Link between the hydration enthalpy of lysozyme and the density of its hydration water: Electrostriction

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Hydration shells around proteins in solution are on average denser than bulk water. Variations in enthalpy are observed during hydration/dehydration of proteins. To explain consistently those phenomena, a common mechanism—electrostriction—underlying the mechanical and contributing to thermal effects is proposed. The mean mass density of the hydration shell of lysozyme derived from the neutron and X-ray scattering is explained as following the compression of water in the fields of the order of 10^9 V m^{-1} due to the charged sites at the boundary of the protein. The mean enthalpy of mixing ΔH^{mean} of lysozyme in water calculated on the basis of the measured mean mass density falls in the middle of the values of the enthalpy of mixing ΔH^{mix} observed in sorption experiments. This testifies that ΔH^{mix} is due in part to the work done by the electrostriction pressure in hydration shell regions situated in high electric fields. The dependence of the sorption enthalpy of exemplary proteins on the number of adsorbed H_2O molecules is also described in terms of electrostriction.

I. Introduction

Characterization of the hydration water of proteins is essential for understanding the protein structure, folding and stability[1,2] as well as their biological functions (see ref. 3 and references therein). Opinions have been expressed that the studies of protein–water interactions are of value for the use of proteins as therapeutic agents[4,5] and for food preservation.[5] The hydration shells of protein molecules are affected by the charged surface sites and the highly polar surface regions. They are usually termed hydrophilic sites and typically cover about 40% of their solvent-accessible surfaces,[2] although they cover about 74%[6] of the surfaces of lysozyme molecules in their native state. The hydration shells around the immersed protein molecules are on average denser than bulk water, as found by small angle neutron scattering (SANS) and small angle X-ray scattering (SAXS) by Svergun *et al.*[7] and Ortore *et al.*[8] An analogous enhancement of the density of a water layer with a nanometre thickness at a charged metallic electrode/aqueous electrolyte interface has been observed earlier and qualitatively explained in terms of electrostriction[9,10] followed by our calculations[11] confirming that this explanation is quantitatively correct.

The local charges of the protein atoms give rise to electric fields. They induce inhomogeneity in the hydration shells through polarization of H_2O dipoles and pulling of additional dipoles into the field, an effect occurring in open fluid systems.[11–13] The latter effect ultimately puts the H_2O molecules (locally) closer together or, which is the same, makes the hydration shell at charged sites denser (electrostricted). The electric field performs work while polarizing the hydration water. This work can be followed by a thermal effect. Hence, the main idea behind the current work comes to mind: to calculate the electric field-related hydration enthalpy. There are two consequences of the work done by the electric field on hydration water. The first one consists of the change in the entropy of water which leads to an electrocaloric effect. It will be shown that it is small enough, comparable to the accuracy of the thermal experiments. The second one consists in the appearance of an electrostriction pressure that compresses hydration water. It is accompanied by a thermal effect. It will be shown that it is large, comparable to the enthalpy of hydration measured. The calculations are performed with the help of the thermodynamic equation of state leading to relations between the density (or specific volume) of water, the electric field and the polarization work providing a contribution to enthalpy. When found, this theoretical enthalpy value based on the mass density data from scattering experiments[7,8] should be compared with these actually measured during hydration in sorption/desorption experiments.[4,5,14] If the comparison will turn out favorable, it will testify that the electrostriction work does indeed provide a non-negligible contribution to the enthalpy of protein hydration measured in the sorption/desorption experiments.[4,5,14,15] In this way the main aim of the current work will be achieved. Further on, the essential feature of the hydration enthalpy, namely its dependence on the number of water molecules adsorbed at the surface of the protein, should be explained. To this aim, we reverse the reasoning and calculate the local fields corresponding to the enthalpy values stemming from the sorption experiments on a number of proteins. One shall discuss whether these fields take reasonable values and do fit the hydration dependence of the enthalpy. An additional comparison with hydration of amino acids in the gas phase is provided.

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A possible experiment checking the validity of the proposed enthalpy-field relation is indicated.

Note that one can investigate the effect of surrounding water on the properties of proteins. The other way round, the properties of the hydration water itself under the influence of the protein surface can be chosen as the subject. It is the latter question that we deal with. Our focus here will be on water at lysozyme as the typical and most investigated globular protein.

An important question arises if our approach is applicable to other proteins apart from lysozyme. We discuss this question on the basis of the values of surface electric fields calculated for two other exemplary proteins with available hydration enthalpy data.

We consider hydration water in the whole range from the full layer coverage of lysozyme molecules in solution[7,8] down to the low humidity regime, with the incomplete layer divided into separate clusters.[4,5,14] The available thermal data concerned only the low hydration regime and noticeable values of enthalpy of mixing were observed only below about a half filling of the hydration shell, whereas the scattering data were taken on fully hydrated lysozyme. We will offer a common look at the data belonging to these two regimes and in general to those at all hydration degrees. In particular, the apparent lack of data of noticeable enthalpy of mixing at high hydration[4] (where, as we argue, sites in no field or in the very low one are hydrated), is explained by pointing out that electrostriction-related enthalpy values lower than the accuracy attained in the recent thermal experiments cannot be registered.[4,5,14]

II. Method

A Enthalpy of mixing in the electric field

We shall calculate two contributions to the enthalpy of mixing water to protein (hydration enthalpy) in the electric field:

$$\Delta H^{\mathrm{mix}} = \Delta H_S + \Delta H_\Pi, \tag{1}$$

where ΔH_S is the contribution to the enthalpy related to the entropy and ΔH_Π represents the contribution to the enthalpy due to electrostriction. Both contributions originate in the work done by the electric field on the dipole moments of H_2O molecules: the former by polarizing them, the latter by putting them closer together. ΔH^{mix} in the form given by eqn (1) formally represents the usual expression for the enthalpy increment as a function of entropy S and pressure. Note that the term containing the pressure increment depends on the electrostriction pressure Π only, since the experiments considered are performed under constant ambient pressure.

