Peptides Adsorbed on Hydrophobic Surfaces—A Sum Frequency Generation Vibrational Spectroscopy and Modeling Study

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Abstract. Sum frequency generation (SFG) vibrational spectroscopy has been used to characterize the interfacial structure of a series of model peptides at the hydrophobic polystyrene–buffer interface. The peptides contain two types of amino acids, one hydrophobic (X) and one hydrophilic (Y). Their sequences are Ac-XYYXXYXXYYXXYX-NH_2 (XY_{14}) and Ac-XYXYXYX-NH_2 (XY_7), respectively, where the X and Y combinations are: leucine (L) and lysine (K); alanine (A) and lysine (K); alanine (A) and arginine (R); and phenylalanine (F) and arginine (R), respectively. One additional peptide was synthesized and characterized, Ac-LKKLLKL-NH_2, referred to as LK_7 α. The XY_{14} peptides showed SFG spectra that were characteristic of the hydrophobic (X) amino acid of the peptide. Comparison with the 7-amino acid peptides shows that the molecular orientation of alanine is more sensitive to changes in sequence and chain length than leucine or phenylalanine. The hydrophilic amino acids are not observed in the SFG spectra of these peptides at the hydrophobic polystyrene interface (with the possible exception of the AR_7 peptide), suggesting the hydrophilic amino acids studied here have a random orientation at this interface. The results of these studies are put into the context of recent SFG studies of proteins adsorbed onto hydrophobic surfaces. Furthermore, our approach to theoretical understanding of interfacial peptide structure is outlined. The results of a molecular dynamics simulation of the LK_{14} peptide on a hydrophobic interface are presented and discussed.

INTRODUCTION

The molecular understanding of protein adsorption on biopolymer surfaces provides the opportunity to develop biocompatible implant systems or prevent unfavorable protein adsorption from the blood onto medical devices, which alters their biological and immunological responses.[1,2] Unfortunately, adsorbed proteins are difficult molecules to study with vibrational spectroscopy on a molecular level due to their size and structural complexity.[3] Therefore, we have turned to a class of small, model peptides that contain two types of amino acids, one hydrophilic and the other hydrophobic.[4–7] In this paper we will review their adsorption onto hydrophobic surfaces studied experimentally by sum frequency generation (SFG) vibrational spectroscopy and outline recent theoretical advances in the understanding of these experiments.

EXPERIMENTAL

Peptide Synthesis

Peptides were synthesized using Rink amide 4-methylbenz-hydrylamine (MBHA) resin (Novabiochem) and standard 9-fluorenylmethyloxycarbonyl (Fmoc) chemistry on an ABI 431A synthesizer (Applied Biosystems). An excess of reagents (9 equivalents) was used in each step, beginning with approximately 200 mg of MBHA. Acetic anhydride was added to the resin-bound peptide prior to peptide cleavage off the resin to acetylate the N-terminus of the peptide. The purity and composition of the peptides were characterized using Liquid Chromatography-Electrospray Ionization-Mass Spectrometry (LC-ESI-MS). Peptides were precipitated in cold methyl-*tert*-butylether and lyophilized.[4]

Two different sequences of peptides were synthesized.[6] One had sequence Ac-XYYXXYXXYYXXYX-NH_2 and is

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referred to as XY_{14}. The other sequence was Ac-XYXYXYX-NH_2 and is referred to as XY_7 β or simply XY_7. There were four XY combinations synthesized in this study: leucine (L) and lysine (K), alanine (A) and lysine (K), alanine (A) and arginine (R), and phenylalanine (F) and arginine (R). One additional peptide was synthesized: Ac-LKKLLKL-NH_2, referred to as LK_7 α. The LK, AK, AR, and FR peptides had different solution solubilities, which decreased in the order AR~AK>LK>FR. The concentrations used in this study are given in Table 1.

