Surface Analysis of Model Peptide Adsorption and Orientation on Self-Assembled Monolayer Surfaces

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Introduction

The interaction of proteins and peptides with surfaces is an area of great interest for current biomedical research. It has been observed that proteins will often partially or fully denature upon adsorption. The purpose of this study is to study protein-surface interactions at a fundamental level using model peptides. The model peptides contained hydrophobic leucine (L) and hydrophilic lysine (K) amino acids arranged in specific sequences to create α-helix or β-sheet secondary structures. The adsorption of these peptides onto well-defined self-assembled monolayers (SAMs) was then studied to isolate the response of these protein structural units with the surfaces. These interactions will then be correlated with the interactions of entire proteins with the model SAM surfaces.

Methods and Instrumentation

Silicon wafer substrates were coated with 10 nm Ti and 80 nm Au by e-beam evaporation. The four types of SAMs on Au were methyl-terminated dodecanethiol, alcohol-terminated 11-mercapto-1-undecanol, carboxylic-acid-terminated 11-mercaptoundecanoic acid (Aldrich), and a fluoro-terminated 1H,1H,2H,2H-perfluorodecanethiol (Oakwood Products). All SAMs were assembled for 24 hours from ethanol solutions and then rinsed copiously with ethanol. The LK α-peptides were made using an automated solid-phase peptide synthesizer. X-ray photoelectron spectroscopy (XPS) studies were performed using Kratos Axis-Ultra DLD spectrometer with a monochromatic Al Kα X-ray source. Compositional survey and detailed (F, O, N, S) scans and high-resolution (C, S) scans were obtained for each sample. The atomic percent of nitrogen was used to determine the amount of peptide deposited on the surface. Static time-of-flight secondary ion mass spectrometry (ToF-SIMS) studies were done using a Model 7200 Physical Electronics Instrument. Positive and negative spectra were both acquired with a total ion dose of less than 2 x 10^{12} ions/cm^2. Near-edge X-ray absorption fluorescence spectroscopy (NEXAFS) spectra were taken at the U7A beamline at Brookhaven National Laboratory.

Results and Discussion

Initial adsorption studies with the 14-mer α-helix showed that the adsorption behavior varied greatly depending on the particular SAM surface. For this reason, isotherm experiments were done to determine ideal adsorption conditions. XPS results showed that adsorption onto methyl and carboxylic acid terminated SAMs from phosphate buffered saline caused adsorption in patches with bare spots. There was no adsorption observed on alcohol-terminated SAMs. It was also observed that the concentration required to achieve a partial peptide layer with some bare spots on methyl SAMs was 100-fold higher that required for carboxylic acid SAMs. Adsorption was investigated for two different buffer salt concentrations to observe the influence of ionic strength on adsorption. On methyl SAMs, it was found that there was less peptide adsorbed at higher ionic strength, possibly due to bundling of the peptides themselves. NEXAFS was used to probe the organization of these peptides. It was found that there was a change in degree of peptide orientation between peptides adsorbed on methyl and carboxylic-acid SAMs. The degree of orientation also depends on the concentration of peptide on the surface, as seen in Figure 1.

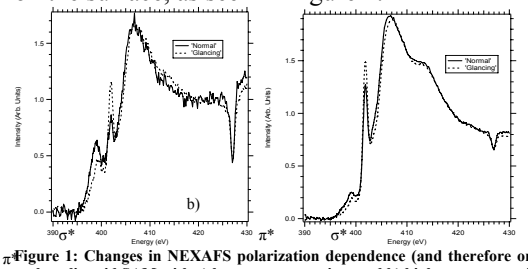

Figure 1: Changes in NEXAFS polarization dependence (and therefore orientation) for a carboxylic acid SAM with a) lower concentration, and b) higher concentration of peptide.

Conclusions

The amount of peptide adsorbed on the surface depends on the type of surface, the concentration of peptide in the adsorption solution, and the buffer salt concentration. It was found that the degree of orientation is dependent both on the type of surface and the amount of peptide present. These results and future work with β-sheet peptides will provide a base for studying the interactions between proteins and more complex surfaces.

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