X-ray Scattering Probes of Br-PEG-Peptide Conformation at the Aqueous-Solid Interface at 13-ID-GSECARS

L. Hanley,[1] C. Crot,[1] M. Schlossman[1], T. Trainor,[2] and P. Eng[2]

[1]University of Illinois at Chicago, Chicago, IL , [2]CARS/University of Chicago, Chicago, IL

Introduction

Many biological, biomedical and environmental science problems hinge upon the behavior of covalently bound molecular species at the aqueous-solid interface. The development of methods for gaining detailed understanding of molecular adsorption at the aqueous-solid interface is of significant interest to a number of related disciplines. Molecular species bound to surfaces are important in biomaterials and medical implants; tissue engineered constructs; DNA and protein array chips; combinatorial drug discovery; and affinity surfaces for chromatography, molecular detection, immunoassays, and disease detection. Both synthetic and naturally occurring molecular adsorbates alter the surface properties of the substrate, which mediate the adhesion of bacteria to surfaces and sorptive properties of the system. X-ray surface scattering can provide structural information with Angstrom resolution of the molecular orientation and conformation at the liquid-solid interface. We are applying long period x-ray standing wave fluorescence (XSW) [1,2] for the conformation analysis of protein, peptide, and other monolayers at the aqueous-solid interface.

Methods and Materials

We applied XSW to probe the conformation of the liquid-solid interface of a 15 residue iodine labeled peptide, a non-specifically Br-labeled-protein (bovine serum albumin), and a Br-polyethylene glycol-RGD peptide (Br-PEG-peptide). This Br-PEG-peptide was synthesized, purified, and characterized in the UIC Protein Laboratory. These experiments showed Br labeling superior to I labeling due to lower fluorescent interferences in the former. The Br label was probed using excitation at the Br K edge at 13.47 keV and both the K_α fluorescence emission at 11.92 keV and the K_β fluorescence emission at 13.29 keV were observed. No contaminants were observed in this spectral region and Br XSW displayed a submonolayer sensitivity (data not shown). Furthermore, specific labeling of the Br was required, as accomplished with the Br-PEG-peptide. Earlier experiments employed siloxane self-assembled monolayers for adsorption, but the final Br-PEG-peptide experiments were performed on an amine film atop a polystyrene spacer layer. The polystyrene layer increases the Br height above the substrate and improves the spatial resolution of the measurements by allowing multiple antinodes to pass through the Br layer. The amine layer was formed by ion-assisted deposition of allyl amine ions [3-5]. Simple x-ray reflectivity was used throughout to demonstrate effective hydration of the adsorbate layer by a humid He atmosphere. This hydration layer is ~100 Å, thick enough to permit solvation of the peptide layer while minimizing scattering from the water layer. The amine coated surface was shown by x-ray photoelectron spectroscopy to display several percent nitrogen at surfaces of initial roughnesses of 4 – 6 Å (RMS), as measured by atomic force microscopy.

Results

XSW data of the hydrated Br-PEG-peptide adsorbed on an amine coated polystyrene surfaces is shown in Fig. 1. The Br-PEG-peptide is a model system expected to stand the Br group away from the surface when the RGD peptide sequence binds to the amine coated surface. The Br-PEG-peptide was adsorbed onto three types of polystyrene films: those that are flat and unmodified and those that are roughened up to a few nm and chemically modified with amine groups by deposition of either 50 or 200 eV allyl amine ions. X-ray reflectivity was used to determine the polystyrene-amine and peptide film thicknesses (data not shown). XSW then monitored the position of the Br atom above the Si surface. Preliminary fits of the data from the November 2003 run indicate that the Br atom is 126 and 138 Å above the PS surface for the 200 and 50 eV allyl amine films, respectively, with width distributions (σ_{Br}) of 9 and 10 Å. The Br-PEG-peptide adsorbed in a monolayer on the amine surfaces, with the RGD peptide adjacent to the amine surface, Br at the aqueous interface, and PEG relatively extended. By contrast, the Br-PEG peptide was disordered and patchy on unmodified polystyrene.

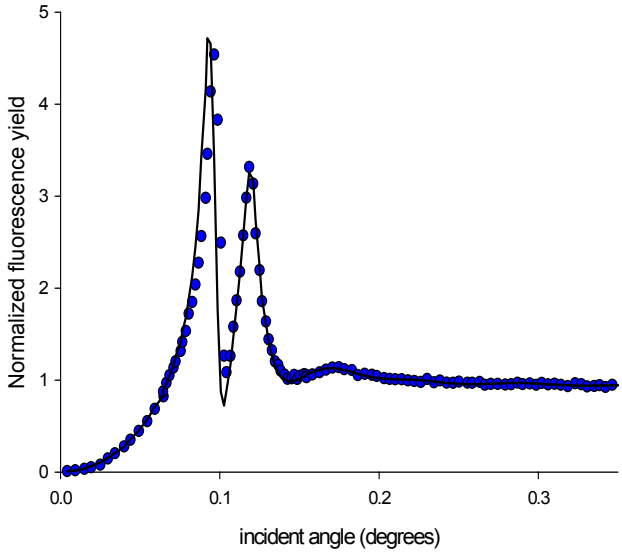

Fig. 1. 14 keV excited fluorescence yield profile for Br-PEG-peptide adsorption for 0.5 hours on amine-coated polystyrene.

Discussion

These results demonstrated the ability to locate the Br distance in Br-PEG-peptide from the polystyrene surface with an accuracy of <10 Å. XSW can therefore be used in conjunction with Br-tagging to follow the conformation of adsorbed peptides by providing the distance and distribution width of the Br atom with respect to the surface.

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