The influence of illumination and ionic strength of a solution on the formation of biosensor structure based on a silicon substrate and glucose oxidase molecules

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Abstract. We report on a change in parameters of a sensitive layer of field effect biosensor based on a silicon transducer and glucose oxidase (GOx) enzyme molecules. Change in the parameters of the enzyme layer occurs due to the white-light illumination of a Si substrate during the GOx adsorption. It was found that the addition of certain value of NaCl salt concentration, which depends on the presence of a polyethylenimine buffer layer on the semiconductor surface, led to a more prominent effect of photo-assisted adsorption of GOx molecules.

1. Introduction

Sensitivity, accuracy and response stability are important characteristics for any sensors. The characteristics of enzyme biosensor based on field effect transistor can be improved by increasing the concentration of enzyme molecules adsorbed onto a semiconductor transducer surface. Orientation of enzyme molecules adsorbed onto a semiconductor substrate is important. For example, if there are no barriers to substrate-enzyme interaction, then the biocatalytic activity of glucose oxidase (GOx) molecules is preserved. Many authors, in particular [1], note that electrostatic interactions play an important role in the fixing and orientation of adsorbed enzyme molecules onto a charged surface. Ionic strength or pH of solution are commonly used to modulate the electrostatic interactions between GOx and surfaces. However, variation of pH or ionic strength leads to a nonmonotonic and unpredictable change in the surface density of GOx molecules.

Previously [2-3], we report on the influence of the white-light illumination of silicon substrate during GOx adsorption on the surface concentration of immobilized GOx molecules. This method is called photo-stimulated adsorption (PSA). The PSA efficiency of GOx molecules significantly depends both on a Si conductivity type [4] and on a buffer polyethyleneimine (PEI) layer on the Si surface.

Here, we present results of determining the influence of salt concentration in GOx solution and electrical potentials of components forming n-Si/SiO₂/GOx and n-Si/SiO₂/PEI/GOx biosensor structures on the PSA efficiency of GOx molecules.

2. Experimental
2.1. Materials and methods
Experiments were performed with single-crystal Si wafers of n-type (ρ = 4 Ω·cm). Initially, the wafers were boiled in a peroxide-ammonia solution and rinsed in deionized water. This treatment led to “reconstruction” of a native oxide layer to SiO$_2$. It can be noted that zero charge point of the SiO$_2$ surface is ca. pH=2-3 [5], above this pH value the surface is negatively charged. Afterwards, wafers were cut into substrates of 8×8 mm$^2$.

GOx molecules from *Aspergillus niger* were used as enzyme molecules. GOx molecules in salt-free aqueous solution have an significant effective negative charge (zeta potential = -21 mV), while at low salt concentration in GOx solution (0.01 M NaCl) the negative zeta potential of GOx reduced to -5 mV (Fig. 1a). It should be noted that isoelectric point of GOx is ca. pH = 4.2 [6]. The results of our experiments confirm the above (Fig. 1b). This information is important for estimating the influence of electrostatic mechanism on the GOx layer adsorbed on the semiconductor substrate under illumination at different NaCl concentration in GOx solution. Therefore, the electrokinetic zeta potential and the hydrodynamic size of GOx molecules were controlled in all GOx solutions (0.5 mg/ml) at different concentrations of NaCl (0; 0.01; 0.05; 0.1; 0.25; 0.5 M). Measurements were performed using Malvern Zetasizer Nano Series (ZS) analyzer.

![Figure 1. Distributions of zeta potential of molecules: (a) PEI and GOx in aqueous solutions and at low salt concentration in GOx solution; (b) GOx at different pH values of aqueous solutions.](image)

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. PEI is positive charged in salt-free aqueous solution (Fig. 1a). This is consistent with data from Ref. [7]. The PEI molecules were adsorbed onto the silicon substrates from the 1 mg/ml aqueous solution during 10 min followed by rinsing in deionized water (ρ=18.2 MΩ·cm) during 10 min and drying in nitrogen flow.

The photo-assisted layer-by-layer adsorption technique suggested in Ref. [8] was used to adsorb GOx from the 0.5 mg/ml aqueous solution onto bare silicon substrates and ones covered with PEI. Two silicon substrates were placed in polyelectrolyte solution. One of the substrates was illuminated during GOx adsorption by a halogen lamp. The optical radiation of the halogen lamp is not absorbed by the GOx solution and does not heat the solution and substrate. However the wavelengths from the spectrum of the halogen lamp are absorbed by Si inducing the photogeneration of nonequilibrium charge carriers. Thus, the surface charge density of Si substrate is change. The results PSA of the GOx on to a silicon substrate were compared with the results of GOx adsorption in the dark.

