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Apolar and Polar Solvation Thermodynamics Related to the Protein Unfolding Process

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ABSTRACT Thermodynamics related to hydrated water upon protein unfolding is studied over a broad temperature range (5–125°C). The hydration effect arising from the apolar interior is modeled as an increased number of hydrogen bonds between water molecules compared with bulk water. The corresponding contribution from the polar interior is modeled as a two-step process. First, the polar interior breaks hydrogen bonds in bulk water upon unfolding. Second, due to strong bonds between the polar surface and the nearest water molecules, we assume quantization using a simplified two-state picture. The heat capacity change upon hydration is compared with model compound data evaluated previously for 20 different proteins. We obtain good correspondence with the data for both the apolar and the polar interior. We note that the effective coupling constants for both models have small variations among the proteins we have investigated.

INTRODUCTION

The understanding of water and water interactions seems to be important to understand protein folding. In particular, the feature of cold unfolding of several small globular proteins seem to crucially depend on the properties of water (Kauzmann, 1959; Makhatadze and Privalov, 1995; Eisenberg and McLachlan, 1986; Phillips and Pettitt, 1995; Lazaridis and Karplus, 1999; Robinson and Cho, 1999) .

In this work we study two separate models for solvation of the apolar and the polar protein interior, respectively, that becomes exposed upon protein unfolding. By the term apolar we mean that the surface has no permanent dipoles, whereas a polar surface consists of permanent dipoles and charges (Privalov and Makhatadze, 1992). Experimentally, one finds that the hydration contribution to the heat capacity is positive for apolar surfaces, whereas it surprisingly becomes negative for polar surfaces (Makhatadze and Privalov, 1990; Privalov and Makhatadze, 1992).

The energy difference between the unfolded and folded apolar interior, with regard to the water, is in this work represented by additional hydrogen bonds. A justification of the apolar solvation model is the ability for water molecules to form an “iceberg” (ice-like shell), due to Frank and Evans (1945), around apolar surfaces and thus create more hydrogen bonds.

However, one should note that use of recently developed experimental techniques such as high-energy neutron scattering (Finney et al., 1993; Finney and Soper, 1994; Turner and Soper, 1994) and x-ray scattering (Filipponi et al., 1997; Bowron et al. 1998a,b) reveals no significant ordering of the water around hydrated apolar surfaces. Nevertheless, we will still use this ice-like shell picture. Reduction of both enthalpy (Olofsson et al., 1984; Naghibi et al., 1986, 1987; Madan and Sharp, 1997) and entropy (Wilhelm et al., 1977; Dec and Gill, 1984) upon apolar hydration seems to be well established from experiments (Makhatadze and Privalov, 1995; Abraham and Marcus, 1986).

The energy difference due to the polar surfaces is modeled as a reduced number of hydrogen bonds compared with bulk water, according to, e.g., Madan and Sharp (1996), whereupon two energy levels are accessible for the water molecules. The latter can be somewhat speculative and simplistic, but we note that two-state models have been applied to protein solvation (Gill et al., 1985; Makhatadze and Privalov, 1988; Madan and Sharp, 1996, 1997; Graziano, 1999). Nevertheless, along polar surfaces the water molecules are strongly bound and quantization is perhaps of importance (as hydrogen is a light element). Anyway, the two-state picture in this connection can be only a first rough approach to a more complex situation.

Finally, we apply equilibrium statistical mechanics to the models and compare the hydration part of the heat capacity change upon unfolding with model compound data from Privalov and Makhatadze (1995), evaluated for 20 different proteins. The data are based upon transfer characteristics for solvation in water of more than 100 low-molecular-weight organic compounds. Makhatadze and Privalov were able to calculate the unfolding hydration effects by assuming that the individual hydration contributions from the different parts of the protein are additive, utilizing the thermodynamic model compound data with which we compare our models.

