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Adsorption of β-Hairpin Peptides on the Surface of Water: A Neutron Reflection Study

Jian R. Lu,*,† Shiamalee Perumal,† Evan T. Powers,*,‡ Jeffery W. Kelly,‡ John R. P. Webster,§ and Jeff Penfold§

Contribution from the Biological Physics Group, Department of Physics, UMIST, PO Box 88, Manchester M60 1QD, U.K., Department of Chemistry and the Skaggs Institute of Chemical Biology, The Scripps Research Institute, 10550 North Torrey Pines Road BCC 265, La Jolla, California 92037, and ISIS Neutron Facility, CLRC, Rutherford Appleton Laboratory, Chilton, Didcot OX11 0QZ, U.K.

Received November 5, 2002; E-mail: j.lu@umist.ac.uk; epowers@scripps.edu

Abstract: Neutron reflectivity has been used to determine the thickness and surface coverage of monolayers of two 14-residue β-hairpin peptides adsorbed at the air/water interface. The peptides differed only in that one was labeled with a fluorophore, while the other was not. The neutron reflection measurements were mainly made in null reflecting water, NRW, containing 8.1% D_2O. Under this isotopic contrast the water is invisible to neutrons and the specular signal was then only from the peptide layer. At the highest concentration of ca. 4 μg/mL studied, the area per peptide molecule (A) was found to be 230 ± 10 and 210 ± 10 Å^2 for the peptides with and without a BODIPY-based fluorophore, respectively. The thickness of the peptide layers was about 10 Å for a Gaussian distribution. With decreasing bulk peptide concentration, both surface excess and layer thickness showed a steady trend of decrease. While the neutron results clearly indicate structural changes within the peptide monolayers with increasing bulk concentration, the outstanding structural feature is the formation of rather uniform peptide layers, consistent with the structural characteristics typical of β-strand peptide conformations. These structural features are well supported by the parallel measurements of the adsorbed layers in D_2O. With this isotopic contrast the neutron reflectivity provides an estimate about the extent of immersion of the peptide layers into water. The results strongly suggest that the 14-mer peptide monolayers were fully afloat on the surface of water, with only the carboxy groups on Glu residues hydrated.

Introduction

Many synthetic peptides with short and intermediate lengths have recently been shown to self-assemble into interesting hierarchical structure organizations of filaments, bundles, and networks[1−5] in aqueous solution. For peptides containing alternating hydrophilic and hydrophobic amino acid side chains, a strong tendency toward formation of β-sheet structures has been widely reported.[6−12] This type of molecular assembly usually leads to the formation of two distinct types of interfaces within the layered structure: a hydrophilic interface comprising polar and charged side chains and a hydrophobic interface comprising hydrophobic side chains. The self-assembly of these peptides is often driven by the combined effect of hydrophobic and electrostatic interactions, in addition to the hydrogen bonding. The stability of the resulting β-sheet and its transition into related model structures are found to depend on primary sequence, solution ionic strength, ion specific interactions, and temperature. Understanding the factors affecting β-sheet formation is critical for the understanding of many of the problems encountered in peptide design and on the predictions of protein folding.[6−12]

Surface and interfacial self-assembly of short peptides on support surfaces, leading to the formation of monolayers, is potentially attractive for applications such as nano-sensors and nano-circuits. However, interfaces such as the air/water and solid/water interfaces represent different energetic balances from bulk solution, and as a result, peptides self-assembled at planar interfaces may adopt different structural conformations.[13] To

* Correspondence should be addressed to these authors. (J.R.L.) Tel: 44-161-2003926. (E.P.) Tel: 1-858-784-9607.
† UMIST.
‡ The Scripps Research Institute.
§ ISIS Neutron Facility.

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realize the potentials afforded by peptide surface assembly, it is important to be able to control the structure and morphology of the peptide layers formed at the interfaces. Understanding the effects of primary sequence and solution conditions on the structural properties of surface layers constitutes an essential step toward the improved fundamental understanding necessary for developing the applications of peptide monolayers. Although peptide layers that are of direct technical relevance are mainly those formed at the solid/solution interface, peptide layers adsorbed or spread at the air/water interface can serve as a template from which films with delicate structural orientations can be prepared, through direct or modified Langmuir−Blodgett (L−B) deposition.[14,15] Knowing the orientation of the molecules in the precusor peptide monolayer at the air/water interface is very important for the formation of functional films with a specific structure.