For the sake of completeness and because we apply here some improved data[16] not available until recently, we will go into the details of the calculations.[17,18]

B Equilibrium condition. Flow of the dipolar H_2O molecules into the hydration shell of a protein

In order to calculate the electrostriction[19] as well as the change in entropy ΔS in a high electric field at a charged or highly polar surface, we will apply a statistical model of the water permittivity $\varepsilon = \varepsilon(T, P, \sigma)$.[17,18] A high electric field

$$E = \frac{\sigma}{\varepsilon\varepsilon_0} \tag{2}$$

performs a work W on H_2O molecules. We use the following notations: E is the electric field strength, σ is the surface charge density and ε_0 is the permittivity of vacuum. The increment $\mathrm{d}W$ in the work done by the field E on a dielectric of a constant volume V amounts to

$$\mathrm{d}W = V\,E\,\mathrm{d}y, \tag{3}$$

where y has the meaning of the dielectric polarization. We recall that

$$y = \varepsilon_0 E(\varepsilon - 1). \tag{4}$$

Taking into account eqn (2) we obtain

$$y = \sigma\left(1 - \frac{1}{\varepsilon}\right). \tag{5}$$

Taking the integral of eqn (3) we obtain the expression

$$W = \frac{V}{\varepsilon_o}\int_0^y \frac{\sigma}{\varepsilon}\,\mathrm{d}y. \tag{6}$$

The work W due to the polarization of water changes the chemical potential by ζ_W

$$\zeta_W = \left(\frac{\partial W}{\partial N}\right)_{\sigma,T,V} \tag{7}$$

with respect to the chemical potential ζ of water outside the field. N is the number of molecules within the volume V. Let us introduce the notation:

$$f = \int_0^y \frac{\sigma}{\varepsilon}\,\mathrm{d}y. \tag{8}$$

One obtains eqn (9):

$$\zeta_W = \frac{V}{\varepsilon_o}\left(\frac{\partial f}{\partial N}\right)_{\sigma,T,V}. \tag{9}$$

To attain the equilibrium in the open system consisting of a subsystem in the field (the hydration shell) and a water reservoir outside the field, the chemical potential gradient between water in the field and that outside the field induces a spontaneous irreversible process: pulling of the dipoles into the field. (Another well-known example of a similar process represents diffusion occurring in the presence of a concentration gradient, which is also accompanied by chemical potential gradient.) We are interested in a new equilibrium state with water density increased in the high field (say, in the hydration shell, or its part, of the protein at hydrophilic sites) due to this process. The work L related to water compression in the field (the electrostriction work) changes the chemical potential by ζ_L.[11] After reaching the equilibrium, the latter compensates the negative increment ζ_W:

$$\zeta_W + \zeta_L = 0. \tag{10}$$

C Thermodynamic equation of state

From eqn (10) taking into account eqn (9) one obtains the following thermodynamic equation of state of H_2O in the electric field, expressed in the variables T, Π, σ:[11,17]

$$-\frac{V}{\varepsilon_o}\left[\left(\frac{\partial f}{\partial y}\right)\left(\frac{\partial y}{\partial \varepsilon}\right)\right]_N\left(\frac{\partial \varepsilon}{\partial N}\right)_\sigma = \int_{P_o}^{\Pi} v(P)\,\mathrm{d}P, \qquad (11)$$

where P_o is the atmospheric pressure, N is the number of water molecules in the field E within the constant volume V (say, that of a portion of the hydration shell), $v(P)$ is the molar volume of water under pressure P and

$$\int_{P_o}^{\Pi} v(P)\,\mathrm{d}P = \zeta_L. \qquad (12)$$

Since the actual dependence of the volume $v = v(\Pi)$ on the local electrostriction pressure Π is not available, the isotherms $v = v(P)$ of H_2O under external pressure P in the absence of a field[20] are applied instead (*cf.* ref. 11). This approximation proved to be quantitatively valid.[11]

The equation of state (11) together with eqns (5), (8) and (12) provide a relation between ζ_L and σ.

The work W done by the electric field E induces a change in entropy ΔS of the subsystem in the field:[17]

$$\Delta S = -\left(\frac{\partial W}{\partial T}\right)_{\sigma,N,V} \qquad (13)$$

or

$$\Delta S = -\frac{V}{\varepsilon_o}\left(\frac{\partial f}{\partial T}\right)_{\sigma,N,V}. \qquad (14)$$

The quantity f (see eqn (8)) depends on the temperature T *via* $\varepsilon(T)$. The expression for the heat $T\Delta S$ due to the electrocaloric effect is:

$$T\Delta S = \frac{TV}{\varepsilon_o}\left[\left(\frac{\partial f}{\partial y}\right)\left(\frac{\partial y}{\partial \varepsilon}\right)\right]_{\zeta_L}\left(\frac{\partial \varepsilon}{\partial T}\right)_y. \qquad (15)$$

D Statistical approach to water permittivity in a high electric field

The partial derivatives in eqn (11) and (15) have been calculated on the basis of a statistical model of water permittivity ε.[18] The latter is needed here due to the difficulties in obtaining experimental data of static ε: the highest electric fields E realizable in condensers filled with liquids are $E \leq 10^7$ V m^{-1} while we are dealing here with the fields of the order of 10^9 V m^{-1} (*vide infra*). The obtained relation $\varepsilon(E)$ of the well-known sigmoid shape has been confirmed by Monte Carlo calculations by Joshi *et al.*[21] and has led to a quantitatively correct description of a number of phenomena in water in high electric field.[17] In brief, a calculation of the statistical mean value $\langle\cos\theta\rangle$ of the cosine of angle θ between the dipole moment of a H_2O molecule and the field direction, with the hydrogen bonds imposing restrictions on the possible orientations of the dipole moments of H_2O molecules, is involved. Namely, the water–water and water–protein hydrogen bonds restrict the number of admissible directions of the dipole moment of a H_2O molecule in the field to just two (*cf.* ref. 18, eqn (2) and Fig. 2 therein, where some of the depicted protons should at present be considered as belonging to protein–water bonds). Since the field is produced by the surface charge density of the protein, the interactions between the protein charge and water dipoles are in this way accounted for as a part of the electrostatic energy of the protein–water bonding.

