ELSEVIER

Journal of Molecular Liquids 124 (2006) 114 – 120

journal of MOLECULAR LIQUIDS

www.elsevier.com/locate/molliq

Lowering of the freezing temperature of water at the protein surface due to electric field

I. Danielewicz-Ferchmin [a], A.R. Ferchmin [b,*]

[a] *Faculty of Physics, A. Mickiewicz University, Umultowska 85, PL-61-614 Poznań, Poland*
[b] *Institute of Molecular Physics, Polish Academy of Sciences, M. Smoluchowskiego 17, PL-60-179 Poznań, Poland*

Received 13 April 2005; accepted 15 September 2005
Available online 27 December 2005

Abstract

A new complementary mechanism of inhibiting water freezing at protein surface is proposed. It is based on the idea that the effect of the local electrostriction pressure Π related with the local electric field in the immediate vicinity of protein molecule on the properties of water is the same as that of the external pressure P. The compressing action of Π can let water attain a state within the region in the $P-T$ phase diagram where it remains liquid below 0 °C. A model approach, recently developed to account for the enhanced density of water in double layers at protein molecules immersed in aqueous solutions, is followed. It is argued that, depending on the values of their surface charge densities, some local regions of surfaces of protein molecules prevent one or two monomolecular layers of the adjacent water from freezing by the effect of the electrostriction pressure.

PACS: 87.15.Da
Keywords: Water; Antifreeze; Protein

1. Introduction

The structural X-ray studies show one or two layers of molecular thickness of water around protein that do not freeze below 0 °C (cf. [1] and references therein). Some proteins are known for their especially effective 'antifreeze' action on water that inhibits freezing of a layer of water surrounding their molecules ('non-freezing' water). It is important to various forms of life: "many organisms are able to survive subzero temperatures at which bodily fluids would normally be expected to freeze" [2]. Generally, one can discern between two somewhat different phenomena: the nanoscopic "non-freezing" (in equilibrium) hydration layers presumably present around most if not all protein molecules, and the more far-reaching, macroscopic, antifreeze action of a few specific proteins (often due to non-equilibrium effects). Our work is devoted to the former one.

How can one in general prevent water from freezing below 0 °C? The answers given for water in contact with protein can vary [2–6]. One of the answers is based on the fact that, during the transition liquid water→ice Ih, the H_2O expands or, in other words, its mass density decreases. A related well-known advice is to apply an external pressure, which would counteract the expansion, and thus inhibit freezing. Indeed, the water $P-T$ phase diagram contains a region below 0 °C where water remains liquid [7,8]. In this phase diagram, at a given temperature T_m, ice at the melting line has a density ρ_i and at the same point of the melting line water has a density ρ_w. It is a textbook matter that it is the difference $\rho_w - \rho_i$, which, following the Clapeyron–Clausius equation, decides upon the slope of the melting line. If by any other physical means, different from pressure, water attained the same value ρ_w as that due to mechanical compression, the system would be at the melting line at the same T_m. It is known that Svergun et al. [9] have found by X-ray and neutron scattering in H_2O and D_2O solutions that the "hydration shell around proteins is denser that the bulk solvent". According to the above argument, this very fact should result in a melting/freezing point T_m of water within this hydration shell different from (actually below) 0 °C irrespective of the mechanism that causes this higher density. However, this is

* Corresponding author. Tel.: +48 61 8695123; fax: +48 61 8684524.
E-mail address: arfer@ifmpan.poznan.pl (A.R. Ferchmin).

0167-7322/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.molliq.2005.09.004

not enough, since the cited result concerns an average over the protein surface and tells nothing on what happens locally. Some inspection on what happens to hydration water locally is due to Merzel and Smith and Smith et al. [10,11]. In particular, they discuss the role played by regions (often termed hydrophilic) with electric charges and the polar ones at the surface of the protein. We shall return to this point in more detail in the following. Now, is there a factor that would play a similar role as the external pressure and enhance water density? The answer is positive: in presence of an inhomogeneous electric field in an open system (in the sense defined in thermodynamics) the local electrostriction pressure Π (cf. [12]) works in the same manner as the external pressure P.