Sum Frequency Generation Vibrational Spectroscopy

Our experimental laser setup, substrate preparation, buffer salt concentration, and experimental geometry have been described in detail elsewhere.[4,8] Deuterated d_8-polystyrene is used in this study to avoid spectral confustion between the substrate and adsorbates. The theory of sum frequency generation has been presented elsewhere.[4,6,9,10] Our spectra are fit to the equation:

$$\left|\chi^{(2)}(\omega_{IR})\right|^2 = \left|\chi^{(2)}_{NR} + \sum_q \frac{\boldsymbol{A}_q}{\omega_{IR} - \omega_q + i\Gamma_q}\right|^2 \qquad (1)$$

where $\chi^{(2)}_{NR}$ is the non-resonant part of the surface nonlinear susceptibility, $\boldsymbol{\chi}^{(2)}$; ω_{IR} is the frequency of the infrared beam; and ω_q and Γ_q are the frequency and damping constant of the qth vibrational mode, respectively. The strength of a vibrational mode, $\boldsymbol{A}_q$, is given by:

$$\boldsymbol{A}_q = N_s \int \mathbf{a}_q f(\Omega) d\Omega \qquad (2)$$

where N_s is the surface density of molecules, $\mathbf{a}_q$ is the amplitude of a molecular vibration, $f(\Omega)$ is an orientation distribution function over Ω, a set of orientational angles that describes a transformation between the laboratory and molecular coordinate system.[11] $\mathbf{a}_q$ can be understood as:

$$\mathbf{a}_q \propto \frac{\partial \vec{\boldsymbol{\mu}}}{\partial Q_q} \otimes \frac{\partial \boldsymbol{\alpha}^{(1)}}{\partial Q_q} \qquad (3)$$

where $\partial\vec{\boldsymbol{\mu}}/\partial Q_q$ and $\partial\alpha^{(1)}/\partial Q_q$ are the infrared dipole derivative and Raman polarizability derivative with respect to Q_q, the classical normal coordinate of the qth vibrational mode, respectively. The previous equations demonstrate what modes are measured in a sum frequency experiment: a mode must be IR and Raman active, and it must be ordered (i.e., a mode that has a random geometrical distribution does not produce a resonant response, as seen by the integral in eq 2). Thus the physical interpretation of the SFG spectra presented here becomes clear: due to specific interactions between the peptide and surface (i.e., hydrophobic or electrostatic) certain vibrational modes will become ordered at an interface, and those modes are seen in the SFG spectra.[4,6,7]

RESULTS

The SFG spectra of the 14-amino acid peptides adsorbed on deuterated d_8-polystyrene are shown in Figs. 1–4.[6] The solid lines in each figure are the results of fitting the spectra to eq 1. Peptide modes are visible for all of the peptides adsorbed on hydrophobic polystyrene surfaces. As we have previously demonstrated, LK_{14} shows three distinct modes on PS-d_8: 2870 cm^{-1}, assigned to a methyl symmetric stretch; 2895 cm^{-1}, assigned to a CH stretch or CH_2 Fermi resonance; and 2935 cm^{-1}, assigned to a Fermi resonance of a methyl mode (Fig. 1).[4] The FR_{14} peptide shows a strong resonance at 3050 cm^{-1}, assigned to the phenyl stretch of the phenylalanine side chains (Fig. 2).[6] The AR_{14} shows similar, yet slightly different results from the LK_{14} peptide: 2870 cm^{-1}, assigned to symmetric stretch of the methyl side chain of alanine and the 2925 cm^{-1} mode, assigned to a Fermi resonance of the methyl side chain of alanine (Fig. 3).[6] The SFG spectrum of the AK_{14} peptide is similar to AR_{14}, with the two modes observed at 2870 cm^{-1} and 2930 cm^{-1} (Fig. 4).[6]

The SFG spectra of the 7-amino acid (β-strand sequences) peptides adsorbed on d_8-polystyrene are shown in Figs. 5–8.[6] The SFG spectra of LK_7 show three resonances: 2870 cm^{-1}, again a symmetric stretch of a methyl group; 2910 cm^{-1}, CH stretch or CH_2 Fermi resonance; and 2935 cm^{-1}, a methyl Fermi resonance (Fig. 5). A peak at 3050 cm^{-1} in the SFG spectra in FR_7 is attributed to the phenyl ring of phenylalanine (Fig. 6).