Our approach has the advantage of reproducing correctly $\varepsilon(E)$ as a function of the field E without any adjustable parameters. We refer the reader to ref. 11, 17 and 18 for details. The derivative $\partial\varepsilon/\partial T$ differs somehow with respect to that given in our earlier work. Thus, we will provide a detailed expression. Taking into account the relation between the permittivity ε and the surface charge density σ given in ref. 18 (eqn (14), (16) and (17) therein) and the fact that the refraction index n depends on the temperature and pressure, one arrives at eqn (16):

$$\left(\frac{\partial \varepsilon}{\partial T}\right)_\sigma = -\frac{\varepsilon + n^2/2}{T(1-gVn^2)} + \left(\frac{\partial n}{\partial T}\right)\frac{2n[\varepsilon - 1 - gV\varepsilon(\varepsilon+2)]}{(n^2+2)(1-gVn^2)} + \alpha_P\frac{gV\varepsilon(\varepsilon-n^2)}{1-gVn^2} \qquad (16)$$

with

$$g = \frac{6k\varepsilon_0 NT(\varepsilon + n^2/2)^2}{[3\sigma V(\varepsilon - n^2)]^2 - [N\mu\varepsilon(n^2+2)]^2}, \qquad (17)$$

where μ denotes the dipole moment of an H_2O molecule and α_P is the thermal expansion coefficient. The partial derivatives in eqn (15) and (16) were calculated for the following data: the data for the permittivity of water in the high field E were taken from ref. 18. The data for the refraction index n were taken from Dewaele's *et al.* work[16] presenting an accurate determination of n of H_2O up to 35 GPa at ambient temperature. These authors[16] applied the Gladstone-Dale relation:

$$n = a + b\rho, \qquad (18)$$

where for H_2O the parameters a and b take the values $a = 1 \pm 0.01$ and $b = 6.05 \pm 0.2$ cm^3 mol^{-1}, respectively. Temperature and pressure enter eqn (18) through the dependence of the mass density ρ on these variables: $\rho = \rho(T,P)$. At $T = 293$ K and at slightly different temperatures, the values of n have been calculated from eqn (18) with the ρ data taken from ref. 22. In the current work, we have calculated $\partial n/\partial T$ with a plausible assumption that although $\rho(T,P)$ does depend on both T and P, the values of the a and b coefficients in eqn (18) do not vary in a narrow range of temperature about 293 K. In our earlier work[17,23] we did not apply eqn (18) and a crude approximation of the value of $\partial n/\partial T$ taken as independent of temperature and pressure has been admitted. In the current work, we have obtained from the calculations $-\partial n/\partial T = 0.75$ K^{-1} for $T = 293$ K. The thermal expansion coefficient of water α_P occurring in eqn (16) has been calculated on the basis of the data taken from ref. 22, $\alpha_P = 2.07 \times 10^{-4}$ K^{-1}. The second and third terms in eqn (16) are negligible with respect to the

first one. In the following (subsection III.A), we will argue that in eqn (1) the term $\Delta H_S = -T\Delta S$ containing all the above contributions is negligible with respect to ΔH_Π.

E On the calculation of local thermodynamic quantities in spatially inhomogeneous systems

A question may arise concerning the validity of the thermodynamic and statistical description of the hydration shell around the protein molecule at the particular chosen parts of the shell. This problem is resolved on the basis of the concept of subsystems of H_2O molecules in the same physical conditions on the statistical ensemble of protein molecules and thoroughly discussed in ref. 13.

In the case of the surfaces of protein molecules one has to do with inhomogeneous charged non-flat dielectric surface either fully hydrated (in solution) or only partly hydrated. High electric fields are generated by the charges of the atoms or highly polar sites at the boundaries of the protein molecules. Similar to the case of hydration shells of ions,[24] the local relative number density is the number of H_2O molecules in a volume cut from a molecular layer at a chosen, chemically and physically well specified, small region of the surface of an average protein molecule divided by the number of H_2O molecules in the same volume of the bulk water. The definition of the local relative mass density is analogous. Since the definition concerns a local feature, it is applicable also to separate water clusters that can form below the H-bond percolation threshold[6,25] at the surface of a partially hydrated protein. One considers many small volumes in the same physical conditions inside (field strength—high, moderate or none), similar to the case of ions in solution. In order to obtain local average quantities such as local mass density, local permittivity, local electrostriction pressure *etc*. one has to calculate the averages over the statistical ensembles of subsystems of such volumes at the mutually corresponding (homologous) surface sites of a macroscopic system of protein molecules.

