalization of cold denaturation

Experimental measurements have shown that small globular protein molecules populate in water and aqueous solutions only two macrostates in thermodynamic equilibrium condition [1–3]: the N-state, representing the ensemble average of folded conformations, and the D-state, representing the ensemble average of unfolded conformations. On this ground, it has been devised a theoretical approach [16–18] that leads to the following expression for the denaturation Gibbs free energy change (note that the N-state is stable when ΔG_d is positive):

$$\Delta G_d = \Delta\Delta G_c + \Delta E_a - T \cdot \Delta S_{conf} \quad (1)$$

where $\Delta\Delta G_c = \Delta G_c$(D-state) – ΔG_c(N-state) represents the difference in the reversible work to create in water a cavity suitable to host the D-state and the N-state, respectively; $\Delta E_a = [E_a$(D-state) – E_a(N-state) + ΔE(intra)] represents the difference in energetic interactions among the D-state and the N-state and surrounding water molecules, and the difference in intra-chain energetic interactions between the D-state and the N-state; ΔS_{conf} represents the conformational entropy gain upon protein denaturation. It is worth noting that Eq. (1) does not contain terms coming from the structural reorganization of water-water H-bonds upon chain denaturation. This happens because water-water H-bond reorganization is characterized by an almost complete enthalpy-entropy compensation and the corresponding Gibbs free energy contribution can be neglected, as emerged from different theoretical routes [22–26].

In any liquid ΔG_c is a purely entropic quantity [27,28], measuring the decrease in translational entropy of liquid molecules for the reduction of the accessible configurational space caused by the solvent-excluded volume associated with cavity creation. Of course, the ΔG_c magnitude depends strongly upon the liquid density and the size of liquid molecules, and water is special in both respects [19,28]. Note that the water accessible surface area [29], WASA, is a reliable measure of the solvent-excluded volume of the cavity, or better of the molecule to be hosted in the liquid. The $\Delta\Delta G_c$ contribution is always positive (i.e., it stabilizes the N-state) because: (a) the ΔG_c magnitude increases with cavity WASA, even though the van der Waals volume, V_{vdW}, of the cavity is kept fixed [30–32]; (b) a marked WASA increase is associated with protein denaturation [33]. Accepting these basic geometric grounds, it is reliable to assume that the polypeptide chain upon unfolding changes conformation-shape, but its V_{vdW} is practically not affected [16–18]. In this respect, it is important to recognize that the volume change associated with protein denaturation is a very small and negative quantity [34,35].

By recalling that the sphere is the 3D object possessing the smallest surface area for a given volume, the N-state of a 138-residue protein is modelled by a sphere of radius $a = 15$ Å, $V_{vdW} = 14{,}137$ Å^3 and WASA = 3380 Å^2 (in calculating WASA, the radius of the rolling water molecule is fixed to 1.4 Å). The corresponding D-state is modelled by a prolate spherocylinder of radius $a = 6$ Å, cylindrical length $l = 117$ Å, $V_{vdW} = 14{,}137$ Å^3 and WASA = 6128 Å^2 (the prolate spherocylinder choice should be qualitatively correct because it has been shown that the D-state of several globular proteins has a prolate ellipsoid shape [36]). All these numbers should be physically reliable [37]. The $\Delta\Delta G_c$ contribution is calculated by means of classic scaled particle theory [38], SPT, formulas for spherical and prolate spherocylindrical cavities in a hard sphere fluid [16], possessing at each temperature the experimental density of water at $P = 1$ atm [39]. The pressure-volume term does not provide a net contribution in classic SPT calculations because the N-state and the D-state have the same van der Waals volume and so $P \cdot [V_{vdW}$(D-state) – V_{vdW}(N-state)] = 0. The effective hard sphere diameter of water molecules has been fixed to the temperature-independent value of 2.80 Å [17]. The latter corresponds to the distance between H-bonded water molecules (i.e., it is not a fitting value), as indicated by the location of the first maximum in the oxygen oxygen radial distribution function of water determined by means of both neutron and X-ray scattering measurements [40,41]. In fact, the distance between water molecules in ordinary ice amounts to 2.76 Å [42].

