Two-state protein model with water interactions: Influence of temperature on the intrinsic viscosity of myoglobin

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We describe a single-domain protein as a two-state system with water interactions. Around the unfolded apolar parts of the protein we incorporate the hydration effect by introducing hydrogen bonds between the water molecules in order to mimic the ''icelike'' shell structure. Intrinsic viscosity, proportional to the effective hydrodynamic volume, for sperm whale metmyoglobin is assigned from experimental data in the folded and in the denaturated state. By weighing statistically the two states against the degree of folding, we express the total intrinsic viscosity. The temperature dependence of the intrinsic viscosity, for different chemical potentials, is in good correspondence with experimental data [P. L. Privalov *et al.*, J. Mol. Biol. **190**, 487 (1986)]. Cold and warm unfolding, common to small globular proteins, is also a result of the model.

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I. INTRODUCTION

Proteins are macromolecules consisting of thousands of atoms. Despite their complexity, Privalov and Khechinashvili [1] showed by a van't Hoff analysis that several small globular proteins (<200 residues) are nearly a two-state system, i.e., either the protein is thermodynamically stable in the folded (native) state, or it is stable in the unfolded (denaturated) conformation.

Proteins are in a compact native state around physiological temperatures and natural chemical environments. An increase of the temperature denaturates the protein, which is quite intuitive from a physical point of view, e.g., thinking about thermal expansion of materials. But, what is rather surprising is that some proteins lose their stability at subphysiological temperatures [2–4]. This is called cold denaturation.

In this work we apply a simple two-state description for a protein, which is a reformulated version of a model proposed by Hansen *et al.* [5] and Bakk *et al.* [6,7]. In the denaturated state water is allowed to access the unfolded regions of the protein. The water molecules in this hydration shell are assigned a bending energy in order to mimic the ''frozen'' structure around an apolar surface [8]. By means of statistical mechanics we calculate an order parameter, which we apply in an expression for the *intrinsic viscosity* (IV). The IV is proportional to the effective hydrodynamic volume of a macromolecule [9], and is *not* equivalent to the *internal viscosity*, where the latter describes a resistance to extension or compression of a macromolecule [10]. Finally we compare the model with experimental data from Privalov *et al.* on sperm whale myoglobin [3].

II. PROTEIN MODEL

To first approximation, a small single-domain globular protein may be regarded as a two-state macroscopic system [1,11]. However, as shown in Ref. [6] the folding of such a protein can be regarded as a multiple process, i.e., a hierarchical folding of M contacts [12], and still be a two-state system from a thermodynamical point of view. Analogous to Zwanzig [13] we assign binary variables $\Psi_j \in \{0,1\}$ corresponding to an open (unfolded) and closed (folded) contact j, respectively. The hierarchical folding implies the constraint

$$\Psi_j \geqslant \Psi_k, \quad k \geqslant j \tag{1}$$

simply because contact $j \leqslant k$ cannot unfold while k is folded. This can further be parametrized by a second set of binary variables $\xi_j \in \{1,-B\}$. The ξ_j variables may be interpreted as a simplified representation of the dihedral angles [9] with only two ''angles'' accessible at each contact. Let ϵ be the energy gain to fold one contact [14], and let the binary variable $\chi_j \in \{1,-C\}$ distinguish between the fully folded state ($\chi_M = 1$) and the intermediate states ($\chi_{j<M} = -C$), respectively. The enthalpy for contact thus becomes

$$E^c = -i\epsilon \Psi_i \xi_i \chi_i, \tag{2}$$

when the unfolded enthalpy is set to zero.

In the two-state limit the intermediate states are unstable, i.e., $C \to \infty$. For simplicity we assume $B \to \infty$, thus the chain-chain enthalpy in Eq. (2) effectively becomes

$$E_i^c = -i\epsilon_c, \quad i \in \{0,1\}, \tag{3}$$

which corresponds to the native state ($i=1$) and the denaturated state ($i=0$) for the complete protein. The protein contact energy ϵ_c is simply the sum of M contact energies ϵ, i.e., $\epsilon_c = M\epsilon$.