In this paper, we shall put together two facts known from experiment and show how they can be correlated. The first one is known as the 'antifreeze' effect of protein on water mentioned above. The second one is known from PVT measurements on H_2O: "It is well known that for H_2O there is a small part of the liquid region with temperatures lower than the temperature of the triple point, $T_{tp}=273.16$ K; this region is bordered by the melting-pressure curves of the ice modifications I, III and V" [8] (cf. Fig. 1). The correlation between these two observations will be drawn on the basis of the idea, based on the growing evidence that the effect of the external pressure P and the electrostriction pressure Π on water is equivalent, in the context of the effect of electrostriction on the

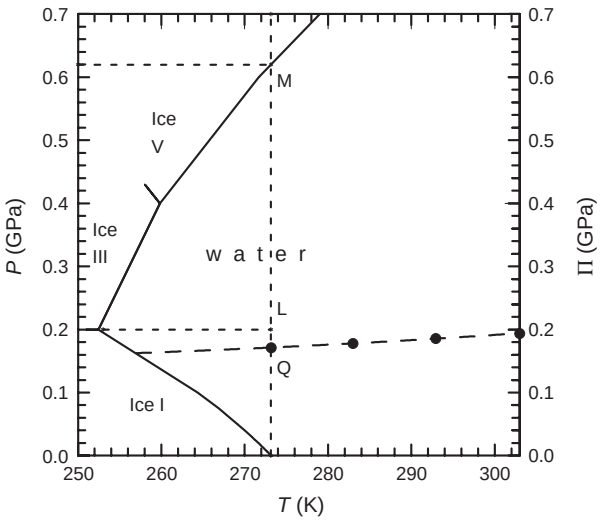

Fig. 1. Part of the $P-T$ phase diagram of H_2O with superimposed cooling line of water under the action of electrostriction pressure Π. A liquid region is seen with temperatures lower than that of the triple point, to the left of $T_{tp}=273.16$ K; this region is bordered from the left by the melting lines of the ice I, III and V. The same melting lines serve as the (left) boundary of the region where water subdue to the action of the electrostriction pressure Π (right-hand scale) is liquid at temperatures T. The dashed line QLM at $T=273$ K borders from the right the region where water remains liquid below 273 K under pressure P or, equivalently, Π. The points L and M have coordinates $L=(273$ K, 0.2 GPa), $M=(273$ K, 0.62 GPa). Four full symbols • represent the electrostriction pressure $\Pi(T)$ as a function of temperature T plotted every 10 K for the local surface charge density $\sigma=0.017$ q Å^{-2}. The symbol at 273 K is marked Q. A long-dashed cooling line is plotted through the symbols down to the crossing with the crystallization line. Drawn in part with the use of data taken from [8].

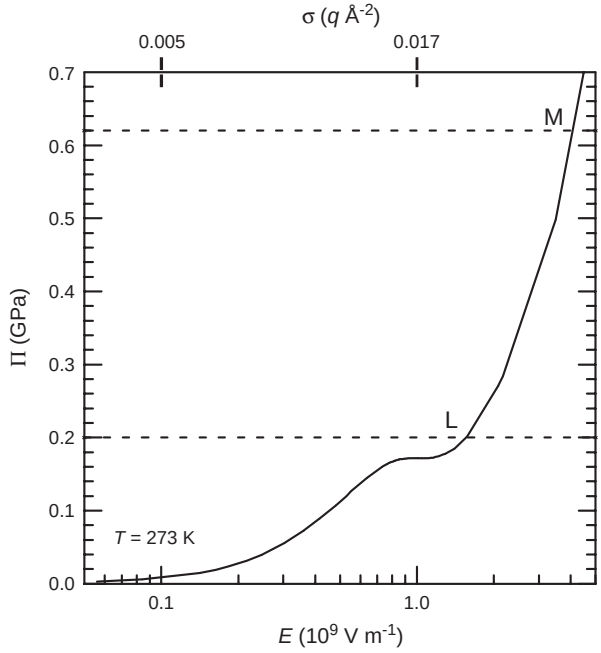

Fig. 2. An exemplary $\Pi(E)$ isotherm at 273 K. The additional upper abscissa axis defines the surface charge density σ in units q Å^{-2}. The points L and M lie at $\Pi=0.2$ GPa and $\Pi=0.62$ GPa, respectively. The crossing of the isotherm with the horizontal dashed line at the point M gives $\sigma=0.0205$ q Å^{-2} ($E=4\times10^9$ V m^{-2}). Another crossing with the horizontal line at the point L gives $\sigma=0.0174$ q Å^{-2} ($E=1.57\times10^9$ V m^{-2}).

structure of water in the electric field of ions [13]. Earlier, this idea has been shown to be quantitatively correct when applied to a calculation of the mass density of various systems including double layers at the charged electrodes, polar crystals and proteins [14–16] (see also [17] for a review). This idea is followed in this work.