HYDRATION UPON PROTEIN UNFOLDING

Water seems to be important to understand protein folding in general (Kauzmann, 1959; Privalov, 1992; Makhatadze and Privalov, 1995; Eisenberg and McLachlan, 1986; Phillips and Pettitt, 1995; Lazaridis and Karplus, 1999; Robin-

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son and Cho, 1999), and furthermore, the peculiar feature of cold unfolding seems to be particularly sensitive to the surrounding water (Privalov et al., 1986; Griko et al., 1988; Chen and Schellman, 1989; Privalov, 1990; Franks, 1995; Graziano et al., 1997; Hansen et al., 1998; Bruscolini and Casetti, 2000; De Los Rios and Caldarelli, 2000; Bakk et al., 2000, 2001c).

The purpose of this work is to study the pure hydration effect upon protein unfolding by making simple models covering some of the essential physics of a more complex system.

Hydration upon unfolding of the apolar interior

The experimental hydration data from Makhatadze and Privalov (1995) are based on the assumption that the hydration contribution to the heat capacity, for each apolar group, is proportional to the accessible surface area of the water molecules. Experimental hydration data from small apolar molecules are used to sum up the total heat capacity change upon protein unfolding.

For the apolar solvation data one should note that the aromatic parts of the protein are partly polar. However, the heat capacity shows the same qualitative behavior as the aliphatic parts; thus, as a simplification the aromatic and aliphatic contributions are here both incorporated in the total apolar contribution. Furthermore, the aromatic parts of the protein contribute to the heat capacity increment by less than 25% of the total apolar contribution.

For the apolar interior that becomes exposed to water upon protein unfolding we will use a refined version of a hydration model first proposed by Hansen et al. (1998, 1999, 2000) and later applications by Bakk et al. (2000, 2001a,b). The refined model was applied by Bakk (2001) in a complete protein folding model and by Bakk and Høye (2002) to model solvation of small apolar molecules. This model has also been applied recently by Bakk et al. (2002) as an effective model to evaluate the total hydration heat capacity increment. However, there we did not explicitly distinguish between the two different kinds of surfaces and their separate contributions, but to be able to account for a maximum in the total hydration heat capacity increment (see Fig. 3) we extended the model by introducing pair interactions between water molecules. In the present work such interactions are not needed to obtain satisfactory results and are thus not included.

Protein unfolding involves a cavity formation in water with a rearrangement of the water molecules surrounding the unfolded protein (Lee, 1985, 1991). When estimating the solvation energy of exposing the interior of a protein to water, one has to calculate the energy difference between hydrated water, associated with the protein, and bulk water (Privalov and Makhatadze, 1992). More precisely, the hydration is defined as the transfer of a solute from a fixed position in the ideal gas phase to a fixed position in the solvent (Ben-Naim and Marcus, 1984), i.e., water in the present case.

Here we will briefly re-derive the refined model used by Bakk and Høye (2002) for apolar surfaces. Due to the ice-like shell analogy of the water around the apolar surfaces (Frank and Evans, 1945) the excess energy is modeled as an increased number of hydrogen bonds compared with bulk water. This is an effective description based on the experimental fact that both enthalpy (Olofsson et al., 1984; Naghibi et al., 1986, 1987; Madan and Sharp, 1997) and entropy (Wilhelm et al., 1977; Dec and Gill, 1984) decrease upon apolar solvation. Each hydrogen bond is modeled in analogy to the distorted hydrogen bond model by Pople (Pople, 1951; Eisenberg and Kauzmann, 1969). The idea is that it costs energy to bend the individual hydrogen bonds. As a simplification, the simple electric dipole, or classical Heisenberg spin in an external field, is used as model. The energy measured per hydrogen bond becomes

$$E_{\mathrm{a}} = -\varepsilon_{\mathrm{a}} \cos \vartheta, \tag{1}$$

where ε_{a} is a bending distortion constant. (The subscript a refers to apolar solvation and below we will use p as subscript for polar solvation.) The angle ϑ is the polar angle.

The idea of representing the solvent by dipoles in protein folding was introduced by Warshel and Levitt (1976) and in later applications by Russell and Warshel (1985), Fan et al. (1999), and Avbelj (2000).