The calculated $\Delta\Delta G_c$ quantity, shown in Fig. 1, is large positive with a parabola-like increasing trend on raising the temperature [17,18]. The $\Delta\Delta G_c$ temperature dependence comes from the combination of two things [17,19]: (a) the small density decrease below the temperature of maximum density, TMD(H_2O) = 4 °C, and the almost constancy of water density over a large temperature range (both caused by the directionality and strength of H-bonds); (b) the increase with temperature of the average kinetic energy of liquid molecules hitting the cavity surface (i.e., the RT factor occurring in all the theoretical formulas to calculate ΔG_c [16,27,38]).

A fundamental assumption of the original version of the approach is that the ΔE_a quantity is close to zero in water, due to a nearly perfect balance between protein-water energetic attractions in the D-state and N-state, respectively, and the intra-protein energetic attractions [16,17]. This implies that the denaturation enthalpy change, ΔH_d, is given by [37]:

$$\Delta H_d = \Delta E_a + \Delta\Delta H_r = \Delta\Delta H_r \quad (2)$$

where $\Delta\Delta H_r$ represents the enthalpy change associated with the structural reorganization of water-water H-bonds occurring as a consequence of protein denaturation. Therefore, the $\Delta\Delta H_r$ contribution should be proportional to the isobaric thermal expansion coefficient of the liquid [37], α_P, and it must be zero at TMD(H_2O), where $\alpha_P = 0$. According to statistical mechanics [43], α_P represents the ensemble correlation between volume fluctuations and enthalpy fluctuations, and the latter in water are usually attributed to the transient H-bond reorganization [42]. It is well established that ΔH_d has a strong temperature dependence [44], and there is a temperature, labelled T_H, at which ΔH_d becomes zero. Analysis of a large data set, consisting of 115 proteins [45], provided $\langle T_H \rangle = 277.5$ K ± 25 K, a value close to TMD(H_2O) [37]. This fact, emerged empirically from the analysis of experimental data, lends support to the reliability of the assumption $\Delta E_a = 0$, and to the idea that the actual contribution to ΔH_d comes from the structural reorganization of water-water H-bonds associated with protein denaturation. A "model" globular protein, tightly corresponding to the "rules" of the theoretical approach, should have T_H = TMD(H_2O). Actually, for several globular proteins $T_H \neq$ TMD(H_2O) and so $\Delta E_a \neq 0$; this

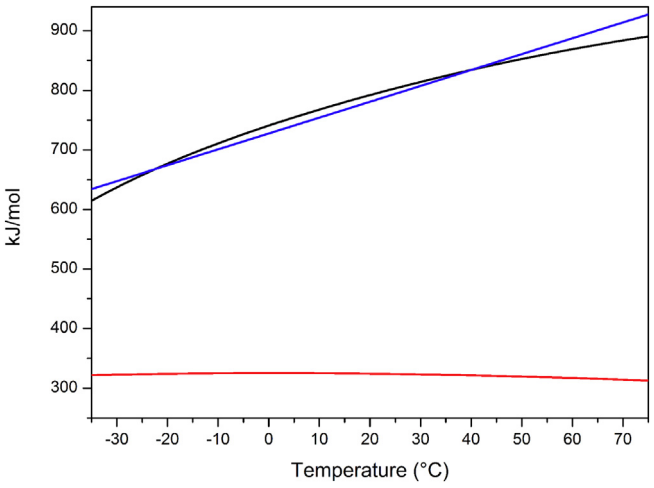

Fig. 1. Temperature dependence of the $\Delta\Delta G_c$ function, calculated via classic SPT, in water (black curve) and CCl_4 (red curve), and of $T \cdot \Delta S_{conf}$ (blue straight line) for the "model" globular protein. See text for further information. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)