III. Results

The results stem from the numerical solutions of eqn (11). Some time ago, by applying a thermodynamic and statistical approach, we have calculated the electric field acting on water within the double layer at an electrode on the basis of its relative mass density d.[26] Later on, we have applied the same approach to find the mean field E at the surface of proteins[12] on the basis of the mean relative density d of hydration water taken from the work by Svergun *et al*.[7] We have presented qualitative arguments that the enhanced density of hydration water may be due to electrostriction in high electric fields close to charged or highly polar sites.[12] In this section, we will find a quantitative relation between d taken from ref. 7 and 8 and the hydration enthalpy of lysozyme. We have found earlier[12] the value of the mean field strength E corresponding to $d = 1.11$ (SANS).[7] One can find the other mean field strength E values from the SAXS[7,8] data with the help of Fig. 1a. The curve plotted in Fig. 1a is based on the data of ref. 20 for hydrostatic pressure P values identified with $\Pi(E) = P$ (*cf*. text following eqn (12)); the electrostriction pressures $\Pi(E)$ were found for the proper values of E with the help of eqn (11).

The next step was to find the chemical potential increment $-\zeta_W$ as a function of E as shown in Fig. 1b. Fig. 1b shows the plot of the calculated chemical potential increment $-\zeta_W$ as a function of the electric field strength E. The curve represents a part of our earlier results (ref. 11, Fig. 2 therein).

A Enthalpy of hydration of lysozyme in solution

The work done by the electric field on water can lead to a thermal effect. Let us look at the entropy-related contribution to the enthalpy. The changes in the heat calculated on the basis of eqn (15) plotted as a function of E are shown in Fig. 2.

ΔS is the change in entropy of water in the field E. In Fig. 2, the electric field strength E varies in the range $0.1 \times 10^9 < E < 3.5 \times 10^9$ V m^{-1}. For the field strength in this range, permittivity ε takes values in the range $77 > \varepsilon > 10$ (see Fig. 6 and ref. 18, Table 2 therein). At the relatively low

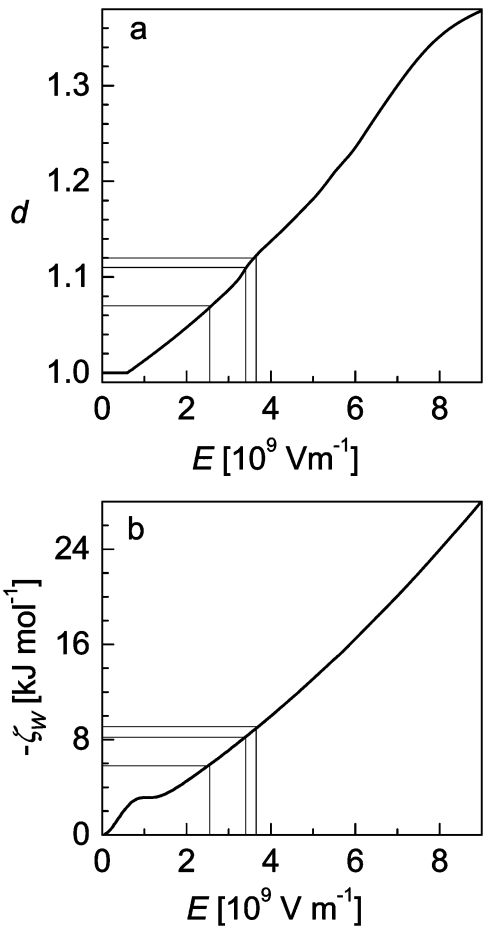

Fig. 1 Solution of the equation of state (11). (a) Relative mass density d of water in the electric field of strength E as a function of E at 293 K. Straight segments show how to find the values of E: 3.6, 3.4 and 2.6 [10^9 V m^{-1}], corresponding to the mean values of the mass density of hydration water of lysozyme: $d = 1.12$ (SAXS),[8] 1.11 (SANS)[7] and 1.07 (SAXS),[7] respectively. (b) Chemical potential increment $-\zeta_W$ as a function of the electric field strength E. Straight segments show how to find the values of $-\zeta_W = \zeta_L \approx -\Delta H_\Pi \approx -\Delta H^{\text{mean}}$: 9.1, 8.2 and 5.8 [kJ mol^{-1}], respectively.

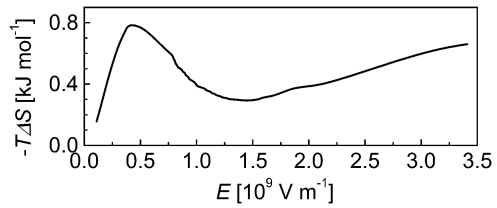

Fig. 2 Calculated values of the heat $-T\Delta S$ due to the electrocaloric effect as a function of the electric field strength E at 293 K.

local field strength values, to the left of the range shown ($E < 0.1 \times 10^9$ V m^{-1}), the changes in $T\Delta S$ tend to zero as $E \to 0$. At high fields, to the right of the range shown ($E > 3.5 \times 10^9$ V m^{-1}), $T\Delta S$ decreases again due to the factor $\partial\varepsilon/\partial T$ entering eqn (15), since $\partial\varepsilon/\partial T \to 0$ as $E > 3.5 \times 10^9$ V m^{-1}. Indeed, for fields higher than $E > 3.5 \times 10^9$ V m^{-1}, the plots of $\varepsilon = \varepsilon(E)$ for different temperatures tend to cover each other (*cf.* ref. 18, Fig. 3 therein), which means that $\partial\varepsilon/\partial T \to 0$ for higher fields. Therefore, $T\Delta S \to 0$ to the right of the range shown in Fig. 2, too. It follows that the value of our calculated entropy contribution $T\Delta S$ (identical to $-\Delta H_S$) to the enthalpy of hydration (eqn (1)) is lower than ≈ 1 kJ mol^{-1} in the whole range of fields.

The electrostriction contribution to the enthalpy of hydration $-\Delta H_\Pi \approx \zeta_L$ for a given value of the field strength E acting on a portion of the hydration shell at 293 K can be found in Fig. 1b. The local relative mass density d of water as a function of the field strength E is shown in Fig. 1a. The function $d(E)$ is found by solving numerically the equation of state (11) (*cf.* text following eqn (12)). With the known value of d one can find the corresponding field strength E in Fig. 1a and with the value of E at hand one obtains the contribution $-\Delta H_\Pi \approx -\zeta_W$ with the help of Fig. 1b.