For simplicity we assume that the denaturated state has g_c chain-related degrees of freedom compared to the thermodynamically unique native state of zero entropy. The present two-state model fulfills the van't Hoff enthalpy relation as shown in Ref. [6], which is also experimentally established for several globular proteins, myoglobin included [1]. As for ϵ_c in Eq. (3) g_c varies little with respect to the temperature [15], thus we assume g_c is independent of the temperature.

Solvation of a molecule in water, in analogy to protein unfolding, is a complex affair. It includes a cavity formation

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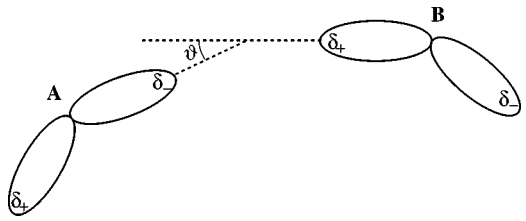

FIG. 1. Schematic illustration of two water molecules (A and B) between which a hydrogen bond is bent an angle ϑ. Only two of the four orbitals, where the polarity is indicated by δ_+ and δ_-, are shown for each molecule. The hydrogen bonds are meant to mimic the ''icelike'' structure of water around the unfolded apolar regions of the protein.

in water, interactions between water molecules and the surface of the solute molecule, and finally a rearrangement of the water around the solute molecule [16]. In this work we will only consider the latter effect.

Proteins consist of apolar[1] as well as polar surfaces [17]. As a simplification, we will in this work only consider the hydration effect upon unfolding around the exposed apolar parts of the protein. It is known from solvation of apolar substances in water that the hydration contribution to the entropy is negative, moreover, it decreases in absolute value for increasing temperature [18]. Frank and Evans [8] attributed this to a gradual melting of an ''ice shell'' around the apolar molecules. In analogy to this, we regard the water in the solvation shell around the unfolded apolar parts of the protein as hydrogen bonded (HB), while upon folding this water is expelled to the bulk, and is there regarded as a ''non-hydrogen-bonded liquid'' [19].

Inspired by Pople [20,21] we define an effective bending energy of one individual hydrogen bond in the solvation shell

$$E_i^{\mathrm{HB}}(\vartheta) = -(1-i)\epsilon_{\mathrm{HB}}\cos\vartheta, \quad \vartheta \in [0,\pi]. \tag{4}$$

The polar angle ϑ is the bending or distortion of a hydrogen bond as illustrated in Fig. 1. One sees from Eq. (4) that it is enthalpically favorable to let water access the unfolded apolar protein surfaces (i.e., $i=0$), otherwise, if water is expelled to the bulk we put this enthalpy to zero. ϵ_{HB} is a bending distortion constant and is supposed to be of the size of breaking one mole of hydrogen bonds and transferring them to bulk water. Némethy and Scheraga [19] estimated 5.5 kJ/mol for this constant, which we will apply in this work. Water molecules in the bulk will also have internal interactions, but with a weaker coupling compared to ice. Thus, the value from Némethy and Scheraga is substantially lower than the widely quoted value 18.8 kJ/mol for breaking one mole of hydrogen bonds and transferring them to vacuum as proposed by Pauling [22]. Each individual water molecule expelled to the bulk is assigned a degeneracy g_{w} in order to take into account the entropy loss of solvated water [18]. Let N be the effective number of hydrogen bonds in the solvation shell around the apolar surfaces of the unfolded protein. Thus, the total degeneracy of the protein is $g_1 = g_w^N$ in the folded state due to the water degrees of freedom, while the degeneracy of the unfolded state is $g_0 = g_c$ due to the chain flexibility. This yields a degeneracy corresponding to state i

$$g_i = g_c^{1-i} g_w^{Ni}. \tag{5}$$

The Hamiltonian for the protein is simply the sum of chain-chain enthalpies [see Eq. (3)] and protein-water interactions [see Eq. (4)]

