Adherence of Packing Defects in Soluble Proteins

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For protein structure to prevail in water, its backbone hydrogen bonds must be shielded from water attack, requiring a cluster of ''wrapping'' nonpolar groups. Thus, underwrapped regions are adhesive, as exogenous removal of surrounding water becomes thermodynamically favorable. Here we measure the average adhesive force exerted by an underwrapped hydrogen bond on a test hydrophobe and thus define a new interactivity constant.

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To prevail in water environments, the structure of soluble proteins must fulfill a building constraint: backbone hydrogen bonds need to be shielded from water attack [1–4]. This protection requires that sufficient nonpolar groups (CH_n, $n = 1, 2, 3$) be clustered in the vicinity of intramolecular hydrogen bonds, wrapping them as needed to exclude surrounding water [1]. Such desolvation enhances the electrostatic contribution and stabilizes the hydrogen bonds [1–5], since in the nonbonded state, the exposed polar amide and carbonyl groups would be hindered from being hydrated. This thermodynamic benefit associated with water removal from preformed structure should make underwrapped proteins adhesive [6]. Here we measure the adhesive force created by an underwrapped hydrogen bond by analyzing the flow-rate dependence of the adsorption uptake of soluble proteins onto a lipid bilayer.

Six soluble proteins from different families and with very different levels of wrapping are investigated across a wide range of hydrodynamic conditions yielding invariably the same value for the adhesive force within the experimental confidence bands. The results have dramatic consequences as they imply that protein interactivity is to a considerable extent related to the structural defects of the monomeric structure. Furthermore, they lead us to infer the existence of an interactivity constant: the adhesiveness of a structural defect.

A measure of hydrogen-bond protection is given by the number of vicinal nonpolar groups [1,6]. As recently shown [1], underwrapped hydrogen bonds (UWHBs) are determinants of protein associations. This is so because water exclusion at the protein-protein interface provides extra stabilization to the preformed hydrogen bonds and, by deshielding the relative charges on the oxygen and nitrogen, it enhances their Coulombic energy. This effect dominates over the opposing thermodynamic cost associated with the removal of residual surrounding water solvating the paired amide and carbonyl [1,2,6]. Since UWHBs are adhesive [1,6], it becomes imperative to determine the attractive force they may exert on a water-removing group.

The desolvation domain for a backbone hydrogen bond is defined as the reunion of two intersecting 6 Å spheres centered at the α carbons of the residues paired by the hydrogen bond. The choice of this domain is justified below, and the statistical inferences are robust to variations (6.4 ± 0.6 Å) in the cutoff values [1,2]. Thus, the extent of intramolecular desolvation of a hydrogen bond, ρ, may be determined by the number of side-chain nonpolar groups in the desolvation domain. An examination of a nonredundant structural database [7] consisting of 2808 soluble proteins gives an average $\langle\rho\rangle = 15.9$, with Gaussian dispersion $\sigma = 3.4$ over all hydrogen bonds. Thus, an UWHB may be statistically defined by the inequality $\rho < 12$.

Previous experiments on adsorption of soluble proteins onto a lipid bilayer [6] revealed a strong negative correlation between the average extent of hydrogen-bond intramolecular desolvation and the adsorption uptake. The results were rationalized assuming that the probability of successful landing on the liquid-solid interface is proportional to the ratio of UWHBs to all hydrogen bonds on the protein surface.

To determine the average magnitude of the attractive force exerted by an UWHB on a nonpolar group we now focus on the flux dependence of the adsorption uptake. The adhesive force is here obtained by measuring the decrease in adsorption uptake as the flow rate is increased. Protein adsorption at a solid-liquid interface is monitored using an optical biosensing device based on evanescent field total reflection [6,8–10].

A flow cell of 1 cm^2 square cross section was mounted on a 5×1 cm^2 waveguide of wide diffraction grating coated with a Langmuir-Blodgett DLPC bilayer (Avanti) [11]. The waveguide became the floor of the cell and the protein solution at $T = 298$ K was maintained at constant bulk concentration 1 μM. The variation in the adsorption uptake at $\tau = 900$ s was

determined by varying the flux ω within the range $(2.0–36.5) \times 10^{-3}$ cm^3/s. The experiment was designed to measure the affinity of specific proteins for lipid bilayers and determine the flux dependence of the adsorption uptake.

The lipid bilayer was deposited onto a 180 nm thick TiO_2/SiO_2-coated waveguide (period 412 nm, Harrick Scientific Corp.). The time dependence of protein uptake was monitored by optical interrogation of the protein adlayer using a polarized light beam produced by a He/Ne laser ($\lambda = 632.8$ nm, Spectra-Physics). Only discrete modes exist in the total reflection spectrum, which was recorded using a Shimadzu instrument (Model PC1200). The number of adsorbed protein molecules per μm^2 was determined from the thickness and refractive index of the adlayer [10]. All measurements were made using a single buffer (0.01 M Hepes, 0.7 mM EDTA, pH 7.1, refractive index $= 1.333\,01$ for $\lambda =$ 632.8 nm).