Table 1. Peptide sequences and concentrations used in this study

peptide	sequence	solution concentration
LK_{14}	Ac-LKKLLKLLKKLLKL-NH_2	0.1 mg/mL
AK_{14}	Ac-AKKAAKAAKKAAKA-NH_2	1 mg/mL
AR_{14}	Ac-ARRAARAARRAARA-NH_2	1.5 mg/mL
FR_{14}	Ac-FRRFFRFFRRFFRF-NH_2	0.05 mg/mL
LK_7 β	Ac-LKLKLKL-NH_2	0.05 mg/mL
AK_7	Ac-AKAKAKA-NH_2	1 mg/mL
AR_7	Ac-ARARARA-NH_2	1 mg/mL
FR_7	Ac-FRFRFRF-NH_2	0.05 mg/mL
LK_7 α	Ac-LKKLLKL-NH_2	0.05 mg/mL

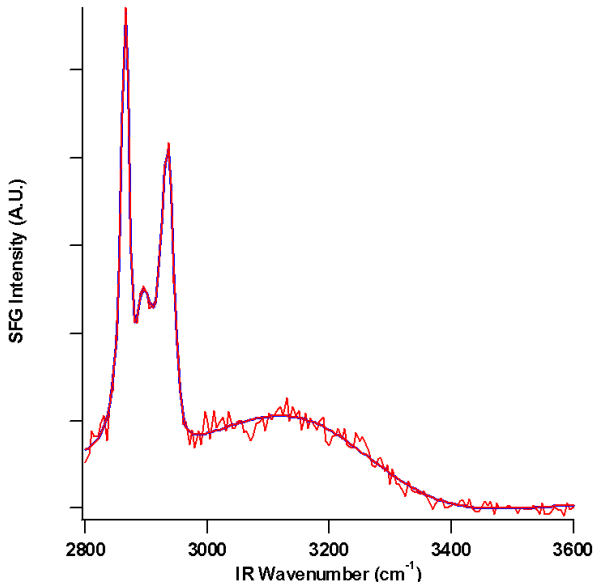

Fig. 1. SFG spectra of LK_{14} adsorbed on hydrophobic deuterated polystyrene. The spectra show three modes from the hydrophobic leucine side chains: 2870 cm^{-1}, a symmetric CH_3 stretch; 2895 cm^{-1}, a CH stretch or CH_2 Fermi resonance; and 2935 cm^{-1}, a CH_3 Fermi resonance.

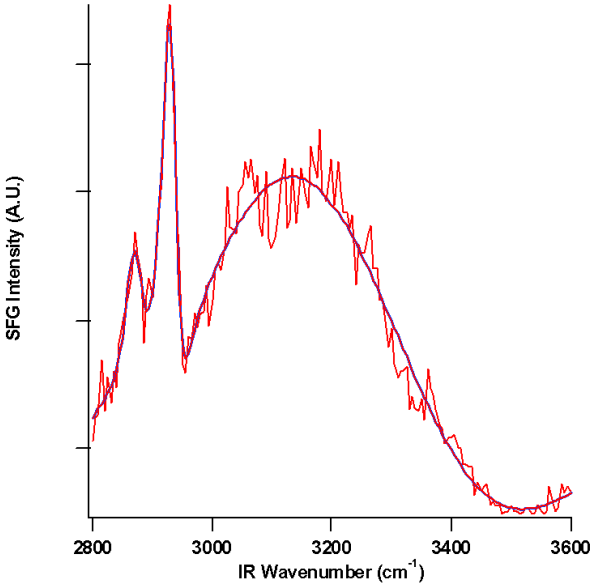

Fig. 3. SFG spectra of AR_{14} adsorbed on hydrophobic deuterated polystyrene. The spectra show two modes from the hydrophobic alanine side chains: 2870 cm^{-1}, a symmetric CH_3 stretch, and 2930 cm^{-1}, a CH_3 Fermi resonance.