A very powerful technique for obtaining structural information about a monolayer is neutron reflectivity (NR), which determines the scattering length density profile of the surface layer. In previous studies,[16−22] we showed that neutron reflection is capable of simultaneous measurement of the thickness and volume fraction of any protein layer in a direction along the surface normal, with depth resolution at the level of 1−3 Å. This high depth resolution, together with the three-dimensional structural information, allows us to assess the structural orientations of protein molecules at a given surface and solution conditions and to determine the extent of deformation and structural unfolding of globular proteins. Peptides of small and intermediate lengths may be predisposed to forming α-helices or β-sheets. These transitions can be strongly affected by surface and solution environment, resulting in a different surface layer thickness and packing density, from which further detailed structural projections and orientations within the peptide layer can be inferred. The 14-mer peptide used in this work is composed of two strands of alternating hydrophobic (Nle and Val) and hydrophilic (Glu) residues attached to a d-Pro-Gly β-turn. This sequence, shown below, forces the peptide to adopt a β-hairpin conformation at the air/water interface, with the hydrophilic and hydrophobic side chains segregated on opposite sides of the molecule.

Peptide F: H-Glu-Cys(DMBDY)-Glu-Nle-Glu-Val-dPro-Gly-Val-Glu-Nle-Glu-Nle-Glu-NH_2

The Cys residue of peptide F is labeled with the 5,7-dimethyl derivative of the BODIPY fluorophore (DMBDY). In previous experiments, this tag enabled fluorescence microscopy experiments to be performed.[14,15] Peptide F can form monolayers on the surface of water either by spreading from a volatile organic solution or by adsorption from an aqueous solution. In the former, the peptide films may not be in thermodynamic equilibrium with the bulk solution, while in the latter equilibrium may not be reached in the time scale of the experiment. Recent fluorescence studies have shown that in adsorbed monolayers of peptide F the early interfacial processes are dominated by dynamic events; depending on the conditions, islands of peptide in the two-dimensional liquid or solid phase can be formed. In contrast, previous AFM studies have been done at the solid/air interface in attempting to provide better structural information of the immobilized peptide layer. The AFM results showed that L−B deposition of the labeled 14-mer peptide from spread monolayers onto mica yielded a well-ordered molecular array in which the molecular area was proposed to be ca. 280 $Å^2$/molecule and the thickness to be 7 Å. The neutron reflection work to be described here aims to complement and extend the early work by providing structural information on the peptide layers under the conditions of equilibrated adsorption. The neutron reflectivity measurements have been made directly from adsorbed surface monolayers in equilibrium with bulk peptide solutions. The present study of the peptide layers on the surface of water will enable direct comparisons to be made between the structures of the peptide layers at the two interfaces. Because DMBDY is quite bulky and hydrophobic, the analogue of peptide F (shown below, different only in that it lacks a Cys residue that can be labeled) was also prepared.

Peptide NF: H-Glu-Nle-Glu-Nle-Glu-Val-dPro-Gly-Val-Glu-Nle-Glu-Nle-Glu-NH_2

The properties of this peptide were studied in parallel.

Neutron Reflection. Neutron reflectivity, $R(\kappa)$, is usually measured as a function of momentum transfer, κ, perpendicular to the reflecting surface where

$$\kappa = \frac{4\pi \sin \theta}{\lambda} \tag{1}$$

where θ is the incidence angle and λ the wavelength of the incidence neutron beam. Through Fourier transformation $R(\kappa)$ is related to the interfacial composition characterized by changes in the scattering length density, $\rho(z)$, perpendicular to the interfacial plane,[23,24] where $\rho(z)$ depends on chemical composition through the following equation:

$$\rho = \sum n_i b_i \tag{2}$$

where n_i is the number density of the element, i, and b_i is its scattering length. Because different isotopes have different b_i values, a variety of neutron reflectivity profiles can be produced for a given chemical structure by isotopic substitution. A common practice in isotopic substitution is to use a mixture of the deuterated and hydrogenated forms of a solvent. Thus, the neutron signal from the peptide layer adsorbed on the surface of water can be optimized by the use of water containing 8.1 vol % D_2O. Under this isotopic contrast, the contribution from bulk solution is completely removed and the solvent is called null reflecting water (NRW).

