

Static Secondary Ion Mass Spectrometry Investigation of the Resistance of Polyacrylamide Graft Coatings to Adsorption of Lysozyme

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INTRODUCTION: Characterization of the adsorption of proteins onto biomedical devices is a key issue in bio-interface science. Preventing the non-specific adsorption of proteins is one approach towards controlling interfacial interactions. Much work has focused on PEO for protein-resistant coatings. In this study we investigated polyacrylamide (PAAm) graft coatings and their interaction with lysozyme. As XPS was unable to detect any evidence of protein adsorption onto PAAm graft coatings, we utilized the higher sensitivity of time-of-flight secondary ion mass spectrometry (ToF-SIMS) to probe for any low amounts of adsorbed proteins.

METHODS: Polyacrylamide graft coatings were produced by surface radical polymerization following attachment of isocyanatoethyl methacrylate onto an amine functionalised surface, produced by plasma polymerization of n-heptylamine (HA) [1]. The PAAm grafted surface was contacted with lysozyme solution (100 µg/L in Phosphate Buffer Saline) at 37 °C for 2 hrs, followed by thorough washing with PBS and de-ionised water. The PAAm graft coating before and after lysozyme contact, as well as lysozyme adsorbed onto Si wafer as reference, were characterised by 10 positive and 1 negative static SIMS spectra. The large quantities of data were processed and interpreted with the aid of Principal Component Analysis [2].

RESULTS AND DISCUSSION: The interaction between PAAm and lysozyme was evaluated by detailed analysis of 3 groups of positive fragments: $[C_mH_n]^+$, $[C_mH_nN]^+$ and $[C_mH_nNO]^+$. The results revealed that a combination of the nitrogen- and nitrogen-oxygen-containing positive ions is best suited for the comparisons. The score plots derived from these fragments are shown in Figure 1. PC1, capturing more than 90 % of the original data variance, clearly separates lysozyme from PAAm and PAAm/Lys. In contrast, PAAm surfaces before and after lysozyme adsorption are not discriminated by PC1. PC2, capturing around 7% of the original data variance, shows some difference between these samples, but also partial overlap.

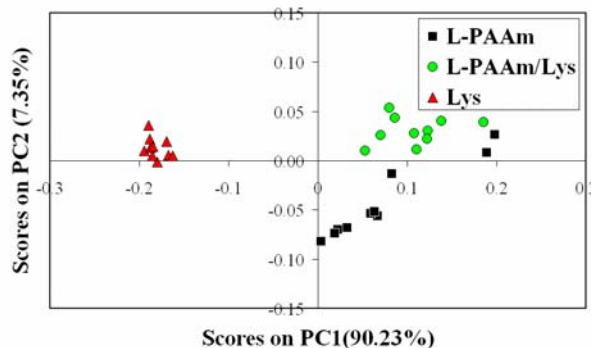


Figure 1: Score plots for L-PAAm, its lysozyme modification and lysozyme derived from combined nitrogen and nitrogen-oxygen based fragments.

The correlation between PC1 and some of the original variables (peaks) is illustrated in Figure 2.

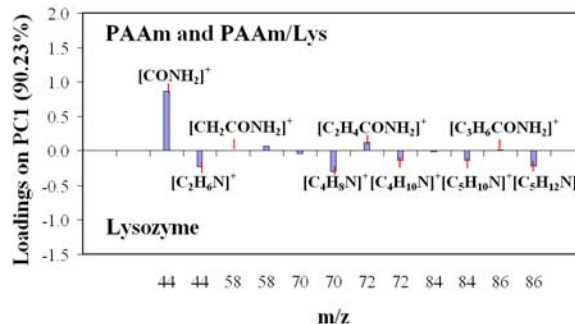


Figure 2: Loadings of individual variables on PC1

$[CONH_2]^+$, $[CH_2CONH_2]^+$, $[C_2H_4CONH_2]^+$ and $[C_3H_6CONH_2]^+$ ions contribute positively to PC1. They are assignable to polyacrylamide fragments. In contrast, most of the $[C_mH_nN]^+$ ions contribute negatively to PC1. They correspond to immonium ions derived from amino acids. The negative ion spectrum shows only a trace amount of sulfur on PAAm/Lys.

CONCLUSIONS: ToF-SIMS can detect some adsorption of lysozyme onto PAAm graft coatings, but the amount is low. The PAAm fragmentation pattern correlates with the linear polyacrylamide structure.

REFERENCES: ¹ H.J. Griesser (1989) *Vacuum* **39**: 485. ² M.S. Wagner, D.J. Graham, B.D. Ratner, D.G. Castner (2004) *Surf Sc* **570**:78-97.