Optimization of capacitive affinity sensors: drift suppression and signal amplification

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Abstract

The detection limit of capacitive affinity sensors based on the gold–alkanethiol system can be improved by optimization of sensor preparation and by signal amplification. The dissociation of the gold–sulfur binding is often a critical point leading to operative errors of such sensors. The stability of self-assembled monolayers prepared with different thiols on gold electrodes in aqueous and organic solvents was studied by the capacitive technique. The results show that monolayers made of 16-mercaptotetradecanoic acid are stable in aqueous solution and can be hardly extracted from a gold surface by ethanol, methanol, or dioxane, while a considerable damage of self-assembled monolayers was observed due to incubation in chloroform or dimethylformamide. In contrast, self-assembled monolayers made from short-chain disulfides or thiols (such as 3,3'-dithio-bis(propionic acid N-hydroxysuccinimide ester) or 11-mercaptoundecanoic acid) displayed a poor stability in aqueous phase. Capacitive affinity sensors based on these short-chain thiols showed a considerable drift of the signal. The use of long-chain thiols resulted in a stable signal; it was applied to compare capacitive effects due to immobilization of different biological molecules and for preparation of different biosensors.

The response of capacitive biosensors can be amplified by formation of a sandwich structure. This principle was illustrated by subsequent adsorption of polyclonal anti-HSA after binding of HSA with a sensor for HSA based on monoclonal antibodies. © 1999 Elsevier Science B.V. All rights reserved.

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1. Introduction

Chemical and biological sensors based on direct detection of analyte–receptor binding (affinity sensors) employ different transducing principles, such as optical, electrical or mechano-acoustical [1–6]. A great progress in the investigation of gold–alkanethiol self-assembled monolayers during the last years [7–18] led to essential progress in affinity sensors based on capacitive transduction [19–27]. The detection of direct binding along with the absence of any additional (bio)chemical system or label for signal transfer (which may result in interfering effects) is a reason for its good selectivity. The width of the detection range of such sensors is determined by the signal-to-
noise ratio, the noise (Fig. 1) also including drift and irreproducibility of the sensor, electrical/optical noise of both sensor and transducer, etc. Therefore, there are two ways to improve the detection limit of affinity sensors. The first consists in a minimization of the signal noise, the second in an increase of the sensor signal.

With respect to affinity sensors based on capacitive transduction (Fig. 2), the first goal can be achieved by a proper choice of a thiol providing the best insulating properties. Secondly, a solvent to be used for further modifications of the electrode surface after adsorption of the thiol, should not cause a desorption of the thiol from the electrode surface. By analogy with known immunological techniques, the capacitive signal can be enhanced by a subsequent adsorption of large molecules on the analyte layer firstly bound to the immobilized receptor layer. These possibilities of improvement of capacitive chemical sensors and biosensors are experimentally verified and discussed in the present paper.

2. Experimental

All measurements were performed with a two-electrode system. The reference electrode was an
Ag/AgCl electrode with a macroscopic surface of about 0.3 cm². Gold electrodes were prepared by UV photolithography combined with “lift-off” technique. Deposition of the gold layer and adhesive sublayers (50 nm Ti, 50 nm Pd and 150 nm Au) on an oxidized silicon surface was performed by r.f. sputtering [27]. The size of the electrodes was 1560 × 1565 μm². To minimize meniscus artifacts [28], the electrodes were connected with contact pads by narrow (10 μm) bands (Fig. 3). The purity of the Au sputtering target was 99.8%. The gold layers display polycrystalline structure with preferred orientation of their grains in the [1 1 1] direction; the surface roughness being less than 3 nm.

Monolayers of ω-functionalized alkane thiols were adsorbed on the gold electrodes, following the usual procedure [21]. The electrodes were cleaned with a hot mixture of piranha solution (1:3 solution of H₂O₂/H₂SO₄), rinsed with water and dried. Caution: piranha solution reacts violently with most organic materials and must be handled with extreme care. Then, the electrodes were immersed into a 5 mM solution of the thiol compound in chloroform for at least 12 h and washed briefly with chloroform or ethanol. Adsorption of 3,3′-dithio-bis(propionic acid N-hydroxysuccinimide ester) (DSP) was also performed from the solution in dimethylacetamide under the same conditions; no differences in the electrode properties were observed. The macroscopic surface of the sensitive area of the electrode was about 2.4 mm².

The electrode capacitance was measured in the frequency range of pure capacitive behavior [21,29] by registration of the 90° component of the electrical current by means of a lock-in amplifier (PAR, model 121) and a home-made current amplifier, the typical amplification being 10⁴ V/A. The amplitude and the frequency of the sine voltage used were 10 mV and 20 Hz correspondingly. At the last stage of the work the automatic impedance analyzer EIS-11 (from Analytical μ-Systems, Regensburg, Germany) was used. The sensitivity of the capacitance measurements being limited by the capacitance drift was typically better than 0.1%. All measurements were performed at room temperature (22±1°C). The electrode potential was +300 mV (ref. Ag/AgCl, 100 mM KCl). To prevent the formation of air bubbles, the electrolyte was degassed under vacuum before the experiment.

