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Understanding Protein-Interface Interactions of a Fusion Protein at Silicone Oil-Water Interface Probed by Sum Frequency Generation Vibrational Spectroscopy

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ABSTRACT

Protein adsorbed at the silicone oil-water interface can undergo a conformational change that has the potential to induce protein aggregation on storage. Characterization of the protein structures at interface is therefore critical for understanding the protein-interface interactions. In this article, we have applied sum frequency generation (SFG) spectroscopy for studying the secondary structures of a fusion protein at interface and the surfactant effect on protein adsorption to silicone oil-water interface. SFG and chiral SFG spectra from adsorbed protein in the amide I region were analyzed. The presence of beta-sheet vibrational band at 1635 cm^{-1} implies the protein secondary structure was likely perturbed when protein adsorbed at silicone oil interface. The time-dependent SFG study showed a significant reduction in the SFG signal of preadsorbed protein when polysorbate 20 was introduced, suggesting surfactant has stronger interaction with the interface leading to desorption of protein from the interface. In the pre-adsorbed surfactant and a mixture of protein/polysorbate 20, SFG analysis confirmed that surfactant can dramatically prevent the protein adsorption to silicone oil surface. This study has demonstrated the potential of SFG for providing the detailed molecular level understanding of protein conformation at interface and assessing the influence of surfactant on protein adsorption behavior.

Introduction

Proteins are generally surface active, and therefore, interact with various interfaces encountered during pharmaceutical manufacturing processes and delivery of these drugs to the patients. The interaction surfaces typically include air-water (i.e., pumping, filling, transportation, etc.), solid-liquid (stainless steel or disposable tank materials, membranes, glass, infusion bags, etc.), and silicone oil-water (syringes, stoppers, etc.). The interactions between protein molecules and an interface can lead to structural alterations of protein, resulting in physical instability to form higher molecular weight species and or subvisible particles.[1-4] Protein aggregation and particulate formation are the leading concerns in the development of protein therapeutics due to their implications in the product safety, immunogenicity, and potency.[5,6]

Silicone oil has been widely used as a lubricant coating in the barrel-plunger assembly and is thought to be a risk for surface-induced protein aggregation in the prefilled glass syringes.[7-9] Adsorption of a protein to the silicone oil constitutes the first step in the silicone oil—induced incompatibilities in therapeutic protein formulations. Hence, a basic understanding of the mechanism of the interaction between protein and silicone oil-water is essential for the prevention of the adverse effects associated with the use of siliconized containers. The protein adsorption at the silicone oil-water interface in the presence of surfactants, and under varied pH and ionic strength has been investigated previously using a variety of techniques, including high-performance size-exclusion chromatography,[2,10] fluorescence,[4] quartz crystal microbalance (QCM),[11,12] atomic force microscopy,[13] and Raman spectroscopy.[14] The formation of high molecule weight species, potentially due to the protein adsorption to silicone oil interface, and the influence of surfactant on protein monomer loss were reported.[9,15] The protein adsorption studies from QCM confirmed that nonionic surfactant can effectively inhibit the protein adsorption to a silicone oil surface in preadsorbed and a mixture of protein surfactant.[11,16] As a result,

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reduction of protein aggregation and subvisible particulate formation, induced by silicone oil in the presence of surfactant, was observed.[2] Agitation-induced aggregation of the antibody in the presence of silicone oil was also reduced at high ionic strength.[9]

Protein adsorbed at the hydrophobic silicone oil-water interface can undergo conformational changes or partial unfolding. Monoclonal antibody adsorption to silicone oil-water interface accompanied by perturbations in protein tertiary structure using fluorescence spectroscopy was reported in the literature.[2,17] However, intrinsic fluorescence spectroscopy based on measuring fluorescence quenching of tryptophan residues in adsorbed protein molecules under a change of hydrophobic environment cannot provide the details on how protein conformation changes. Overall, the connections between protein adsorption and the alteration of protein conformation, especially for the protein secondary structure at silicone oil interface with or without surfactant and its impact on protein aggregation induced by interface are not well understood.

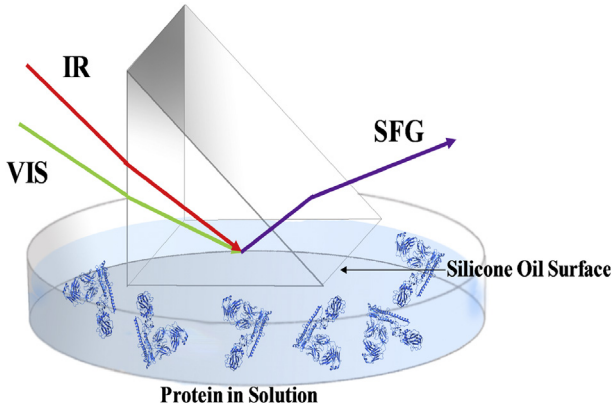
Characterization of protein secondary structures at interfaces is challenging due to the lack of a sensitive and selective surface method. For the bulk measurements, vibrational circular dichroism,[18] NMR,[19] FTIR/Raman spectroscopy, and more recent Raman optical activity[20] allow characterization of protein secondary structures. However, these methods are not surface selective. Although total internal reflection Fourier transfer infrared spectroscopy can detect the IR signals of molecule from an interface, its IR spectrum interferes with that of protein in the solution because the penetration depth of the evanescent IR wave at a surface is much higher than the monolayer level.[21] Moreover, strong water background with OH bending mode at 1640 cm^{-1} typically overlaps with the protein amide I band which makes the identification of protein secondary structural change at an interface difficult with total internal reflection Fourier transfer infrared spectroscopy.

