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Letters

Dynamic Surface Activity by Folding and Unfolding an Amphiphilic α-Helix

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We describe a rationally designed peptide with tunable surface activity, where the dynamics of surface activity are an outcome of helical folding. Our rationally designed model peptide is surface-active only as an α-helix. We apply circular dichroism to show that the folded population can be controlled with changes in electrolyte concentration, and we apply pendant bubble tensiometry to explore dynamic surfactant activity. This study shows a peptide that responds to environmental stimuli with dynamic folding and surface activity. Extending this concept to selective binding peptides will lead to new tools, where dynamic surface activity is coupled to targeted binding.

Introduction

The ability to control the surface tension in a dynamic fashion has applicability in a wide range of fields. To date, others have examined how to control surface tension dynamically with redox-active[1] and light-switchable[2] low-molecular-weight surfactants. The goal of this work is to rationally design a peptide that captures this behavior by coupling folding to surface activity, resulting in the dynamic amphiphilicity of peptide-based helices. This notion of transient behavior is common in natural systems, where dynamic structural transitions of proteins have been shown to be necessary for enhanced selectivity and kinetics.[3−9] For example, a fluctuating transcription factor protein slides across nonspecific sequences of DNA until the target sequence is found. The target sequence stabilizes transcription factor protein into a well-ordered state.[10] Rationally designing peptides to control surface activity depending on the folded state has a distinct advantage in that peptides can be designed with the ability to bind a wide variety of targets. Therefore, peptide folding and surface activity can be coupled to selective binding.

This work demonstrates that a peptide can fold and unfold to control surface tension and the pendant bubble technique can be used to measure the dynamics. We use circular dichroism to characterize the bulk-phase ensemble average folded state as a function of salt concentration, and we use the pendant bubble method to characterize the dynamics of the process, namely, surface activity with folding. These tools provide a proof of concept for a simplified peptide design that couples folding to amphiphilicity. The model peptide can serve as a platform for future designs that will incorporate the selectivity inherent in helical biological molecules. The next generation of peptide designs will respond to particular stimuli, applying sequences

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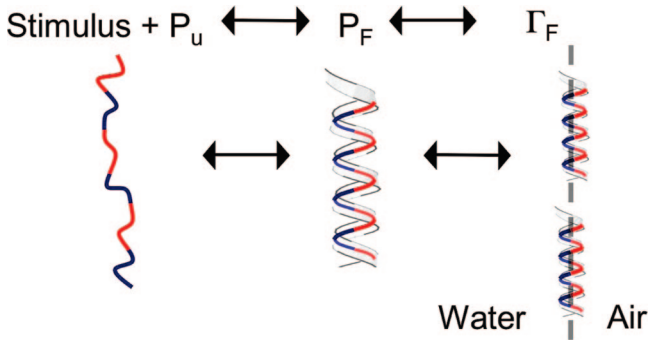

Figure 1. Folding-initiated interfacial dynamics. The peptide (red = hydrophobic, blue = hydrophilic) is ensemble average unfolded (P_U). The sequence folds in the presence of a stimulus into an α-helical conformation (P_F). The α-helical conformation is amphiphilic. Folding will promote interfacial adsorption (Γ_F, concentration of folded peptide at the interface).

taken from nature such as selective DNA binding, that define the switching behavior via selective ligand binding.

Figure 1 shows the concept of a dynamic surface-active peptide, where folding is a consequence of an environmental cue. In contrast to designing for static binding with a fixed target site, the novelty of our design approach is that binding behavior results in structural transitions. Peptides are well suited for the complexity associated with the study of transient surface activity because they have more degrees of conformational freedom than do simple surfactants and more control of sequence and molecular weight distributions over polymers.

Tunable amphiphilicity is an attractive tool for applications ranging from separation processes[11] to drug delivery.[12] Over the past decade, the Abbott laboratory has explored a redox-based switch to control the hydrophobilicty of the surfactant-like systems. These ferrocene-containing amphiphiles tune the oxidation state to yield an amphiphilic architecture.[1] Another tunable system involves photoresponsive surfactants where an azobeneze group defines the hydrophobic region, allowing UV light to control the cis and trans conformations.[2,13] In recent insightful studies, Cicciarelli et al. apply the pendant bubble technique to illuminate the reaction dynamics and transport phenomena of several molecular variations in the azobenzene system.[14,15] The work presented here also uses the pendant bubble technique to examine the dynamic surface activity of an amphiphilic peptide α-helix, where we tune the chemical potential by a folding "reaction" instead of redox reactions or light induced cis/trans isomerizations.