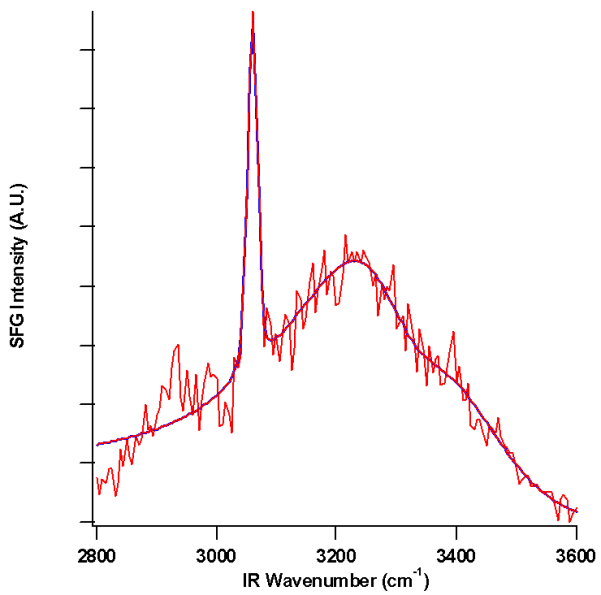

Fig. 2. SFG spectra of FR_{14} adsorbed on hydrophobic deuterated polystyrene. The spectra show one mode from the hydrophobic phenylalanine side chains. This mode at 3050 cm^{-1} is assigned to the υ_2 mode of the phenyl ring.

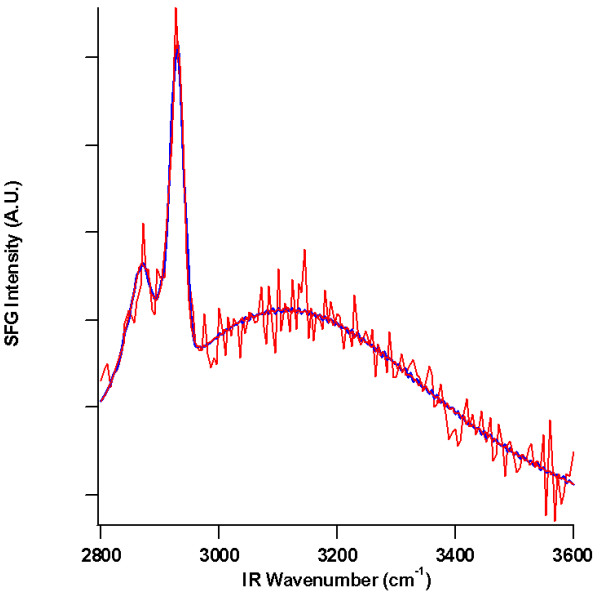

Fig. 4. SFG spectra of AK_{14} adsorbed on hydrophobic deuterated polystyrene. The spectra show two modes from the hydrophobic alanine side chains: 2870 cm^{-1}, a symmetric CH_3 stretch, and 2925 cm^{-1}, a CH_3 Fermi resonance.

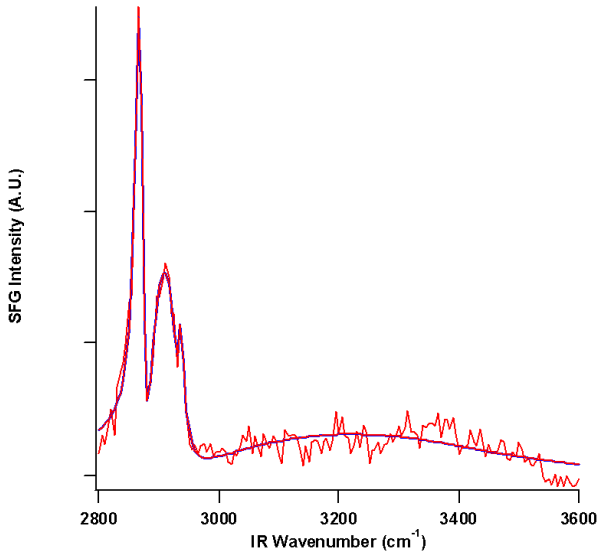

Fig. 5. SFG spectra of LK_7 β adsorbed on hydrophobic deuterated polystyrene. The spectra show three modes from the hydrophobic leucine side chains: 2870 cm^{-1}, a symmetric CH_3 stretch; 2910 cm^{-1}, a CH stretch or CH_2 Fermi resonance; and 2935 cm^{-1}, a CH_3 Fermi resonance.

