Communication

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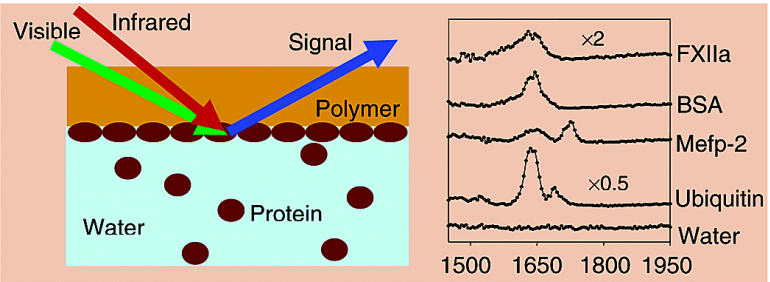

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Detection of Amide I Signals of Interfacial Proteins in Situ Using SFG

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Understanding protein adsorption is a crucial step in developing biomaterials or controlling marine biofouling.[1] In the last few decades, protein adsorption has been extensively studied by various experimental techniques.[1] However, molecular level in situ detection of interfacial protein structures is still difficult. Recently, research on protein adsorption using sum frequency generation (SFG) vibrational spectroscopy has revealed some important molecular level information about structures of proteins at interfaces.[2] Until now, all SFG studies on protein adsorption have been limited to the detection of C−H stretching modes to determine the orientation and order of hydrophobic side chains of interfacial proteins. From such studies, "hydrophobic" or "hydrophilic" conformations of interfacial proteins have been deduced, but more detailed structural information on the entire interfacial protein is yet to be acquired.

Vibrational spectroscopic techniques such as infrared spectroscopy and Raman scattering have frequently been employed to study protein structures in the bulk environment.[3] Such studies indicate that amide vibrational bands are very sensitive to protein secondary structures. Attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR) has been utilized to detect secondary structures of interfacial proteins in situ. However, due to the limited surface sensitivity of the technique, amide signals from bulk solution proteins and water signals may strongly interfere with amide signals from the interfacial proteins, making the in situ detection of such structures difficult. In this Communication, we demonstrate the novel observation that it is feasible to collect amide signals from polymer/protein solution interfaces in situ using SFG, allowing for more detailed molecular level information of entire interfacial protein structures.

The evident advantage that SFG has over ATR-FTIR in the study of interfacial protein structure is that SFG can probe protein conformations at interfaces with inherent surface/interface selectivity.[2] Usually, protein molecules in solution have a random orientation distribution and the SFG process is forbidden under the electric-dipole approximation; thus bulk proteins will not generate SFG signals. Another advantage is that amide I signals can be acquired directly, without subtracting the water background. Theoretically, the water bending mode should be insignificant as compared to the C═O stretching mode of adsorbed proteins in SFG spectra.[4] In addition, because the second-order nonlinear optical susceptibility tensor has more elements than the linear susceptibility tensor, SFG has the potential to provide more structural information for interfacial proteins than does linear spectroscopy.

To our knowledge, no reports on SFG observation of amide signals of interfacial proteins have been published previously. To collect weak amide signals in situ, we have adopted a "near" total reflection experimental geometry using a CaF_2 prism (Figure 1).

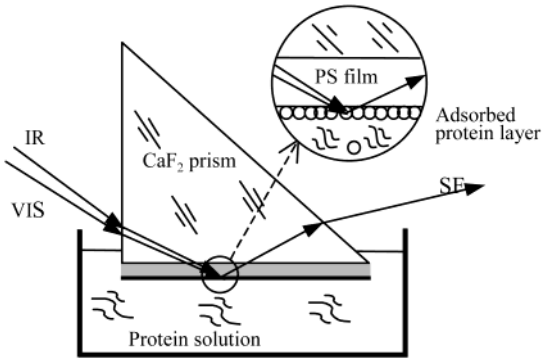

Figure 1. SFG experimental geometry.

Using this geometry, SFG signal intensity can be greatly enhanced through a substantial increase in the Fresnel coefficients of the polymer/protein solution interface.[5]

The SFG setup in our lab has been previously described.[6] Polystyrene (PS) was purchased from Scientific Polymer Products Inc. and was used as received. The PS films were made by spin coating a 2 wt % solution in toluene on CaF_2 prisms (ESCO products) at 2500 rpm spin speed. The surface structure of such PS films has been extensively studied.[6] Before being spin coated, the prisms were cleaned by Ar plasma and then rinsed in toluene. Fatty acid free bovine serum albumin (BSA) and ubiquitin (His-tagged) were purchased from Sigma and ASLA, respectively. Mussel protein mefp-2 was donated by the Waite group,[7] and factor XIIa (FXIIa) was purchased from Enzyme Research Laboratories. The concentrations of all protein solutions were ∼50 ppm. BSA and ubiquitin solutions were made by dissolving the proteins into deionized water. Mefp-2 was dissolved into phosphate buffer solution (ion intensity ≈ 0.1 M, pH ≈ 2.3) to prevent possible cross-linking between proteins.[8] The concentrated FXIIa (850 ppm) phosphate buffer solution (pH ≈ 7.4) was diluted by deionized water.

