ToF-SIMS study of Fibronectin orientation/conformation on self-assembled monolayers.

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Statement of Purpose: Protein adsorption and orientation plays a critical role in many biomedical applications. Fibronectin (FN) is an extra cellular matrix protein that is involved in many cell processes such as adhesion, migration and growth. The orientation and conformation of FN adsorbed onto surfaces can therefore have a critical effect on cell-surface interactions. In this study the adsorbed orientation and conformation of the 7-10 fragment of FNIII was studied on three different model surfaces (self-assembled monolayers (SAM) of C_{11} alkanethiols on Au, -CH_3, -NH_2, and -COOH terminated SAM.) The effect of different surface chemistries on binding and adsorption configuration was investigated using X-ray photoelectron spectroscopy (XPS) and time of flight secondary ion mass spectrometry (ToF-SIMS). A trehalose coating was used to inhibit the conformation changes do to dehydration of the sample when the adsorbed protein films are dried. Surfaces with similar sub-monolayer protein coverage were studied with ToF-SIMS and principal component analysis (PCA) used to determine changes in the protein fragmentation when adsorbed on various surfaces. These results are related to different orientation or conformations of the fragment on the different surfaces.

Methods: Self-assembled monolayers (SAM) on Au were prepared with thiols obtained from Assemblon (Bothel WA), 1-dodecanethiol (HS-$(CH_2)_{11}$-CH_3), 11-mercaptoundecanoic acid (HS-$(CH_2)_{10}$-COOH), 11 amino-1-undecanethiol (HS-$(CH_2)_{11}$-NH_2), these thiols will hereafter be referred to as CH_3, COOH and NH_2. The Fibronection fragment, $FNIII_{7\text{-}10}$ was produced and purified by Prof. García's research group at Georgia Institute of Technology as described preciously(1).
The fibronectin fragment adsorption is done at room temperature and the freshly prepared SAM's are incubated in $FNIII_{7\text{-}10}$ solution for 30 min. Trehalose protected samples are rinsed in 0.1% D(+)-trehalose solution instead of water. Care is taken to keep all samples immersed in aqueous solution until after the trehalose protection.
XPS experiments were done on a Surface Science Instruments S-probe spectrometer using monochromatic Al Kα X-ray source and a 55º takeoff angle. ToF-SIMS data was acquired on an ION-TOF TOF.SIMS 5-100 instrument using Bi_3^+ primary ion source. Data were collected using an ion does below the static SIMS limit of $1{\cdot}10^{12}$ ions/cm^2. Principal component analysis (PCA) was used as a part of the ToF-SIMS data analyzes as described previously(2).

Results: When $FNIII_{7\text{-}10}$ was adsorbed on the different surfaces from same solution a large difference was seen in the coverage for the different SAMs. But for comparison of the fragment orientation on different surfaces a comparable surface coverage is essential. The coverage was estimated using XPS and ToF-SIMS and the highest coverage was found on the CH_3 SAM but the lowest on the COOH SAM. Looking at the amino acid peaks for the three different surfaces the PCA analyses separates the CH_3 from the two charged surfaces where the most significant difference is in the hydrophobic amino acids peaks that have less intensity on the CH_3 SAM.
In the hydrated state the $FNIII_{7\text{-}10}$ fragment is expected to have more hydrophilic amino acids on it's surface while the hydrophobic amino acids are more towards the middle of the protein were they are shielded from the aqueous solution but once the protein is dried the more hydrophobic regions migrate to the vacuum/protein interface. Here we use trehalose to protect the structure of the hydrated state. Three samples with same protein coverage are prepare for each surface one protected with trehalose, one dried after rinsing and a third which is dried but later exposed to trehalose. Figure 1 shows an example PCA analysis of the trehalose SIMS data for $FNIII_{7\text{-}10}$ on C_{11}-CH_3 SAM. Principal component #2 separates the trehalose protected sample from the dried sample and the sample dried before the trehalose protection. On the loading plot the negative loadings correspond to the trehalose protected fragments but the positive loadings to the two dried samples. The negative loadings are mainly fragments from the hydrophilic amino acids while the hydrophobic amino acid fragments load positively.

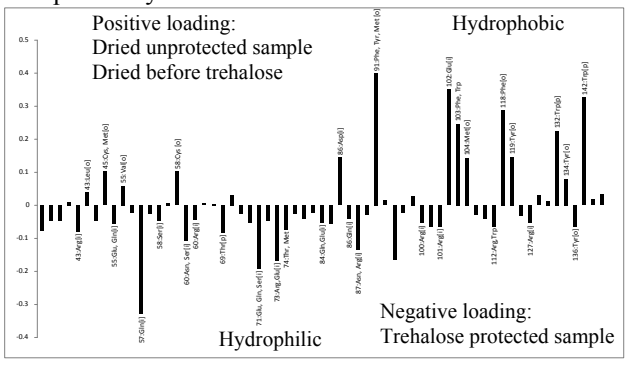

Figure 1. PC2 loading plot for $FnIII_{7\text{-}10}$ on CH_3 SAM. shows how the three samples differ hydrophilic amino acid peaks loading with the protected sample and hydrophobic amino acids loading with unprotected protein.

Conclusions SIMS and XPS is used to determine the surface coverage and conformation of $FnIII_{7\text{-}10}$ on three different SAM's. The denaturing of the $FnIII_{7\text{-}10}$ is seen in the SIMS data on CH_3 SAM emphasizing the potential of the SIMS technology on determining surface bound protein conformation.

References:

1. Michael KE. Langmuir 2003;19:8033-8040.
2. Wagner MS. Appl. Surf. Sci. 2006;252:6575-6581.