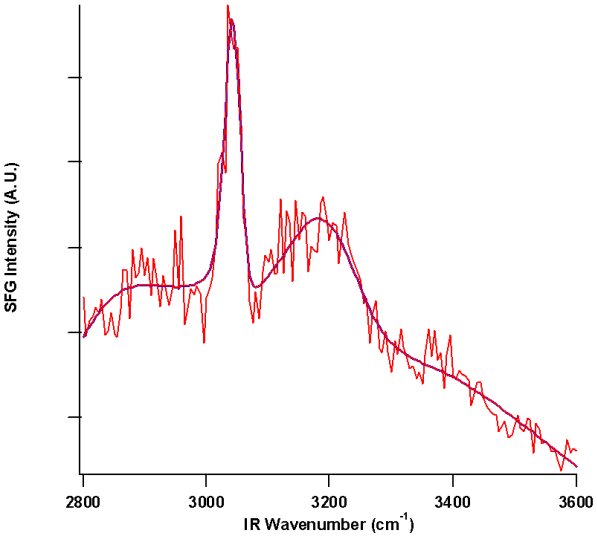

Fig. 7. SFG spectra of AR_7 adsorbed on hydrophobic deuterated polystyrene. The spectra show one mode from the peptide at 3030 cm^{-1}. The origin of this mode is not certain, but could be assigned to the arginine side chain (see text for details).

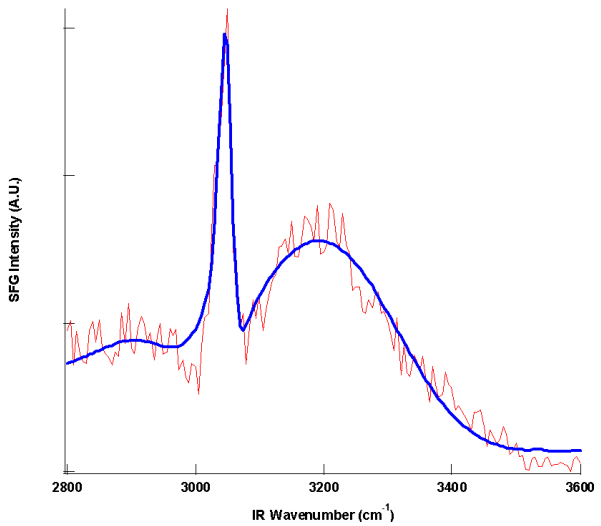

Fig. 6. SFG spectra of FR_7 adsorbed on hydrophobic deuterated polystyrene. The spectra show one mode from the peptide side chains at 3050 cm^{-1}. This mode at 3050 cm^{-1} is assigned to the υ_2 mode of the phenyl ring of phenylalanine.

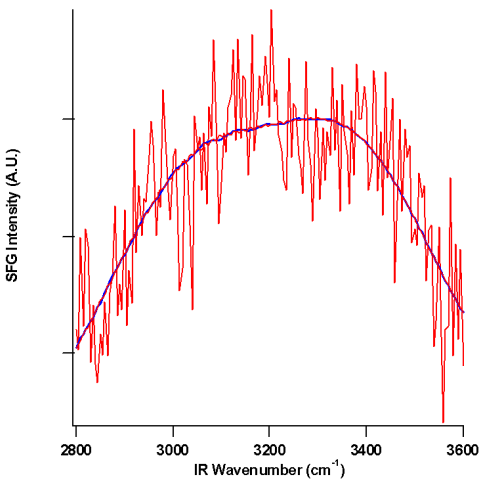

Fig. 8. SFG spectra of AK_7 adsorbed on hydrophobic deuterated polystyrene. The spectra show no modes from the adsorbed peptide itself; rather, interfacial water is observed. The spectra here is fit with two modes at ~3200 cm^{-1} and ~ 3400 cm^{-1}.

The SFG spectra of AR_7 do not show modes associated with alanine, but a strong CH stretching mode at 3030 cm^{-1} (Fig. 7). Previous work of Larsson has assigned a CH mode at 3030 cm^{-1} to the arginine side chain.[12,13] In contrast, there are no peptide modes visible in the SFG spectra of AK_7 (Fig. 8). Figure 9 shows the SFG spectra of the 7-amino acid LK α peptide, with sequence Ac-LKKLLKL-NH_2. The two LK_7 spectra have three similar modes: 2870 cm^{-1}, assigned to the CH_3 symmetric stretch; a mode around 2900 cm^{-1}, assigned to a CH resonance or CH_2 Fermi resonance; and 2935 cm^{-1}, assigned to a Fermi resonance of a methyl group.[6]

