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Protein hydration and the huge electrostriction

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Abstract

Recent experiments indicated the existence of hydration shells about biomolecules with densities markedly higher than that of bulk water. The compression is due to the pull of the dipoles of H_2O molecules, necessary to achieve the thermodynamic equilibrium, from bulk water into the high field (approx. 10^9 V/m) region at the surface of the protein molecule. The electric field values at the surfaces of the biomolecules are calculated on the basis of the known densities. The reverse calculation of the limiting density values on the basis of known electric field distributions is performed, too. The results compare favourably with experiment.

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1. Formulation of the problem

Comprehensive characterisation of the physical properties of water at the surface of protein molecules is a subject of current interest (cf. [1,2] and citations therein). Three types of proteins: chicken egg white lysozyme, thioredoxine reductase from *Escherichia coli*, and ribonucleotide reductase protein R1 from *E. coli*, were investigated by Svergun et al. [1] in parallel by X-ray and neutron scattering in H_2O and D_2O solutions. These authors found that the 'scattering density in the border layer was typically 1.05–1.25 times that of the bulk, suggesting that the hydration shell around proteins is denser than the bulk solvent'. Also, Perkins [3] notes that the water in the hydration shell of a protein is 'electrostricted'. Ebel et al. considered as 'likely' that the temperature variation in volume observed in their hydration study of rabbit muscle aldolase in solutions with sugar was due to electrostriction [4]. Merzel and Smith [2] argued that the density of the surface water layer was determined by both the topography (cf. [5]) of the protein surface and the electrostatic field generated by the protein atom partial charges. We are interested in the latter case.

The most highly populated field values generated by the protein atom partial charges are given in Ref. [2] in the units that have a dimension of surface charge density σ, namely $\sigma = 0.005 - 0.03$ q Å^{-2}, where q denotes the elementary charge,

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1 q Å^{-2} = 16 C/m^2. The related electric field strength E is:

$$E = \sigma / \varepsilon \varepsilon_o \quad (1)$$

where ε–permittivity (see Section 2.1 below), ε_o–permittivity of vacuum. Thus, E is related to σ and ε.

Generally, when approaching the problem of the hydration of proteins, the attention is concentrated on the interaction of the surface water layer with its neighbourhood on the *inner* side, the protein. We propose a novel look at this question. Keeping in mind these interactions, we shall deal with the surface water layer and its neighbourhood 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 here is, which aspects of this water system determine the high density of the surface water layer? We can anticipate the results and say that it is the huge electrostriction [6,7], which causes this compression effect. Similar effects occur also in double layers at the surfaces of charged or highly polar solids in aqueous electrolytes [8,9]. In this work, on the basis of the thermodynamic equation of state (cf. [10]), we show 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.

2. Method

To achieve a proper description of the behaviour of water layers in high electric field at a quantitatively correct level, we shall pass two essential steps. The first one is to get a quantitatively correct account for the dielectric behaviour (permittivity) of water, treated as a dipolar hydrogen-bonded substance. This is done by applying a simple statistical model, which previously proved its usefulness in describing hydrogen-bonded liquids [11]. The second one is the realistic approach to the huge electrostriction properties of water layer in high field treated as a subsystem in contact with water in no field. This is done by applying an equilibrium condition leading to the thermodynamic equation of state (cf. Ref. [10]), which previously proved its usefulness ([6,10]) in describing the density of water layers at surfaces of charged electrodes or highly polar crystals ([8,9], respectively).

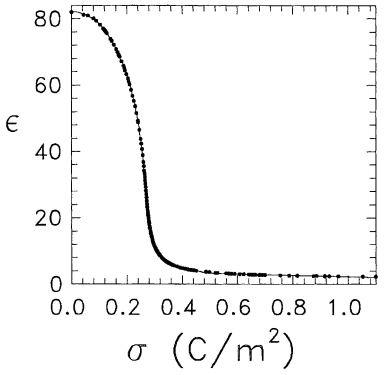

Fig. 1. Permittivity ε as a function of the surface charge density σ. The points marked are obtained at $T = 293$ K on the basis of Ref. [7], Eq. (2) therein. The lines follow the fitting polynomials (see Appendix A).