Although the principal relationship between $R(\kappa)$ and $\rho(z)$ is very straightforward, neutron reflectivity profiles are usually analyzed by means of the optical matrix formalism, which has

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been described in detail elsewhere.[25,26] The main difficulty in Fourier transforming these reflectivity profiles, as indicated previously, is that the data were measured over too narrow a κ range to allow for a reliable determination of phases. A typical modeling procedure usually starts with an assumption of a structural model for the adsorbed layer, followed by calculation of the reflectivity based on the optical matrix formula. The calculated reflectivity is then compared with the measured data. The structural parameters are then varied in a least-squares iteration until a best fit is found. The structural parameters used in the fitting are the number of layers, thickness (τ), and corresponding scattering length density (ρ) for each layer (related to layer composition as in eq 2). For a uniform layer adsorbed on NRW, the area per molecule (A) for a peptide can then be calculated from

$$A = \frac{\sum m_p b_p}{\rho \tau} \tag{3}$$

where $\sum m_p b_p$ is the total scattering length for the peptide.

In the cases where ρ_w is nonzero and the peptide layer is predominantly immersed in water, the uniform layer model will still apply. Equation 3 needs to be modified to take into account the contribution of scattering length arising from the water associated with the peptide layer. However, in a more general situation where the adsorbed peptide layer is partially immersed in water, the upper part of the layer is out in air and the lower part of the layer is predominantly immersed in water. This uneven water distribution can be approximated to a two-layer model, with the upper part of the layer completely dry and the lower layer fully immersed in water. Although eqs 2 and 3 are developed under the condition of uniform layer distribution, they are directly applicable to each of the sublayers when more than one layer is required to model the density distribution profiles. The total adsorbed amounts are obtained by summing over the sublayers used in the fitting procedure. The choice of the number of sublayers is dependent upon the extent of inhomogeneity across the interface. However, in general the minimum number of layers that will successfully fit the data is chosen. In a mixed layer system the volume fraction of each component within the layer is expressed as

$$\rho = \phi_p \rho_p + \phi_w \rho_w \tag{4}$$

where ρ_p and ρ_w are the scattering length densities of protein and water and ϕ_p and ϕ_w their respective volume fractions.

Results and Discussion

The surface adsorption of the two 14-mer peptides was first measured in null reflecting water. Under this isotopic contrast, the entire specular neutron signal was only from the adsorbed layers. For all the concentrations studied, the reflectivity was mainly detected over the low momentum transfer (κ) region below 0.1 Å^{-1}. At $\kappa > 0.1$ Å^{-1} it rapidly fell off to the background level which was associated with incoherent scattering from the solvent. The attainment of constant background and the fast signal decay were indications of the formation of thin interfacial layers. Figure 1 shows the reflectivity profiles after subtraction of constant background. For each reflectivity

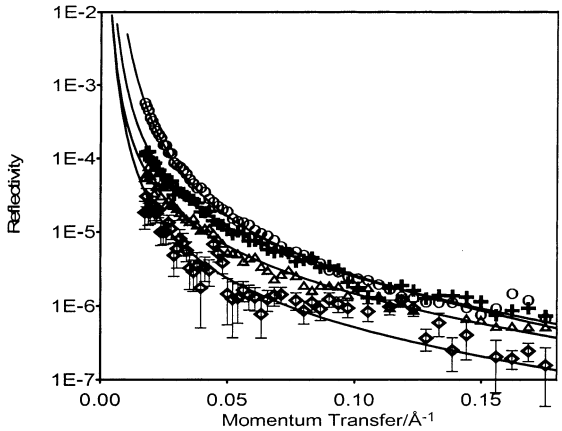

Figure 1. Neutron reflectivity measured from the 14-mer peptide without the fluorophore label (peptide NF) adsorbed at the air/NRW water interface employing peptide concentrations of 3.83 (○), 1.53 (+), 0.77 (△), and 0.38 μg/mL (◇). The continuous lines represent uniform layer fits with structural parameters given in Table 1.

the exact background was obtained by averaging the measured reflectivity between 0.3 and 0.5 Å^{-1}. Differences between reflectivity profiles after background subtraction reflect changes in layer thickness and composition. Increase in the slope of the reflectivity reflects thickening of the peptide layer, while increase in the level of reflectivity indicates the increased amount of adsorbed peptide. Figure 1 shows that change in bulk solution concentration mainly results in a shift of the level of reflectivity. In contrast, the shape of the reflectivity varies much less.