Characterizing the physical properties of water at the surfaces of protein molecules is a subject of current interest (cf. [9–11] and citations therein) irrespective of the 'non-freezing' water problem. Three proteins were investigated by Svergun et al. [9] in parallel by X-ray and neutron scattering in H_2O and D_2O solutions. As mentioned above, a denser shell of water around proteins has been observed. Also, Perkins [18] noted that water in the hydration shell of a protein is "electrostricted". Merzel and Smith [10] argued that the density of the surface water layer is determined by both the topography of the protein surface and the electrostatic field generated by the protein atoms partial charges. We are interested in the latter one and ask the question what happens under the action of the electric field to the layer of water below 0 °C (or, which is very close in the temperature scale, the triple point temperature $T_{tp}=273.16$ K) at the protein surface.

When approaching the problem of the hydration of proteins, the attention is often concentrated on the interaction of the surface water layer with its *inner* side, namely the protein. We have proposed a novel look at this question [16]. Keeping in mind these interactions, in the following, we deal with the surface water layer and its neighborhood on the *outer* side, and point out that the surface water layer forms a common system with the remaining water localized outside the electric field.

The question asked is which aspects of this water system determine the behaviour of the surface water layer, in particular its density and phase. We can anticipate the results and say that it is the enhanced density due to the huge electrostriction [14,15], which, at a given temperature, decides which phase this water layer belongs to. Similar effects occur also in double layers at the surfaces of charged or highly polar solids in aqueous electrolytes [19,20]. Referring to the thermodynamic equation of state [16,17,21], we recall the mechanism of the huge electrostriction in water: It is the pull of the dipolar water molecules into the field, leading to a thermodynamic equilibrium between a water shell in the field and the rest of water outside the field, which causes the increase of water density in the electric field. With the knowledge of the values of the electric fields acting at the surfaces of proteins, the corresponding values of the electrostriction pressure Π are found (Fig. 2). Finally, the effect of Π is discussed with stress on the protein surface properties necessary to let the adjacent water remain liquid below 0 °C.

2. The reasons why the dipolar water molecules flow into the hydration envelope

As argued in our previous work, water placed in a local high electric field of strength E, generated by the protein atom partial charges forms a common system with the remaining water localized in a weak field or outside the field. Between these subsystems there is no barrier, which would hinder the mass transport. For the sake of completeness, we shall briefly repeat some of the earlier argument [16].

The thermodynamic law describing the state of the system under discussion is the equilibrium condition with respect to the mass transport between the regions within and outside the field. It follows from the condition of equality of the chemical potentials ζ:

$$\zeta^{\mathrm{i}} = \zeta^{\mathrm{o}}. \tag{1}$$

The superscripts i and o mark the quantities inside and outside the high field, respectively. (A discussion of where the parts i and o of the system are positioned close to the protein shall be presented below.) The chemical potential of a water molecule, placed in a high electric field at the expense of the work W needed for its reorientation, is reduced by ζ_W with respect to that of a molecule outside the field. Due to this local reduction in value of the chemical potential, there arises a chemical potential gradient between the subsystems "i" (hydration shell) and "o" (bulk water). This gradient induces a spontaneous irreversible process: the pull of the dipoles into the field. Each electric dipole belongs to a water molecule, hence the pull of the dipoles into the field is accompanied by a mass transport from subsystem out "o" into that in "i" the field. The mass transport makes the subsystem "i" (hydration shell) more and more dense until the compression work, denoted L, per molecule, or the related chemical potential increment ζ_L, compensates the increment ζ_W,

$$-\zeta_W = \zeta_L. \tag{2}$$

This equation represents the equilibrium condition of the system with respect to the mass transport.