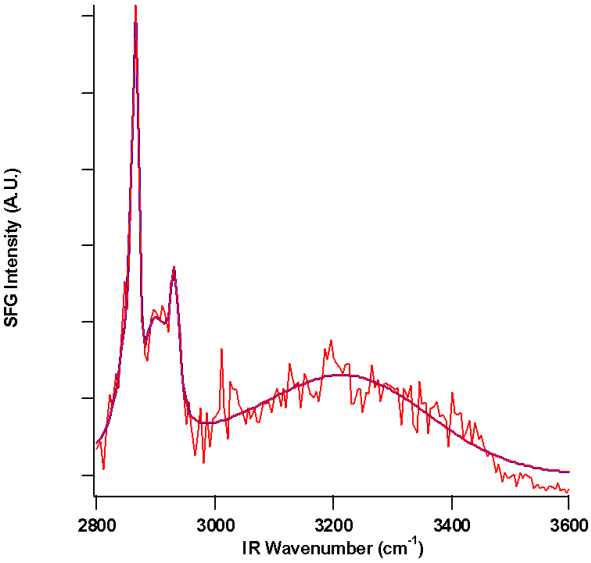

Fig. 9. SFG spectra of LK_7 α adsorbed on hydrophobic deuterated polystyrene. The spectra show three modes from the hydrophobic leucine side chains: 2870 cm^{-1}, a symmetric CH_3 stretch; 2900 cm^{-1}, a CH stretch or CH_2 Fermi resonance; and 2935 cm^{-1}, a CH_3 Fermi resonance.

DISCUSSION

In this section, we will briefly discuss the historical background of SFG studies on biomolecules at interfaces, then put the results outlined above into the context of these studies. We will subsequently explain how theoretical studies can aid in the interpretation of these spectra.

SFG Studies on Biomolecules: From Proteins to Peptides

SFG was first demonstrated as a surface-specific vibrational spectroscopy in 1987 by Shen and coworkers.[14,15] There are a number of recent reviews of this technique and the many interfaces studied by it in the literature,[16–18] and we will not attempt to give a comprehensive review here. The first study of proteins at interfaces was performed by the Cremer group, which examined bovine serum albumin adsorption on silica.[19] Since then, a number of groups have performed studies of various proteins adsorbing onto various surfaces and have demonstrated how a number of parameters (e.g., surface hydrophobicity) influence protein interfacial structure, with much of the work done by the Chen group.[20,21] Reviews are now in the literature concerning the application of SFG to biological molecules at interfaces.[22–24] However, determining the orientation of individual amino acids has proven difficult due to the large

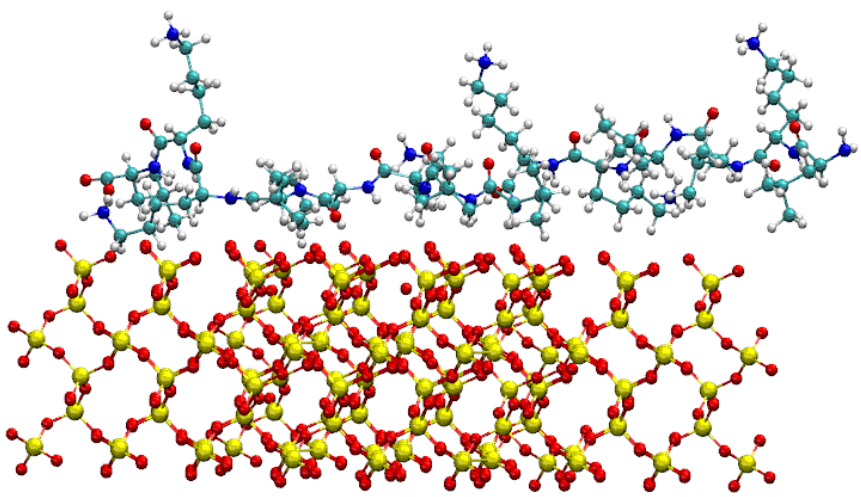

Fig. 10. An LK_{14} peptide adsorbed at the surface of a SiO_2 surface with no partial charge separation. The peptide's initial configuration was alpha helical, persisting for several picoseconds of simulation time. This conformation remains for simulation times up to 1 ns.