$$\mathcal{H}_i(\vartheta) = E_i^c + E_i^{\mathrm{HB}}(\vartheta), \tag{6}$$

whereupon the canonical partition function becomes

$$\begin{aligned} Z &= \sum_{i=0}^{1} g_i e^{-E_i^c/(RT)} \left(\int_0^\pi d\vartheta \sin\vartheta e^{-E_i^{\mathrm{HB}}(\vartheta)/(RT)} \right)^N \\ &= g_c \left[\frac{2RT}{\epsilon_{\mathrm{HB}}} \sinh\{\epsilon_{\mathrm{HB}}/(RT)\} \right]^N + g_w^N e^{\epsilon_c/(RT)} 2^N \\ &= 2^N g_w^N e^{\epsilon_c/(RT)} (r+1) \equiv \sum_{i=0}^{1} Z_i. \end{aligned} \tag{7}$$

$R = 8.31$ J/(K mol) is the molar gas constant, T is the absolute temperature, and the function r is defined as

$$r \equiv [aTe^{-\mu/T} \sinh(b/T)]^N, \tag{8}$$

where $a \equiv R g_c^{1/N}/(\epsilon_{\mathrm{HB}} g_w)$, $\mu \equiv \epsilon_c/(NR)$, and $b \equiv \epsilon_{\mathrm{HB}}/R$. The power of N in Eq. (7) is due to the N hydrogen bonds that are supposed to act individually. This is a coarse simplification because ice is supposed to have long-range order [21].

In Sec. III below, which concerns the intrinsic viscosity, we will need a quantity or a measure of the degree of folding. Thus, we define an order parameter[2] n for the system. According to the previous notation where $i=0$ and $i=1$ corresponds to a denaturated and a native protein, respectively, we weigh the two states by the corresponding Boltzmann weights Z_i defined in Eq. (7). The order parameter becomes

$$n \equiv \frac{\sum_{i=0}^{1} i Z_i}{\sum_{i=0}^{1} Z_i} = \frac{1}{r+1}. \tag{9}$$

Up to this point the model is general, only restricted to single-domain (small) proteins exhibiting two states. For positive values of a and b, which is valid throughout this work, $r>0$ is a consequence for all temperatures. Moreover,

[1] *Apolar* means that the molecule exhibits no permanent dipole moment, as opposed to *polar*.

[2] *Order parameter* in physics is equivalent to *reaction coordinate*, commonly used in chemistry and protein literature.

the order parameter has the following limits: $\lim_{r\to 0} n=1$ and $\lim_{r\to\infty} n=0$. Thus, we have constructed an order parameter confined to the interval $n\in[0,1]$, which statistically describes the degree of folding.

III. INTRINSIC VISCOSITY

We now have the ''machinery'' to describe the IV. First we will calculate IV separately in the native and denaturated state. By quantifying the population of the two states by the order parameter [see Eq. (9)], we are finally able to express the total IV for myoglobin as a sum of native and denaturational IV weighed against the degree of folding.

IV is in general defined as [24]

$$[\eta]\equiv\lim_{c\to 0}\frac{\eta'-\eta}{c\,\eta}, \tag{10}$$

which is the limit of zero concentration c of the *reduced viscosity* [25]. η' is the macroscopic viscosity (water + protein), η is the viscosity of pure solvent (water), and c is the protein concentration. For a fixed conformation IV is independent of the solution. However, the conformation will strongly depend on the solution, e.g., pH. Thus, in this respect IV will implicitly depend upon the solution, as discussed further in Sec. IV.