Several other groups have explored the nature of amphiphilic peptides in synthetic and natural systems. A key parameter in understanding how to control the formation and stability of secondary structure is to define a periodic pattern in the sequence. Work from Degrado and Lear on peptides containing only leucines (hydrophobic) and lysine (hydrophilic) highlight the role of periodicity in the design of folding. Sequences composed of LKKLLKL repeats and LKLKLKL are chemically similar but exhibit distinct secondary structures because of the distribution of the hydrophobic groups. LKKLLKL defines an amphiphilic helix, and LKLKLKL defines an amphiphilic β-strand. This periodic architecture is responsible for the stability of the secondary structure.[16] Many aspects of this sequence have been explored. Several studies look at the potential applications for antimicrobial activity[17,18] and hemolytic activity as a function of sequence length.[19,20] There are also some studies that explore the dynamic aspects of surface-active helicies, including the ability to partition into liposomes[21] and the rate of adsorption to surfaces.[22] We should note that there are few studies that address the transient amphiphilicity, measuring dynamics in response to stimuli.

Another sequence known as the GALA peptide has been applied by the Szoda group to mimic the activity of viral hemagglutanin. The sequence is composed primarily of repeating glutamic acids, alanines, and leucines (thus GALA), and several papers carefully explore the mechanism of pore formation, the rates of membrane permeabilization, the effect of environmental cues such as pH, and the role of sequence.[23−28]

Additionally, extensive study on naturally occurring amphiphilic peptides precedes much of the work described for the synthetic systems. Natural analogues can be found in antimicrobial peptides.[18,29−32] or viral hemagglutinin.[33] It has been hypothesized that both of these systems use structural transformations based on an α-helical motif to penetrate the cell membrane. Whereas these antimicrobial peptides certainly exhibit interesting behavior, the work primarily focuses on the ability of sequences to penetrate cell walls. In contrast, our work focuses on the development of a simple model peptide to measure the dynamics of folding coupled to amphiphilicity using the pendant bubble apparatus, where the long-term objective is a molecular architecture that possess tunable surface activity coupled to specific binding.

Applying dynamic structure to the rational design of biological molecules allows one to consider this new objective of tunable surface activity. The following sections detail our effort to design and characterize a peptide capable of folding and unfolding to change the surface activity. We describe a minimal allosteric model peptide design (HEAKELLKEWAKLLKKLLKEAKE) that can later be modified to investigate peptide motifs where structural transitions can actively respond to specific target molecules.

Materials and Methods

Materials. The model peptide sequence is synthesized and purified with HPLC by The Rockefeller University Proteomics Resource Center and is stored in the fridge at −20 °C. The peptide is used without any modification. All aqueous solutions are prepared using

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clean water from a Milli-Q water purification system at pH 7. Sodium chloride (≥990 and ≤1010 ppm Na) obtained from Fisher Scientific (NJ) is used for preparing peptide solutions in salt. All peptide solutions are prepared freshly for each experiment.

Methods. Circular dichroism (CD) spectroscopy is used for determining the ensemble average secondary structure of the peptide. The measurement is applied to estimate the fraction of molecules in a given secondary structure conformation. The application of this characterization tool is well established. Briefly, ellipticity is measured by passing circularly polarized light through a solution containing an optically active peptide and measuring the difference in the absorption of left circularly polarized light versus right circularly polarized light. The transmission of circularly polarized light is measured as a function of wavelength. The instrument output, ellipticity in millidegrees, is converted to mean residue ellipticity (degree cm^2 dmol^{-1}), $[\theta]$, by using the following conversion

$$[\theta]_{\mathrm{MRE}} = \frac{100\theta}{Cln}$$

where C is the molar concentration, l is the path length in cm, and n is the number of residues in the peptide. We determine the secondary structure by scanning in the far-UV region (190−250 nm), where the peptide bond is the chromophore.