number and types of amino acids in a protein.[3] Clearly, a study of individual amino acids at hydrophobic and hydrophilic surfaces would be an ideal method to elucidate the interfacial structure of amino acids. However, this has proven somewhat difficult at the solid/liquid interface,[5] although amino acids have been well characterized at the carbon tetrachloride/water interface.[25] Therefore, we have turned to a series of short, model peptides that contain two types of amino acids in order to elucidate how individual amino acids change orientation at hydrophobic surfaces as a function of the peptide chain length and sequence. Our studies demonstrate that the molecular orientation of alanine is more sensitive to peptide chain length and sequence than the more hydrophic leucine or phenylalanine amino acids.[6]

The 14 amino acid peptides adsorbed on hydrophobic polystyrene studied here provide characteristic spectroscopic signatures of the hydrophobic amino acid contained in the peptide. The LK_{14} (Fig. 1) and FR_{14} (Fig. 2) SFG spectra are nearly identical to the spectra observed for leucine and phenylalanine at the carbon tetrachloride/water interface, respectively.[25] The LK_{14} SFG spectra are also very similar to the SFG spectra of leucine at the air/water interface.[26] A comparison with the work of Kim et al.,[27] who studied polyalanine at the air/water interface, shows that the SFG spectra of AR_{14} (Fig. 3) and AK_{14} (Fig. 4) from this study are characteristic of the alanine side chains. This is further supported by the observation that the AR_{14} and AK_{14} spectra are nearly identical.

The 7-amino acid peptides (β sequence) show some interesting trends.[6] The LK_7 peptide (Fig. 5) shows the three characteristic peaks associated with leucine. The observation that intensity ratios between the CH_3 symmetric mode and CH_3 Fermi resonance mode are slightly different for the LK_7 (Fig. 5) than for the LK_{14} (Fig. 1) suggests that the leucine side chains in each peptide adopt slightly different orientations at the polystyrene interface. The LK_7 α (Fig. 9) also shows these three peaks, again with the intensity ratio between the CH_3 symmetric mode and CH_3 Fermi resonance being slightly different than the LK_7 β. The SFG spectra of the FR_7 peptide (Fig. 6) are nearly identical to FR_{14} peptide (Fig. 2), indicating that the orientation of phenylalanine is not sensitive to changes in peptide sequence or chain length. The peptides containing alanine show the opposite effect. The AK_7 peptide (Fig. 8) shows no modes in SFG spectra from the peptide, and the AR_7 spectra (Fig. 7) are completely different than the AR_{14} (Fig. 3). The two characteristic modes of alanine observed in AR_{14} are not present in the SFG spectra of AR_7; instead a mode at 3030 cm^{-1} is observed. This mode is postulated to originate from the side chains of arginine.[6] Regardless of the exact origin of this mode, the results obtained from the AR and AK experiments reveal that the average orientation of alanine is sensitive to the sequence and chain length of a peptide.

The use of the model peptides described here allows for a molecular-level understanding of how individual amino acid residues order at hydrophobic interfaces. The results presented here demonstrate that the orientations of the most hydrophobic amino acids studied here (phenylalanine and leucine) are insensitive to peptide chain length and sequence. However, the more hydrophilic alanine residues' orientation is much more sensitive to the sequence and chain length of a peptide. The most hydrophilic side chains are not observed in the SFG spectra presented here (with the possible exception of the AR_7 peptide), suggesting that the hydrophilic residues of a peptide have random orientation at a hydrophobic surfaces.

Theoretical Models of Peptide Adsorption on Hydrophobic and Hydrophilic Surfaces

Although SFG reports directly on orientational symmetry-breaking near interfaces, inferring microscopic structure from its frequency dependence is not straightforward. Indeed, for solutes that are sensitive to rearrangements in water's hydrogen bond network, even linear spectra can be difficult to interpret. One need look no farther than Raman scattering by HOD in D_2O to find long-standing debates over the molecular implications of shifts in frequency and intensity.[28] Recent theoretical calculations have offered new insight into those trends and have provided a microscopic basis for understanding their physical origins.[28–30] Computer simulations promise to become a similarly powerful counterpart to SFG, but theoretical developments necessary to realize this potential have not been fully developed.