It is assumed that the hydrogen bonds are independent of each other, and the partition function that follows from Eq. 1 is (φ is the azimuthal angle)

$$Z_{\mathrm{a}} = \int_0^{2\pi} d\varphi \int_0^{\pi} d\vartheta \sin \vartheta e^{-\mathrm{E_a}/(RT)} = \frac{4\pi RT}{\varepsilon_{\mathrm{a}}} \sinh(\varepsilon_{\mathrm{a}}/(RT)), \tag{2}$$

where $R = 8.314\ \mathrm{JK^{-1}mol^{-1}}$, the molar gas constant, is used instead of Boltzmann's constant by which ε_{a} becomes energy per mole of hydrogen bonds.

With N_{a} additional hydrogen bonds per protein compared with bulk water, the internal energy per mole of proteins becomes $U = N_{\mathrm{a}}\partial(\ln Z_{\mathrm{a}})/\partial\beta$, with $\beta = (RT)^{-1}$, which yields the corresponding specific heat:

$$\Delta C_{\mathrm{a}} = \frac{\partial U}{\partial T} = N_{\mathrm{a}}R\left[1 - \left(\frac{\varepsilon_{\mathrm{a}}}{RT \sinh(\varepsilon_{\mathrm{a}}/(RT))}\right)^2\right]. \tag{3}$$

Hydration upon unfolding of the polar interior

Hydration of the polar interior upon protein unfolding is a challenging problem, and here we will present only a crude model. A crucial difference compared with apolar surfaces is that on polar surfaces dipolar water molecules are acted upon by electric fields from electric charges and dipoles located on these surfaces. Modern techniques such as high-energy neutron scattering show that ionic charges actually disrupt the characteristic structure of bulk water (Leberman and Soper, 1995). So in this work we simply assume that the solvation of a polar surface is a two-step process. In step 1, upon solvation, hydrogen bonds are more broken compared with bulk water (Madan and Sharp, 1996; Robinson and Cho, 1999). In step 2, the water molecules (dipoles) can choose between two distinct energy levels. A possible justification for this is the presumably strong bonds between the polar surface and the water molecules, by which quantization of the motion of the light hydrogen atoms may be of importance; i.e., we apply a two-state model, which is the simplest model for quantized energy levels.

Our specific choice of such a two-state model along with broken hydrogen bonds may be considered somewhat speculative, but to model, analyze, and better understand polar solvation data such a model can still be useful. This model for polar solvation (step 1 and step 2) also lacks several features of polar/ionic solvation as discussed in, e.g., Sharp and Honig (1990), Yang et al. (1992), Marcus (1994), and Dominy (2000). However, our purpose with this model is to try to describe and capture some key features of polar solvation.

We will now discuss the above two steps a bit. The heat capacity for a hydrogen bond around an apolar surface is modeled in the previous subsection (Eq. 3). So we here for simplicity apply the same model for breaking a hydrogen bond, but now we fix the electric field coupling constant to the mean value we will calculate in the next section based upon 20 different proteins, i.e., $\varepsilon_a \rightarrow \langle\varepsilon_a\rangle$. Thus, the heat capacity change for breaking N_{1p} hydrogen bonds per protein, analogous to Eq. 3, is

$$\Delta C_{1p} = -N_{1p}R\left[1 - \left(\frac{\langle\varepsilon_a\rangle}{RT\sinh(\langle\varepsilon_a\rangle/(RT))}\right)^2\right]. \qquad (4)$$

Note here that the minus sign is included as the hydrogen bonds are removed.

With an energy difference $2\varepsilon_p$ (the factor 2 is chosen for convenience) between the two energy levels (and ground state energy set to zero), the partition function of step 2 above becomes

$$Z_{2p} = 2\cosh(\varepsilon_p/(RT)). \qquad (5)$$

From this the heat capacity for N_{2p} independent strongly bound water dipoles per mole of proteins becomes

$$\Delta C_{2p} = N_{2p}R\left(\frac{\varepsilon_p}{RT\cosh(\varepsilon_p/(RT))}\right)^2. \qquad (6)$$

We will not require that the number of broken hydrogen bonds in water per protein upon unfolding (N_{1p}) in step 1 equals the number of dipoles (N_{2p}) in step 2, but we assume that these numbers are proportional; i.e.,

$$\omega_p = \frac{N_{2p}}{N_{1p}} \qquad (7)$$

is a given number.