Using this technique, our peptide structure is characterized as a function of salt (sodium chloride). A peptide solution of 0.5 g/L is prepared in water, and it is titrated with a 2 M NaCl solution. After adding NaCl to the solution, a series of CD experiments are conducted. This allows us to calculate the equilibrium constant of the "reaction" between folded and unfolded peptide, which we discuss in the following section. This is repeated for NaCl concentrations in the solution, ranging from 0 to 1 M.

Pendant Bubble Tensiometry. This is used to measure the surface tension relaxation and equilibrium tensions. We have used an apparatus similar to those described by Pan et al. and Ferri et al.[34,35] A collimated beam of light passes through a pendant bubble and forms a silhouette of the bubble that is captured by a CCD video camera. The pendant bubble is generated in an aqueous solution with the peptide at a known bulk concentration. A 14-gauge stainless steel inverted needle (0.063 in. i.d.; 0.083 in. o.d.) is connected to a motor-driven syringe thorough a three-way solenoid valve. A pendant bubble (diameter ∼1−3 mm) is formed at the tip of the inverted needle by pushing air through the valve, tubing, and inverted needle. After the bubble is formed, sequential digital images are then taken of the bubble. For a high-concentration (0.1−0.01 g/L) range, the time interval is around 5−15 s. For a lower-concentration interval, the time interval is around 20−30 s. Each image is processed to determine the bubble edge coordinates, and the surface tension is obtained from the shape by comparing to the theoretical shape. The theoretical shape of the pendant bubble is derived according to the Laplace equation that relates the pressure difference across the curved fluid interface to the shape

$$\gamma\left(\frac{1}{R_1} + \frac{1}{R_2}\right) = \Delta P$$

where γ is the surface tension, R_1 and R_2 are the two principal radii of curvature of the surface, and ΔP is the pressure difference across the interface. Using this technique, the peptide is characterized as a function of peptide concentration in 1 M NaCl solutions. The peptide is also characterized as a function of salt at a fixed concentration of peptide, 0.32 g/L.

Results and Discussion

Peptide Design. Engineering biologically inspired peptides with controllable molecular architectures that demonstrate nativelike structure and activity requires a careful consideration of the dominant inter- and intramolecular interactions.[36] Moreover, the model peptide described here should be designed for a transient 2° structure coupled to transient surface-activity. To accomplish this goal, we apply two simple rules for the *de novo* design of well-nucleated 2° structure. First, secondary structure propensity refers to the intrinsic inclination of individual amino acids to a given secondary structure, where side-group van der Waals and steric interactions tend to restrict an amino acid to particular ϕ and Ψ conformations.[37] Second, periodicity refers to the nonlocal interactions, where sequence is "programmed" with a recurring pattern that defines the secondary structure.[16,38] α-Helices have a periodicity of 7 (3.5 amino acids per turn), meaning that the amino acids at i and $i + 7$ define the refrain. For example, choosing valine at these positions results in a hydrophobic face pattern and promotes an α-helical conformation, even if valine has a low helical propensity.[39] Another key design parameter is that the model peptide needs to be in a partially folded state, where the ΔG of folding is on the order of kT and the peptide can "fish" for a target molecule. Our model sequence does not have a particular target. Rather, it is natively disordered on the basis of simple electrostatic charge repulsion, allowing us to robustly test the dynamics of folding and surface activity. The seven positively charged lysines are shown in blue in Figure 2.