Six commercially available proteins (Sigma) were investigated, β lactoglobulin (pdb.1beb, $N = 156$), human apomyoglobin (pdb.2hbc, $N = 146$), hen egg-white lysozyme (pdb.133L, $N = 130$), human β_2 microglobulin (pdb.1i4f, $N = 100$), apolipoprotein A-I (pdb.1av1, $N =$ 201), and monomeric human insulin (pdb.6ins, $N = 50$). As an illustration, the UWHBs for myoglobin, lysozyme, and β_2 microglobulin are shown in Figs. 1(a)–1(c). Their numbers n_{UW}, n_W of UWHB's and well-wrapped backbone hydrogen bonds on the protein-surface are the following: $n_{UW} = 3$, $n_W = 34$ (apomyoglobin); $n_{UW} = 13$, $n_W = 34$ (lysozyme); and $n_{UW} = 9$, $n_W = 17$ (β_2 microglobulin). The parameters for the remaining three proteins are the following: $n_{UW} = 3$, $n_W = 106$ (β lactoglobulin); $n_{UW} = 66$, $n_W = 121$ (apolipoprotein A-I); and $n_{UW} = 14$, $n_W = 30$ (monomeric insulin). The adsorption uptake for the six proteins is given in Fig. 2 as a function of the flux. This information over a vast range of hydrodynamic conditions is now used to determine the average magnitude of the adhesive force per UWHB, and establish its constancy.

The parabolic hydrodynamic profile of the flow velocity vector $\mathbf{v}$ at each point of the cell cross section Ω is approximated by the average value $v = w/1$ cm^2, the fluid velocity averaged over the 1 cm^2 cross section. We distinguish a 6 Å thick bottom layer in the cell, where protein molecules may adhere to the lipid bilayer so that their UWHBs find proper desolvation. The cutoff value 6 Å (~ 4 water molecules in length) is a typical spatial threshold for solvent-organizing interactions [2,12]. Any protein molecule in the bottom fluid layer may be readily attracted with a nonzero force towards the coating lipid bilayer.

The number $N(\tau)$ of adsorbed molecules at time t (= 900 s) is given by

$$N(\tau) = [\tau v/L] f(v) P(n_{UW}, n_W, T) N, \qquad (1)$$

where $f(v)$ = fraction of molecules that reach the immobile bottom layer of the fluid "in time" ($t < L/v$); $P(n_{UW}, n_W, T)$ = conditional probability of a successful (sticky) landing at temperature T given that the bottom layer has been reached in time $t < L/v$; and N = average number of protein molecules in solution in the cell at any time (3.01×10^{15} mol).

