Kinetics of photo-stimulated adsorption of enzyme molecules onto $n$- and $p$-type silicon

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Abstract. We report on a change in the surface density of glucose oxidase (GOx) molecules on surface of Si/SiO$_2$/polyethyleneimine structures depending on type of Si conductivity, adsorption time and white-light illumination of a substrate during GOx adsorption. It is clearly shown by AFM images that the dependence of GOx adsorption on adsorption time and illumination is different for $n$-Si and $p$-Si substrates. The results can be explained by the electrostatic interaction between GOx molecules and the surface charge of Si, which depends on illumination.

1. Introduction
Adsorption of enzyme molecules onto surface of a semiconductor transducer is critical for selectivity, sensitivity, and other important parameters of biosensors. Enzyme molecules (for example, glucose oxidase (GOx)) can be used to fabricate a receptor layer on the surface of an ion-sensitive semiconductor transducer. Previous experimental data of GOx adsorption and molecular simulation results of other enzymes [1, 2] have shown that electrostatic interactions play an important role in controlling the enzyme adsorption.

Despite the realization of the biological significance, enzyme adsorption mechanisms and kinetics are still not fully understood, and several important questions remain unanswered. For example, the kinetics of adsorption of enzyme molecules to a solid surface, depending on changes in the surface charge density (SCD), is poorly understood. Remote methods of influencing the SCD of a substrate during the immobilization of enzyme molecules are interesting. Semiconductors as a solid substrate allows us to remotely change SCD value affecting the electrostatic interaction between enzyme molecules and the substrate. Obviously, the remote change of the semiconductor SCD can be achieved by either a field effect using external electric field or a super band-gap illumination.

Previously, we have shown the effect of the ionic strength of a solution and the photoelectron processes in Si during GOx adsorption on the electrostatic interaction between enzyme molecules and the semiconductor surface [3, 4]. However, studies on the enzyme adsorption kinetics onto a semiconductor, depending on its conductivity type and changes in the surface charge density caused by illumination, have not been carried out before.

Weighing or indirect methods characterizing adsorption onto a solid substrate by changing solution parameters are often used to measure the adsorption kinetics. However, these methods allow us to obtain information on the amount of adsorbed substance only, but not on the distribution of adsorbed molecules on the substrate surface, their conformation, and the processes of molecules coagulation. AFM measurements can clearly show the kinetics of adsorption of enzyme molecules and allow us to draw...
conclusions about coagulation processes and the regular/random distribution of GOx molecules using photo-stimulated adsorption (PSA).

Thus, in our work, AFM was used to study the PSA kinetics of GOx molecules onto the surface of both p- and n-type silicon.

2. Experimental section

2.1. Materials and methods
Experiments were performed with single-crystalline Si wafers of n-type ($\rho \approx 4 \Omega \text{ cm}$) and p-type ($\rho \approx 8 \Omega \text{ cm}$). Initially, the wafers were boiled in a peroxide–ammonia solution and rinsed in deionized water. This treatment leads to “reconstruction” of a native oxide layer to SiO$_2$. It can be noted that above pH = 2.3 the surface is negatively charged [5]. Afterwards, wafers were cut into substrates of 8×8 mm$^2$.

GOx molecules from *Aspergillus niger* were used as enzyme molecules. At pH > 4.2 the GOx molecule has an effective negative charge [6]. The size of the GOx molecule is 6.0×5.2×7.7 nm$^3$ [6]. A cationic polyelectrolyte polyethylenimine (PEI) with a molecular weight of 25 kDa was used to increase the adsorption of negatively charged GOx onto the silicon substrates. The PEI molecules were adsorbed onto the silicon substrates from the 0.5 mg/ml aqueous solution during 10 min followed by rinsing in deionized water ($\rho \approx 18.2 \text{ M\,\Omega \,cm}$) during 10 min and drying in nitrogen flow.

The photo-stimulated layer-by-layer adsorption technique suggested in [7] was used to adsorb GOx from the 0.5 mg/ml aqueous solution onto silicon covered with PEI. Two silicon substrates were placed in GOx solution. One of the substrates was illuminated during GOx adsorption by white-light of a halogen lamp (Philips 13186 EPX/EPV) with illuminance level of about 20000 lx. Adsorption time was varied from 10 to 180 min. The optical radiation of the halogen lamp was not absorbed by the GOx solution and did not heat the solution and substrate. However, the radiation of the halogen lamp absorbed by Si induced the photogeneration of nonequilibrium charge carriers. Thus, the surface charge density of Si substrate was changed. The PSA results of the GOx onto a silicon substrate were compared with the results of GOx adsorption in the dark.

