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Building of an immunosensor: How can the composition and structure of the thiol attachment layer affect the immunosensor efficiency?

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Abstract

Immunosensors, based on the immobilization of a model rabbit antibody on mixed self-assembled monolayers and Protein A as a linking agent on gold transducers, were elaborated and characterized at each step by modulated polarization-infrared spectroscopy (PM-IRRAS) and occasionally by atomic force microscopy (AFM) and quartz crystal microbalance (QCM). By testing two different mixed SAMs comprising 11-mercaptoundecanoic acid (MUA), together with either decanethiol (C9CH3) or mercaptohexanol (C6OH), the role of the chemical composition and structure of the antibody attachment layer upon the sensor performance was demonstrated.

Keywords: Mixed SAM's; Immunosensor; Thiol attachment; IRRAS; QCM

1. Introduction

Many research fields involve the study of interaction of biomolecules with solid surfaces in particular for new biosensor elaboration. A biosensor can be described as a sensitive interface including a bioreceptor coupled with a transducer able to detect binding events between the bioreceptor and the analyte. Biosensors provide a rapid and convenient alternative to conventional analytical methods for detecting and in some cases measuring, an analyte in a complex medium. Different classes of biosensors are classically distinguished, among them immunosensors that exploit the ability of an antibody to recognize its associated antigen in a very complex medium (Wink et al., 1997).

The quality of the biointerface governs both the sensitivity and the specificity of the biosensor. Therefore, its design is the key step during the biosensor elaboration process. Ideally, the bioreceptor should specifically recognize and bind target species at the lowest possible concentration. It is well known that proteins and antibodies in particular, may loose part of their biological activity when immobilized on a surface due to a combination of two factors: change in conformation and/or unfavourable orientation of the molecule (Norde, 1986). As a consequence, controlling both the amount and orientation of the bioreceptor on the transducer while preserving its bioactivity is the most critical issue in biosensor research.

Immobilization of bioreceptors (in particular antibodies) via self-assembled monolayers (SAMs) or mixed SAMs enables to investigate biorecognition phenomena at a molecular level and fulfils the criteria mentioned above. Let us remind that alkylthiols spontaneously chemisorb on gold surfaces and form densely packed, crystalline-like thiolate films in a very reproducible manner (Bain et al., 1989; Nuzzo et al., 1990a; Porter et al., 1987; Truong and Rowntree, 1996; Whitesides and Laibinis, 1990). When dealing with ω-functionalized thiols, various chemical moieties can thus be strongly bound at the solid–liquid interface and materials with new surface properties can be designed for the binding of biomolecules (Lahiri et al., 1999; Su and Li, 2004; Ulman, 1996). In some cases, the use of mixed SAMs,

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formed for instance by co-adsorption of mixtures of two thiols, has been shown to prevent denaturation and thus improve the bioactivity of a protein immobilized on such layers in comparison with the protein immobilized on a pure SAM (Frederix et al., 2003; Ge and Lisdat, 2002; Guiomar et al., 1999; Wirde et al., 1999). Mixed SAMs are generally constituted of one thiolate with a functional headgroup (like a carboxylic acid) at a low mole fraction and of another "diluting" thiolate at a high mole fraction. The second thiol first reduces the surface concentration of functional groups and thus minimizes steric hindrance, partial denaturation of the protein (Guiomar et al., 1999) and non-specific interactions that can produce interference signals (Frederix et al., 2003; Ge and Lisdat, 2002). Second, the diluting thiolate can also be used to tailor the overall physico-chemical properties of the interface (such as its hydrophobic/hydrophilic character). Characterization of surfaces covered with mixed SAMs is still the subject of numerous studies (Chen et al., 2000, 2001; Hobara et al., 1999; Li et al., 2003).

In this study, we report the construction of an immunosensor, taking the rabbit IgG/anti-rabbit IgG couple as a model system. The biointerface consisted of a layer of antibody immobilized by bioaffinity onto Protein A, often used as a linker agent for biosensor elaboration (Babacan et al., 2000; Oh et al., 2003; Pribyl et al., 2003); Protein A was covalently linked to two mixed SAM systems where 11-mercaptoundecanoic acid is the "active" thiol. We have found that the nature of the diluting thiolate had a noticeable influence not only on the overall amount of immobilized Protein A but also on its affinity towards rabbit IgG and finally on the properties on the resulting immunosensor.

