

On the Thermal Expansion of Water and the Phase Behavior of Macromolecules in Aqueous Solution

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Dedicated to Professor Gérard Demazeau on the occasion of his 65th birthday

Water is crucial for the existence of life as we know it, and many have wondered what makes water so special. Here we point out the analogies between the pressure-temperature dependence of the isobaric thermal expansion of water (α_p) and the pressure-temperature phase behavior of macromolecules in aqueous solutions. We suggest that α_p could be the key to understand why water is to be the so-called ‘matrix of life’.

Key words: Water, Thermal Expansion, Polymers, Protein Stability, Phase Diagram

Introduction

Although the role of water in the biosphere has been recognised for a long time, this role is not often related with the physical properties of water [1–6]. From a macroscopic, planetary viewpoint one could easily draw the conclusion that the hydrosphere mainly functions as a thermostat, a device that keeps the temperature of this planet within a range that is suitable for the stability of a number of biomacromolecules such as proteins, nucleic acids, lipids and sugars. On going from the planetary to the molecular scale one becomes aware of the more fundamental role of water in the functioning of these biomolecules and their chemical reactions. In reactions such as photosynthesis and breathing, water is a partner in the chemical transformations. Water is also essential in determining the structural properties of DNA [7]. In the case of enzymes it has been found that a minimum amount of water is needed for their function and activity [8]. Moreover, the breakdown of the hydration layer by an increase in temperature has been correlated with the onset of protein denaturation [9, 10]. From the viewpoint of protein dynamics, Frauenfelder and co-workers have pioneered a model in which the main changes in the conformation of a protein are coupled to the dielectric relaxation of the bulk solvent, whereas

local movements are coupled to the properties of the water in close contact with the protein [11]. The above examples illustrate that many experiments point at the role of the hydration water in the vicinity of the macromolecule rather than at the possible role of the bulk water in determining the biological properties of macromolecules.

Clearly, water not only represents what is often called the matrix of life, it also has a great influence on the dynamic and thermodynamic behavior of macromolecules. However, as pointed out by Finney: “Although much effort has gone into trying to understand the ways in which water is involved in these processes – for example, protein folding and stability, enzyme-substrate interactions – there has been much less focus on trying to identify the specific molecular characteristics of water that ‘Nature’ exploits...” [12]. In this paper we wish to draw attention to the correlation between the effect of temperature and pressure on the isobaric thermal expansion of water and the temperature-pressure phase diagram of proteins, water-soluble polymers, starch and microorganisms. It is argued that the thermal expansion of water is the key to our understanding of the role of water in phenomena such as protein unfolding and the liquid-liquid phase separation of polymer-water mixtures.

Physical Properties of Water

Water is a liquid at ambient temperature and pressure. Though this seems an obvious thing to state, many molecules of a similar molar mass are gases (*e. g.* H₂S). The liquid nature of water is due to hydrogen bonding and the strength of this bonding, which is several times the thermal energy kT . Hydrogen bonds, however, also introduce directionality, resulting in a quasi-tetrahedral geometry of the local environment of the water molecule. Many of the so-called anomalous properties of water arise from this directionality [12, 13]. Here we consider three anomalies related to the response functions of water. Both the isothermal compression β_T and the isobaric heat capacity C_p increase upon cooling, whereas the thermal expansion α_p decreases upon cooling (see, for instance, [14]). These response functions are defined as:

$$\alpha_p = \frac{1}{V} \left(\frac{\partial V}{\partial T} \right)_p \quad (1a)$$

$$\beta_T = -\frac{1}{V} \left(\frac{\partial V}{\partial p} \right)_T \quad (1b)$$

$$C_p = \left(\frac{\partial H}{\partial T} \right)_p \quad (1c)$$

Moreover, β_T and C_p are proportional to the fluctuations in volume and enthalpy, respectively, whereas α_p is the cross-term between the enthalpy and volume fluctuations.

$$\langle \delta V^2 \rangle = k_B T V \beta_T \quad (2a)$$

$$\langle \delta H^2 \rangle = k_B T^2 C_p \quad (2b)$$

$$\langle \delta H \delta V \rangle = k_B T^2 V \alpha_p \quad (2c)$$

where δ denotes the fluctuation of a given variable about its mean value.