Thus, the total heat capacity change for the polar solvation, which is the sum of step 1 and step 2 above, becomes

$$\begin{aligned}\Delta C_p &= \Delta C_{1p} + \Delta C_{2p}\\ &= N_{1p}R\left[\omega_p\left(\frac{\varepsilon_p}{RT\cosh(\varepsilon_p/(RT))}\right)^2 - 1\right.\\ &\qquad \left. + \left(\frac{\langle\varepsilon_a\rangle}{RT\sinh(\langle\varepsilon_a\rangle/(RT))}\right)^2\right]. \qquad (8)\end{aligned}$$

CALCULATIONS AND DISCUSSION

We want to investigate the hydration heat capacity change upon unfolding of the apolar interior and the polar interior, by relating results in Eqs. 3 and 8 to model compound data on 20 different proteins evaluated by Makhatadze and Privalov (1995).

It can be noted, as already remarked at the end of introduction, that the model compound data are based upon transfer characteristics for the solvating process in water of more than 100 low-molecular-weight organic compounds (Makhatadze and Privalov, 1995). The latter data are based upon the Ben-Naim definition of the solvation process of a molecule (Ben-Naim and Marcus, 1984), i.e., transferring the molecule from a fixed position in the ideal gas phase into a fixed position in water, which considers only effects associated with insertion of the solute molecule into water. Thus, in the solvation process, effects associated with differences in translational motions of the molecules in the gas phase and in the water soluted phase or effects associated with interactions between the molecules in the two phases are not included. Based upon the solvation data from these small organic substances and assuming that the apolar and polar hydration data of a given protein can be represented as a sum of these smaller contributions to the heat capacity, Makhatadze and Privalov (1995) evaluated the hydration contribution to the heat capacity upon unfolding of the 20 different proteins considered here. These data are termed as model compound data. Because the hydration effect does not include all contributions upon the protein unfolding process, it has not yet been possible to measure experimentally the hydration effect upon unfolding directly, not even for the total surface (apolar plus polar). Thus, in this respect, model compound data will serve as the best alternative in lack of direct experimental data. When we in this work study the hydration of apolar and polar surfaces upon protein unfolding, the models will thus represent an average of these surfaces.

TABLE 1 Best-fit parameters, according to Eq. 3, for the apolar hydration data

Protein	ε_a (kJ/mol)	N_a ($\times 10^3$)	ΔA_a ($\times 10^3$ Å^2)	ϕ_a (Å^2)
ROP	5.9	1.7	5.6	3.2
SH3 domain	5.9	0.86	2.8	3.2
BPTI	5.7	0.73	2.4	3.3
CI-2	6.0	0.89	2.9	3.2
Eglin c	5.8	0.88	2.9	3.3
G protein	5.8	0.73	2.4	3.3
Tendamistat	5.9	0.95	3.1	3.2
Ubiquitin	6.0	1.2	3.9	3.2
RNAse T1	5.7	1.5	4.9	3.3
Met-J	5.9	3.3	11	3.2
Cytochrome c	5.8	1.7	4.9	2.9
Barnase	5.8	1.7	5.6	3.3
RNAse A	5.9	1.6	5.3	3.3
Lysozyme	5.9	2.0	6.4	3.3
Interleukin-1β	5.9	2.4	7.9	3.3
Myoglobin	5.8	2.8	8.4	3.0
T4 lysozyme	5.9	2.7	8.6	3.2
Papain	5.9	3.9	13	3.3
Chymotrypsin	5.9	3.9	13	3.2
Pepsinogen	5.9	6.9	22	3.3
Mean ± SD	5.9 ± 0.1			3.2 ± 0.1

The difference in accessible surface area between the unfolded and the folded apolar parts of the protein (ΔA_a) is obtained from Makhatadze and Privalov (1990). The parameter ϕ_a is defined in Eq. 9.