It can be shown that IV for a compact macromolecule of arbitrary shape can be written by heuristic means as [24]

$$[\eta]_1=\nu(\overline{V}_p+\delta\overline{V}_w), \tag{11}$$

where ν is the Simha factor containing all the shape dependence, $\overline{V}_p$ and $\overline{V}_w=1.0$ cm^3/g are the partial specific volumes of protein and pure water, respectively, and δ is the hydration ratio. From Eq. (11) one sees that IV can be regarded as an effective measure of the size of a macromolecule. In this work we study sperm whale metmyoglobin that has the following data: $\overline{V}_p=0.75$ cm^3/g [9], $\nu=2.8$ and $\delta=0.35$ [24]. Thus, according to Eq. (11), IV for myoglobin in the *native* state (1) is

$$[\eta]_1=3.1\ \text{cm}^3/\text{g}, \tag{12}$$

i.e., independent of temperature. The data, leading to Eq. (12), is measured at 20 °C. However, to first approximation we assume that Eq. (12) is valid at all temperatures.

IV in the denaturated state is a bit more complicated, where we *may* regard the protein as a random coil [26,27]. Flory [28] proposed

$$[\eta]_{\text{Flory}}=\frac{\Phi\langle r^2\rangle^{3/2}}{M} \tag{13}$$

for the intrinsic viscosity of a non-free-draining coil. $\Phi=3.62\times 10^{21}$ is a universal constant, $\langle r^2\rangle$ is the mean-square end-to-end distance, and M is the molecular weight. According to the data from Privalov *et al.* [3] there seems to be a pronounced temperature dependence of IV in the denaturated state (see upper curve in Fig. 2). Moreover, it is known that

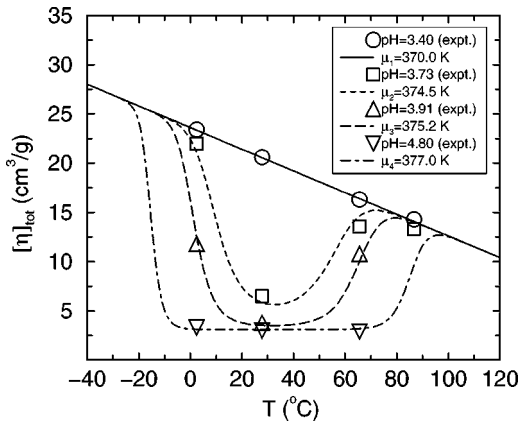

FIG. 2. Temperature dependence of myoglobin intrinsic viscosity at different chemical potentials μ. The curves are based upon Eq. (15) where $a=2.59\times 10^{-3}$ K^{-1} and $b=662$ K [19]. μ_1 corresponds to a denaturated protein, while μ_4 corresponds to the native state in the horizontal region between -10 °C and 80 °C. Experimental (expt.) data at various pH′s from Privalov *et al.* [3].

facilitation of rotational degrees of freedom in the backbone will cause a decrease of the dimension [27,29], hence, $\langle r^2\rangle$ decreases with increase in temperature, as well. Thus, according to Eq. (13), the IV also decreases. In order to incorporate the latter effect, we do a linear regression of data on unfolded myoglobin.[3] This implies the following temperature[4] dependent expression on IV of *denaturated* myoglobin

$$[\eta]_0=23.6-0.11T\ \text{cm}^3/\text{g}. \tag{14}$$

At 25 °C $[\eta]_0=20.9$ cm^3/g, noteworthily close to the value 20.1 cm^3/g from Tanford [26] obtained in 6 M guanidinium HCl.

From the calculated IV of native and denaturated myoglobin in Eqs. (12) and (14), respectively, we weigh the two states by the order parameter defined in Eq. (9). We put native IV proportional to the degree of *folding*, n, and denaturational IV proportional to the degree of *unfolding*, $(1-n)$, whereupon the total IV becomes

$$[\eta]_{\text{tot}}=[\eta]_1 n+[\eta]_0(1-n). \tag{15}$$

The two states, folded and unfolded protein, correspond to the limits $\lim_{n\to 1}[\eta]_{\text{tot}}=[\eta]_1$ and $\lim_{n\to 0}[\eta]_{\text{tot}}=[\eta]_0$, respectively. The order parameter n depends both on the temperature and on the chemical environments, as discussed in the section below.