Given the value of the mean relative mass density $d = 1.07$ of the hydration shell of lysozyme in solution (fully hydrated) found by SAXS by Svergun *et al.*,[7] we are led to the field value $E = 2.6 \times 10^9$ V m^{-1} (see Fig. 1a) and the value $\zeta_L = 5.8$ kJ mol^{-1} of the electrostriction work done on one mole of the hydration water by this field. Hence, the value of the mean enthalpy of mixing $\Delta H^{\text{mean}} \approx -\zeta_L \approx \Delta H_\Pi = -5.8$ kJ mol^{-1}. This is marked by the bottom full circle in Fig. 3. Since the calculated ΔH^{mean} values should be compared with the experimental data of ΔH^{mix} taken as approximately equal to ΔH_Π with the neglected small contribution ΔH_S, we neglect the latter for consistency's sake in this case, too.

For the mean relative water density $d = 1.11$ of hydration water found by SANS[7] one obtains[12] the field value $E = 3.4 \times 10^9$ V m^{-1} (see Fig. 1a) and ultimately one finds the value of the mean enthalpy of mixing $\Delta H^{\text{mean}} \approx -\zeta_L \approx \Delta H_\Pi = -8.2$ kJ mol^{-1} (*cf.* eqn (1)). This is marked by the middle full circle in Fig. 3.

For the value of the mean relative water density $d = 1.12$ found by SAXS[8] corresponding to the field value $E = 3.6 \times 10^9$ V m^{-1} (see Fig. 1), one finds the value of the mean enthalpy of mixing $\Delta H^{\text{mean}} \approx -\zeta_L \approx \Delta H_\Pi = -9.1$ kJ mol^{-1} (*cf.* eqn (1)). This is marked by the top full circle in Fig. 3. We have taken only the d value obtained by Ortore *et al.*[8] under atmospheric pressure from their study of the hydrostatic pressure effect on d.

Let us recall that since it is the *value* E of the electric field vector and not its direction which matters, the results do not depend on the sign of E. Hence, the sign of the electric charges does not matter either. Local fields close to the mean ones mentioned herein before and consequently similar properties of hydration water (*e.g.*, the mass density) can be found within the first hydration shells of Li^+ ions.[24]

B Enthalpy of sorption of water at the surface of solid lysozyme

The enthalpy ΔH^{sorp} of H_2O vapor sorption on dry proteins[15] is:

$$\Delta H^{\text{sorp}} = \Delta H^{\text{mix}} + \Delta H^{\text{cond}}, \qquad (19)$$

where ΔH^{mix} is the molar enthalpy of mixing of water in lysozyme.[4,15] We follow the remark by Smith *et al.*[5] that since the sorption experiments deal with water vapor, the enthalpy of condensation ΔH^{cond} should be taken into account. The ΔH^{sorp} data have been read by this author from figures in the corresponding publications (ref. 4, 5 and 14) and subsequently the value of the enthalpy of condensation $\Delta H^{\text{cond}} = -44$ kJ mol^{-1} was subtracted to get ΔH^{mix}. In Fig. 3, the values of $-\Delta H^{\text{mix}} = -\Delta H^{\text{sorp}} + \Delta H^{\text{cond}}$ thus obtained are marked as a function of the mass % of the adsorbed water per mass of lysozyme. At higher hydration, after a number of experimental runs, the enthalpy of mixing values indiscernible from zero within the data accuracy are sometimes reached.[5,14] Luthra *et al.*[4] call ΔH^{mix} "the heat of protein–water interaction" and consider it to be equal to zero at high hydration. Even before reaching the high hydration limit (or after leaving it during desorption) some processes changing the structure of the protein can occur reverting the tendency in the ΔH^{sorp} variations due to an additional term $\Delta H^{\text{conformational}}$ attributed to conformational changes in the protein.[4] Since in the current paper we are interested in the properties of hydration water and not the protein itself, we will take into account only four data points corresponding to lower hydration levels of lysozyme and bovine serum albumin BSA available in ref. 4, Fig. 3 and 5 therein, and five data points for immunoglobulin IgG (ref. 4 Fig. 7 therein) referred to hereafter.

C Comparison of the theory with experiment

With the knowledge of the values of the mean relative mass density d of the water shells at the surfaces of lysozyme molecules in solution found by neutron scattering (SANS),[7] and X-ray scattering (SAXS),[7,8] we were able to calculate the values of the mean enthalpy of hydration. The calculations were performed on the basis of the equation of state of water (eqn (11)) in electric field and a statistical model of the electric permittivity of water. Following ref. 12, we have admitted that the mean relative water density d of the H_2O molecular layer at lysozyme reported by Svergun *et al.*[7] (see also ref. 8) is mainly due to the action of the electric field E stemming from the surface charge density σ of the protein molecules. Subsequently, we have obtained the values of E and the related quantities such as ΔH_S, $\Delta H_\Pi \approx -\zeta_L$ and finally the mean molar enthalpy of mixing ΔH^{mean} of water in lysozyme. The latter *calculated* quantity is compared with the ones *measured* during sorption[4,5,14] of water at the surface of the same

substance (lysozyme) (Fig. 3). The calculated mean enthalpy values marked as the full circles in Fig. 3 fall well within the range of the experimental values of the enthalpy of mixing found during sorption experiments on the same protein. So, the calculated enthalpy provides a non-negligible contribution to the total (measured) enthalpy of mixing. *This is the first main result of our work*. It confirms that the electrostriction work provides a non-negligible contribution to the hydration enthalpy of lysozyme (see also section "IV Discussion" hereafter).