Practical strategies for calculating the response functions that determine SFG have been devised and demonstrated.[31] They require not only a scheme for determining instantaneous oscillator frequencies but also numerical integrations weighted by complicated orientational factors. These convolutions, together with interference of resonant and non-resonant contributions, obscure any simple connection between spectral intensities and the distribution of microscopic environments. Understanding of bulk vibrational spectra has benefited greatly from such connections, which draw special attention to electric fields generated by aqueous surroundings.[32–34] Lacking a direct correspondence, one can nonetheless calculate SFG spectra for complex systems, provided a representative ensemble of molecular configurations. Agreement with experiment provides a measure of confidence in the intermolecular structures generated by simulation.

Competing requirements of physical realism and computational tractability heavily constrain the choice of microscopic models useful for describing polypeptide adsorption. An empirical force field must account for conformational variability, fluctuations in solvent density that underlie the hydrophobic effect, and intrinsic interfacial affinity of individual amino acids. Two distinct approaches can be pursued toward this end. A minimalist view would depict the polypeptide as a connected chain with varying degrees of hydrogen bonding potential along its backbone. Solvent would be represented by a smoothly varying density field capable of drying at hydrophobic surfaces. The substrate itself would be caricatured as uniformly hydrophobic or hydrophilic, or as a statistically patterned mixture of the two. This kind of model is ideal for posing general questions about the role of hydrophobicity in polymer adsorption, for investigating correlations among multiple solutes, and for exploring broad ranges of parameters and molecular compositions. Making contact with experiment, however, is made difficult by coarse-graining over local structures that might influence spectroscopic probes. Furthermore, mimicking experimental conditions requires simultaneous parameterization of many effective interaction strengths.

As a first step we have pursued an alternate route, which attempts a more detailed representation of molecular arrangements. Specifically, we model the motions of all atoms in the peptide and solvent, as determined by Newton's equations of motion and the empirical AMBER 94 force field. The substrate appears as a fixed external field, which we constructed from the atomic structure of a quartz sheet with partial charges assigned to each Si and O atom. By varying these partial charges, we can vary the substrate continuously between hydrophobic and hydrophilic extremes.

Proper treatment of the hydrophobic effect near extended interfaces requires a careful choice of simulation geometry. In particular, boundary conditions that suppress fluctuations in net solvent density can inhibit formation of a vapor-like layer above hydrophobic substrates. This kind of bias can be avoided in several ways, for example by sampling an open ensemble that permits exchange of molecules and/or volume with an external bath. Because the dynamics of such exchange is artificial in most protocols, we chose instead to enforce conditions of liquid–vapor coexistence by placing an ideal hydrophobic sheet and a thin layer of vapor on the top surface of our simulation cell (opposite the substrate). Integrating molecular equations of motion for this system does not curtail the natural range of local density variations, even though its total volume is fixed.

We have focused computational efforts to date on a single LK_{14} peptide situated above a hydrophobic substrate. SFG data suggest that helical ordering should not be observed in this case,[7] and our simulations concur. Initial conditions in which the peptide adopts perfect helical structure rapidly evolve toward disordered conformations that associate strongly with the substrate. Secondary structure all but disappears within tens of picoseconds and does not reappear within the nanosecond timescale of our calculation. This preliminary result does not, however, unambiguously implicate the substrate in loss of helical order, since LK_{14} does not exhibit strong helicity in bulk solution at low concentrations and low ionic strength.[7,35] In order to clarify the role of surface, we are currently investigating effects of salt, in the form of a phosphate buffer known to stabilize secondary structure in bulk, as well as interactions among multiple peptide solutes at the surface.

CONCLUSION

SFG has been used to characterize the adsorbed structure of a class of model peptides. The results show that on hydrophobic polystyrene surfaces, side chain vibrational modes are observed in the SFG spectra of these peptides. The modes observed for the 14-amino acid peptides are characteristic of the hydrophobic side chains of the peptide. With the possible exception of AR_7, no hydrophilic amino acid side chains are observed in the SFG spectra. Additionally, the molecular orientations of phenylalanine and leucine are less sensitive to peptide sequence and chain length than alanine. Our approach to achieving a molecular level theoretical understanding of the experiments presented here has been outlined. Finally, our initial results of the interfacial structure of the LK_{14} peptide have been presented.

Acknowledgments. Professor Levine is the experimentalist's dream of a theorist; he listens to experimental details and his involvement in extracting the key concepts of an experiment and then developing models to describe the phenomena permit the creation of new science. It has been my privilege to interact with him in the field of surface science throughout the years.

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