16-Mercaptohexadecanoic acid and 11-mercaptopoundecanoic acid were purchased from Analytical μ-Systems (Regensburg, Germany). Deionized water was additionally purified by passing it through a Millipore-Milli-Q system, the final resistivity being at least 18 MΩ·cm. Monoclonal anti-HSA (mouse IgG2a, purity 26% from total protein), polyclonal anti-HSA (rabbit IgG-fraction of antiserum, purity 38% from total protein), HSA (purity 97–99%), BSA (purity 97–99%), protein A, avidin and 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) were from Sigma. Amino-terminated 24'-mer oligonucleotides were from MWG-Biotech (Ebersberg, Germany). The LDL-receptors were provided by
Dr. T. Bajari (University of Heidelberg). 3,3’-Dithio-bis(propionic acid N-hydroxysuccinimide ester) and dimethylacetamide were from Fluka. N-hydroxy-succinimide (NHS) and all other reagents and solvents were from Merck.

Chemical activation of the carboxy groups of the adsorbed ω-functionalized alkanethiols were performed by the EDC or NHS methods according to [30]. The electrolyte consisting of 100 mM KCl and 5 mM phosphate buffer was used. The immobilization of the receptor layer was carried out at pH 5.0 or 6.4. The antigen–antibody reactions were performed at pH 7.2.

3. Results and discussion

To study a possible extraction of alkanethiols from self-assembled monolayers by organic solvents during the chemical modification of alkanethiol ω-terminal groups, capacitance changes (Fig. 2) after incubation of the thiol covered electrodes in different organic solvents were investigated (Table 1).

No capacitance changes were observed after incubations of electrodes, covered by 16-mercaptohexadecanoic acid, in aqueous solution, and only a small increase was observed after incubation in methanol, ethanol or in dioxane. However, on contact of these electrodes with DMF or chloroform, a considerable increase of the electrode capacitance was detectable. These large effects cannot be interpreted in terms of a structural modification of the self-assembled monolayers, but rather point to a desorption of thiols from the monolayer. To exclude the extraction of thiols from the self-assembled monolayers, DMF and chloroform based techniques were not used in further work.

Previous investigation had shown that an extraction of alkanethiols by aqueous solutions may occur [21]. This results in a complete loss or a drift of the sensor signal. This is illustrated by comparison of the capacitance drift (Fig. 4) and sensor properties (Fig. 5) of electrodes covered by 16-mercaptohexadecanoic acid, by 11-mercaptoundecanoic acid and by 3,3’-dithio-bis(propionic acid N-hydroxysuccinimide ester) (DSP) which are commonly used for preparation of biosensors. The desorption of DSP occurred in an hours time-scale (Fig. 4, curve 1), while the desorption of 11-mercaptoundecanoic acid was much slower (Fig. 4, curve 2), and no desorption of 16-mercaptohexadecanoic acid was detected even over 4 days (Fig. 4, curve 3). Assuming a capacitance of bare gold electrode to be of 25 μF/cm², one can use

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Incubation time (h)</th>
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<tr>
<td></td>
<td>0.25</td>
</tr>
<tr>
<td>Water (100 mM KCl, 5 mM phosphate, pH 5.1, 6.4 or 7.2)</td>
<td>0</td>
</tr>
<tr>
<td>Dioxane</td>
<td>1.2</td>
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<tr>
<td>Dimethylformamide</td>
<td>5</td>
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<tr>
<td>Chloroform</td>
<td>27.4</td>
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<td>Methanol</td>
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<td>Ethanol</td>
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Fig. 4. Comparison of stability of adsorbed monolayers from 3,3’-dithio-bis(propionic acid N-hydroxysuccinimide ester) (1), 11-mercaptoundecanoic acid (2), and 16-mercaptophexadecanoic acid (3) expressed as capacitance changes due to long-time incubation in the electrolyte at pH 7.2.
Fig. 5. Drift of capacitance during immobilization of receptors on the monolayer of 11-mercaptoundecanoic acid ((a) and (e)) and binding of analytes ((b) and (c), (f) and (g)) to these receptors. No drift was observed for an analyte binding with receptors immobilized on the monolayer of 16-mercaptohexadecanoic acid (d). Receptors: polyclonal anti-HSA ((a) and (b)), monoclonal anti-HSA (d), and HSA ((e) and (f)). Immobilization of proteins was performed at pH 5.0, the antigen–antibody reaction at pH 7.2. Additions of proteins are marked by arrows, the concentration values in the figures indicate the respective concentrations in the cell. The curves in (c) and (g) were obtained from (b) and (c) correspondingly, by subtraction of the signal drift.
the Frumkin’s model of two parallel capacitors to estimate the amount of desorbed molecules. The increase in the capacitance of DSP-coated electrodes from 5 to 10 μF/cm² during 4 h incubation in aqueous solution is equivalent to a desorption of about 25% of thiols.

