Monitoring Surface-Bound Protein Conformational Changes Using Cold-Stage Time-of-Flight Secondary Ion Mass Spectrometry

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Statement of Purpose: Dehydration of protein films attached to solid surfaces is typically accompanied by structural rearrangements as well as loss of bioactivity. These changes can be monitored by time-of-flight secondary ion mass spectrometry (ToF-SIMS) and surface plasmon resonance (SPR). In this study, ToF-SIMS was coupled with a variable temperature sample stage to monitor the conformational changes that occur when a surface-bound protein goes from a hydrated to a dehydrated state. The changes in bioactivity due to dehydration were investigated using SPR,

Methods: The ToF-SIMS and SPR experiments were conducted on a surface-bound protein system, a histagged humanized anti-lysozyme variable fragment (HuLys Fv) coordinated on a Ni^{2+}-loaded nitrilotriacetic acid (NTA) surface.[1] Positive ToF-SIMS data from the protein surfaces were acquired with an ION-TOF TOF.SIMS 5-100 system (ION-TOF GmbH, Münster, Germany) using a pulsed 25-keV Bi_3^+ primary ion source. For each spectrum, the analysis area was 100 x 100 μm^2 and the total ion dose was maintained below the static limit ($<10^{12}$ ions/cm^2). Hydrated samples were mounted onto a variable temperature sample stage, rapidly frozen by liquid N_2 cooling, and then evacuated. To remove frozen water and dehydrate the protein films, the sample temperature was sequentially raised in 10°C increments from -90 to +20°C. The sample was equilibrated at each temperature for 10 min[2]. Samples were then cooled down to -150°C for spectra acquisition unless otherwise specified. SPR studies were conducted on a home-built SPR liquid biosensing system.

Results: Two surface coverages of HuLys Fv protein films, low coverage (~100 ng/cm^2) and high coverage (~200 ng/cm^2), were investigated with ToF-SIMS over the temperature range of -90 to +20°C . Applying PCA to the ToF-SIMS data, the spectral differences resulting from two surface coverages and various heat treatments can be determined. Fig. 1 shows a scores plot of the first two principal components (PC), PC1 versus PC2, which captured 75% and 19% of the variance in the whole dataset. As can be seen, all spectra are separated into three groups: (1) all high coverage samples ( ) overlap on the right center of the plot, (2) low coverage at -90°C and -80°C ( ) are located toward the left bottom of the plot, and (3) low coverage at -60°C and +20°C ( ) are located toward the left top of the plot. As a control experiment, the pre-dried samples (x) overlapped with the spectra at -60°C and +20°C with the same coverage. The ellipses drawn around each group indicate the 95% confidence interval of that grouping.

Score trends observed in the plot suggest both surface coverage and heat treatment affected the secondary ion spectra in different ways. First, the spectra from the samples with high coverage ( ) had higher positive PC 1 scores than those with low coverage ( ), indicating spectral difference was mainly dependent on the surface coverage. Second, low coverage spectra obtained at -60°C and +20°C had higher PC2 scores than the spectra obtained at -90°C and -80°C, indicating the heat treatment also affect the low coverage spectra.

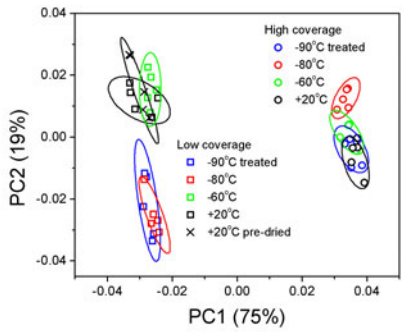

Figure 1. PCA results from HuLys Fv protein films.

The shift in PC2 scores for low coverage samples can be further associated with protein mobility and dehydration events. At the temperature below about -80°C, the protein molecules are frozen into their hydrated conformation. As the temperature is raised, the surface-bound proteins become more mobile and susceptible to structural changes, such as conformational changes that expose hydrophobic amino acid residues.

The antigen binding capacity of surface-bound HuLys Fv before and after dehydration was measured by SPR. At the low coverage, the antigen binding capacity on the dried protein film was roughly 50% lower than that on the fresh film. As comparison, high coverage dried samples lost ~20% binding capacity. The loss of HuLys Fv bioactivity on the dried protein film was attributed to an irreversible disruption of protein native conformation during the drying process. The high coverage samples exhibited less loss of bioactivity, consistent with the smaller conformational changes observed by PCA.

Conclusions: In this study, the structure and bioactivity of the adsorbed protein films upon dehydration were investigated using cold-stage ToF-SIMS and SPR. Combined with PCA, cold-stage ToF-SIMS showed that upon dehydration, low surface coverages of HuLys Fv had significant conformational changes, whereas high surface coverage inhibited these structure transitions and better preserved the native protein structure. These results were confirmed by SPR, which showed that the dehydration-induced conformational changes reduced the antigen binding activity by 50% and 20% for the low and the high coverage, respectively.

References: 1. Cheng, F.; Gamble, L. J. and Castner, G.D. *Ana. Chem.* **2008**, 80, 2564-73. 2. Lewis, K. B. and Ratner, B. D., *J Colloid Interf Sci* **1993**, 159, 77-85.