2.1. Permittivity ε of water as a function of surface charge density σ

The most highly populated field values generated by the protein [2] are so high (approx. 10^9 V/m) that the knowledge of dependence of the permittivity ε on electric field strength becomes necessary. The value of the strength E of the field generated by the protein atom partial charges is related to σ and ε through Eq. (1). The permittivity ε occurring in Eq. (1) is obtained on the basis of a statistical model approach to the water permittivity proposed earlier [11,12]. The resulting relation between the permittivity ε and the surface charge density is shown in Fig. 1 (see also the fitting polynomials in Appendix A).

2.2. Why do the dipolar water molecules flow into the hydration envelope?

Water placed in a local high electric field of strength E, generated by the protein atom partial charges (see the schematic Fig. 2, subsystem '*i*'),

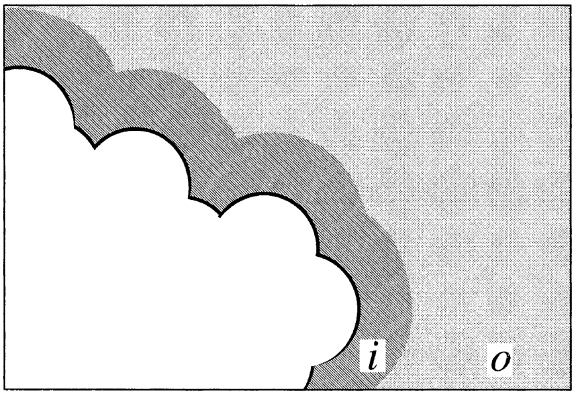

Fig. 2. A thermodynamic system divided into subsystems: '*i*' (hydration envelope) situated in a high electric field E and '*o*' (bulk water) in the field $E \to 0$ (schematic).

forms a common system with the remaining water localized in a weak field or outside the field (Fig. 2, subsystem '*o*'). Between the subsystems '*i*' and '*o*' there is no wall (barrier), which would hinder the mass transport.

The thermodynamic law describing the state of this system 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:

$$\zeta^i = \zeta^o \tag{2}$$

The superscripts i and o mark the quantities inside and outside the field, respectively (Fig. 2). The chemical potential of 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. (Yet, another well-known example of a similar process represents diffusion occurring in the presence of a concentration gradient, which is also accompanied by a chemical potential gradient). 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 '*o*' into '*i*'. 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

$$-\zeta_W = \zeta_L \tag{3}$$

When ζ_W and ζ_L are explicitly written, the equilibrium condition of the system with respect to the mass transport (Eq. (3)) takes the form of a thermodynamic equation of state for our subsystem '*i*' (Ref. [7], Eq. (1) therein). This in turn leads to the interrelation, shown in Fig. 3, between the surface charge density σ (corresponding to the field generated by the protein atom partial charges) and the relative mass density d of the adjacent layer of water molecules—the hydration envelope.

3. Results

With the data in Fig. 3 at hand (see also Appendix B), one can look for the (relative) mass density d of the hydration envelope, provided that the field generated by the protein atom partial charges is known. There is also a possibility to proceed in the reverse order (Fig. 4, Appendix C).

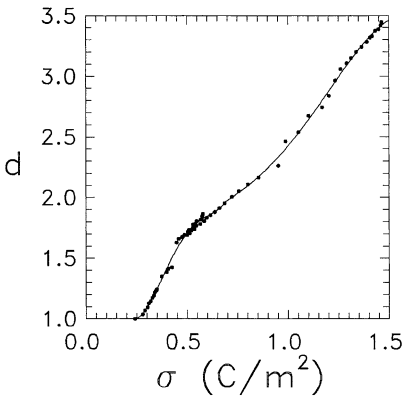

Fig. 3. The relative mass density d values as a function of the surface charge density σ. The points marked are obtained at $T = 293$ K on the basis of Ref. [7], Eq. (1) therein. The lines represent polynomial fits to the data in three separate ranges (see Appendix B).

