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Applied Surface Science 252 (2006) 6801–6804
applied surface science
www.elsevier.com/locate/apsusc

Quantitative ToF-SIMS study of surface-immobilized streptavidin

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Received 12 September 2005; accepted 15 February 2006
Available online 2 May 2006

Abstract

ToF-SIMS analysis with principal component analysis (PCA) has been used for quantitatively studying the interaction between streptavidin and biotin on a dendrimer surface. A poly(amidoamine) dendrimer surface was used as a model amine surface for biotinylation. The surface streptavidin density was systematically varied and independently quantified using the surface plasmon resonance (SPR) technique. A good linear correlation of streptavidin density was observed between the ToF-SIMS and SPR results.

Keywords: Quantification; Dendrimer; Streptavidin; ToF-SIMS; SPR; PCA

1. Introduction

Quantitative analysis of biomolecules on surfaces is essential to numerous applications of biosensor and biomaterial engineering [1,2]. In particular, a quantitative study of protein interactions in various environmental conditions is needed. Recently, static time-of-flight secondary ion mass spectrometry (ToF-SIMS) has become an increasingly popular way of investigating adsorbed proteins due to its chemical specificity and surface sensitivity [3–7]. However, there are few systematic quantification studies of protein–ligand interactions due to the complex matrix effect, improper orientation and denaturation of the protein, and low molecular secondary ion efficiency from biomaterials [3–6].

In the present work, we used the ToF-SIMS technique with a principal component analysis (PCA) to produce a quantitative study of surface protein density in the protein–ligand interaction. As a model system, the interaction of the streptavidin and biotin on the dendrimer surface was studied as a function of streptavidin concentration. Instead of a self-assembled monolayer (SAM) surface, a G3 poly(amidoamine) (PAMAM) dendrimer surface was used for making the biotin-functionalized surface due to its property of elevating the surface density of the protein. The surface streptavidin density was determined by the surface plasmon resonance (SPR) technique and compared with the ToF-SIMS results to obtain a correlation curve.

2. Experimental

2.1. Sample preparations for ToF-SIMS analysis

An overall sample preparation was depicted in Scheme 1. Gold substrates were prepared by successively evaporating a 20 Å thick film of Ti and a 400 Å thick film of gold onto a Si wafer. The gold substrates were cut into 10 mm × 10 mm pieces and cleaned for 5 min in a piranha solution (1:4) 30% H_2O_2:concentrated H_2SO_4 (v/v) (*Caution*: the piranha solution reacts violently with most organic materials and must be handled with extreme care). The cleaned gold substrates were washed sequentially with DI water and absolute grade ethanol. For the formation of a SAM, the substrates were immersed overnight in a 2 mM ethanol solution of mercaptoundecanoic acid (MUA) at room temperature. The SAM-forming gold surface was then activated by immersing it in an ethanol solution of EDAC (0.1 M) and PF_5 (0.2 M) for 30 min. The PF_5-activated surface was rinsed with ethanol and water, and transferred into a methanol solution of PAMAM G3 dendrimer (22 μM, based on the primary amine concentration). After a 2 h