D Thermal effect during protein hydration explained

Enthalpy of mixing depends on the coverage by water of the protein molecules during sorption runs in a characteristic way (Fig. 3): it is large at the beginning of the hydration process of the initial dry protein (low hydration) and decreases during subsequent hydration runs down to negligible values at higher hydration.[4] Desorption provides similar dependences (Fig. 3 and 4).[4] In this subsection, we will describe this behavior in terms of electrostriction.

We will discuss in some detail the picture that arises from our finding that the enthalpy of sorption of water at the surface of lysozyme can be understood partly in terms of the electrostriction of water and extend it to two other exemplary proteins (BSA[4] and IgG[4]) to demonstrate that our approach is not limited to a single protein.

The character of the ΔH^{mix} variations seen in Fig. 3 and 4 resembles that of consecutive energies of binding water molecules one after another to amino acid and other protein-forming chemical groups in the gas phase, as measured in mass spectrometry (MS). For example, Wincel[27] has found by MS that the hydrogen-bond energies for protonated amino acids $AAH^+ (H_2O)_n$, where AA = Gly, Ala, Phe and Pro, decrease with rising n. The same tendency has been found by MS for

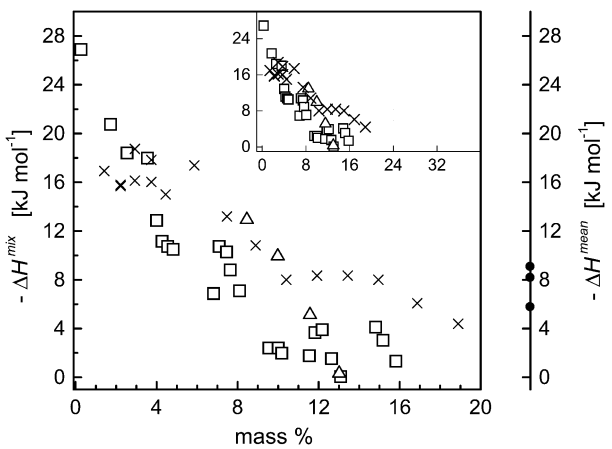

Fig. 3 Enthalpy of mixing of water in lysozyme at 293 K, as a function of water to lysozyme mass % derived from the data by Bone,[14] Smith *et al.*[5] and Luthra *et al.*[4] marked by ×, □ (absorption) and △ (desorption), respectively. Insert shows the same $-\Delta H^{mix}$ data in the range up to the complete hydration of all polar sites.[5] The full circles on an additional right-hand-side axis show three values of $-\Delta H^{mean}$ calculated on the basis of the mean relative mass density d of the hydration shell of lysozyme in water solution from SAXS,[7] SANS[7] and SAXS[8] data, from the bottom to the top, respectively.

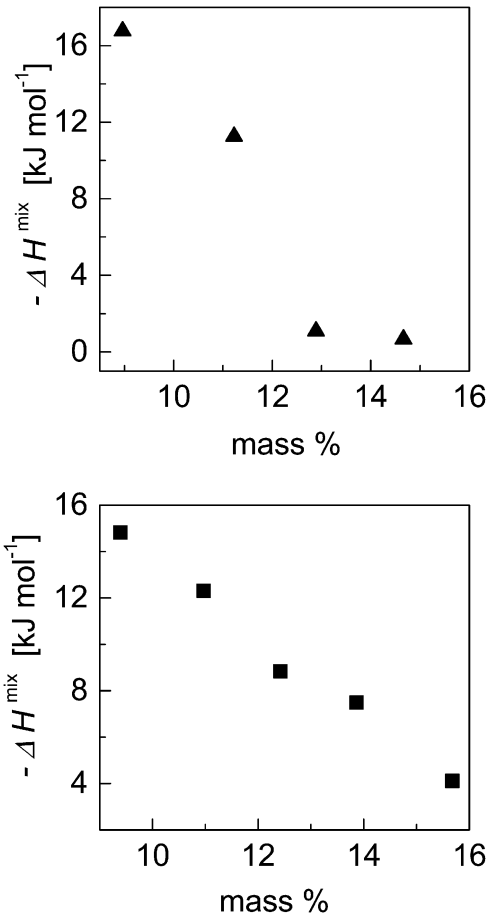

Fig. 4 Enthalpy of mixing of water in proteins BSA (upper figure) and IgG (lower figure) at 293 K, as a function of water to protein mass % derived from the water desorption data by Luthra *et al.*[4] marked by ▲ and ■, respectively.

n-decylamine and arginine.[28] According to Liu *et al.*[29] it can arrive so since "the electrostatic interactions with successively ligated water are successively weaker". Note that it means essentially the same as our suggestion (see hereafter) that positioning consecutive water molecules at less and less charged sites leads to weaker electrostriction-related enthalpy contributions.

To the enthalpy of hydration of a portion of water adsorbed during a particular experimental run one can associate a definite electric field strength value (*cf.* Fig. 5) characterizing that region of the surface of the protein where this portion of water has been deposited. To be specific, note that the accuracy of estimation of the value of $-\Delta H_S$ is not important, since it falls within the spread of measured values of the enthalpy of mixing ΔH^{mix} seen in Fig. 3.