Figure 2 shows SFG spectra collected from different PS/protein solution interfaces using the experimental geometry shown in Figure 1. Representative proteins investigated include BSA, ubiquitin, mefp-2, and FXIIa. Ubiquitin is a "simple" protein which serves as a standard for NMR studies. Albumin is the most concentrated protein in blood, FXIIa is involved in blood coagulation,[9] and mefp-2 is one of the mussel adhesive plaque proteins. Studies on interfacial structures of such proteins will aid in the understanding of biocompatibility and biofouling control. Before the PS surface was contacted with protein solutions, ssp (s-polarized signal beam, s-polarized visible input, and p-polarized infrared input) SFG spectra were collected from PS in air, in water, and in buffer solution using the same geometry. There are no discernible signals in the frequency range displayed in Figure 2. The SFG spectrum from the PS/water interface is shown in Figure 2 as a reference. It is clear that the

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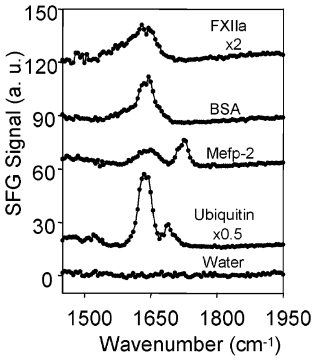

Figure 2. SFG spectra collected from interfaces between PS and various protein solutions.

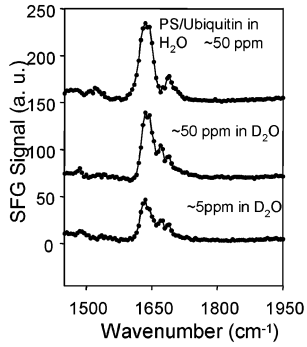

Figure 3. SFG spectra collected from interfaces between PS and ubiquitin H_2O solution, ubiquitin D_2O solution, and D_2O solution with a much lower concentration.

SFG spectral intensities and features of the amide I bands from the different proteins are quite different, indicating that the coverage, orientation, and secondary structures of various interfacial proteins are different.

The absence of a discernible SFG signal from the PS/water interface indicates that water bending modes do not generate detectable SFG signals at this interface. However, we still need to prove that the SFG signals shown in Figure 2 are from the proteins, not the interfacial water molecules oriented by protein molecules. Figure 3 compares the ssp SFG spectrum collected from the PS/ubiquitin−D_2O solution (∼50 ppm) interface and that from the PS/ubiquitin−H_2O solution (∼50 ppm) interface. These two spectra are quite similar. This result indicates that SFG signals are not contributed appreciably by the water bending mode, but by the interfacial ubiquitin molecules, because the spectra collected from the H_2O and D_2O solutions otherwise should be quite different. The small spectral difference seen may be due to a change of some C═O- -HO hydrogen bonding to C═O- -DO bonding in the interfacial protein molecules. In addition, we collected an ssp spectrum from a PS/ubiquitin−D_2O solution of a much lower concentration. This spectrum is not very different from the original spectrum with the more concentrated ubiquitin solution interface. This latter finding demonstrates that the SFG spectrum is not affected appreciably by the concentration of protein in solution; otherwise the signal should be much weaker. The intensity difference between the spectra is due to different ubiquitin interface coverage or interfacial structures.

As mentioned in Figure 2, different proteins have different amide I signals, with a range of intensities at the PS/protein solution interface. Ubiquitin has the strongest amide I SFG signal, while the FXIIa signal is weakest. Mefp-2 has a broad spectral feature around 1650 cm^{-1}, but no single distinct peak. Its strongest peak is around 1720 cm^{-1}, which may be the C═O stretching mode of side chain COOH groups. The BSA spectrum is narrower and stronger than that of FXIIa. The diversity of amide I SFG spectra among these different proteins may be a phenotypic characteristic of protein differences in size, native conformation, and other properties. BSA is a large, "soft" protein, while ubiquitin is relatively small and stable. FXIIa is a two chain molecule composed of a heavy chain and a light chain, held together by a disulfide bond,[9] and mefp-2 is acidic and highly cross-linked. The different amide I SFG spectra indicate that it is feasible to study protein secondary structure changes at interfaces by SFG. Protein secondary structures at interfaces can be compared to their corresponding native structures in solution to better understand their behavior at interfaces.

This Communication demonstrates the feasibility of SFG studies to monitor interfacial protein structures. More detailed quantitative structural information about interfacial proteins deduced from SFG amide signals needs to be worked out. Peak assignments, peak fitting, and theoretical simulation are under study and will be presented in the future. NMR has been developed into a powerful technique to study molecular structures and dynamics of proteins in bulk environments by many scientists in the last few decades. We believe that continued success in probing interfacial structures of important proteins will contribute to the development of SFG into a powerful technique to reveal structures and dynamics of proteins at interfaces in situ.

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