2.2. Atomic Force Microscopy measurements
The topography of the surface was measured using atomic force microscopy (AFM) by NTEGRA Spectra (NT-MDT Spectrum Instruments, Russia) in a tapping mode with scan frequencies of 0.3-1 Hz using HA_NC/WoC cantilevers of ETALON series with resonance frequency of 140 ± 10% kHz. The Gwyddion software was used for statistical analysis of AFM data. The measurements have not been corrected for the convolution with the tip shape. Therefore, the lateral size of enzyme molecules observed by AFM does not correspond to their real size due to the artifacts appearance (“profile broadening” effect due to the tip-sample convolution). However, using results from [8], we can recognize individual molecules of GOx on the scan.

3. Results and discussion
Figure 1 shows AFM-images of adsorbed GOx molecules onto the surface of p-Si structure either in the dark or under illumination for each value of the adsorption time (10, 30, 60, and 180 min), similar images were obtained also for n-Si (Fig. 2). It is seen that adsorption of GOx molecules onto the surface of Si/SiO$_2$/PEI structure increases with adsorption time and this does not depend on the silicon conductivity type or illumination level. The GOx adsorption efficiency can be estimated using a mean surface coverage calculated as the relative part of area covered with GOx molecules. Table 1 displays the mean surface coverage (±standard dev.) dependence on adsorption time in the dark and under illumination.

In the case of GOx adsorption onto p-Si/SiO$_2$/PEI, significant adsorption rate during the first hour and its acceleration by illumination were observed. In the case of GOx adsorption onto n-Si/SiO$_2$/PEI, a very low adsorption rate during the first hour of adsorption and illumination of the substrate during adsorption further slowed down the adsorption process. However, after 3 hours, the surface coverage values with GOx molecules for both p-type and n-type were comparable.
Figure 1. AFM-image of $p$-Si/SiO$_2$/PEI/GOx structures, which GOx adsorbed during 10 min (a, e), 30 min (b, f), 60 min (c, g) and 180 min (d, h). Top line corresponds to GOx adsorption in the dark, while down line corresponds to PSA.

Thus, in order to achieve a high value of surface coverage with GOx molecules in case $p$-Si/SiO$_2$/PEI substrate, the adsorption time is crucial. In case of $n$-Si/SiO$_2$/PEI, photo-stimulation of the substrate during GOx deposition allows one to achieve the same surface coverage, but in a shorter time. It is an important practical result.

Different kinetic dependences for $n$-Si and $p$-Si can be explained by different electrostatic interactions between negatively charged in solution enzyme molecules and the substrate surface charge. This leads to different values of rate constants for adsorption and desorption processes. These constants can also be controlled by illumination, because illumination induces changes in the surface charge density of the substrate.

Figure 2. AFM-image of $n$-Si/SiO$_2$/PEI/GOx structures, which GOx adsorbed during 10 min (a, e), 30 min (b, f), 60 min (c, g) and 180 min (d, h). Top line correspond to GOx adsorption in the dark, while down line correspond to PSA.
Table 1. The mean percentage of the surface coverage (±standard deviation) after GOx adsorption in the dark and under illumination depending on the GOx adsorption time.

| GOx adsorption conditions | p-Si/SiO₂/PEI/GOx | n-Si/SiO₂/PEI/GOx |
|---------------------------|-------------------|-------------------|
|                           | 10 min | 30 min | 60 min | 180 min | 10 min | 30 min | 60 min | 180 min |
| in the dark               | 0.30±0.1 | 0.29±0.1 | 0.48±0.1 | 4.85±0.5 | 0.40±0.1 | 2.21±0.5 | 3.26±0.5 | 5.4±0.5 |
| under illumination        | 0.10±0.1 | 0.09±0.1 | 0.25±0.1 | 4.9±0.5  | 0.58±0.1 | 3.27±0.5 | 4.47±0.5 | 5.6±0.5 |

The same maximum values of the surface coverage with GOx molecules for n-Si and p-Si can be explained by the charge of GOx molecules, which depends only on values of pH and ionic strength of the solution. In our experiments, pH and ionic strength of the solution did not specifically change and the charge of individual GOx molecule was the same in all experiments. At a certain surface coverage with GOx molecules (ca. 5%), an electric field blocks further immobilization of enzyme molecules (the desorption rate constant prevails).

4. Conclusion
In the present study, we have investigated the kinetics of photo-stimulated adsorption of GOx molecules onto silicon substrates of different conductivity types. It was found that illumination increased the adsorption rate of GOx during the first hour in case of n-Si/SiO₂/PEI structure by about 50%. In the case of GOx adsorption onto p-Si/SiO₂/PEI structure, significant covering of the surface with enzyme molecules is possible only after 3 hours. In the first hour, illumination reduces the enzyme adsorption onto a p-type semiconductor. The obtained results were explained by illumination-induced amplification (for n-Si-based substrate) or attenuation (for p-Si-based substrate) of the electrostatic interaction between the enzyme molecules and semiconductor substrate.

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