For our current purposes $\Delta H_S < 1$ kJ mol^{-1} can simply be neglected when dealing with the sorption data the spread of which exceeds one kJ mol^{-1}. Therefore, by subtracting the value of the condensation enthalpy ΔH^{cond} from the ΔH^{sorp} data of ref. 4, 5 and 14, one obtains approximately the enthalpy of electrostriction: $\Delta H^{sorp} - \Delta H^{cond} = \Delta H^{mix} \approx \Delta H_{\Pi}$. So, our suggested recipe for finding the value of the local electric field strength E related to a particular value of enthalpy says: take a definite value of ΔH^{mix} marked in

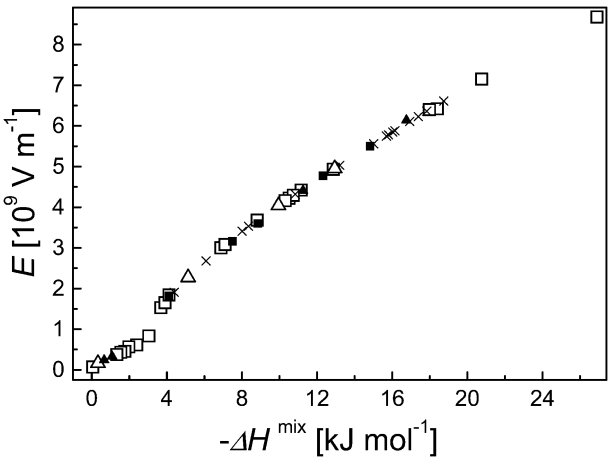

Fig. 5 The calculated electric field strength E as a function of the enthalpy of mixing of water in protein $-\Delta H^{mix}$. The latter is approximately equal to ζ_L—the electrostriction work per mol of water. The symbols for lysozyme[4,5,14] are the same as in Fig. 3, for BSA[4] and IgG[4]—the same as in Fig. 4.

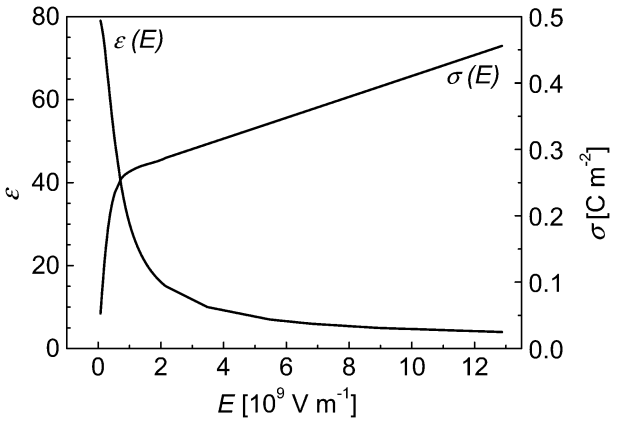

Fig. 6 Dielectric permittivity ε of water at 293 K (left scale[18]) and surface charge density σ (right scale, *cf.* eqn (2)) as a function of the electric field strength E.

Fig. 5 and look for the corresponding value of the field strength E.

Let us discuss the high-, medium- and close-to-zero-enthalpy of mixing regimes one after another.

It has been ascertained by Smith *et al.*[5] that at low hydration, at the beginning of the sorption experimental runs, the charged or highly polar (hydrophilic) sites are hydrated. The magnitude of the sorption enthalpy ΔH^{sorp} decreases as the degree of hydration (mass %) increases during adsorption of consecutive portions of water.[5] As known from the experimental data by Smith *et al.*[5] (*cf.* Fig. 6a therein), the highest observed absolute values of the enthalpy of sorption ΔH^{sorp} start with the first run of exposure of the sample to the flow of the nitrogen carrier gas containing water vapor. During the first run water vapor has the whole dry surface at its disposal and is likely adsorbed at the energetically most favorable sites. The adsorbed water molecules lower the energy of the system at the most if the highest electrostriction work L is done on them. We suggest that this occurs in regions where the protein surface shows the highest surface charge density σ and consequently gives rise to the highest electric fields (*cf.* Fig. 6).

This is equivalent to saying that the sites with the highest surface charge density σ giving rise to the highest fields within the adjacent hydration shell correspond to the highest enthalpy of sorption (*cf.* Fig. 5). For example, the highest absolute value of enthalpy of hydration read by this author in Fig. 6a of ref. 5 amounts to 71 kJ mol^{-1}. One finds that this corresponds to $-\Delta H^{mix}$ = (71 − 44) kJ mol^{-1} = 27 kJ mol^{-1}. Assuming that $-\Delta H^{mix}$ is entirely due to electrostriction, which as we know can well not be the case, one finds in Fig. 5 the value of $-\Delta H^{mix} = \zeta_L$ = 27 kJ mol^{-1} at $E = 8.65 \times 10^9$ V m^{-1} and the corresponding local surface charge density is σ = 0.39 C m^{-2}; the permittivity of water in such a field amounts to ε = 5.1[18] (*cf.* Fig. 6). The local water mass density at this site provides a large contribution (*cf.* Fig. 1a) to the mean d value.

For the sake of comparison, note that similar conditions are encountered for instance in the double layer at the electrode investigated in the X-ray experiment by Toney *et al.*[9,10] Namely, in the *second* layer of water molecules at the electrode charged to a potential of +0.52 V,[9,10] the field strength amounted to $E = 7 \times 10^9$ V m^{-1}.[26] Yet another example of similar conditions can be found in the first hydration shells of the Hg^{2+} ions.[24]

Let us now look at the situation during the next sorption runs. Subsequent experimental runs[5,14] lead to H_2O adsorption at the sites that provide lower and lower magnitude of ΔH^{mix} (*cf.* Fig. 3 and 4). On the other hand, it is seen in Fig. 5 that the calculated electric field strength E decreases with the decreasing magnitude of the electrostriction work per mole of water ($\approx \Delta H^{mix}$). We suggest that during the consecutive experimental runs water is adsorbed at the regions of the surface of protein giving rise to gradually lower and lower fields, since the sites with higher E, being energetically more favorable, have already been occupied during the preceding runs. During those runs the less charged sites are hydrated with lower local mass densities d of water shell in their neighborhood that provide lower contributions to the mean density of the whole shell.