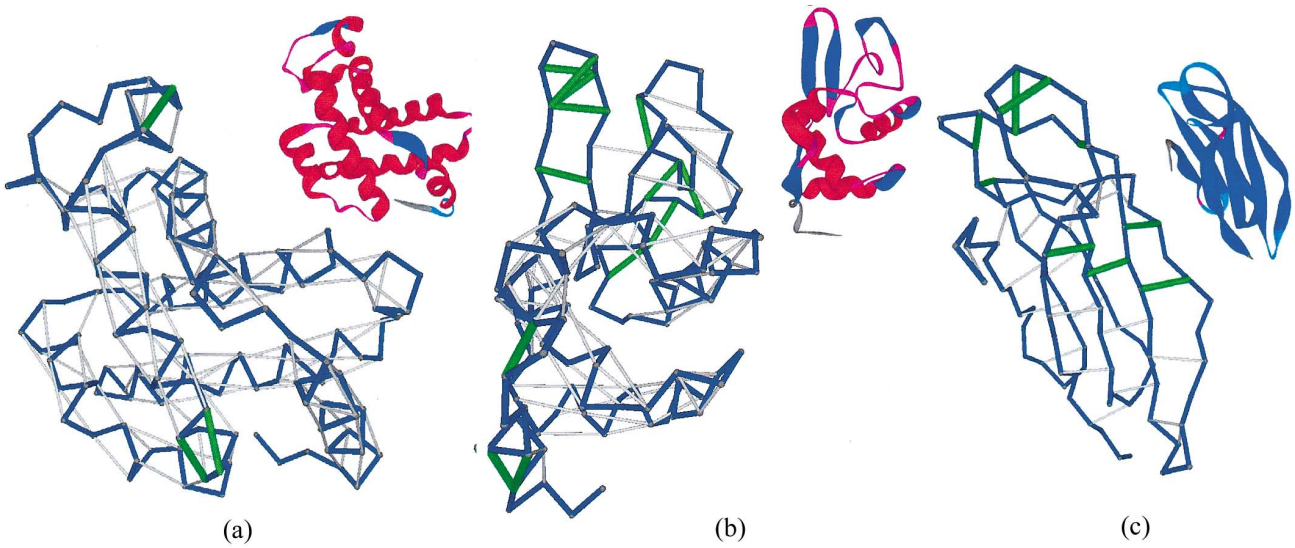

FIG. 1 (color). Pattern of UWHB's in the crystal structure of human apomyoglobin (a), lysozyme (b), and β_2 microglobulin (c). The ribbon representations are an aid to the eye. The backbone is represented as a blue virtual-bond polygonal joining α carbons, the well wrapped backbone hydrogen bonds are shown as grey segments joining the α carbons of the residues involved, and the UWHBs ($\rho < 12$) are displayed as green segments.

Thus, we get

$$P(n_{UW}, n_W, T) = (1 + qe^{\Delta U/kT})^{-1} \approx n_{UW}e^{-\Delta U/kT}[n_W + n_{UW}e^{-\Delta U/kT}]^{-1}, \quad (2)$$

where q^{-1} is the fraction of the protein surface that would yield a successful landing, here estimated at n_{UW}/n_W and ΔU = average decrease in Coulomb energy associated with the desolvation of an UWHB upon adhesion, with k = Boltzmann's constant.

The probability $f(v)$ of penetrating the bottom layer of the fluid is

$$f(v) = \int_\Lambda d\mathbf{r} \int_{\Omega/\Lambda} d\mathbf{r}_0 \int_{[0,L/v]} dt[m\xi^2/(2\pi kT\Gamma(t))] \exp[-m\xi^2||\mathbf{r} - \mathbf{r}_0||^2/2kT\Gamma(t)], \quad (3)$$

where $\mathbf{r}$ is the two-dimensional position vector exploring the cell cross section Ω due to Brownian two-dimensional diffusion orthogonal to the flow direction [13]; Λ is the 6 Å $\times$ 10^8 Å cross section of the bottom layer; $\xi = 6\pi R\eta/m$ with m = molecular mass, R = hydrodynamic radius [14], and η = bulk viscosity; and $\Gamma(t) = 2\xi t - 3 + 4e^{-\xi t} - e^{-2\xi t}$. The physical picture is best grasped in terms of the representation given in Fig. 2.

The validity of the model represented by Eqs. (1)–(3) is established by noting that the value $\Delta U = 3.91 \pm 0.67$ kJ/mol, the only parameter in the model, consistently fits within the confidence band the apomyoglobin adsorption data across the entire ω range and also fits within the confidence band the adsorption uptake for the other five proteins over the whole ω range (Fig. 3).

In order to compute the force $\|\mathbf{F}\|$ exerted by the surface on a single protein molecule at a 6 Å distance of the UWHB, we take into account that the drag, stemming from the solvent-ordering effect imposed by the hydrophobic-solvent interface, is exponentially decreasing in $r = \|\mathbf{r}\|$ with characteristic length $\lambda = 1.8$ Å [2,12]. Thus, the work done by the force $\mathbf{F}$ to decrease by ΔU the Coulomb energy of the UWHB as a result of the protein-surface association may be calculated from the energy balance equation:

$$\Delta U = 3.91 \text{ kJ} \times (6.02 \times 10^{23} \text{ mol})^{-1} = \int_{[4\text{ Å},\infty]} \mathbf{F}(\mathbf{r}) \cdot d\mathbf{r} = \int_{[4\text{ Å},\infty]} \phi e^{-r/\lambda} dr, \quad (4)$$

where 4 Å is the lower bound for the proximity of a test hydrophobe to an UWHB. This bound can be determined with 0.1 Å precision by noting that the inherent geometry of a protein chain is such that 4 Å is the minimum distance a side-chain nonpolar group can approach a