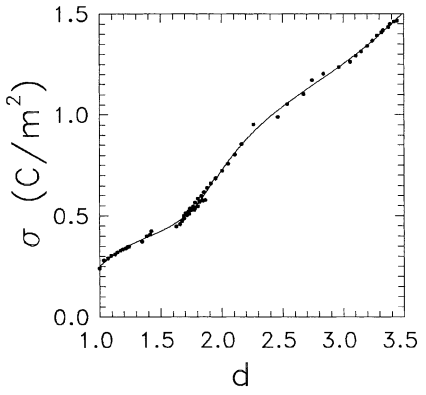

Fig. 4. The surface charge density σ as a function of the relative mass density *d*. The points marked are obtained at $T=293$ K on the basis of Ref. [7], Eq. (1) therein. For $d=1$, σ can take any value in the range $0 \le \sigma \le 0.24125$. For $1 \le d$ polynomials have been fitted to the $\sigma(d)$ relation (See Appendix C).

On the basis of the known structures of proteins, one can calculate the charge-generated fields in their hydration envelopes. Such a task has been undertaken in Ref. [2]. We exploit it for our purposes. Merzel and Smith [2] have applied their values of fields (surface charge densities σ) as a starting point for their MD simulations. We apply the same data as Merzel and Smith as a starting point for the calculation of hydration shell density *d* by our method. In this work, our earlier relation between *d* and σ is for the first time applied to the protein hydration envelope. Our primary concern is to gain insight into the work of our method when applied to this problem and to find out if it leads to reasonable results.

From the whole range of most populated field values found for lysozyme by Merzel and Smith [2], we take only the limiting values. We expect that any field values calculated on the basis of known densities of the hydration envelope of the same protein (lysozyme) should fall between them; this we consider as a criterion of the correctness of our approach. In Fig. 5a, there are the limiting values $(0.11\times10^9$ V/m) and $(19.36\times10^9$ V/m) of the field given in Ref. [2]. We take the values of mean relative density *d* of hydration water found by Svergun et al. [1] for chicken egg-white lysozyme, thioredoxine reductase from *E. coli* and ribonucleotide reductase protein R1 from *E. coli*. We find the corresponding field mean *E* values with the help of Fig. 4 and Eq. (1) and plot them in Fig. 5a. We observe that not only the resulting mean value of *E* for lysozyme (which is a must), but also the calculated mean values of *E* for two other proteins fall within the above-mentioned range. Similarly, starting from the values of mean volume v per H_2O molecule given in Refs. [3,13], which represent mean values of many kinds of proteins, we arrive

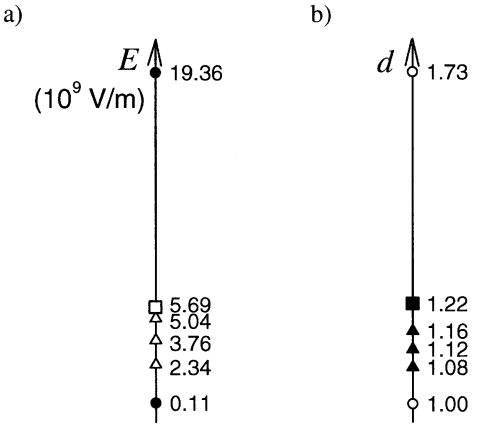

Fig. 5. (a) Electric field strength *E* values in the protein hydration envelope. ●–the lower $(0.11\times10^9$ V/m) and upper $(19.36\times10^9$ V/m) limits of the most highly populated field values generated by the protein (chicken egg-white lysozyme) atom partial charges [2]. Δ–*average* values of *E* (2.34, 3.76 and 5.04×10^9 V/m) calculated in this work on the basis of $\sigma(d)$ plot (Fig. 4) and Eq. (1) from the average relative density *d* values given in Ref. [1] for chicken egg-white lysozyme, thioredoxine reductase from *E. coli* and ribonucleotide reductase protein R1 from *E. coli*, respectively. □–*mean* value of *E* calculated on the basis of the *mean* volume of water molecule at the surface of a protein (a mean value found for many proteins in Refs. [13,3]). Full symbols—literature data, open symbols–calculated in this work. (b) Relative mass density *d* values in the protein hydration envelope. ○–the lower (1.00) and upper (1.73) values of *d* calculated in this work corresponding to the limits of the most highly populated field values generated by the protein (chicken egg-white lysozyme) atom partial charges [2]. ▲–the average relative density *d* values (1.08, 1.12 and 1.16) given in Ref. [1] for chicken egg-white lysozyme, thioredoxine reductase from *E. coli* and ribonucleotide reductase protein R1 from *E. coli*, respectively. ■–*d* from volume of water molecule at the surface of a protein (a mean value for many proteins given in Refs. [13,3]). Full symbols–literature data, open symbols–calculated in this work.