Our approach explains why apparently no noticeable ΔH^{mix} has up to now been observed during sorption at about a half of the protein boundary. Namely, if large contributions to the enthalpy of mixing water to protein come from the electrostriction-related thermal effects, the latter are negligible in low fields and absent from regions in no field. In particular, this concerns the fact that Luthra *et al.*[4] apparently observed no noticeable ΔH^{mix} at high hydration. During the sorption runs done in this range, the additional H_2O molecules are placed at "less polar" binding sites,[5] in the fields essentially lower than $E = 0.6 \times 10^9$ V m^{-1}, corresponding to an accuracy of ±2.2 kJ mol^{-1} attributed by Luthra *et al.*[4] to their enthalpy data, or at places in no field. These regions provide negligible (lower than the experimental accuracy[4]) contributions to the enthalpy of mixing, if any.

For the sake of comparison, note that similar conditions are encountered in the first hydration shells of the univalent anions and cations (ref. 24, Table 1 therein) except Li^+.

In brief, the sorption experiments on several proteins show a characteristic behavior: the values of the enthalpy of mixing ΔH^{mix} water to protein are high at low hydration and

gradually fall down to the low (or vanishing) ones at high hydration.[4,5,14] This agrees with the tendency observed by MS in gas phase hydration of amino acids.[27–29] We suggest that the higher and lower values of ΔH^{mix} should be interpreted as due to higher and lower electrostriction, related to electric fields (Fig. 5) at the places at the surface of protein with a higher local charge or polarity and places with low or no polarity, respectively. During the hydration runs the former are hydrated first and the latter at the end.

Thus, the phenomenon of electrostriction leads to a description of the dependence of the hydration enthalpy on the number of adsorbed H_2O molecules that agrees semi-quantitatively with experiment[4,5,14] and to the related electric field distribution. *This is the second main result of the current paper*. It concerns at least the three exemplary proteins discussed herein, but extending it to the other ones seems plausible. Namely, there exists a distribution of surface charge density on every protein and the magnitudes of the electric fields strength around them should not differ much from each other. BSA and IgG share with lysozyme the property of the order of drying the surface sites from the less polar to the highly charged ones, with the corresponding variations in the related enthalpy of mixing values. However, unlike the case of lysozyme, the thermal effects at BSA and IgG cannot at present be confronted with an independent measurement of the mean hydration layer density. Such measurements would therefore be welcome.

Note that our description of the dependence of hydration enthalpy on the degree of protein hydration has a semi-quantitative character only. A complete check would require an experiment-based knowledge of the electric field distribution at the protein surface. Such knowledge could be gained, *e.g.*, by applying the surface force apparatus that already served to find local charge distributions at the surfaces of other biomolecules.[30]

IV. Discussion

Some evidence of the nature of water–protein bonding could be deduced from the study of hydration of the protein-forming chemical components. For example, Wincel[27] remarks that the hydrogen bond of the protonated $-NH_3^+$ group with H_2O "is largely due to electrostatic forces". It could be compared to charge–charge interactions of the charge distributions obtained by density functional methods for protonated primary amines with the restriction that "electrostatics is not the only factor contributing to the water binding energy".[29] One realizes that it is not easy to say to what extent the direct bonding interactions are reflected in the electrostriction-related enthalpy stemming from the protein charge–water dipole interactions within our model accounting for hydrogen bonds in a more crude way and applied to a more complex problem. One can only state that it reflects essentially the same electrostatics as in the hydration of protein-forming chemical groups treated by more sophisticated methods and does contribute to the total enthalpy. The comparison presented in Fig. 3 certifies that it should not be neglected.

One can ask if there are any other contributions to hydration enthalpy having a different origin. What comes to mind is the phenomenon of percolation.[6,25] To our knowledge, no data of ΔH^{mix} have been given[5,14] corresponding to sorption above about a half of the full coverage of protein by water. It is not too far from that of the H-bond percolation transition (*cf.* ref. 6 and 25). Nevertheless, we argue that this coincidence should be considered as an accidental one. The formation of H-bonds between consecutive water molecules deposited during sorption that leads to an interconnected 2D percolation cluster and eventually to the full covering of the protein represents the contribution to the water–water interaction energies and not to the water–protein ones. Hence, they contribute to the condensation enthalpy only.

V. Conclusion

We have discussed the electric field-dependent hydration of lysozyme observed in different scattering and water sorption experiments within the same theoretical approach. It has been assumed that the hydration water density found in the former experiments is related to the enthalpy measured in the latter ones by the phenomenon of electrostriction in the field of charges at the surfaces of protein molecules. We have found that the enthalpy of mixing calculated on the basis of the neutron and X-ray scattering data[7,8] is close to the mean value of the enthalpy measured in the sorption experiments on lysozyme.[4,5,14] This suggests that the calculated enthalpy of mixing provides a non-negligible contribution to the measured one.

Note that in our calculations no fitting parameters were applied.

The sorption experiments on several proteins show a characteristic behavior: the high value ΔH^{mix} of the enthalpy of mixing water to protein at low hydration and the low one (or none) at high hydration.[4,5,14] This is well described in terms of electrostriction as follows: the former correspond to hydrating the highly charged or polar places at the surface of protein, while the latter refer to hydrating the places of low or no polarity. Indeed, the enthalpy-field relations derived herein show that a higher electrostriction in a higher field corresponds to a higher thermal effect and a lower electrostriction leads to a lower thermal effect. To our knowledge, detailed experimental data on local charge distributions and their populations at the surfaces of proteins needed to perform a full quantitative check of the enthalpy-field relations are not available. Such data could be found, for example, by applying the surface force apparatus.[30]

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