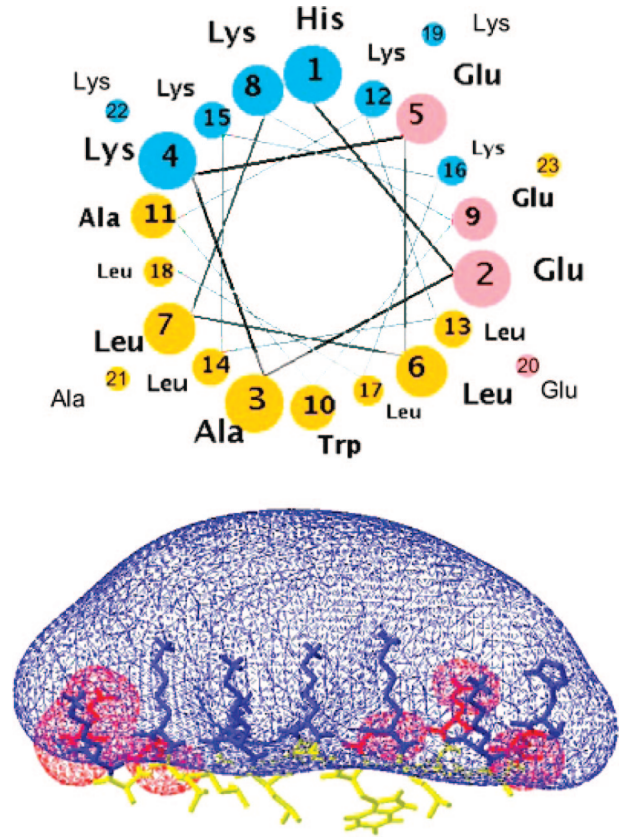

Figure 2. Model peptide. Helical wheel (top) for the model peptide system, where hydrophobic (yellow), basic (blue), and acidic (red) amino acids are highlighted. This peptide is amphiphilic only in the folded helix. The side view of the peptide (bottom) is shown, where the blue cloud represents the region of concentrated electrostatic repulsion.

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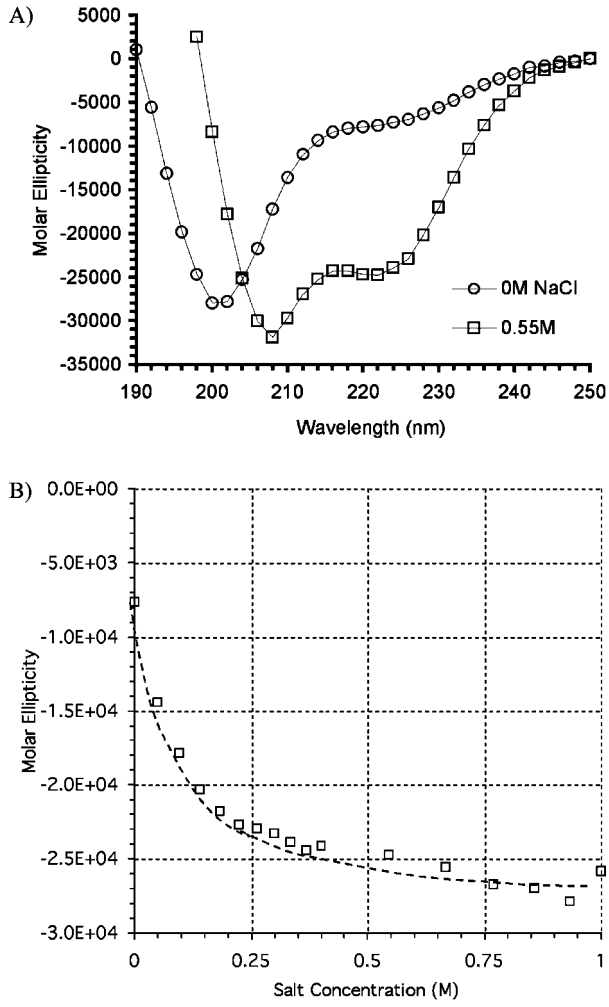

Figure 3. Circular dichroism. (a) The open circles represent the disordered form of the peptide in DI water, and the open squares are the helical amphiphilic conformation in 550 mM salt solution. (b) Mean residue ellipticity at 222 nm as a function of salt concentration. Lower values represent increased helicity.

Circular Dichroism. Circular dichroism (CD) spectroscopy is applied to measure the ensemble-average secondary structure, namely, α-helices. Using circular dichroism, the characteristic spectrum of a random coil and α-helix are deconvoluted to quantify the population of each state. CD for the model sequence is shown in Figure 3a. The characteristic CD spectrum for an α-helix shows minima at 208 and 222 nm. In deionized water (blue), the data show a typical spectrum for a random coil (10% α-helical). The predominantly populated state is natively disordered. The overall charge on this peptide is +3; therefore, the intramolecular electrostatic repulsion results in an elongated state in deionized water. As the charges are screened by electrolytes (550 mM NaCl), decreasing the Debye length from ~300 to 0.4 nm, an ordered structure emerges (red, 70% α-helical). At an approximately physiological electrolyte concentration of 95 mM NaCl, the peptide shows a 63% helical structure. Experiments that were run at physiological conditions, 37 °C and 95 mM NaCl, did not show significantly different helicity. This value was predicted using established secondary structure predictors, such as GOR4 (65.4% α-helical)[40] or JUFO (70.4% α-helical).[41] This data corroborates the hypothesis that one can design a peptide sequence that can be programmed to fold under specific conditions. Still, the consideration of a percentage value for helicity can be deceptive because the value represents the mean helicity of an ensemble of fluctuating conformations with time.