Quantitative information about the structure and composition of the peptide layers was obtained by fitting the data to models using the optical matrix approach, as outlined previously.[25,26] The reflectivity data were subsequently analyzed using the uniform layer model fits. The continuous lines shown in Figure 1 were calculated assuming a thickness (τ) of 9 ± 3 Å for the layer adsorbed at the lowest peptide concentration of 0.38 μg/mL to 13 ± 2 Å at the highest concentration of 3.8 μg/mL. The thickness derived in the model fitting corresponded to the minimal χ^2, a residual term indicating the deviation between the measured and calculated data.

As can be seen in eqs 3 and 4, the model fitting simultaneously produces scattering length density (ρ) and thickness (τ). The difference in ρ reflects the different amounts of adsorbed peptide in terms of area per molecule (A). The change of A with the bulk peptide concentration is shown in Figure 2. It can be seen that change in the concentration over the two higher concentrations results in little variation in A. However, a further decrease in the bulk concentration to the two lower concentrations causes substantial reduction in layer thickness and the associated increase in the area per molecule. The structural parameters obtained from the model fitting are given in Table 1.

The above calculations require the total scattering length, whose value was obtained from the primary sequence of the peptide given previously. Because the peptide contains labile hydrogens on the main peptide chain and the amino acid side groups, which readily exchange with D_2O, due consideration should be given to the effect of H/D exchange on the total scattering length of the peptide. Because the peptide chain is fully exposed, the encapsulation associated with hydrophobic

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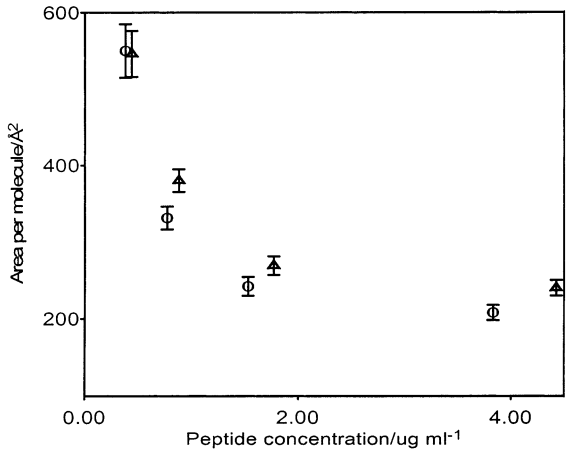

Figure 2. Variation of area per peptide with bulk concentration for the 14-mer peptide with (△) and without (○) fluorophore attachment.

Table 1. Structural Parameters Obtained from Single Layer Fits to Reflectivities Measured from the 14-mer Peptide without Fluorophore Adsorbed in NRW

concentration (μg/mL)	uniform layer thickness (τ/Å)	Gaussian thickness (σ/Å)	area per peptide (A/Å^2)	volume fraction (ϕ_p)
4.42	13 ± 2	11 ± 2	240 ± 10	0.67
1.77	12	10.5	270 ± 15	0.64
0.88	10	8.5	380 ± 20	0.54
0.44	8 ± 3	7 ± 3	540 ± 30	0.48

effect and hydrogen bonding that hinders the exchange process in the case of globular protein structures[27,28] is not expected. H/D exchange is thus expected to be complete upon dissolution of the peptide. As the scattering lengths are -3.74×10^{-5} Å for H, 6.5×10^{-5} Å for C, 5.8×10^{-5} Å for O, and 9.4×10^{-5} Å for N, b_p was calculated to be 3.43×10^{-3} Å. As the NRW water contains 8.1% D_2O and the scattering length for D is 6.67×10^{-5} Å, b_p is 3.48×10^{-3} Å for the peptide in NRW and is 4.06×10^{-3} Å in D_2O. The volume of the peptide was estimated by adding all the residues and backbone fragments together,[29,30] giving a value of ca. 1900 Å^3. The scattering length density (ρ_p) was subsequently calculated to be 1.85×10^{-6} Å^{-2} for the peptide in NRW and 2×10^{-6} Å^{-2} in D_2O. In comparison, because DMBDY is a bulky group, it will contribute to the total size and scattering length of the peptide. The value of b_p for the 14-mer peptide with the DMBDY attachment was calculated to be 4.35×10^{-3} Å in NRW and 5.01×10^{-3} Å in D_2O. The corresponding values of ρ_p were found to be 2.1×10^{-6} Å^{-2} in NRW and 2.3×10^{-6} Å^{-2} in D_2O. The accuracy of these values is affected by the estimated molecular volume, but it is reassuring to find that these values are within 0.3×10^{-6} Å^{-2} of those obtained for lysozyme and HSA (human serum albumin) when NRW is the solvent. On the other hand, in D_2O, ρ_p is substantially lower, indicating that there are fewer labile hydrogens in the two peptides than in lysozyme and HSA.[16−21]