2. Materials and methods

2.1. Chemicals

n-Decanethiol (C9CH3), 6-mercaptohexanol (C6OH), 11-mercaptoundecanoic acid (MUA), *N*-hydroxysuccinimide (NHS), 1-(3-dimethylaminopropyl)-*N*'-ethylcarbodiimide hydro-chloride (EDC) were purchased from Aldrich (St.-Quentin Fallavier, France). Rabbit IgG, goat anti-rabbit IgG, goat anti-mouse IgG and bovine serum albumin (BSA) were purchased from Pierce (Perbio, Brebières, France). All solvents were reagent-grade. Reagents were used without any further purification. Experiments were carried out at room temperature.

2.2. Formation of the mixed SAMs

Glass substrates (11 mm × 11 mm) coated successively with 50 nm thick layer of chromium and a 200 nm thick layer of gold were purchased from Arrandee (Werther, Germany). The gold-coated substrates were annealed in a butane flame to ensure a good crystallinity of the topmost layers, as recommended by the company and rinsed in a bath of absolute ethanol during 15 min before adsorption. The substrates were immersed in binary mixtures of 2.5 mM of MUA and 7.5 mM of C9CH3 or 7.5 mM of C6OH in absolute ethanol for 3 h and thorough rinsed in ethanol and dried under a flow of clean air.

2.3. Covalent immobilization of Protein A

The substrates were treated with a solution of NHS (20 mM) and EDC (10 mM) in ultrapure water for 2 h following by an immersion in a solution of Protein A (50 mg/L) in 10 mM PBS pH 7.4 for 2 h. The residual NHS esters were blocked with 1 M ethanolamine pH 9.0 for 20 min. After washing with ultrapure water, the substrates were finally immersed in a 1% (w/v) solution of BSA in 10 mM PBS pH 7.4 for 2 h.

2.4. Binding of antibody

Protein A-coated substrates were immersed in a solution of rabbit IgG (100 mg/L) in PBS for 1 h, washed with water and dried under a flow of clean air.

2.5. Tests of specificity

Goat anti-mouse IgG or goat IgG (non-specific antigen) (10 or 30 mg/L in PBS, respectively; 150 μL) was spotted on the immunosensor and incubated for 1 h. After extensive washing with water and PM-IRRAS analysis, goat anti-rabbit IgG (specific antigen) (10 or 30 mg/L in PBS; 150 μL) was spotted on the immunosensor and incubated for 1 h.

2.6. PM-IRRAS measurements

The FTIR instrument used in our experiment is a commercial NICOLET Nexus spectrometer. The external beam was focused on the sample with a mirror, at an optimal incident angle of 75°. A ZnSe grid polarizer and a ZnSe photoelastic modulator, modulating the incident beam between p and s polarizations (HINDS Instruments, PEM 90, modulation frequency = 37 kHz), were placed prior to the sample. The light reflected at the sample was then focussed on a nitrogen-cooled MCT detector. The sum and difference interferograms were processed and Fourier-transformed to yield the differential reflectivity $\Delta R/R = (R_p - R_s)/(R_p + R_s)$ which is the PM-IRRAS signal. Sixty-four scans were recorded at 8 cm^{-1} resolution for each spectrum.

2.7. AFM imaging

The AFM was a PicoLE by Molecular Imaging (Scientec, Palaiseau, France) used in an acoustic mode in air at 22 °C. Images of 500 nm × 500 nm were obtained at 0.75 Hz with a resolution 512 × 512 pixels, with the following characteristics:

Probe characteristics Ultrasharp NSC15/noAI. Radius of curvature: less than 10 nm; tip height: 15 . . . 20 μm; full tip cone angle: less than 30°; cantilever length: $L \pm 5$ μm: 230; cantilever width: $w \pm 3$ μm: 35; cantilever thickness: 4.0 μm; resonance frequency: 325 kHz; force constant: 40 N/m.

2.8. QCM measurements

AT-cut planar quartz crystals (14 mm diameter) with a 9 MHz nominal resonance frequency (Matel Fondhal France) were