at a field E value falling within this range (Fig. 5a). Hence, our criterion of correctness of our approach formulated above is fulfilled. In this way, we have completed one of the aims of our work, namely we have found the electric fields acting on the hydration envelope of a biomolecule on the basis of its relative density d (or equivalently, mean volume v per water molecule).

Let us now turn to the second goal of the present work, which is the calculation of the water density within the hydration shell enhanced due to the flow of additional water molecules from the region outside the field (bulk water). The data on relative density d (or mean volume v per water molecule) are available from literature [1,3,13]. It remains to compare them with these we have found starting from the electric field E values within the hydration envelope. These values are comprised between the relative densities 1.00 and 1.73 found in this work with the help of Fig. 3 and Eq. (1) for the limiting values of the distribution of mostly populated field values for lysozyme (cf. Ref. [2] and Fig. 5a). As seen in Fig. 5b, all the values of water density d in the protein hydration shells collected from literature fall within the range $1.00 \leq d \leq 1.73$ calculated in this work.

We can summarize as follows:

1. Our approach applied to the protein hydration envelope leads to reasonable values of hydration water density (if field values are known) or the other way round, to reasonable values of fields originating from electric charges within the biomolecules and acting on the hydration shell, provided that the density of the latter is known.
2. The electric fields E at the outer surface of biomolecules, and their hydration shells densities d, taken from literature or calculated in this work, take relatively close values for different proteins.

4. Discussion

In this work we have undertaken one of the subjects discussed in the paper by Merzel and Smith, concerning the dependence of the density in the hydration envelope on the surface charge density (or electric field) created by the protein atom partial charges. We find an analogy with the subject of our earlier papers concerning the density of the double layers at the charged surfaces and ion hydration shells. In both cases, the subject of investigation represents a charged (or highly polarized) surface immersed in an aqueous electrolyte. Merzel and Smith state: 'We do find clear density effects if a simple view of the protein is taken, in which it is considered to be an envelope with an associated electrostatic field generated by the protein atom partial charges' and 'a clear electrostatic effect is demonstrated here' (Ref. [2]). It is just this 'simple view' of the protein, but with addition of the bulk aqueous electrolyte surrounding the hydrated protein molecule, which is adopted in this work.

This is consistent with our earlier approach to the hydration phenomenon. In our opinion, we have made a further step in the description of the envelope properties: namely, the new quantitative relation between the density effects in an envelope and the associated electrostatic field generated by the protein atom partial charges. This relation has the form of a thermodynamic equation of state (Ref. [7], Eq. (1) therein). The *quantitative relations* ($d(E)$ or $E(d)$) between the water shell density and the electric field strength (Figs. 3 and 4) follow therefrom. Similar effects occur also in double layers at the surfaces of charged or highly polar solids in aqueous electrolytes [8,9]. Let us recall that the fundamental assumption leading to these relations is that the surface water layer forms a common system with the remaining water localized outside the electric field (Fig. 2). Within our approach, it is the huge electrostriction [6,7] that is responsible for the high density (compression) effect. Also, the observation (Ref. [2]) that 'the dipole orientation perturbations are highly correlated with the water density, i.e. high density regions are those with dipoles more parallel to each other' agrees qualitatively with our findings, since the 'high density regions' are within our approach those subdue to the high field, which forces the dipoles to align in one common direction, and hence makes them parallel to each other. Such a configuration of dipoles bears the name of a 'dielectric saturation' and is accompanied by lowering of the electric permittivity seen in Fig. 1 for high σ values.