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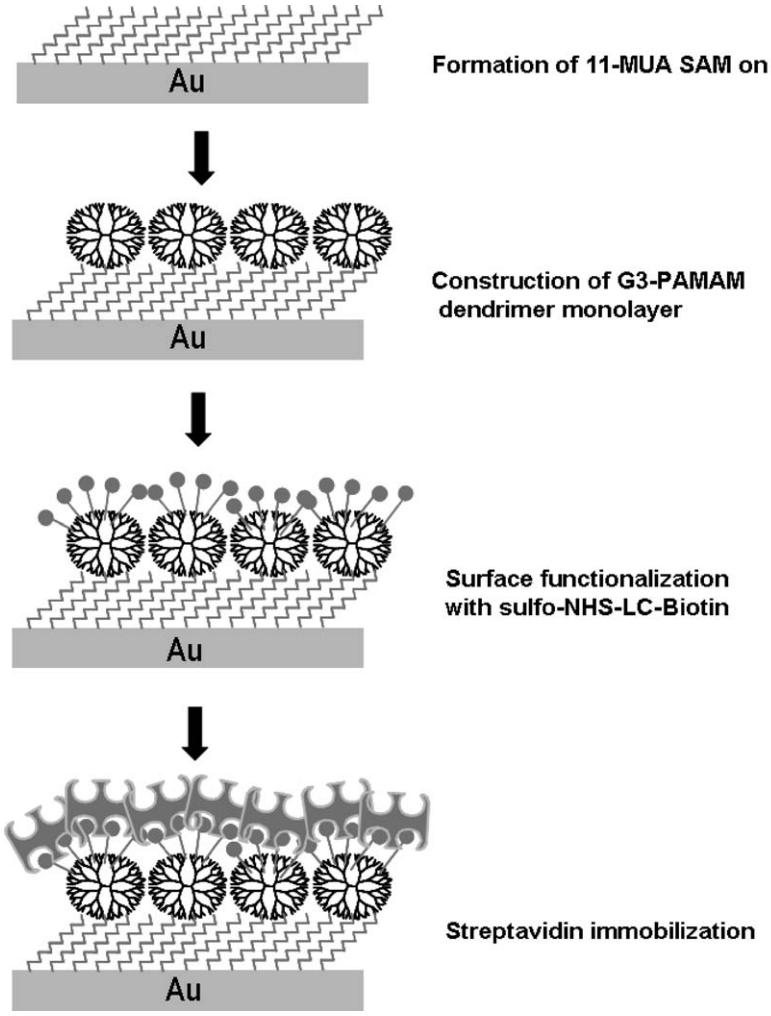
doi:10.1016/j.apsusc.2006.02.203



Scheme 1. Schematic of all analytes sequentially constructed onto gold.

incubation at room temperature, the amine-functionalized substrate was biotinylated by immersing it into a solution of sulfo-NHS-LC-biotin in 0.05 M bicarbonate buffer of pH 8.5 for 30 min. To hydrolyze the remaining reactive esters, the substrate was immediately subject to a solution of sodium bicarbonate buffer of pH 9.5 for 20 min, followed by washing with water. For quantitative analysis, the biotinylated substrates were immersed in dilute streptavidin solutions (0.1–20 μg/mL) in 10 mM PBST (phosphate buffer saline containing 0.05% Tween-20, pH 7.4) for 40 min, followed by a thorough washing in PBST and water, dried under a stream of nitrogen, and used immediately thereafter.

2.2. ToF-SIMS and PCA

TOF-SIMS was carried out using a TOF-SIMS V instrument (ION-TOF GmbH, Germany) with 25 keV Au^+ primary ions (average current of 0.8 pA, pulse width of 16.8 ns, repetition rate of 5 kHz). The analysis area of 500 μm × 500 μm was randomly rastered by primary ions for the spectrum analysis. The primary ion dose was maintained below 10^{12} ions/cm^2 to ensure static SIMS condition. Mass resolution was usually higher than 5000 at *m/z* 197 in both the positive and negative modes. Positive and negative ion spectra were internally calibrated by using H^+, H_2^+, CH_3^+, $C_2H_3^+$, and $C_3H_4^+$ peaks and H^-, C^-, CH^-, C_2^-, and C_2H^- peaks, respectively. The total ion currents were used for the normalization of peaks intensities. A PCA of ToF-SIMS data was performed using PLS_Toolbox v. 3.5 (Eigenvector Research, Manson, WA) for MATLAB (MathWorks Inc., Natick, MA). The raw data were normalized to the total secondary ion counts and mean-centered before the PCA process.

2.3. SPR analysis

SPR spectroscopy was performed with a Biacore X instrument (BIAcore, Sweden) and gold sensor chips (BIAcore). The chip surface was first cleaned with 0.1 M NaOH containing 0.1% Triton-X for 5 min. The procedures for preparing the SAM and dendrimer surfaces were similar to those described in Section 2.1. After drying with a N_2 stream, the dendrimer-modified chip was attached to a chip socket and docked into a SPR instrument, followed by pre-washing with DI water for system-stabilizing and on-chip-cleaning. Biotinylation was carried out by passing a 2 mM solution of sulfo-NHS-LC-biotin in bicarbonate buffer (0.05 M, pH 8.5) for

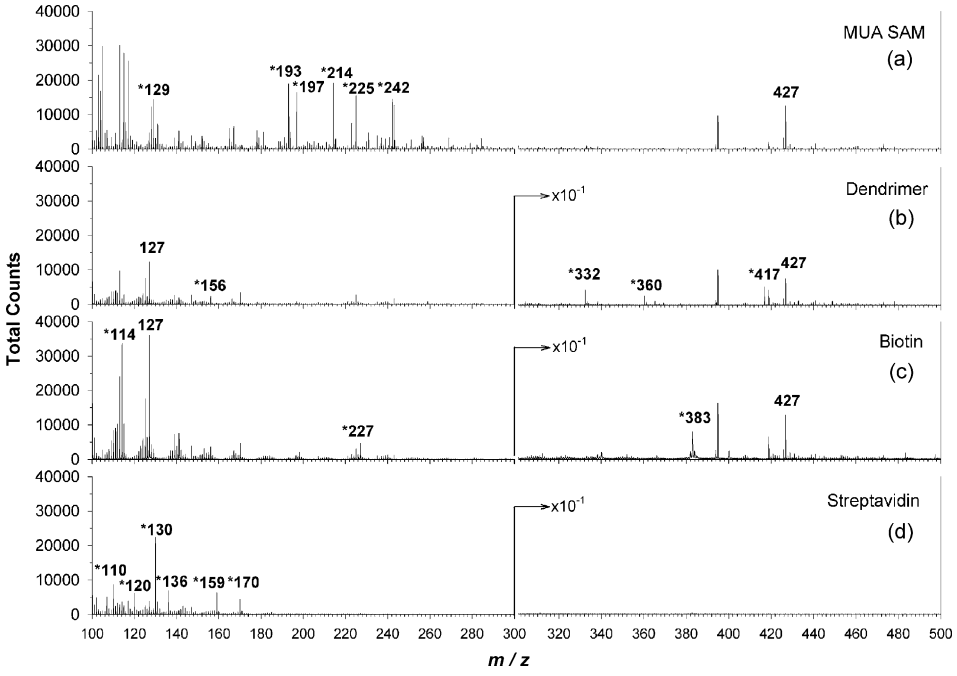


Fig. 1. Positive ion ToF-SIMS spectra of the surfaces obtained from each sample preparation step: (a) MUA-SAM, (b) G3 PAMAM dendrimer, (c) biotinylated dendrimer and (d) streptavidin bound onto the biotinylated surface. Characteristic peaks of each surface are labeled with an asterisk.

30 min over the sensing surface. All procedures in SPR were maintained at a flow rate of 3 μL/min at 25 °C. After biotinylation, the system was sequentially returned to DI water, and excess NHS esters were deactivated by washing with a pH 9.5 bicarbonate buffer for 20 min. To analyze the streptavidin binding, the biotinylated surface was pre-rinsed with PBST for 10 min, and various loadings of dilute streptavidin solutions (0.1–20 μg/mL in PBST) were sequentially injected through two microfluidic channels over the surface for 40 min. After washing with PBST, a relative SPR angle shift for streptavidin interaction was estimated.

3. Results and discussion

ToF-SIMS measurements of the surfaces were separately performed at each step of the sample preparation. Fig. 1 shows positive ToF-SIMS spectra obtained from the MUA-SAM, G3 PAMAM dendrimer, biotinylated dendrimer and streptavidin-immobilized surfaces. All the surfaces were distinguishable by their unique characteristic peaks, which are labeled with an asterisk in Fig. 1. For our quantitative analysis, characteristic secondary ion peaks of streptavidin were selected based on existing literature and were used for comparison with the results of the SPR measurements.

After the surface streptavidin density was systematically varied on the biotinylated dendrimer surface and determined by the SPR measurements, they were correlated with the results of the ToF-SIMS measurements (Fig. 2a). Among the characteristic secondary ions of streptavidin, the ToF-SIMS intensities of only three of the peaks correlated well with the surface streptavidin densities as shown in Fig. 2a. These peaks were *m/z* 130 ($C_9H_8N^+$, Trp), 136 ($C_8H_{10}NO^+$, Tyr) and 159 ($C_{10}H_{11}N_2^+$, Trp).

For a systematic ToF-SIMS data interpretation, a PCA was performed on the ToF-SIMS data set of six characteristic secondary ions signals of streptavidin (i.e. *m/z* 110, 120, 130,

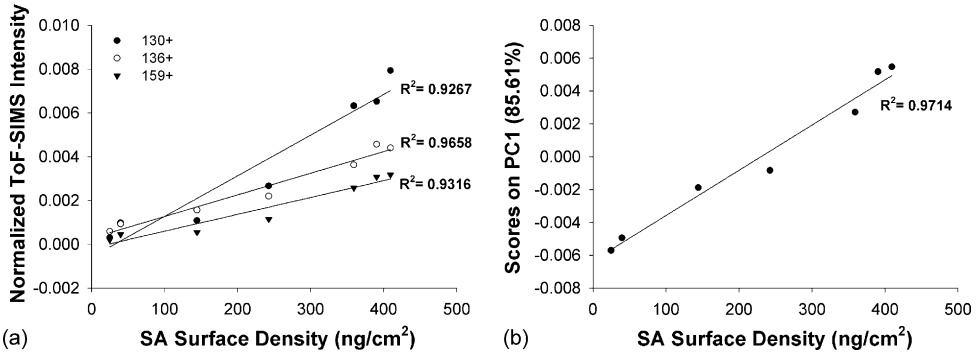


Fig. 2. Correlation between surface streptavidin densities as determined by the SPR method and (a) normalized ToF-SIMS intensities of 130 ($C_9H_8N^+$, Trp), 136 ($C_8H_{10}NO^+$, Tyr), and 159 ($C_{10}H_{11}N_2^+$, Trp) or (b) scores on PC 1 from PCA of ToF-SIMS data for the surface-immobilized streptavidin.

136, 159 and 170). Instead of using the individual intensities of the secondary ions from the streptavidin (Fig. 2a), the scores on PC 1 were compared to the surface streptavidin densities for quantitative analysis. Fig. 2b shows the scores plot of PC 1 as a function of the surface streptavidin densities. Interestingly, there was a good linear correlation between the scores on PC 1 from PCA of the ToF-SIMS data for the surface-immobilized streptavidin and the surface streptavidin densities. It would be useful to study the reasons behind this good correlation between the ToF-SIMS and SPR results, even with the significant matrix effects and conformation effects on the ToF-SIMS data [7]. We will investigate these effects on the interaction of streptavidin and biotin on the surface as a function of surface streptavidin density in a future study. A PCA of the ToF-SIMS data would be helpful to recognize the subtle and overall changes in the ToF-SIMS results [8].

4. Conclusions

We have shown that it is possible to conduct a quantitative ToF-SIMS study of surface protein density in the protein–ligand interaction. The interaction between streptavidin and biotin on the dendrimer surface was systematically studied as a function of the surface streptavidin density using the SPR and ToF-SIMS techniques. We found good linear correlations between the surface streptavidin densities and the normalized intensities or scores on PC 1 of characteristic streptavidin secondary ions.

Acknowledgement

This work was supported by the R&D Program of Fusion Strategies for Advanced Technologies of MOCIE.

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