TABLE 2 Best-fit parameters, according to Eq. 8, for the polar hydration data

Protein	ε_p (kJ/mol)	N_{1p} ($\times 10^3$)	ω_p	ΔA_p ($\times 10^3$ Å^2)	ϕ_p (Å^2)
ROP	2.4	5.6	1.7	3.7	0.67
SH3 domain	2.4	1.9	1.6	1.4	0.78
BPTI	2.6	2.0	1.7	1.7	0.85
CI-2	2.4	3.1	1.8	1.6	0.53
Eglin c	2.7	1.1	1.4	1.4	1.3
G protein	2.6	1.1	1.3	1.4	1.2
Tendamistat	2.8	1.5	1.4	2.1	1.4
Ubiquitin	2.5	2.1	1.5	1.9	0.92
RNAse T1	2.5	3.0	1.6	2.8	0.92
Met-J	2.6	6.4	1.5	7.0	1.1
Cytochrome c	2.6	2.6	1.4	3.4	1.3
Barnase	2.6	3.1	1.5	3.1	1.0
RNAse A	2.9	2.1	1.2	4.1	2.0
Lysozyme	2.5	5.2	1.7	4.2	0.81
Interleukin-1β	2.4	5.9	1.6	4.1	0.79
Myoglobin	2.4	7.7	1.7	4.8	0.62
T4 lysozyme	2.4	7.7	1.7	5.4	0.70
Papain	2.5	10	1.7	7.8	0.75
Chymotrypsin	2.5	10	1.7	6.8	0.66
Pepsinogen	2.5	16	1.6	11	0.69
Mean ± SD	2.5 ± 0.1		1.6 ± 0.1		0.95 ± 0.34

The difference in accessible surface area between the unfolded and the folded polar parts of the protein ΔA_p is obtained from Makhatadze and Privalov (1990). The parameters ω_p and ϕ_p are defined in Eqs. 7 and 10.

The apolar hydration model (Eq. 3) with N_a and ε_a chosen as free parameters, and the polar hydration model (Eq. 8) with N_{p1}, ω_p, and ε_p chosen as free parameters, were both fitted to the model compound data from Makhatadze and Privalov (1995) by a least-squares fit procedure.

In Tables 1 and 2 we list the least-squares fittings of parameters for 20 different proteins of the apolar and polar model compound data, respectively.

We find that the quality of the fitting is good, especially the apolar part, as can be seen from Figs. 1, 2, and 3. The relative deviation between the least-squares fittings and the model compound data are limited to ~0.5% for the apolar data and ~2% for the polar data. For the other proteins present in Tables 1 and 2, corresponding figures can be drawn, and we find relative errors within the same limits. Thus the figures represent the typical accuracy for all the proteins considered in this work.

One notes that the parameter ε_a is very stable for all of the 20 proteins considered. The mean value $\langle \varepsilon_a \rangle = 5.9$ kJ/mol (see Table 1) compares well with the estimated value of breaking one mole of hydrogen bonds in ice, which was estimated by Némethy and Scheraga (1962) to be 5.5 kJ/mol. We note that Silverstein et al. (2000), using a two-state Muller's model (Muller, 1990), estimated 10 kJ/mol for breaking one mole of hydrogen bonds in the first solvation shell of several noble gases.

For the apolar model it is of interest to check whether the change in accessible surface area ΔA_a is proportional to the increase in the number of hydrogen bonds N_a, i.e., proportional to the hydration heat capacity change upon unfolding. (For calculation of ΔA_a see Makhatadze and Privalov (1990).) We note here that it is usually assumed that the first solvation shell is the one responsible for the heat capacity