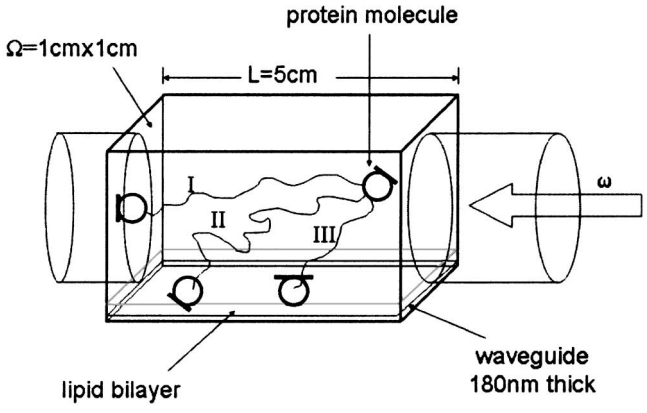

FIG. 2. Representation of the adsorption experiment. The soluble protein molecule enters the cell and faces one of three possible outcomes as a result of its Brownian motion: (I) it does not land in time before exiting; (II) it lands on the bilayer and the landing is successful because the bilayer faces the adhesive UWHB; and (III) it lands in time but the landing is not sticky since the bilayer faces a well-wrapped side of the protein. The UWHB is represented as a thick segment.

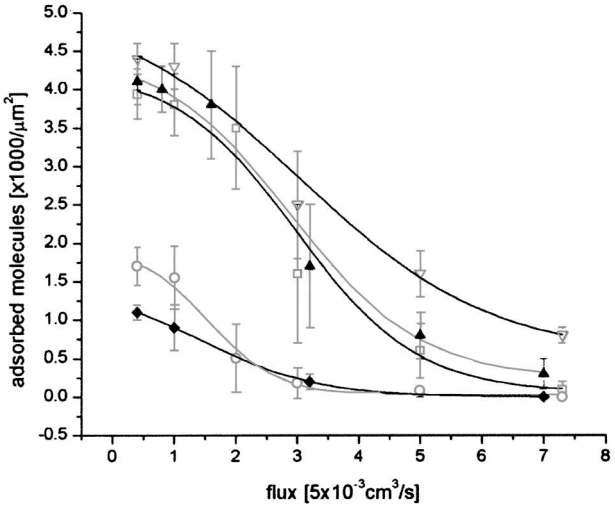

FIG. 3. Adsorption uptake at 900 s for β lactoglobulin (filled diamonds), apomyoglobin (open circles), lysozyme (open squares), β_2 microglobulin (filled triangles), and apolipoprotein A-I and monomeric human insulin (open triangles) onto the DLPC bilayer. The values for the apolipoprotein and insulin are practically indistinguishable, with the expected values for one protein lying within the dispersion of the other, and vice versa. The uptake is given in thousands of molecules per μm^2 and plotted as a function of the flow rate ω. The dispersion in the ordinate values results from 15 repetitions of the adsorption experiment under the same hydrodynamic conditions and bulk composition. The solid lines represent the theoretical computation fixing ΔU at 3.91 kJ/mol. The thicker solid lines correspond to β lactoglobulin, lysozyme, and apolipoprotein A-I.

backbone hydrogen bond [1,2,6]. Thus, the scaling constant ϕ is estimated at 218 ± 37 pN, which yields an adhesive force of $\|\mathbf{F}\| = 7.78 \pm 1.2$ pN for a single UWHB at 6 Å distance. An error analysis encompassing the precision in the integration limit yields $\phi = 218 \pm 44$ pN, and $\|\mathbf{F}\| = 7.78 \pm 1.5$ pN for a single UWHB at 6 Å distance.

The adsorption uptake values for the six unrelated proteins with highly diverse extents of structural wrapping consistently lie within the confidence bands of the theoretical prediction over a vast range of hydrodynamic conditions and are fitted accordingly by Eqs. (1)–(4), revealing the existence of an interactivity constant. This constant measures the adhesiveness of a structural defect.

Protein binding sites have not been associated with secondary structure motifs but have been often attributed to exposed hydrophobic patches, although hydrophobic residues are rarely found on the surface of a protein because of the large entropic cost of making a solvent cavity around them [1,6]. To ensure that our results imply a causal relation between hydrogen-bond underwrapping and adhesiveness, we determined the local ratio of hydrophobic to hydrophilic residues in 300 $Å^2$ patches covering the surface [1,15] of the proteins investigated. The ratio was found to be between 0:7 and 1:7 with a predominance of the former, which arose on average in 8.6 of every 10 patches interrogated, regardless of the protein investigated.

The adhesion of the proteins cannot be attributed to electrostatic interactions between hydrophilic groups on the protein surface and the polar phospholipid heads: such contributions are extremely weak in water (78 times weaker than in vacuum, since the bulk water permittivity is 78 times higher than that of vacuum [2]). This situation is supremely illustrated by the dismantling of the crystal lattice of ionic solids, like NaCl, in water. On the other hand, if the polar group occurs near an UWHB on the protein surface, then the dehydration demands of the structural defect act synergistically with the electrostatics: the favored removal of water surrounding the UWHB also enhances the electrostatics. In the end, adhesion is still attributable to UWHBs, which become fully desolvated only after penetrating into the nonpolar region of the bilayer.

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