Similar neutron reflectivity measurements have been made using the 14-mer peptide with the DMBDY group (peptide F),

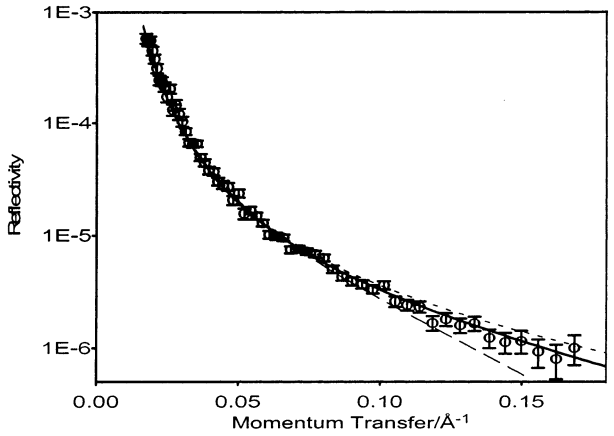

Figure 3. Sensitivity of the fit to layer thickness as demonstrated by comparing the reflectivities calculated at 8 Å (short dashed line) and 20 Å (long dashed line) with the best fit of 13 Å (solid line). The area per peptide was fixed at 230 Å^2.

and the same data analysis approach was used to extract structural parameters. Again, the thickness of the layers was calculated to be between 8 and 13 Å, indicating that within experimental error the attachment of the DMBDY group had little measurable effect on layer thickness. The corresponding area per molecule was also found to decrease with increasing bulk concentration, and the values are also plotted in Figure 2 for comparison. It appears that the DMBDY attachment has caused a slight increase in A. This increase could result from the bulky size of this group and is consistent with differences found in the limiting molecular areas of these peptides as recently determined from surface pressure−area isotherms. However, it should be noted that the difference in the total scattering length used here was calculated assuming stoichiometric attachment of DMBDY to the thiol group. An incomplete reaction or subsequent detachment/deterioration would mean possible overestimate of b_p and hence a greater A; this, however, is unlikely, as the linkage between the DMBDY and the peptide is robust and the peptide is initially 100% labeled.

We commented previously that although the neutron reflectivity measurements are sensitive to the area per molecule, it is less sensitive to the layer thickness and the possible inhomogeneity within the layer along the surface normal direction. As an example, we show in Figure 3 the effect of thickness variation on the calculated reflectivities. As can be seen from Figure 3, the change in layer thickness mainly affects the reflectivity at the relatively high κ, where the signal starts to drop substantially. For all data measured in this work, the reflectivity profiles fall into the background at $\kappa > 0.12$ Å^{-1}. The signal could however be significantly improved if the hydrophobic side chains were deuterated. This enhanced reflectivity level would make the measurement more sensitive to the thickness of the layer.[23,24]

Although the uniform layer model is mathematically the simplest to implement, it may not be the best to represent the distribution of the peptide chains. As discussed previously, the adsorbed layers are usually loosely packed and contain structural disorder arising from thermal roughness and side chain projections. These effects tend to make the actual layer distribution ($n(z)$) better represented by the Gaussian function with its width σ defined by

$$n(z) = n_o \exp(-4z^2/\sigma^2) \quad (5)$$

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Table 2. Structural Parameters Obtained from Single Layer Fits to Reflectivities Measured from the 14-mer Peptide with Fluorophore Adsorbed in NRW