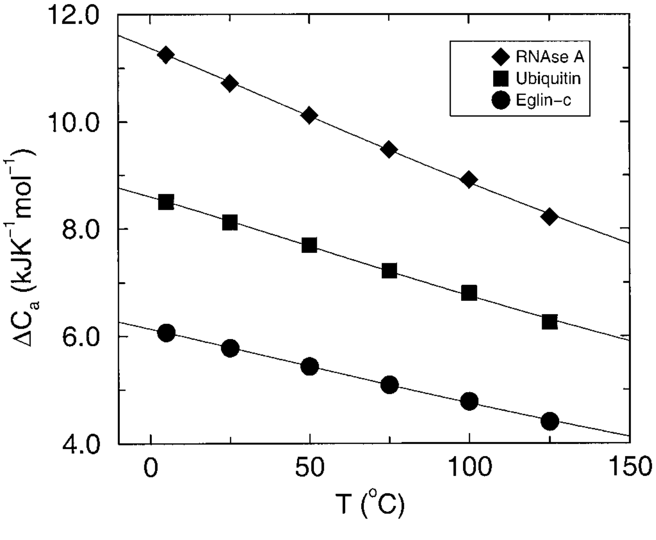

FIGURE 1 Data for the purely apolar hydration heat capacity change upon unfolding per mole of proteins for RNAse A, ubiquitin, and eglin c. Model compound data are obtained from Makhatadze and Privalov (1995). The continuous lines (——) are best fits from the theoretical estimate in Eq. 3. Parameters are listed in Table 1.

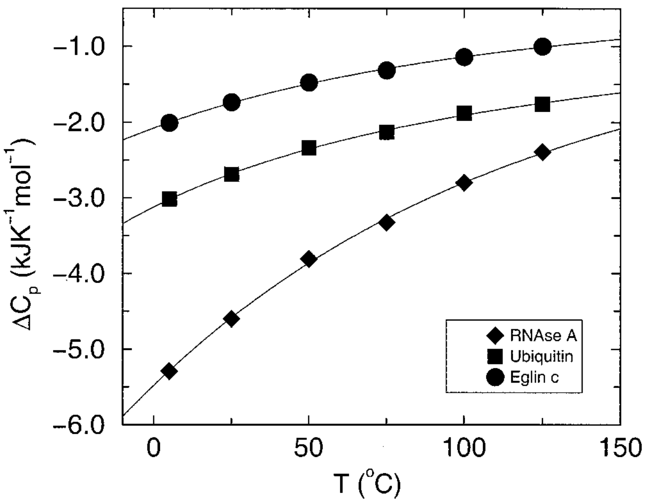

FIGURE 2 Heat capacity change upon aqueous unfolding of the polar interior part for the same proteins as in Fig. 1. Model compound data are also here obtained from Makhatadze and Privalov (1995). The continuous lines (——) are best fits from the theoretical estimate in Eq. 8. Parameters are listed in Table 2. Note that the heat capacity change is negative for the polar hydration, in contrast to apolar hydration.

change upon apolar solvation (Naghibi et al., 1986, 1987; Makhatadze and Privalov, 1988; Muller, 1990; Silverstein et al., 2000). Within our model we find it reasonable that N_a is proportional to ΔA_a. Thus, the ratio

$$\phi_a = \frac{\Delta A_a}{N_a}, \tag{9}$$

is evaluated, and a very stable value (~3.2 Å^2), with minor deviations for chymotrypsin and myoglobin, is found as indicated in Table 1.

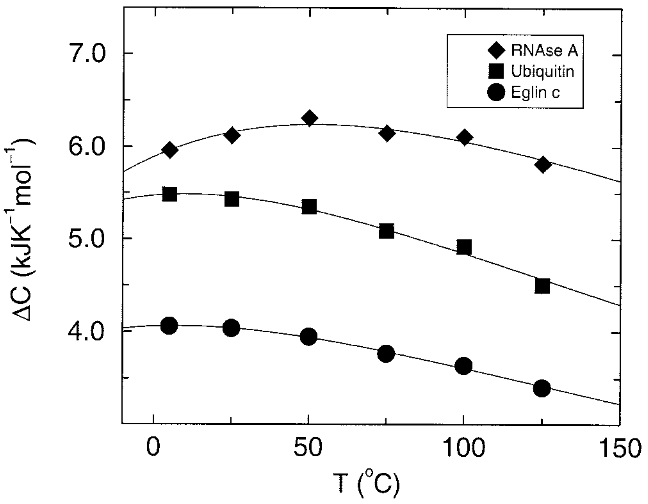

FIGURE 3 The total hydration heat capacity change upon unfolding for the same proteins that as in Figs. 1 and 2. The continuous lines (——) are the sums of the theoretically estimated lines in Figs. 1 and 2. Likewise the model compound data, evaluated by Makhatadze and Privalov (1995), are the sums of the model compound data of Figs. 1 and 2.