IV. CALCULATIONS AND DISCUSSION

It seems to be reasonable to only incorporate hydration effect of the first solvation shell [30,23], according to Cohn and Edsall [31] who state that roughly one monolayer of

[3]Experimental data from Privalov *et al.* [3].

[4]Temperature here and in Figs. 2 and 3 in units of °C.

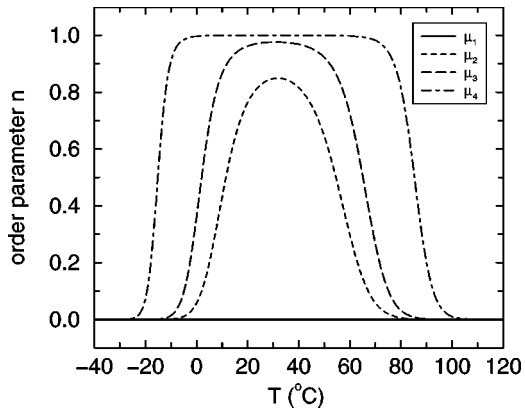

FIG. 3. Temperature dependence of the order parameter n defined in Eq. (9). All parameters correspond to Fig. 2. The order parameter measures the degree of folding. Thus, for μ_1 the protein is folded around physiological temperatures (30 °C), while for $\mu \lesssim \mu_4$ the protein is denaturated at all temperatures. Note that n is decreasing with decreasing μ.

water around apolar molecules is required to explain hydrodynamic data.

If we use an estimated value 8.4×10^3 Å^2 for the difference of the accessible surface area of the denaturated and the native apolar parts of myoglobin [15], together with an estimated value 9 Å^2 for the effective surface area of one water molecule [23], it is roughly 930 water molecules around the unfolded apolar regions of a myoglobin molecule in the first solvation shell. Let it be effectively one hydrogen bond per water molecule that forms or makes the ''freezing action'' in the hydration shell, thus $N=930$ in Eq. (8). Note that the latter number is the *excess* number of hydrogen bonds in the solvation shell compared to bulk water. Thus, N is not a very fundamental constant, merely a rough estimate. The estimated value of $\epsilon_{\mathrm{HB}}=5.5$ kJ/mol [19] implies $b=662$ K in Eq. (8).

Consequently, only two parameters remain ''adjustable'' in the protein model [see Eq. (7)] and thus in $[\eta]_{\mathrm{tot}}$ [see Eq. (15)], namely, a and μ. It is likely to believe that a change in the parameter μ is equivalent to a change in the chemical environments (pH, denaturant concentration, etc.), because μ is proportional to the chain-chain contact enthalpy ϵ_c, which reasonably depends upon, e.g., pH. On the other hand, the parameter a contains chain and water entropies in addition to the hydrogen-bond-bending constant ϵ_{HB}, which are presumably more stable parameters upon a change in the chemical environment compared to μ. Thus, we call the effective parameter μ the *chemical potential*.

In Fig. 2 we plot the intrinsic viscosity vs temperature for different μ and compare them to experimental data from Privalov *et al.* [3]. The curve corresponding to μ_1 exhibits the characteristic temperature dependence of an unfolded protein. In Fig. 3, where the corresponding order parameter vs temperature is plotted, one sees that μ_1 corresponds to $n=0$ for all T, i.e., it is only $[\eta]_0$ that contributes to $[\eta]_{\mathrm{tot}}$. This is nothing but the temperature dependent intrinsic viscosity of a free-draining coil expressed in Eq. (14). We note that the assumption of linear dependence on denaturational IV in Eq. (14) is a good approximation to experimental data.

The curve corresponding to μ_4 in Fig. 2 is horizontal in a broad temperature range from approximately 0 °C to 60 °C. This corresponds to $n=1$ as seen in Fig. 3. Thus, $[\eta]_{\mathrm{tot}} \rightarrow [\eta]_1$ implying a native protein in this temperature region, and is in fairly good correspondence to experimental data. However, in the experiments there seems to be a slight decrease of IV in the region discussed, probably due to a melting of the native structure analogous to the denaturational IV.