The thermodynamics of the equilibrium folded population can be established in the following manner. For the model sequence, one can plot the ellipticity at 222 nm as a function of salt concentration. Shifts in the populations of the folded and unfolded states are described by the following equation

$$\sigma \equiv \frac{F_n}{F_u} = \frac{\eta_u - \eta_x}{\eta_x - \eta_n} \tag{2}$$

where the equilibrium constant, σ, is the ratio of folded peptide to unfolded peptide, F_n/F_u, determined from the ellipticities η_n (folded), η_u (unfolded), and η_x (intermediate). By performing the same measurement as a function of salt concentration, (Figure 3b), we can approximate the equilibrium constant for our model peptide. Moreover, future systematic variations in sequence can be applied to establish the free energy of folding with regard to the position of the charged groups or the concentration of specific targets.

Pendant Bubble. The pendant bubble technique is applied to examine the dynamic behavior of folding. The interfacial phase behavior is resolved by the axisymmetric analysis of the bubble shape. The bubble is inflated ($t = 0$), and the adsorption of folded peptides to the interface changes the surface tension as a function of time.

Data on the model peptide verifies that the pendant bubble is capable of detecting changes in surface activity as a function of time, where the formation of a depletion layer near the bubble interface gives nonequilibrium behavior. Figure 4a shows these results with increasing peptide concentration. Each line shown here represents a separate concentration of peptide at a constant salt concentration of 1 M NaCl. At this salt concentration, the equilibrium constant is given by $\sigma = 25$. This experiment was done both dynamically and at very long times to establish equilibrium (data not shown). As we increase concentration, we see a faster decrease in surface tension, whereas longer studies shows us that the equilibrium values are not concentration-dependent. We tested three decades of peptide concentrations and observed no variation in the equilibrium surface tensions, implying nearly irreversible adsorption.

Dynamic pendant bubble experiments are also taken with changing salt concentration (Figure 4b). As expected from the equilibrium bulk phase folded populations (Figure 3), we see an increase in the adsorption rate of the peptides at higher salt concentrations. Again, this agrees with the hypothesis that the folded structures are surface-active but the less folded populations are not. In contrast to the fixed salt concentration data (Figure 4a), at longer times, this data does show decreasing values of equilibrium surface tension with increased salt concentration. The equilibrium results suggest that there is also screening at the interface, where amphiphilic helical peptides are capable of packing closer at the interface because of a shorter Debye length.

We quantify the nature of the dynamics by evaluating how quickly the interface reaches 90% of the equilibrium surface tension. We define this time as t_{90}. The evaluation of t_{90} allows us to estimate the relative contributions of bulk diffusion compared to surface adsorption dynamics. The folding time scales do not define the dynamics of this system. Folding is not rate-limiting

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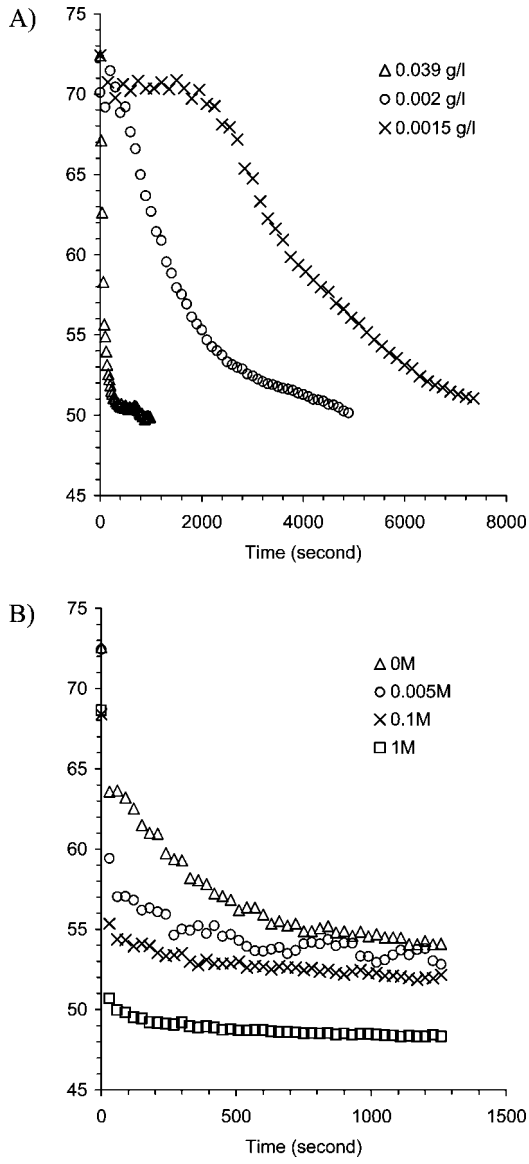