The change in the chemical potential ζ_W is calculated in a way similar to that described earlier [14,22,21]. In the immediate neighbourhood of the protein molecule (first layer of water molecules), the field strength E is

$$E = \frac{\sigma_{\mathrm{o}}}{\epsilon_{\mathrm{o}}} = \frac{\sigma}{\epsilon\epsilon_{\mathrm{o}}}, \tag{3}$$

where σ_{o} is the surface charge density at a chosen portion of the protein surface, ϵ is the permittivity and ϵ_{o} is the permittivity of vacuum. The work W done by the electric field is [14,21]:

$$W = \frac{V}{\epsilon_{\mathrm{o}}}\int_0^y \frac{\sigma}{\epsilon}\mathrm{d}y, \text{ where } y = \sigma\left(1 - \frac{1}{\epsilon}\right). \tag{4}$$

V=const. is the volume of the system and $V\mathrm{d}y$ is the increment of the electric polarization of the whole system. The increment of the grand potential Ω is:

$$\mathrm{d}\Omega = -S\mathrm{d}T + EV\mathrm{d}y - N\mathrm{d}\zeta_L, \tag{5}$$

where N is the number of water molecules in the volume V. Let us introduce the notation

$$f = \int_0^y \frac{\sigma}{\epsilon}\mathrm{d}y. \tag{6}$$

The work W performed leads to a change $\Delta\Omega$ in the value of the grand potential Ω of water

$$(\Delta\Omega)_{T,V,\zeta_L} = \frac{V}{\epsilon_{\mathrm{o}}}f. \tag{7}$$

For ζ_W–the change in ζ as a result of the work W–one obtains

$$\zeta_W = \left(\frac{\partial\Omega}{\partial N}\right)_{T,V,\zeta_L}. \tag{8}$$

The increment

$$\zeta_W = \frac{v^{\mathrm{o}}N^{\mathrm{o}}}{\epsilon_{\mathrm{o}}}\left(\frac{\partial f}{\partial N}\right)_{\zeta_L}, \tag{9}$$

where $v^{\mathrm{o}} = V/N^{\mathrm{o}}$ and N^{o} is the Avogadro's number. It follows from Eq. (9) that

$$\frac{\zeta_W}{v^{\mathrm{o}}} = \frac{1}{\epsilon_{\mathrm{o}}}\left[\left(\frac{\partial f}{\partial y}\right)\left(\frac{\partial y}{\partial \epsilon}\right)\right]_{\zeta_L} N^{\mathrm{o}}\left(\frac{\partial \epsilon}{\partial N}\right)_y. \tag{10}$$

The derivatives in Eq. (10) are obtained on the basis of a statistical model approach to the permittivity of hydrogen bonded liquids (including water) proposed earlier [23].

Let us now consider the change in the chemical potential ζ_L due to the compression work L, which, according to Eq. (2), shall compensate ζ_W. The compression work L is calculated [14,24] by integrating the area under the isotherm $V = V(P)$ with P—pressure:

$$L = \int_{P^{\mathrm{o}}}^{P^{\mathrm{i}}} V(P)\mathrm{d}P. \tag{11}$$

The change in the chemical potential ζ_L due to this work is

$$\zeta_L = \frac{\partial}{\partial N}\int_{P^o}^{P^i} V(P)\mathrm{d}P = \int_{P^o}^{P^i} v(P)\mathrm{d}P, \tag{12}$$

where P^o denotes the atmospheric pressure. One can re-write Eq. (2) in the form

$$-\frac{\zeta_W}{v^o} = \frac{\zeta_L}{v^o}, \tag{13}$$

which, taking into account Eqs. (10) and (12), is the same as

$$-\frac{N^o}{\epsilon_o}\left[\left(\frac{\partial f}{\partial y}\right)\left(\frac{\partial y}{\partial \epsilon}\right)\right]_{\zeta_L}\left(\frac{\partial \epsilon}{\partial N}\right)_y = \frac{1}{v^o}\int_{P^o}^{P^i} v(P)\mathrm{d}P. \tag{14}$$

The integrals on the right hand sides of Eqs. (12) and (14) have been found by substituting the isotherms [25] $v=v(P)$ of water in the liquid state under pressure P in the absence of field. Of course, the use of the data measured in the absence of the field introduces an approximation. Its validity can only be judged a posteriori, as has been done with a positive result [14]. The upper integral limit (P^i, see Eq. (12)) was matched so as to fulfill Eq. (14). This is equivalent to putting the pressure value P^i in the field equal to the local electrostriction pressure value Π:

$$-\frac{N^o}{\epsilon_o}\left[\left(\frac{\partial f}{\partial y}\right)\left(\frac{\partial y}{\partial \epsilon}\right)\right]_{\zeta_L}\left(\frac{\partial \epsilon}{\partial N}\right)_y = \frac{1}{v^o}\int_{P^o}^{\Pi} v(P)\mathrm{d}P. \tag{15}$$