The p, T -dependences of α_p , β_T and C_p were first determined by Bridgman and further refined by others [15, 16], and are shown in Fig. 1. One can immediately see that β_T and C_p both decrease with increasing pressure, regardless of the temperature. In contrast, α_p increases with pressure at temperatures below $\sim 44^\circ\text{C}$, reaching a maximum at ~ 400 MPa, but decreases at temperatures above 44°C . At higher pressures α_p shows a weak, slightly negative pressure dependence. Presumably this is due to the fact that water behaves more like a normal liquid at high pressure,

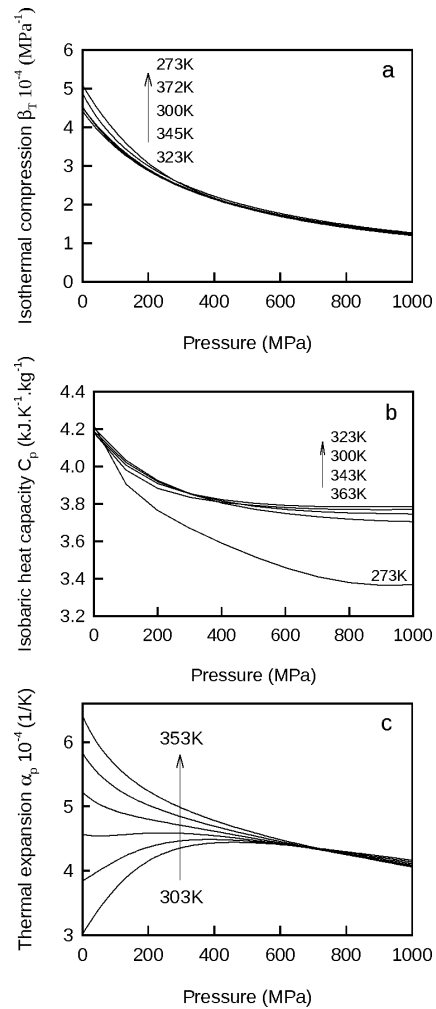


Fig. 1. Pressure-temperature dependence of the isothermal compression β_T (a), the isobaric heat capacity C_p (b), and the thermal expansion α_p (c) of water. The temperature increases in steps of 10 K in (c). These graphs were calculated using the NIST/ASME Steam Properties Database Version 2.1.

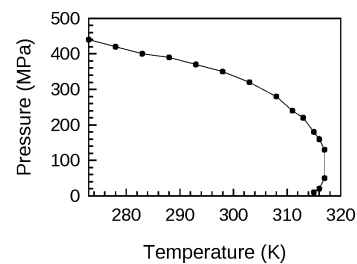


Fig. 2. An alternative representation of the p, T -dependence of α_p . The contour of the ellipse represents the p, T -combinations where $(\partial\alpha_p/\partial p)_T = 0$. Within the ellipse $(\partial\alpha_p/\partial p)_T > 0$, and outside the ellipse $(\partial\alpha_p/\partial p)_T < 0$.

as high pressures disrupt the tetrahedral-like geometry of the water network. Fig. 2 illustrates the p, T -dependence of α_p in another way, obtained by plotting the points where $(d\alpha_p/dp)_T = 0$. The significance of this representation will be discussed below.

Temperature-Pressure Effects on Aqueous Solutions of Macromolecules

Most biological molecules will undergo a change in conformation in response to chemical and physical stress factors such as urea, pH, pressure and temperature. The physical basis of the denaturing action of pressure and temperature has been described in detail elsewhere [17, 18].

The thermodynamic description of the correlation between the cold, heat and pressure unfolding of proteins in water contains the difference in heat capacity ($\Delta C_p'$), compression ($\Delta\beta'$) and thermal expansion ($\Delta\alpha'$) between the native and the unfolded state of the protein in solution [19, 20]:

$$\begin{aligned} \Delta G(p, T) &= G_D(p, T) - G_N(p, T) = \\ \Delta G^o - \Delta S^o(T - T_o) &+ \Delta C_p' [(T - T_o) - T \ln(T/T_o)] \quad (3) \\ + \Delta V^o(p - p_o) - \frac{\Delta\beta'}{2}(p - p_o)^2 &+ \Delta\alpha'(T - T_o)(p - p_o) \end{aligned}$$

where $G_D(p, T)$ ($G_N(p, T)$) is the Gibbs energy of the unfolded (native) state of the protein, ΔG^o , ΔV^o and ΔS^o refer to the changes in Gibbs energy, the volume and entropy, respectively, at the reference conditions, taken to be p_o (0.1 MPa) and T_o (298 K). The precise definitions of $\Delta\alpha'$, $\Delta\beta'$ and $\Delta C_p'$ can be found in [20].

What is of interest for the present discussion is to point at the elliptic outline of the p, T -stability diagram, shown in Fig. 3, which is obtained when plotting the pressure-temperature conditions where the Gibbs free energy change ($\Delta G(p, T)$) is zero, *i. e.* the condition at which the protein undergoes the native–unfolded transition. Within the elliptic contour the protein is in the native conformation ($\Delta G > 0$), outside the contour the protein is unfolded ($\Delta G < 0$). The intersection of the boundary with the temperature axis at lower temperatures points at the cold unfolding of proteins, whereas the corresponding point at high temperature is due to the heat-induced unfolding of the protein.