Figure 4. Pendant bubble. Surface tension as a function of time is plotted (a) at various total peptide concentrations and a fixed salt concentration of 1 M NaCl and (b) at various salt concentrations and a fixed peptide concentration of 0.32 g/L.

because short peptides will fold and unfold on time scales on the order of milliseconds,[42] and the phenomenon observed here is limited by time scales on the order of seconds to hundreds of seconds. Figure 5 shows log t_{90} plotted against the log of the total peptide concentration, C_o, in the pendant bubble experiment. Far from the bubble, the folded peptide concentration is simply the total peptide concentration multiplied by $\sigma/(1+\sigma)$, where σ is from eq 2.

The slope for Figure 5 is ~1.35. Surface tensions are found to increase with surface concentration according to pendant bubble expansion experiments (data not shown). Using the assumption of irreversible Langmuir adsorption as described above, we assume that the adsorption-limited dynamics will follow $d\Gamma/dt = \beta C_s(\Gamma - \Gamma_\infty)$, where Γ represents the amount of peptide adsorbed, Γ_∞ represents the maximum amount of peptide that can adsorb, β is the adsorption coefficient, and C_s is the sublayer concentration of folded peptide. In the limit of fast diffusion, this

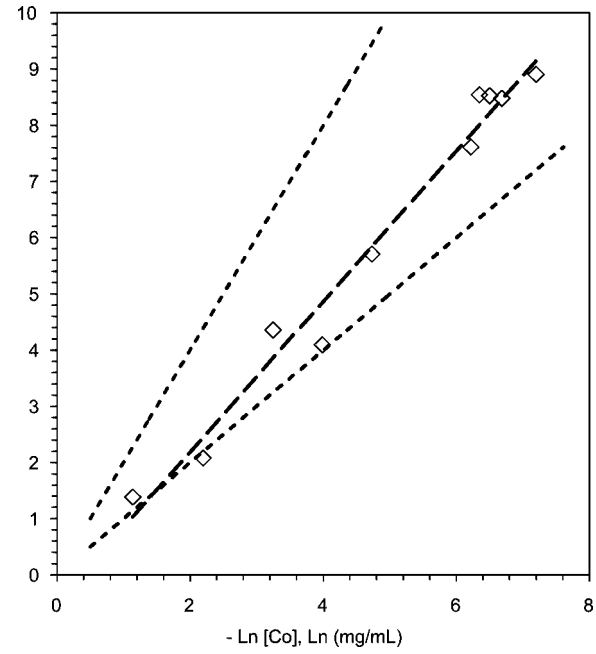

Figure 5. Values for the time where the surface tension reaches 90% of the minimum surface tension, $-\ln(t_{90})$, against the log of total peptide concentration. The slope is 1.35. Slopes of 1 (adsorption limited) and 2 (diffusion limited) are also plotted.

leads to a slope of 1 in Figure 5, where the scaling of the log of $t_{90} \sim -\ln(C_s) \sim -\ln(\sigma/(1+\sigma)C_o)$.