This is equivalent to saying that the external pressure applied without electric field would produce water compression comparable to that due to the local electrostriction pressure. Note that a similar position has also been adopted by other authors, e.g., a neutron scattering experiment with isotopic substitution on a 10 M NaOH solution was interpreted as to "indicate that ions in aqueous solutions induce a change in water structure equivalent to the application of high pressure" [13].

Eq. (15) is the so-called rigorous or thermodynamic equation of state of the general form

$$f(T,E,\Pi) = 0, \tag{16}$$

and contains the variable Π — the local electrostriction pressure. With given temperature and electric field E (or surface charge density σ), it can be solved to give the electrostriction pressure Π [14–17]. Further on, the value of the mass density of water subdued to the action of Π can simply be found from the tabulated data of water density under external pressure P admitting the values $\Pi=P$. It should be stressed that the electrostriction pressure Π is a real thermodynamic parameter, the action of which leads to measurable consequences, e.g., compression of water. This is to say that, as mentioned above, the electric field E affects water in a manner similar to that of external pressure P. The calculations [14,15,21] of the mass density of water in the double layers at the electrodes and polar crystals have lead to quantitatively correct values when compared with those measured by Toney et al. [19] and Chu et al. [20]. This gives us confidence that this shall also be the case in the problem discussed here.

3. Results and discussion

Merzel and Smith and Smith et al. [10,11] have found that the strength of the field around a protein is correlated to the water mass density over the most highly populated field values (corresponding to σ in the range 0.005–0.03 q Å^{-2}, cf. Ref. [17], Table 7 therein), where q denotes the elementary charge, $1q$ Å^{-2}=16 C m^{-2}, with a higher field strength accompanying the higher density. This, within the present approach, should be interpreted in terms of water density enhancement by different local electrostriction pressures acting at various specific portions of the surface of a protein molecule. The related electric field strength E is given by Eq. (3). Thus, E is related to σ and ε. The former is characteristic of the protein surface [10,11] and the latter is the field-dependent permittivity of water [23]. Accordingly, the fields at the surface of protein are of strengths in the range $10^8<E<10^{10}$ V m^{-1} [10,16,17]. For E within this range, the permittivity ε reveals an abrupt fall at about 10^9 V m^{-1} [23]. Within the hydration layer put into such fields, the local electrostriction pressure Π takes values in the range $0.01<\Pi<1$ GPa. This follows from the equation of state Eq. (15) (see [21,22] for details), which was a basis for plotting the isotherm shown in Fig. 2. This is an exemplary isotherm for 273 K. Fig. 2 is to be compared with Fig. 1, where the melting line for H_2O is plotted on the basis of the data of Ref. [8], showing also a part of the liquid region with temperatures lower than the temperature of the triple point, T_{tp}=273.16 K (not indicated). In both Figs. 1 and 2, the ordinate axes are in the same scale.

3.1. Cooling under pressure and cooling under electrostriction pressure

As discussed in the Introduction, we are persuaded that pressure P affects water in quantitatively the same manner as the electrostriction pressure Π, at least as long as it concerns the mass density of the liquid phase. With this idea in mind, we shall plot in the same diagram (Fig. 1) the crystallization line (solid line) of water under pressure in $P-T$ coordinates and the cooling line $\Pi(T)$ (long-dashed line) of water under electrostriction pressure. On condition that a point in Fig. 1 lies in the region of liquid water, to the right of the freezing lines bordering various solid ice phases, one can consider it as a (Π, T) point with P (left scale) equivalent to Π (right scale) since both correspond to the same mass density of water. Let us consider an example illustrating what happens to water when cooled to temperatures below T=273 K. From Fig. 1, it can be realized that a part of the liquid region with temperatures lower than 273 K exists for pressures $P<0.62$ GPa to the left of the line passing through the points marked Q, L and M. For water under pressure P, it is easy to maintain the pressure unchanged, P=const., while cooling. The dashed line starting at point L at 273 K and P=0.2 GPa and going horizontally to the left until it reaches the freezing line is a good example of such a process.