concentration (μg/mL)	uniform layer thickness (τ/Å)	Gaussian thickness (σ/Å)	area per peptide (A/Å^2)	volume fraction (ϕ_p)
3.83	13 ± 2	11 ± 2	210 ± 10	0.65
1.53	12	10.5	240 ± 15	0.65
0.77	10	8.5	330 ± 20	0.57
0.38	9 ± 3	8 ± 3	550 ± 30	0.38

where n_o is the maximal number density and z is the distance normal to the surface layer. In the case of surfactant distributions, there is evidence from computer simulation that the Gaussian model is more appropriate.[31] For egg white lysozyme adsorbed on the surface of water at pH 7, our previous neutron reflection has convincingly shown that the layer is better described by the Gaussian distribution.[16] We have also shown[32,33] that the relationship between σ and τ is $\sigma = (\sqrt{3}/2)\tau$. Thus, according to the Gaussian model, the width of the peptide chains is some 10% narrower. This means that for the thickest layer of 13 Å derived from the uniform layer model its thickness is reduced to 11 Å in the Gaussian model. The values of σ and τ so obtained for both peptides at the four concentrations studied are listed in Tables 1 and 2.

The discussion given above assumes no contribution to the thickness from capillary waves. The presence of thermal fluctuation on a liquid surface tends to broaden the distribution of the interfacial region. The contribution can be estimated using the following simple equation:[32,33]

$$\sigma^2 = \sigma'^2 + \varpi^2 \tag{6}$$

where ϖ is the contribution from thermal roughness and σ' is the intrinsic layer thickness after roughness removal. Schwartz et al.[34] have shown that the amplitude of the thermal motion in a pure liquid is inversely proportional to the square of its surface tension. For pure water at 25 °C, its surface tension is around 72 mN/m and its root-mean-square amplitude is 2.8 Å. In terms of the Gaussian distribution used here, this is equivalent to 6.5 Å. From the Langmuir film experiment carried out previously for peptide F,[14] the surface tension at the highest peptide concentration is about 63 mN/m, giving a thermal roughness of 6.9 Å. Taking the total layer thickness of 11 Å, the use of eq 6 gives the value of σ' of 8.6 Å. Given the inaccuracy in the depth resolution of the technique as already explained, this value is consistent with the expected mean thickness for a β-sheet peptide monolayer.

The reflectivity profile measured from the peptides adsorbed at the D_2O/air interface contains information about the peptide distribution and its relative location to the surface of water. Because the peptide layer is very thin, the reflectivity obtained under this condition is likely to be dominated by the contribution from the solvent. However, comparison of the reflectivity profiles measured from the two peptides with and without the fluorophore with that from pure D_2O gives a clear indication

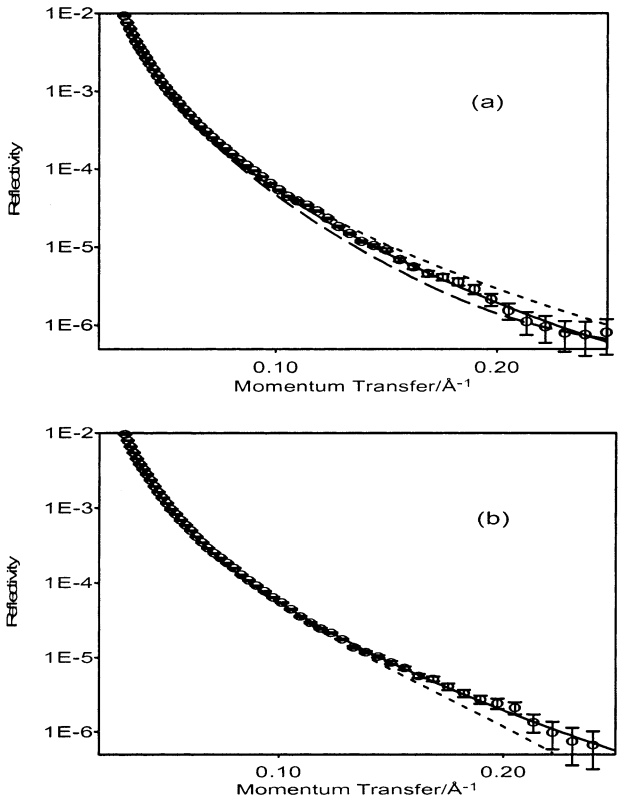

Figure 4. Neutron reflectivity measured from adsorption of 14-mer peptides at the air/D_2O interface. In (a) the best uniform layer fit of 11 Å from the 3.83 μg/mL peptide NF was compared with the calculated reflectivities with a thickness of 7 Å (short dashed line) and 15 Å (long dashed line). The layer was assumed to be fully afloat. In (b) the solid line represents the best uniform model fit of 11 Å dry layer to the reflectivity measured at 4.42 μg/mL peptide F. The dashed line assumes the whole layer is fully immersed in D_2O.

of suppression, showing that despite the dominance of D_2O the reflectivity profiles still offer useful information about the structure of the peptides.