In the diffusion-limited case, the surface concentration, scales as $\Gamma \sim 2(\sigma/(1+\sigma))C_0(Dt)^{1/2}$. This leads to time scales for the diffusion-limited adsorption of $t_{90} \sim [\Gamma_{90}/(\sigma/(1+\sigma))C_o]^2/D$. For this case, we would expect to recover kinetics where $\ln(t_{90}) \approx -2\ln(C_o)$. The diffusion-controlled case would yield a slope of 2. In Figure 5, slopes of 1 and 2 are shown with dashed lines. A slope of 1.35 indicates that the adsorption of the peptide to the interface dominates the dynamic process. This is in contrast to surfactants where diffusion typically dominates.[43]

Applying the assumption that folding kinetics is fast and adsorption kinetics limits the process, we can also evaluate the influence of changing salt concentrations at constant total peptide concentration (Figure 4b). In this case, we used t_{95} rather than t_{90} because these processes have much faster relaxation times, making it difficult for us to assess a value for t_{90} accurately. We anticipate the rate of adsorption to the interface to increase with increasing salt concentration. This increased rate of adsorption is controlled by an increase in the population of folded peptides at higher salt concentrations, where the assumption of fast diffusion fixes the sublayer concentration to $(\sigma/(1+\sigma))C_o$. Figure 6 shows $\ln(t_{95})$ plotted against $-\ln[(\sigma/(1+\sigma))C_o]$, where the σ term is determined from Figure 3b using eq 2. If the assumptions made regarding diffusion and folding are correct, then the same slope should be recovered in this plot. The slope here is found to be 1.40. These experiments corroborate our central hypothesis that we can dynamically control surface activity by controlling the folded and unfolded states of the peptide. Additionally, this simplistic analysis shows that the pendant bubble can effectively quantify the adsorption dynamics of the peptide system.

A more complete model for the dynamic pendant drop experiment is currently being developed. The model is based on existing models for the diffusion and adsorption of surfactants

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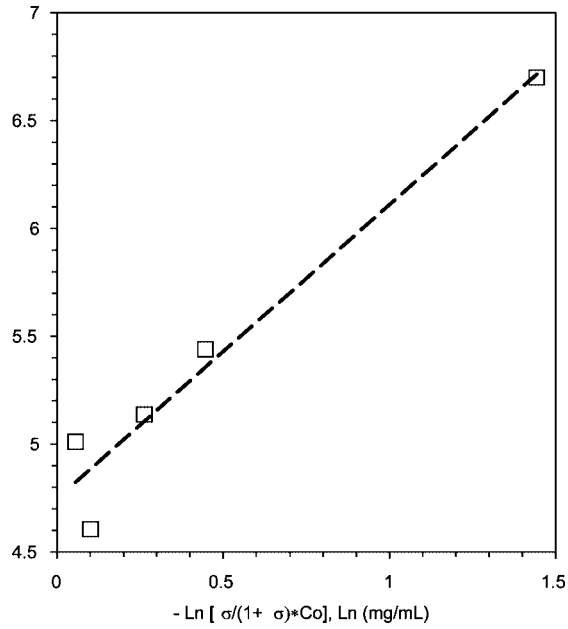

Figure 6. For various salt concentrations, $-\log(t_{95})$ is plotted against the folded peptide population, given by σC_o. The slope of $\ln(t_{95})$ vs $-\ln(\sigma/(1+\sigma)C_o)$ is 1.40.

to the pendant bubble interface,[44,45] but it takes into account the dynamics of folding and unfolding. Using this model, we observe that the nonequilibrium concentrations of folded and unfolded peptides in the depletion layer adjacent to the bubble determine the rate of change of the surface tension. This numerical analysis is the subject of a separate contribution.

Conclusions

We have shown using circular dichroism and dynamic pendant bubble experiments that the rationally designed peptide shown in Figure 2 exhibits dynamic surface activity that is coupled to the folding of the peptide into an amphiphilic helix. We also show that the peptide folding is controlled by an environmental cue, namely, changes in the salt concentration. These results will provide a starting point for the design of the next generation of peptides, where selective binding coupled to surface activity will be a platform for novel diagnostic and separation tools. These designs combine the inherent specificity and dynamic potential of biological molecules to surface-active architectures.

Future studies explore two particular aims: (1) DNA selectivity and (2) modeling effort that combines folding kinetics, diffusion to the interface, and adsorption dynamics. Engineering dynamic molecules, where surface activity is coincident with the inherent specificity, will have far-reaching benefits for the design of biomimetic tools, particularly in scenarios where fast rates and selective binding in a sea of similar molecules are essential.

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