Generally, changes of transition temperatures as a function of pressure are described by the Clapeyron equation, defining the relationship between pressure

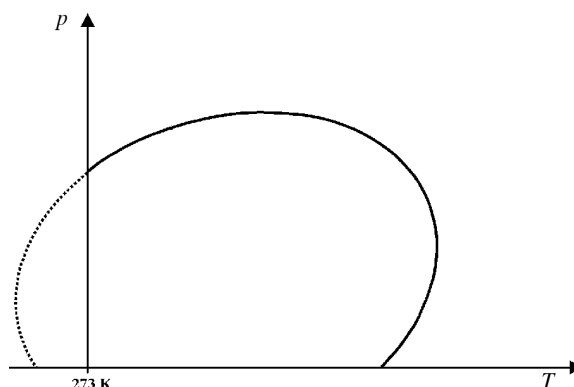


Fig. 3. A schematic representation of the pressure-temperature stability diagram of proteins. This diagram is also representative for other aqueous solutions such as those of synthetic polymers and starch. See text and [20] for further details.

and temperature along the transition line:

$$\left. \frac{dT}{dp} \right|_{transition} = T \frac{\Delta V}{\Delta H} \quad (4)$$

with ΔV and ΔH being the volume and enthalpy changes occurring at the transition, respectively.

Combining Eqs. 3 and 4, the elliptic outline of the native–unfolded conformational transition of proteins as seen in Fig. 3 can be described [19, 20], placing at the same time constraints on the values of the coefficients $\Delta\alpha'$, $\Delta\beta'$ and $\Delta C_p'$ as required to obtain the elliptic outline.

Note that Eq. 4 is identical in functional form as the classical Clapeyron equation describing the phase transitions in pure components with the provision that ΔV and ΔH refer to the change of the volume and enthalpy accompanying the phase transition at the particular pressure and temperature. For simple liquids the transition temperature generally varies linearly with pressure, although conditions for an ellipse-like dependence have been discussed before ([21] and references therein).

Other biological systems that display an elliptical p, T -stability diagram are starch [22], gelatin [23] and, as shown by a recent molecular dynamics study, the r(GCUUCGGC) ribonucleic acid (RNA) tetraloop [24]. Notable exceptions are the double-stranded deoxyribonucleic acid (DNA) [25, 26] and lipids [27], where dT/dp is independent of pressure and temperature. An elliptical diagram is also obtained when plotting the inactivation isokineticity lines of microorganisms in aqueous suspension [28, 29].

The elliptical p, T -diagram is, however, not limited to biological macromolecules. For instance, the p, T -phase behavior of water-soluble polymers such as poly(*N*-vinylisobutyramide), which undergo a liquid-liquid phase separation at higher pressures and temperatures, can also be described by an elliptical diagram, where the one phase homogeneous state is located within the ellipse [30]. Interestingly, salts can have a significant effect on the liquid-liquid phase separation behavior, which is intimately connected to the solvent-polymer interaction and the influence of salt on these interactions [30, 31].

In this latter case of solutions and mixtures, Eq. 4 is frequently used to discuss the pressure dependence of the liquid-liquid phase behavior, with ΔV and ΔH involving here the changes in volume and enthalpy of mixing of both coexisting phases at the liquid-liquid phase transition [30, 32]. Note, however, that in this case Eq. 4 can *never* be valid and can only be used to predict the *sign of* dT/dp when certain simplifying assumptions are made [33–35]. Although the exact Clapeyron equation for the mixture and solution case is available [33, 34], it has not been applied in detail so far, most likely because of the difficulty to obtain the experimental data on partial molar volumes and enthalpies of the coexisting phases, required to correctly predict dT/dp [33].

To summarise this section it should be emphasised that water is an essential reaction partner in all these phenomena. In the absence of water proteins and small organisms are found to be extremely stable towards pressure and temperature effects [36, 37]. Moreover, water is the only common feature of these structurally and chemically diverse macromolecules (and organisms) and their various types of phase behavior. It is, however, noteworthy that non-aqueous systems can also display similar elliptical phase diagrams ([21] and references therein). Presumably interactions introducing directionality are also involved in these cases.