This exemplary cooling line is chosen so that it reaches the lowest temperature of 253 K of non-frozen water under pressure attainable in this manner. One can conceive that exactly the same process of cooling with constant electrostriction pressure Π cannot so easily be realized. Indeed, one can find the values of Π at a given portion of the protein surface with a given value of surface charge density σ (assumed to remain temperature-independent) from a function $\Pi(\sigma)$ like that represented by the solid line in Fig. 2. Now, this solid line represents an isotherm (in this case, at 273 K). When cooling, for another T value one needs another isotherm and when it differs from the former one, another value of Π acting on water at the same place at the protein molecule (same σ) is obtained. It follows that the realizable cooling line for a specific surface charge density σ kept constant deviates from the horizontal one. We shall present an example for a chosen value of σ.

3.2. Cooling in constant field—an example

What happens when temperature of water at a specific portion of the surface of protein attains values below 273 K? For a chosen surface charge density $\sigma = 0.017\ q\ \text{Å}^{-2}$, this is illustrated with the help of the four points in the $\Pi = \Pi(T)$ plot Fig. 1 found on four consecutive isotherms above 273 K (cf. [21], Table 1 therein) with 10 K distance between them. Through the four points $\Pi(T)$ for $T = 273$ (marked Q), 283, 293 and 303 K a long-dashed line (cooling line) has been drawn down to the freezing temperature T_f at its crossing with the border of the Ice I phase. Let us stress that this represents the argument in favour of the thesis that water at this portion of protein surface does freeze only below 273 K. Just for this long-dashed line, for $\sigma = 0.017\ q\ \text{Å}^{-2}$, the distance between consecutive isotherms (cf. [21], Fig. 1 therein), represented by differences between ordinates Π of the points marked • in Fig. 1, is the largest in the electrostriction pressure scale Π. This happens at, and close to, the plateau apparent in Fig. 2. At other values of σ, the deviation of the actual cooling line from a horizontal one shall be lower. In this way, one has found the upper limit of the shift in pressures of the cooling line with temperature, which amounts to ca. 0.008 GPa per 10 K. The upper limit of its relative deviation from a horizontal line in the pressure scale found in this manner is less than 10%. Thus, calculation of cooling in constant electric field leads to a value of the melting/freezing temperature T_m only slightly different from that found for constant electrostriction pressure.

3.3. Electric characteristics of protein surface that lower the freezing temperature of water

As already noted, the values of Π encountered in the hydration layers of proteins lie within the range $0.01 < \Pi < 1$ GPa of electrostriction pressures. The latter range can be divided into two separate ranges: $0.01 < \Pi < 0.62$ GPa, in which the crystallization temperature of water is lower than 273 K, and $0.62 < \Pi < 1$ GPa, in which the reverse is true. Hence, on condition of having Π of due magnitude ($\Pi < 0.62$ GPa) at the surfaces of biomolecules, which as mentioned above is the case, below 273 K the water layer at their surfaces can be prevented from freezing (into ice Ih). Note that, to the limiting value $\Pi = 0.62$ GPa, there correspond the values of σ and E given in Table 1, row 3. The lowest temperature of about 20 K below 273 K at which water remains liquid under pressure is encountered for $\Pi = 0.2$ GPa. This happens for the corresponding E and σ values given in Table 1, row 2. The maximum 'non-freezing' effect due to the physical mechanism invoked in this paper is expected there.

To summarize, at the surface of biomolecules one encounters such regions ($\sigma < 0.0205\ q\ \text{Å}^{-2} = 0.328\ \text{C m}^{-2}$, equivalent to the field $E < 4 \times 10^9\ \text{V m}^{-1}$, giving rise to $\Pi < 0.62$ GPa) in which the electrostriction pressure shifts the melting temperature of the hydration layer to values lower than 0 °C and also the regions in which the reverse effect is expected ($\sigma > 0.0205\ q\ \text{Å}^{-2}$, equivalent to $E > 4 \times 10^9\ \text{V}^{-1}\ \text{m}^{-1}$, giving rise to $\Pi > 0.62$ GPa). The net result depends on the detailed structure of a particular protein. Let us recall that we have dealt with the equilibrium effect concerning portions of one or two monomolecular layers of H_2O at the surface of protein molecule. Indeed, the effect under consideration depends on the noticeable compression of water and that has been observed at protein boundary by Svergun et al. [9] by X-ray and neutron scattering in the first water layer. The dependence of water density on the distance from the charged surface outwards has been estimated at a charged electrode, for which compression of two adjacent water layers of molecular thickness [19] has been observed by X-ray scattering and water densities at the distances of the first and second H_2O molecular layers from the electrode have been found [15,19].