The β-hairpin structure of peptides F and NF is supposed to segregate the hydrophobic and hydrophilic residues. However, it should be noted that only the ends of the Glu residues are truly hydrophilic. Because of this, it is possible that the entire peptide except for the carboxylates is located above the surface of the water, in the air phase. If so, the adsorption can be described by assuming a layer of peptide adsorbed on the top surface of D_2O. The thickness and composition of the peptide layer can be taken to be the same as that obtained from the corresponding NRW solution, except that the labile hydrogen exchanges with D_2O need to be taken into account. For the adsorption of peptide NF at 3.83 μg/mL, the layer scattering length density increases from 1.2×10^{-6} Å^{-2} to 1.3×10^{-6} Å^{-2} when in D_2O. The best fit, shown as a continuous line in Figure 4a, gives a thickness of 11 Å for the layer. The sensitivity to the thickness is demonstrated by comparing the reflectivity with those calculated with the layer thickness of 7 Å (short dashed line) and of 15 Å (long dashed line), respectively.

The optimal values of thickness from the uniform layer model on the surface of D_2O are 2−3 Å shorter than those derived from the NRW data from the same model but are comparable to the values obtained from the Gaussian model. The difference may largely arise from the different sensitivity to the thickness with different water substrates. A similar approach applied to

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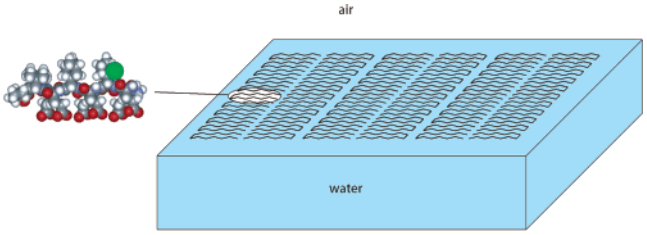

Figure 5. Schematic representation of the adsorption of peptide F on the surface of water.

the D_2O profile for peptide F at 4.24 μg/mL again gave the optimal thickness value of 10−11 Å. The results thus provide a consistent picture of smaller thickness when the same uniform model is applied on the surface of D_2O. This observation is broadly consistent with previous findings from surfactant systems.

It should also be added that the uniform layer model is only approximate, and it is unlikely to be appropriate for the description of the association of the hydrophilic side chains with D_2O. However, the fact that the uniform layer model containing no measurable association of D_2O fits the reflectivities well suggests that the mixing and association of the hydrophilic groups with D_2O may not be significant. Ideally, the contrast variation requires the use of a two-layer model, with the top sublayer describing the hydrophobic region in the air and the hydrophilic region immersed in D_2O. We have tested this model but found that the D_2O profiles were overall insensitive to the extent of immersion in water. Figure 4b compares the fittings where it was assumed that the whole peptide layer is either fully afloat (solid line) or fully immersed (short dashed line). The reflectivity was measured in D_2O for peptide F adsorbed at 4.24 μg/mL. As can be seen from Figure 4b, the reflectivity starts to deviate only below 10^{-5}, though the difference is clearly measurable.

Neutron reflectivity profiles in D_2O have also been measured at lower peptide concentrations for both peptides. As expected, the reflectivity becomes close to that of the pure D_2O as the peptide concentration decreases, but the fitting to the reflectivity profiles measured over the intermediate concentrations of 1−2 μg/mL in D_2O also suggested the formation of a single layer of 6−10 Å on the surface of water, thus giving consistent support to the variation of peptide layer structure with bulk concentration.