Because of the strong drift, all attempts to detect capacitance changes of DSP-covered gold electrodes due to immobilization of HSA or anti-HSA and due to a subsequent binding of corresponding proteins (anti-HSA or HSA) were unsuccessful. Gold electrodes covered by 11-mercaptopoundecanoic acid displayed a lower drift and allowed us to detect an increase of the effective thickness of the insulating layer due to immobilization of receptors (Fig. 5(a) and (e)) and subsequent analyte binding (Fig. 5(b) and (f)). A subtraction of the drift signal (assuming a constant drift rate) resulted in capacitive signals (Fig. 5(c) and (g)) which can be used for analytical purpose. However, the signal correction decreases the detection limit, accuracy and reliability of such analysis. This problem can be overcome by using long-chain thiols. The results show that there is no drift, not only in case of gold electrodes covered by 16-mercaptophexadecanoic acid, but also for electrodes with immobilized receptors and during analyte–receptor binding. As an example, the capacitive effects of anti-HSA based biosensor due to subsequent additions of HSA is presented in Fig. 5(d). The graph was obtained in a direct experiment without any subsequent corrections.

The results also allow one to exclude the loss of the sensor properties due to destroying of alkanethiol monolayer and suggest a quite general technique for preparation of capacitive affinity sensors. Taking into account these requirements, capacitive sensors for different biological analytes were developed. The capacitive effects due to non-specific adsorption of proteins on non-modified carboxy-terminated alkanethiol monolayers were at least five times less than due to binding with receptor modified monolayers. Fig. 6 illustrates a new application of this principle to make the sensor for immunoglobulins. In this case, protein A, providing a binding of Fc region of immunoglobulins [31], was used as a receptor. Addition of immunoglobulins led to the decrease of the electrode capacitance. The amplitude of the effect depends on the immunoglobulin concentration, the saturation values were in the range 0.7–1.2% for addition of polyclonal antibodies, and in the range 2.2–2.8% for addition of monoclonal antibodies.

In addition to its analytical perspectives, the changes in capacitance that occur as a result of protein immobilization can be used to compare the effective thickness of immobilized protein monolayers. Only low (1.5–3%) capacitive effects were observed due to immobilization of oligonucleotides and polyclonal antibodies while the immobilization of monoclonal antibodies or protein A resulted to higher (6–9%) capacitive effects (Fig. 7). Except polyclonal antibodies, a correlation between molecular weight and capacitive effect was observed.

A further increase in the signal-to-noise ratio in capacitive sensors can be achieved by amplification of the signal by a specific binding of large molecules to free binding sites of an analyte. This principle was applied to an immunosensor for HSA (Fig. 8, curve 1) which was prepared by covalent immobilization of monoclonal anti-HSA on gold electrodes covered with 16-mercaptophexadecanoic acid using the EDC coupling technique. Addition of HSA to the electrolyte led to the decrease of the capacitance. As soon as a stationary capacitance was observed, the sensor was rinsed with water and placed into the electrolyte containing no HSA. Then polyclonal antibodies for HSA were added to the solution. Such a “development procedure”, formally used in the quartz crystal micro-
balance [32], resulted in a further decrease of the electrode capacitance by a factor of 1.5–1.75. So far, this amplification effect is not high enough to be of real practical use, but it confirms the suggested idea.

A similar approach can probably be used not only for signal amplification, but also for distinguishing between specific binding of antigens to immobilized monoclonal antibodies and non-specific adsorption of antigens onto the receptor layer. One can expect that in the latter case, some binding sites of the adsorbed antigen are exposed to the aqueous phase. Subsequent addition of monoclonal antibodies (of the same type as the antibodies immobilized on the electrode) will result in the formation of sandwich structure leading to a decrease in the capacitance. This test has been performed, however, no decrease of the capacitance was observed (Fig. 8, curve 2). It allows us to suggest that HSA is bound by immobilized anti-HSA through specific binding sites only.

4. Conclusion

We find the following requirements to be of significance while preparing affinity chemo- and biosensors, based on the gold–alkanethiol system: (i) the use of organic solvents, especially DMF and chloroform at the stage of receptor immobilization should be minimized or even excluded; (ii) the use of thiols with a large hydrophobic part (of at least 11 methylene groups for aliphatic thiols). Otherwise, the sensors display a considerable signal drift. The signal can be amplified by the formation of a sandwich structure. These statements were tested on the sensors with capacitive transducing, but one can expect similar effects for all sensors based on the gold–alkanethiol system employing any transducing principles.

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