In Fig. 1, we show the apolar part of the hydration heat capacity model compound data for 3 of the 20 different proteins considered in this work, namely, RNAse A, ubiquitin, and eglin c together with the theoretical fit based on Eq. 3. These proteins are chosen as typical examples that can give a picture of the quality of the fits.

In Fig. 2, experimental data for the polar parts of the hydration of RNAse A, ubiquitin, and eglin c are drawn together with the theoretical estimates of Eq. 8, which fit the experimental data well. The parameters resulting from this latter fitting procedure are listed in Table 2.

In analogy to the discussion of ε_a, we note here that the level spacing parameter ε_p (see Eq. 5) is stable around 2.5 kJ/mol. To a first approximation this means that the parameters ε_a and ε_p are constants that may be used to predict thermodynamic data of proteins other than those considered in this work.

A feature of the polar model is the parameter ω_p. As seen in Table 2 its mean value $\langle \omega_p \rangle = 1.7 \pm 0.1$; i.e., the number of broken hydrogen bonds (N_{1p}) are proportional to the number of dipoles that enter the two-state model (N_{2p}), as one can expect.

In analogy to the parameter ϕ_a of the apolar solvation model, one can also investigate the ratio

$$\phi_p = \frac{\Delta A_p}{N_{1p}}, \tag{10}$$

i.e., the accessible polar surface area per broken hydrogen bond. From Table 2 the ϕ_p equals (0.95 ± 0.34) Å^2, and note that the standard deviation of this parameter is relatively large compared with those for ε_a, ε_p, and ω_p. This difference in behavior for ϕ_p may reflect a dependence of polar solvation upon the specific characters of the surface; i.e., the behavior of the water molecules depends upon parameters such as polarity and charge on the protein surface. This is in contrast to apolar solvation, which seems to reflect a more intrinsic effect of the water; i.e., there is only one kind of apolar surface.

In Fig. 3, the total hydration heat capacity change for RNAse A, ubiquitin, and eglin c are shown as the sum of the corresponding heat capacity change for the apolar and polar interiors of Figs. 1 and 2.

It can be added that the main intention of our hydration models for apolar and polar surfaces are to capture key features of hydration effects upon protein unfolding. They thus represent only the part related to water interactions of a more complete description of the protein unfolding process. To our knowledge there are no independent results available, besides the ones by Makhatadze and Privalov (1995), with which we can compare our results. However, molecular dynamics (Mancera and Buckingham, 1995) and Monte Carlo simulations (Jorgensen and Nguyen, 1993; Matubayasi and Levy, 1996) have been performed for smaller molecules using more detailed and thus more complex models. Also, integral equation theory has been performed for small molecules (Pratt and Chandler, 1977; Hirata et al., 1982). But these works did not consider heat capacities (Silverstein et al., 1999). Madan and Sharp (1996, 1997; Sharp and Madan, 1997) have by their Monte Carlo simulations on a random network model made some progress toward predicting the solvation heat capacity of different small substances. But they did not consider the temperature dependence of the heat capacity. However, compared with Madan and Sharp, Silverstein et al. (1999) made a simpler numerical model where they were able to study the heat capacity versus temperature. But as far we can see, our results cannot be directly related to the latter as the systems and models are different.

CONCLUSION

We have proposed models for the hydration of the apolar and the polar protein interior that becomes exposed to water upon unfolding. To our knowledge, there are no previous models describing apolar and polar protein hydration data with the same accuracy over such broad temperature range (5–125°C).

Hydration of the apolar surfaces, using an ice-like shell analogy, is modeled as an increased number of hydrogen bonds compared with bulk water. Hydration of the polar surfaces is modeled as a lack of hydrogen bonds compared with bulk water. In addition, the dipolar water molecules are supposed to be strongly bound to ionic and polar parts along the protein surface. These strong forces acting on the hydrogen bonds are assumed to lead to quantization, which is represented by a two-state system.

Compared with model compound data evaluated by Makhatadze and Privalov (1995) on 20 different proteins, the models fit well. We note that the coupling parameters ε_a and ε_p (see Eqs. 1 and 5) have only small variations among the proteins considered.

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