5. Conclusion

We have exploited the hitherto almost unnoticed analogy between dense water layers at charged metal electrodes [8] or at the surfaces of highly polar oxides [9], on the one hand, and the compressed hydration shells at surfaces of protein molecules, on the other hand. We have pursued two goals. The essence of the first one has been to find the values of *mechanical quantities*, such as the relative mass density d and volume per H_2O molecule v, describing the state of water forming the shell surrounding a biomolecule, on the basis of *electric quantities*, such as electric field strength E or surface charge density σ, known from literature [2]. The second one was just the reverse, to find the values of *electric quantities*, such as E or σ, having at our disposal the *mechanical* ones, such as d [1] or v [13,3]. We have found that the resulting *calculated* values of the quantities in question, both electric and mechanical, fall well into the ranges of the *measured* ones found in literature. The consistency found between the calculated and literature data suggests a leading role of electrostatics in the hydration of biomolecules.

Appendix A: Fitting polynomials to Fig. 1 data

The lines (Fig. 1) represent the fitting polynomials. Thus, the value of permittivity ε for a given σ can be found either graphically, directly from Fig. 1 or numerically, by applying the polynomial fits. We have found two different polynomial fits of 6th order in σ to the data in Fig. 1: one for the range $0 \le \sigma \le 0.276$ and another one for $0.276 \le \sigma \le 0.5$. In the range $0.5 \le \sigma \le 1.1$ a linear fit has been applied. The explicit mathematical form of these polynomials is: $\varepsilon \cong A_0 + A_1\sigma + A_2\sigma^2 + A_3\sigma^3 + A_4\sigma^4 + A_5\sigma^5 + A_6\sigma^6$. The coefficients A_n are collected in Table A.1.

Table A.1
Coefficients of the sixth order fitting polynomials in σ to the data marked in Fig. 1

	$0 \le \sigma \le 0.276$	$0.276 \le \sigma \le 0.5$	$0.5 \le \sigma \le 1.1$
A_0	81.9802	20176.7	4.31522
A_1	27.2991	−309124	−2.03292
A_2	−1743.52	1968 150	0
A_3	28159.2	−6658030	0
A_4	−256956	12614 500	0
A_5	1040 020	−12687000	0
A_6	−1589360	5290 700	0

Appendix B: Fitting polynomials to Fig. 3 data

The lines (Fig. 3) represent the fitting polynomials. Thus, the value of relative density d for a given σ can be found either graphically, directly from Fig. 3 or numerically, by applying the polynomial fits. We have found polynomial fits to the data in three separate ranges of the independent variable σ. The explicit mathematical form of these polynomials is: $d \cong B_0 + B_1\sigma + B_2\sigma^2 + B_3\sigma^3 + B_4\sigma^4$. The coefficients B_n are collected in Table B.1.

Table B.1
Coefficients of the fourth order fitting polynomials in σ to the data marked in Fig. 3

	$0 \le \sigma \le 0.24125$	$0.24125 \le \sigma \le 0.6865$	$0.6861 \le \sigma \le 1.5$
B_0	1	4.50748	−0.350567
B_1	0	−37.7997	9.50673
B_2	0	138.513	−16.2021
B_3	0	−198.623	12.9949
B_4	0	100.741	−3.52612

Appendix C: Fitting polynomials to Fig. 4 data

The (inverse) dependence $\sigma(d)$ is shown in Fig. 4. We observe that the surface charge density takes the values in the range $0 \le \sigma \le 0.24125$ for $d = 1$. For $1 \le d \le 1.95$ a polynomial of the 4th order in d has been fitted. For $1.95 \le d \le 3.5$ yet another polynomial of the 4th order in d has been fitted to the $\sigma(d)$ relation. Again, the fitting polynomials

Table C.1
Coefficients of the fourth order fitting polynomials in d to the data marked in Fig. 4

	$1 \le d \le 1.95$	$1.95 \le d \le 3.5$
C_0	−1.08387	−8.25642
C_1	1.90354	10.8419
C_2	−0.0968121	−4.83164
C_3	−0.747045	0.96734
C_4	0.27497	−0.0697664

are written. The explicit form of these polynomials is: $\sigma \cong C_0 + C_1 d + C_2 d^2 + C_3 d^3 + C_4 d^4$. The coefficients C_n are collected in Table C.1.

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