3.4. Remarks on the method

We shall discuss two questions. First, how to discern between molecules belonging to either subsystem i (in the high field) or the other one marked o. Second, if taking the statistical mean values in our calculations is justified.

The electric field decreases continuously with the distance from a charged or polar portion of the protein boundary. Therefore, it would seem hard to find the boundary between the subsystems i and o. In the context of the present work, the practical rule is to look for the highest value of the strength of the field E, which is not yet accompanied by any noticeable compression of water. It marks the boundary between subsystems i and o. Note that the limiting field need not be known to a good accuracy since E falls initially down so abruptly with distance from the protein boundary that two consecutive H_2O molecules may feel fields differing by an

Table 1
Three values of electrostriction pressure Π encountered in Figs. 1 and 2 with the corresponding electric field strength E and surface charge density σ

Π (GPa)	E (10^9 V m^{-1})	ε	σ (q Å^{-2})	σ (C m^{-2})
0.01	0.11	80	0.005	0.08
0.2	1.57	20	0.0174	0.278
0.62	4	9.25	0.0205	0.328

The values of permittivity ϵ have been taken from Ref. [23].

order of magnitude, and hence can belong to different subsystems. The exemplary result is that two H_2O molecular layers form the subsystem i in the Toney et al. [19] experiment with uniformly charged Ag electrode (cf. also Ref. [15]). Apparently, the subsystem i included on average only one molecular layer at the protein boundary in the Svergun et al. [9] experiment. However, since the average over the protein boundary involves contributions from high surface charge densities as well as such of low or no polarity, one can speculate on the possibility of having locally two H_2O compressed molecular layers i at the highly charged places, and only the further ones belonging to the o subsystem.

We shall discuss the statistical calculation of average quantities as applied to the case of water layers at large protein molecules, in close analogy to those encountered at hydrated ions and charged electrodes. Strong electric field generated by the charges at protein boundary decay rapidly with growing distance from the charges in a similar way as it decays at ions [24] or charged or polar crystal surfaces [14]. As a consequence, the water molecules within the first hydration shells of proteins are in the fields of a considerably higher strength than those outside them. If the surface of a protein was uniformly charged, the calculation of the properties of water layer of molecular thickness in the theory of electrolytes at an electrode discussed in Ref. [14] could simply be repeated. Since this is not the case, one can mentally divide the boundary of a protein into portions with the same surface charge density and the adjacent water layer into corresponding portions in the same field. The number of such places at a whole statistical ensemble of protein molecules flowing in water is sufficiently large to be considered as a macroscopic one. Taken together, the selected parts of hydration shells at the places with the same surface charge densities form a subsystem of water molecules in the same physical conditions, although they do not have a common macroscopic boundary, like the hydration shells of ions [24]. The subsystems of water molecules in the same electric fields form macroscopically large ensembles of molecules in the same physical conditions and thus can be subjected to the procedure of statistical averaging leading to equilibrium values of their thermodynamic parameters. Although dispersed in space, each of such sets of the portions of hydration shells in equal fields can be treated in much the same way as a layer of molecular thickness in the theory of electrolytes at an electrode discussed in Ref. [14]. Thus, one is allowed to derive thermodynamic quantities concerning the dispersed in space, but otherwise macroscopic, set of portions of hydration shells by statistical methods.

4. Conclusion

In this paper, we have argued that water in the hydration layers of protein molecules can reside at local electrostriction pressures of the order of 10^{-1} GPa, which is accompanied by a lowering (or rising, depending on the local charge density on the surface of the biomolecule) of the freezing temperature despite maintaining the ambient external pressure. We have related these findings with the problem of 'antifreeze' protein properties extending to one or two molecular layers of water at its surface. We have exploited the hitherto almost unnoticed analogy between the phases to which belong dense hydration water shells at surfaces of protein molecules compressed by the huge *electrostriction pressure* present in open systems [16] on the one hand and the phases of water under high applied *external pressure* at subzero temperatures on the other hand.