The curves μ_2 and μ_3 in Fig. 3 have both maxima $n<1$, thus only a *fraction* of the proteins are native. Here we note that an intermediate value of the order parameter, let us say $n=0.8$, does *not* mean that the protein is partly folded, but means *statistically* that 80% of an ensemble of proteins are folded, while 20% are unfolded. Actually, the curves corresponding to μ_2 and μ_3 is a crucial test of the validity of the model, because the corresponding experimental data clearly deviates from a straight line, as a consequence of the mixture of native and denaturated proteins that contribute to different intrinsic viscosities. In sum, our model seems to resemble the experimental data quite well.

The curve corresponding to μ_4 in Fig. 2 exhibits the characteristic temperature dependence of cold and warm destabilization. This is better seen in Fig. 3, where μ_4 corresponds to a native protein in an intermediate region around physiological temperatures (-10 °-80 °C), while it is denaturated outside this temperature region. Cold and warm unfolding is a common feature to small globular proteins [4,3,32]. The specific values of the chemical potential are all around $\mu=375$ K, which corresponds to $\epsilon_c=2900$ kJ/mol [see Eq. (8)]. It is interesting to compare this to the estimated values from Makhatadze and Privalov [15] on enthalpies of internal interactions $\Delta_N^U H^{\mathrm{int}}=7600$ kJ/mol, where van der Waals's (vdW) interactions contribute $\Delta_N^U H^{\mathrm{vdW}}=1200$ kJ/mol and hydrogen bonding contributes $\Delta_N^U H^{\mathrm{HB}}=6400$ kJ/mol. The latter three values are nearly constant between 5 °C and 100 °C. It is reasonable that $\Delta_N^U H^{\mathrm{HB}} > \epsilon_c > \Delta_N^U H^{\mathrm{vdW}}$, because in addition to disruption of the internal van der Waals's bonds the broken internal hydrogen bonds are likely to partly reappear as water-protein interactions. The latter enthalphy contribution is only partly because the specific water structure determines the possible hydrogen bond combinations towards the protein surface.

In a future expansion of the model it may be interesting to look at the apparent decreasing dimensionality with increasing temperature for both native and denaturational IV, which is more expressed for the latter. This may be attributed to a gradual melting of the structure due to some excitement of soft vibrational modes implying an effective smaller dimension [17]. If we were able to incorporate such interactions in the protein model, the parameter fit onto $[\eta]_0$ and $[\eta]_1$ may then turn out to be redundant—resulting in a more complete model.

To the author's knowledge regarding experiments on the temperature dependence of the IV, myoglobin is the only studied protein over such a broad temperature range and in different chemical environments as in Ref. [3]. Thus, we

hope that the present paper may stimulate experimental work on IV for other proteins, especially those that exhibit cold unfolding, in order to check the generality of the model.

V. SUMMARY

Single-domain proteins have thermodynamically two stable states, the native and the denaturated [1,11]. We apply a two-state description and incorporate the hydration effect upon unfolding by a model that mimics the ''icelike'' shell around the unfolded apolar surfaces as an increased number of hydrogen bonds compared to bulk water. By means of equilibrium statistical mechanics we calculate an order parameter (reaction coordinate) for the system, measuring the degree of folding.

In order to express the IV we do a linear fit onto experimental data on myoglobin of native and denaturational IV, respectively. The total IV for the native state is supposed to be linearly dependent on the order parameter and proportional to the degree of unfolding for the denaturated state.

The total IV exhibits good correspondence with experimental data from Privalov *et al.* [3]. For large chemical potentials the protein is native around physiological temperatures (30 °C), whereupon it becomes unstable at lower as well as higher temperatures. Cold and warm destabilizing action, common to small globular proteins, is a consequence of the model.

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