2.2. Measurements
The characteristics of the nano-sized GOx layer adsorbed on a Si/SiO$_2$ or Si/SiO$_2$/PEI substrate depend on the electrostatic interaction between molecules of the adsorbate and the adsorbent. Thus, the electric potential was measured both for GOx molecules in solution and for Si/SiO$_2$ substrates before and after adsorption of PEI nanolayer.
The influence of NaCl concentration on the charge and potential of GOx in an aqueous solution was investigated using the electrophoretic light scattering method (Zeta Sizer Nano-ZS). The method allowed us to appreciate not only the effective charge of GOx in solutions with different NaCl concentration, but also a degree of agglomeration of GOx molecules in solutions, caused by Na$^+$ and Cl$^-$ counter ions.

The topography of the surface samples was measured by atomic force microscopy (AFM) method. The contact potential difference between the probe and the sample were measured by the Kelvin probe method (KPM). Measurements of KPM allow to appreciate changes of the surface electric potential of the samples after processing and applying nanolayers of polyelectrolyte molecules.

The measurements were carried out according to the 2-pass technique on the NTEGRA Spectra (NT-MDT Spectrum, Russia) installation in the semi-contact mode with scan frequencies of 0.2-0.5 Hz using HA_NC/W2C cantilevers of ETALON series with resonance frequency of 140±10% kHz. The Gwyddion software was used for statistical analysis of AFM and KPM data. When analyzing the AFM-images was taken into account that lateral size of enzyme molecules observed by AFM does not correspond to the real size of the enzyme molecule due to the effect of the probe on the object resulting in the artefacts appearance (“profile broadening” effect due to the tip-sample convolution). However, using the results from Ref. [9], we can recognize individual molecules of GOx on the scan.

3. Results and discussion

Figure 2 shows average diameters and zeta potential of GOx molecules in aqueous solution at different NaCl concentrations. Earlier in Ref. [10], the biocatalytic activity of GOx molecules at various salts in solution was studied. It was shown that the selected NaCl concentration range does not result in loss of a biocatalytic activity of GOx molecules in solution.

![Figure 2](image)

*Figure 2. Dependence of the average size of GOx molecules and the modulus of the zeta potential on NaCl concentration in an aqueous solution.*

Our research (Fig. 2) shows that small NaCl concentration (0.01 M) significantly reduces the charge of GOx molecules in solution. However, further increase of C$_{NaCl}$ neither changes the potential nor the charge sign of GOx. With a further increase of salt concentration, an increase in the hydrodynamic size of GOx molecules to 10 nm is observed. It can occur due to the screening of intermolecular repulsion, which results in GOx aggregate formation in solution.

Figure 3 shows AFM-images demonstrating the result of GOx adsorption onto the bare silicon surface in case of adsorption in the dark and under illumination. Similar images were obtained after GOx adsorption from solutions at NaCl concentrations indicated in section 2.1, as well as after GOx adsorption onto Si with PEI monolayer.

The values of average height of irregularities, surface potential and mean surface coverage with GOx molecules for each sample were obtained using Gwyddion software (Fig. 4-6).
According to the Figure 4, PSA of GOx molecules onto the bare Si surface is effective at high NaCl concentrations in solution, while PSA of GOx molecules onto surface of the n-Si/SiO$_2$/PEI structure is effective at low C$_{NaCl}$.

The results correlate with values of surface coverage versus NaCl concentration presented in Figure 5. The surface coverage with GOx molecules was calculated as a percentage of area covered with GOx molecules from the total substrate area. Growth of NaCl concentration leads to increase in the surface coverage with GOx molecules adsorbed onto bare Si substrate by an order of magnitude compared to a salt-free solution. The deposition of a PEI buffer layer leads to an increase in the surface coverage for all samples compared to the surface of bare Si, while growth of NaCl concentration leads to decrease in the surface coverage with GOx molecules.

Illumination of Si during GOx adsorption increases the value of surface coverage. The increase in the surface coverage is greatest at 0.01 M NaCl for structure with PEI layer and at 0.1 M NaCl for structure without it. With a further increase of salt concentration, the difference between the GOx adsorption in the dark and under illumination is reduced. Above C$_{NaCl}$ = 0.1 M, the effect of illumination is effectively negligible.
Figure 5. Surface coverage with GOx molecules on the surface of (a) Si/SiO$_2$ and (b) