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Spectroscopic and microscopic examination of protein adsorption and blocking of non-specific binding to silicon surfaces modified with APTES and GOPS

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Abstract

X-ray Photoelectron Spectroscopy (XPS), Atomic Force Microscopy (AFM) and Time of Flight Secondary Ion Mass Spectrometry (TOF-SIMS) were applied to characterize two series of silicon nitride surfaces, modified either with (3-aminopropyl)triethoxysilane (APTES) or (3-glycidoxypropyl)trimethoxysilane (GOPS), prior to and after immobilization of rabbit gamma globulins (rIgG) at different concentrations and blocking with bovine serum albumin (BSA). Higher rIgG amount was adsorbed to surfaces silanized with APTES rather than GOPS, resulting in different behavior to subsequent blocking. There was no increase in total protein surface density due to blocking with BSA for surfaces with already high protein coverage. Apparently, BSA molecules were partly exchanged with rIgG ones, in case of APTES and higher rIgG concentrations, or attached to free surface sites, for GOPS modified surfaces.

Keywords: protein adsorption, APTES, GOPS, TOF-SIMS, AFM, XPS

1. Introduction

Silicon sensor surfaces are commonly modified with silanes, in order to provide a suitable interface between silicon-based transducer and biochemical environment [1]. Active surfaces of protein biosensors are fabricated in a multi-stage process involving, besides silanization, immobilization of receptor proteins

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– often through physical adsorption – followed by blocking of non-specific binding through additional adsorption of a 'neutral' protein. Sensitivity and specificity of biosensors are determined by molecular arrangement of these protein nanolayers. Therefore their characterization is of considerable interest [2].

In this work we compare, using three different spectroscopic and microscopic techniques, namely XPS, TOF-SIMS and AFM, protein nanolayers formed after adsorption of rIgG (using concentrations ranging from 33 to 660 nM) and blocking with BSA (using a 10 mg/mL solution) on silicon surfaces pre-modified with two silanes, APTES and GOPS.

2. Results and discussion

A distinct analysis of surface chemistry of two series of silicon nitride surfaces, modified with GOPS and APTES, respectively, is provided with XPS sampling the outermost ~8 nm of the surface (Fig. 1). High resolution spectra of N1s core-level consist of the signals characteristic for the Si_3N_4 substrate (binding energy 397.5 eV) but also for amine (NH_2, from APTES and proteins, 399.8 eV) and protonated amino groups (NH_3^+ from APTES, 401.4 eV). The latter are hardly present (Figs. 1b, d, f), indicating that the APTES films were formed with amine groups pointing away from rather than towards the substrate. Protein adsorption is reflected in the increased contribution of amine groups at expense of reduced Si_3N_4 lines (Fig. 1). Therefore, the creation of protein/silane/silicon structure is confirmed. The increment in amine group contribution to the XPS spectra is larger for the surface modified with APTES (Figs. 1b, d) rather than GOPS (Figs. 1a, c), revealing more effective immobilization of rIgG.

In addition, XPS spectra allow for a preliminary comparison of rIgG coated surfaces prior to and after blocking with BSA. For the surfaces modified with GOPS, the relative contribution of the NH_2 line is slightly increased due to blocking and the Si_3N_4 signal is somewhat less pronounced (cf. Figs. 1c and e). The opposite tendency is visible for the surfaces modified with APTES (cf. Figs. 1d and f).
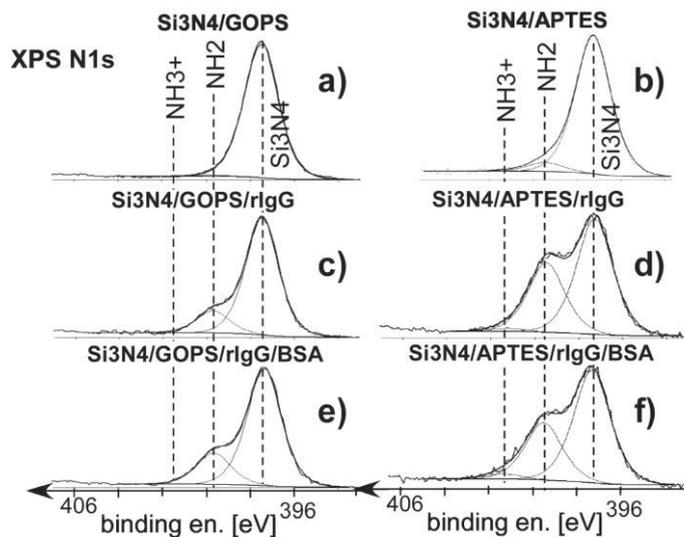

Fig. 1. High resolution N1s core-level XPS spectra of two series of Si_3N_4 surfaces modified with GOPS (a, c, e) and APTES (b, d, f). Distinct contributions from Si_3N_4 and amine (NH_2 and protonated NH_3^+) groups are visible, characteristic for silanized substrates (a, b) and protein overlayers formed due to coating with rIgG (660 nM) (c, d) and blocking with BSA (e, f).

To characterize further the protein nanolayers formed on silanized Si_3N_4, TOF-SIMS was applied working in the so called static mode, known for superior surface sensitivity (sampling depth of 1-2 nm) and chemical specificity (mass resolution $m/\Delta m > 6500$). Representative positive ion TOF-SIMS spectra

recorded for two series of Si_3N_4 surfaces, modified with APTES and GOPS, respectively, prior to and after immobilization of rIgG (from a 660 nM solution) and blocking with BSA are presented in Fig. 2.
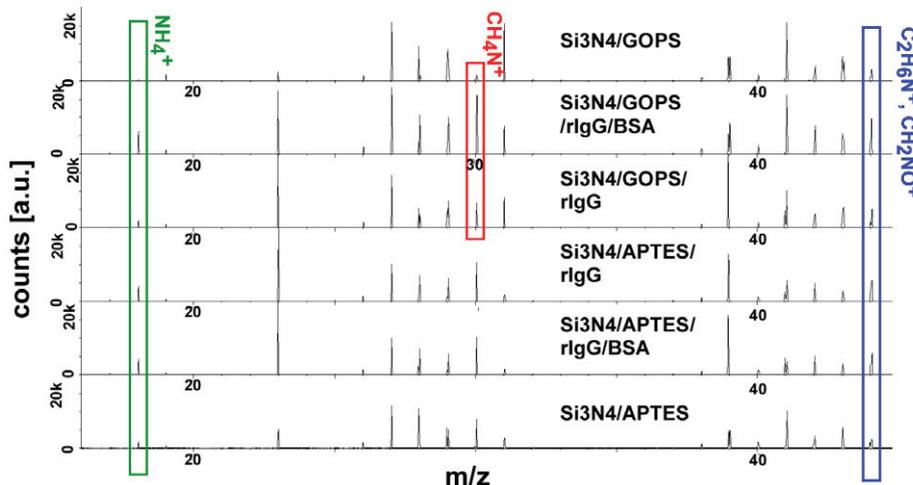

Fig. 2. Representative positive ion TOF-SIMS spectra of the Si_3N_4 surfaces, modified with APTES and GOPS, respectively, prior to and after immobilization of rIgG (660 nM) and blocking with BSA. Signals characteristic for proteins are marked with rectangles.

A more detailed TOF-SIMS analysis is presented in Fig. 3 for rIgG layers adsorbed from solutions with different concentrations. Normalized TOF-SIMS intensities of two secondary ions are compared, reflecting fragments of amino acids: tyrosine (Fig. 3a) and serine (Fig.3b), present in rIgG (4.1% of Tyr, 11.9% of Ser) and BSA (4.7% of Tyr, 3.1% of Ser [3]). The results of this analysis are similar to that revealed by other secondary ions identifying proteins (see e.g. Fig. 2) confirming the features signaled by XPS. In particular, the adsorption of rIgG proteins was higher to the surfaces modified with APTES (open circles in Fig. 3) rather than to those with GOPS (solid circles). In addition, different behavior (indicated with arrows) to subsequent blocking (diamonds) was observed, depending on initial surface coverage.
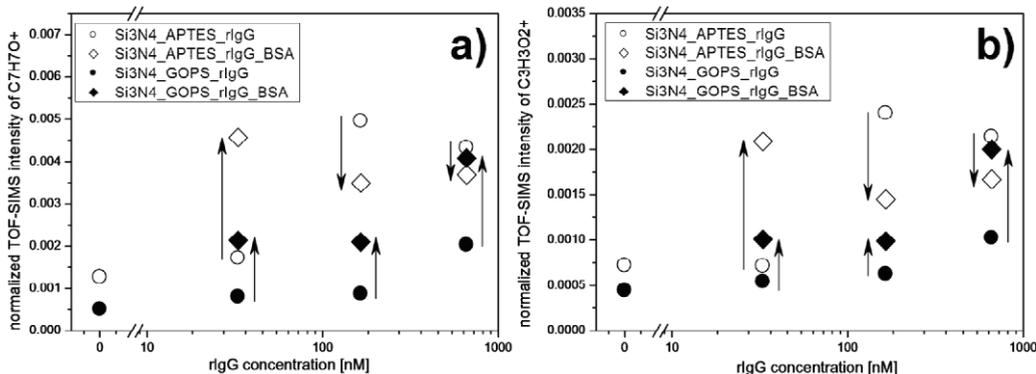

Fig. 3. Intensity of secondary ions characteristic for proteins $C_7H_7O^+$ (left, m/z = 107.05, from Tyr) and $C_3H_3O_2^+$ (right, m/z = 71.01, from Ser) indicates higher rIgG adsorption to Si_3N_4 surfaces modified with APTES (open circles) rather than GOPS (solid circles) and different behavior (indicated with arrows) to subsequent blocking with BSA (diamonds).

The results of spectroscopic characterization of the analyzed surfaces are echoed by the conclusions from microscopic AFM examination, illustrated in Fig. 4. Surface features with apparent protein size are more coarsely packed on the rIgG coated surfaces modified with GOPS rather than APTES (cf. Figs. 4a

and c, Figs. 4e and g), suggesting lower adsorption of rIgG. As a result, blocking of GOPS modified surfaces with BSA resulted in an increase of surface features packing (cf. Figs. 4a and b, Figs. 4e and f) in contrast to the situation on the surfaces silanized with APTES (cf. Figs. 4c and d, Figs. 4g and h).
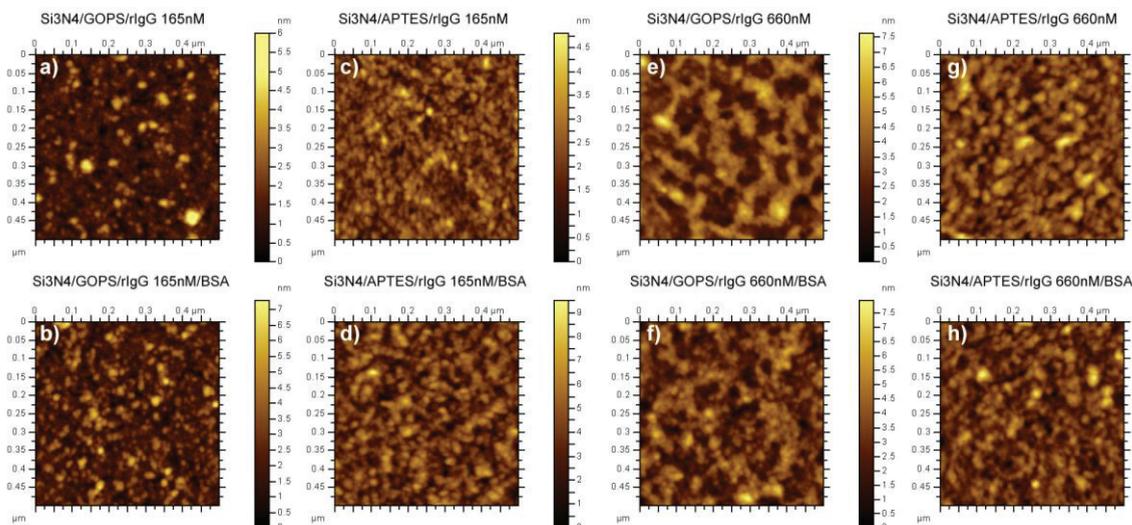

Fig. 4: AFM images of Si_3N_4 surfaces after adsorption of rIgG from 165 nM (a, c) and 660 nM (e, g) solutions, followed by subsequent blocking with BSA (b, d, f, h). The blocking resulted in visibly increased protein coverage for rIgG coated surfaces modified with GOPS (a-b and e-f) rather than for those modified with APTES (c-d and g-h).

3. Conclusions

The combined analysis of silanized silicon nitride by XPS, AFM, and TOF-SIMS showed that surfaces modified with APTES provided more effective immobilization of rIgG as compared to GOPS. In addition, there was no increase in total protein surface density due to blocking for surfaces with already high protein coverage. This suggests that BSA attachment to free surface sites is not the main scenario here in contrast to surfaces with low surface density of rIgG. On the other hand, for the lowest rIgG concentration used, and especially for GOPS modified surfaces, blocking resulted in significant increase of total protein coverage. Apparently, BSA molecules were partly exchanged with rIgG ones, for APTES, or attached to free surface sites, for GOPS modified surfaces. These results demonstrate the power of microscopic and spectroscopic techniques to provide insight on the protein layer compositions on biosensor surfaces and help to determine the most suitable functionalization method.

Acknowledgements

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