Recently, sum frequency generation (SFG) vibrational spectroscopy, a nonlinear optical technique, has been developed into a powerful tool to characterize biological macromolecular structures at solid-liquid and air-liquid interfaces, such as protein secondary structure, orientation, kinetics of folding, proton exchange of proteins at interfaces.[22,23] SFG and chiral SFG vibrational spectroscopy are capable of detecting molecular signatures (e.g., the amide I and N–H stretching vibrational modes) of proteins and peptide molecules at the interface with a sub-monolayer level.[22,23] The unique surface-specificity and selectivity of SFG makes it promising for studying protein-interface interactions *in situ* and in real time, particularly in therapeutic protein formulations. In this article, we have for the first time applied SFG vibrational spectroscopy to probe the interfacial structural changes of a fusion protein at the silicone oil-water interface. The SFG vibrational spectra in the amide I region from adsorbed protein at the silicone oil interface were measured and analyzed with regard to protein secondary structures. By monitoring the time-dependent SFG signals of adsorbed molecules at a monolayer level, we have conducted *in situ* analysis of the influence of surfactant, polysorbate 20, on protein adsorption behavior at silicone oil surface. Our results have shown the utility of SFG vibrational spectroscopy as a powerful surface tool that can provide a mechanistic understanding of protein-interface interactions and surfactant-mediated modulation of protein adsorption at interfaces *in situ* and in real time, especially at the oil-water and air-water interfaces.

Materials and Methods

Materials

Highly purified fusion protein (BMS-A) was provided by Bristol-Myers Squibb (New Brunswick, NJ) in a stock formulation containing 50 mg/mL protein, 10-mM sodium phosphate buffer, 250-mM sucrose, 25-mM arginine at pH 6.5. Medical grade silicone oil was purchased from Dow Corning Corporation (Midland, MI). All other chemicals including highly pure polysorbate 20, toluene, and methanol were obtained from Sigma-Aldrich (St. Louis, MO) and used as received.



Scheme 1. SFG geometry used in this study.

Preparation of Silicone Oil Surface

Right-angle CaF_2 prisms were purchased from Altos Photonics, Inc. (Bozeman, MT). Prisms were soaked in toluene for 24 h and in 1% Contrex AP solution (Decon Laboratories, King of Prussia, PA) for 10 min for cleaning. The prisms were then thoroughly rinsed with deionized water (18.2 MΩ cm) and dried under gaseous nitrogen. The clean prisms were treated with O_2 plasma (Glen 1000P) for 30 s and immediately coated with silica (100 nm thick), resulting in a hydrophilic surface. Detailed coating parameters have been reported previously.[24] Silicone oil was dissolved in a mixture of toluene and methanol, and spin coated onto silica-coated CaF_2 prism surfaces at 1500 rpm for 10 s, and then at 3000 rpm for 20 s. To create a thinner oil layer, a lint-free wipe was used to wipe away most of the silicone oil after spin coating. Silicone oil coating was confirmed by measuring the SFG spectrum of methyl group from silicone oil molecule (polydimethylsiloxane).

SFG Measurement

The SFG instrument used in this study was purchased from EKSPLA (Vilnius, Lithuania). The details of instrument setup, measurements, and SFG theories have been reported previously.[23,25-38] Briefly, SFG is a second-order nonlinear optical vibrational spectroscopy and is intrinsically surface/interface sensitive. The schematic illustration of the geometry of SFG experimental data collection used in this study is shown in Scheme 1. As shown in Scheme 1, 2 laser beams, one 532 nm visible picosecond pulsed laser beam and one picosecond frequency-tunable IR beam, overlap spatially and temporally at the substrate (on bottom surface of a silica-coated CaF_2 prism) surface coated with silicone oil thin film. SFG signal from the silicone oil/protein solution interface was collected after placing the protein solution (1 mg/mL) in contact with the silicone oil surface. In SFG experiments, the polarization combinations of ssp (s-polarized output SFG signal, S-polarized input visible beam, and p-polarized input IR beam) and psp were used to collect SFG spectra with the total internal reflection geometry. The chiral SFG spectrum collected with the psp polarization provides the chiral vibrational signatures of a molecule at interface.[23,28,29] Time-dependent change in SFG signal intensity