We conclude that in favourable conditions (specific protein structure) yet another effect described in this paper, in addition to those suggested earlier [2–5], can contribute to the equilibrium 'non-freezing' water phenomenon very close to the protein molecules.

Let us stress that our approach accounts only for equilibrium (thus excluding, e.g., supercooling) and static (thus not referring to possible kinetic hindrances to freezing [6]) phenomena. Also, it applies only to the thinnest water layers at the protein of thickness not exceeding one nanometer.

The other proposed hitherto possible ways leading to inhibition of water freezing at the protein [2–6] are neither confirmed nor questioned here; rather, the present work proposes a complementary mechanism. Also, we have only pointed to the possibility of a new mechanism of antifreeze action of some proteins on water, leaving any detailed discussion of how specific proteins differ in this respect to a future study.

References

[1] R. Dorbez-Sridi, R. Cortès, E. Mayer, S. Pin, J. Chem. Phys. 116 (2002) 7269–7275.
[2] S.P. Graether, C.M. Slupsky, P.L. Davies, B.D. Sykes, Biophys. J. 81 (2001) 1677–1683.
[3] H. Hatakeyama, T. Hatakeyama, Thermochim. Acta 308 (1998) 3–22.
[4] J. Wolfe, G. Bryant, K.L. Koster, CryoLetters 23 (2002) 157–166.
[5] M.M. Harding, P.I. Anderberg, A.D.J. Haymet, Eur. J. Biochem. 270 (2003) 1381–1392.
[6] L.M. Sander, A.V. Tkachenko, Phys. Rev. Lett. 93 (2004) 128102-1–128102-4.
[7] D. Eisenberg, W. Kauzmann, Structure and Properties of Water, Oxford University Press, London, 1969.
[8] W. Wagner, A. Pruß, J. Phys. Chem. Ref. Data 31 (2002) 387–535.
[9] D.I. Svergun, S. Richard, M.H.J. Koch, Z. Sayers, S. Kuprin, G. Zaccai, Proc. Natl. Acad. Sci. U. S. A. 95 (1998) 2267–2272.
[10] F. Merzel, J.C. Smith, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 5378–5383.
[11] J.C. Smith, F. Merzel, C.S. Verma, S. Fischer, J. Mol. Liq. 101 (2002) 27–33.
[12] H.S. Frank, J. Chem. Phys. 23 (1955) 2023–2032.
[13] F. Bruni, M.A. Ricci, A.K. Soper, J. Chem. Phys. 114 (2001) 8056–8063.
[14] I. Danielewicz-Ferchmin, A.R. Ferchmin, J. Phys. Chem. 100 (1996) 17281–17286.
[15] I. Danielewicz-Ferchmin, A.R. Ferchmin, A. Szlaferek, Chem. Phys. Lett. 288 (1998) 197–202.
[16] I. Danielewicz-Ferchmin, E. Banachowicz, A.R. Ferchmin, Biophys. Chem. 106 (2003) 147–153.
[17] I. Danielewicz-Ferchmin, A.R. Ferchmin, Phys. Chem. Liq. 42 (2004) 1–36.
[18] S.J. Perkins, Biophys. Chem. 93 (2001) 129–139.
[19] M.F. Toney, J.N. Howard, J. Richer, G.L. Borges, J.G. Gordon, O.R. Melroy, D.G. Wiesler, D. Yee, L.B. Sorensen, Nature (Lond.) 368 (1994) 444–446.

[20] Y.S. Chu, T.E. Lister, W.G. Cullen, H. You, Z. Nagy, Phys. Rev. Lett. 86 (2001) 3364–3367.
[21] I. Danielewicz-Ferchmin, A.R. Ferchmin, Chem. Phys. Lett. 398 (2004) 186–189.
[22] I. Danielewicz-Ferchmin, A.R. Ferchmin, Phys. Chem. Chem. Phys. 5 (2003) 165–171.
[23] I. Danielewicz-Ferchmin, A.R. Ferchmin, Phys. Chem. Chem. Phys. 6 (2004) 1332–1339.
[24] I. Danielewicz-Ferchmin, A.R. Ferchmin, Physica B 245 (1998) 34–44.
[25] Sydney P. Clark (Ed.), Handbook of Physical Constants, revised ed., The Geological Society of America, New York, 1966.