Correlation Between Physical Properties of Water and the Pressure-Temperature Behavior of Macromolecules

One of the dominant intermolecular forces determining the stability of biological macromolecules is the hydrophobic effect, which arises entirely from the structure of water [4]. A loss of the tetrahedral geometry of water will therefore result in a weakening of this effect, which in turn makes water a better solvent

for the non-polar moieties that tend to cluster in a biological molecule in its native (folded) state. Despite the fact that water lacks any long-range order, the existence of two metastable states of water has been recognised. This is the so-called two-state model of water, in which water is considered to be a mixture of low- and high-density states, often referred to as ice-Ih- and ice-II-type water, respectively [38–40]. A shift in the balance of the fractions of these two types of water has been suggested to provide a thermodynamic explanation for the unfolding of proteins by a number of researchers [38–41].

If one compares the p, T -dependence of α_p of water with that of, for instance, protein stability, (Figs. 2 and 3) one immediately notices a striking resemblance between both curves. Given the fact that both β_T and C_p do not show such an elliptical p, T -dependence we suggest that the shape of the phase diagram of macromolecules is, at least in part, determined by the pressure-temperature dependence of the thermal expansion of water. Though one should be careful to seek for correlations based on graphical similarities, there is also a thermodynamic argument in favour of the proposed correlation. The thermal expansion is the cross-term between pressure and temperature effects, *i. e.* between volume and enthalpy fluctuations (Eqs. 2c and 3). In addition the pressure-temperature dependence of the thermal expansion can be related to the structure of water, as it results from two competing effects: angular variation and changes in the amplitude of anharmonic intermolecular vibrations [5, 12]. Hence the pressure-temperature dependence of α_p will determine the fractions of ice-Ih and ice-II, which, as described above, can be related to protein stability. In this respect it is worthwhile to note that the line that separates the low- and high-density states of water in the density-temperature phase diagram of SPC/E water also follows a contour similar to that of the p, T -dependence of α_p [42].

At this point we should mention a possible fly in the ointment. As mentioned before, the phase behavior of solutions and mixtures is not determined by the pure component properties, but is uniquely determined by the mixture properties. A specific example for which this has been investigated in some detail is the poly(vinylmethylether)/water system. It has been experimentally shown that the pressure dependence of the phase separation temperature is determined by the subtleties of the compositional curvature of the volume of the mixture at a given composition [33], and all this

suggests that there is no direct thermodynamic connection between α_p of pure water and the liquid-liquid phase behavior. However, in support of our view we note that excess enthalpies and volumes of aqueous systems may depend significantly on the structural features of water [34] and as such the properties of water are still, *via* the solvent-polymer interactions, responsible for the peculiar behavior of aqueous systems. Also note that in contrast to polymer mixtures, where one investigates the whole concentration range between $0 \leq \phi \leq 1$ (with ϕ the volume fraction of the polymer), biological systems are generally studied under quite dilute conditions ($\phi \leq 0.05$), and hence water is the main component in the mixture.

In recent years there has been a trend to focus on the properties of the hydration layer surrounding the macromolecule [8, 9, 31, 43]. Yet we suggest that the properties of the bulk solvent may just be as important, as Fig. 2 represents the thermal expansion of the bulk solvent. Alternatively, the p, T -dependence of the thermal expansion of the hydration water may be analogous to that of bulk water. However, we believe neither model is mutually exclusive. Different techniques, with inevitable differences in sensitivity and accuracy, may suggest a correlation between the phase behavior of the macromolecule and both the bulk solvent and the hydration layer [44]. Moreover, although it has been shown that the onset of thermally-induced protein unfolding coincides with the disruption of the hydration layer, this does not necessarily imply that this event is in itself sufficient to explain unfolding [9, 10]. Instead it is expected that any disruption of the hydration layer is to be accommodated by the bulk solvent, as the water molecules that were part of the hydration

layer will tend to hydrogen bond with those in the bulk phase [40]. Finally, it should be pointed out that much may depend on the definition of the hydration layer. In this respect it is worth noticing that at the hydration levels required for the onset of enzyme activity the protein surface is not completely covered with water molecules [8].

Conclusion

We propose that there is a correlation between a particular property of (bulk) water, the isobaric thermal expansion α_p , and the p, T -phase behavior of proteins and other macromolecules in aqueous solution. The proposed correlation has a thermodynamic basis which requires one to take into account the pressure dependence of thermodynamic variables. In addition, there is a structural argument related to the two-state model of water. The existence of this correlation provides additional evidence that the properties of bulk water play a role in determining the behavior of macromolecules in aqueous solution. Despite the large focus in the literature on the hydration of macromolecules, in our opinion any correlation between the behavior of macromolecules and water should reconcile the p, T -dependence of both the bulk and the hydration water. Our suggestion that α_p is “the specific molecular characteristic of water that ‘Nature’ exploits” [12] remains, however, to be further explored.

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