The characteristic structural feature of the peptide layers observed from neutron reflection can be schematically summarized in Figure 5. The main finding is the formation of a uniform peptide monolayer, which is predominantly afloat on the surface of water, with the carboxylic groups hydrated only. The area of this monolayer responds to the dilution of bulk peptide concentration, and the limiting area per molecule is some 230 Å, close to that estimated by AFM study from the same peptides deposited on mica. Because of this, we have assumed that the lateral packing and the orientation between the hairpin and the tails are similar to those observed at the solid substrate surface. This conformational similarity is likely to be true because the water surface offers a greater extent of flexibility. The change in the thickness may indicate responsive variation of the projection of side chains with surface area per peptide. The schematic in Figure 5 does not take into account the capillary wave effect.

Conclusions

Although neutron reflectivity has been used for studying adsorption of proteins and synthetic polymers, this is the first time the technique has been used to determine the limiting area per molecule and thickness of an adsorbed peptide layer. Although the signal obtained with the 14-mer peptide layers in NRW was relatively weak, it was sufficient for the determination of layer thickness, volume fraction, and hence the area per molecule. The reflectivity profiles in D_2O showed less variations because the signal mainly arose from the solvent, but these profiles offered further reliable assessment of the thickness of the layer, thus complementing the information obtained from NRW. The limiting areas per molecule for peptides F and NF are broadly consistent with those found from surface pressure−area isotherms.

The neutron data show that the 14-mer peptides both form uniform layers on the surface of water and that the two peptides have very similar surface properties. Thus, the attachment of the DMBDY group does not strongly affect the surface adsorption. For both peptides, surface excess and layer thickness vary with bulk concentration. The thickness of some 7−9 Å supports the formation of a β-sheet conformation and is consistent with the layer thickness determined previously by AFM measurements on Langmuir−Blodgett films. The increase in the layer thickness with bulk concentration indicates the variation of the projection of the side chains with increasing volume fraction of the peptide within the layer.

The D_2O measurements studied independently suggest that the peptide monolayers stay almost entirely in the air, with the hydrophobic side chains completely dry and the hydrophilic side chains solvated only at their tips. Such conformational structure has direct implications on the hydrogen bonding relating to labile hydrogens on the peptide backbone. The interfacial monolayer formed under such conditions may self-assemble more easily because the process is likely to be promoted by hydrogen bonding with each other, and dehydration would not become an energetic barrier.

Experimental Section

Neutron reflection experiments were performed at Rutherford Appleton Laboratory near Oxford, UK, using reflectometer CRISP, and the experimental arrangement was similar to that described in ref 35. Four Teflon troughs were placed in parallel in a sealed Perspex container. The container was mounted on an antivibration bench. Its height and the horizontal position were adjusted through two computer-controlled step motors so that solution surface in each trough could be aligned. The precise sample alignment was made using a laser with its beam on the same path as the incoming and exiting neutron beam. Each trough had a surface area of 15 cm × 5 cm and required some 50 mL of solution to produce the positive solution meniscus to avoid the obstruction of the trough edges to the beams. However, the exact volume required in each case depended on the surface tension and hence the peptide concentration. The beam-illuminated area was typically around 10 cm × 3 cm, and its exact size was defined by the horizontal and vertical slits placed before the sample container.

The reflectometer used a white neutron beam with wavelengths ranging from 0.5 to 6.5 Å. Calibration was made using the D_2O measurement at the highest beam incidence angle of 1.5°. Each

(35) Lee, E. M.; Thomas, R. K.; Penfold, J.; Ward, R. C. *J. Phys. Chem.* **1989**, *93*, 381.

reflection experiment was then carried out at three incidence angles of 0.5°, 0.8°, and 1.5°, and the resulting reflectivity profiles were combined to cover a momentum transfer (κ) range between 0.012 and 0.5 $Å^{-1}$. For each reflectivity profile, a constant background was subtracted using the average reflectivity between 0.3 and 0.5 $Å^{-1}$. For a given solution, the time-dependent adsorption was monitored by repeating reflectivity measurements at different time intervals. It was found that for almost all the systems studied here no time-dependent adsorption was detected some 30−60 min after the placement of the solutions in troughs. All neutron reflectivity profiles were measured after ca. 2 h solution equilibration.

Peptides F and NF were both synthesized as C-terminal amides by standard, Fmoc-based solid phase peptide synthesis. The Cys residue of peptide F was fluorescently labeled by treating the peptide with the iodoacetamide derivative of the 5,7-dimethyl BODIPY group (DMB-DY). Both procedures have been described in detail previously.[14]

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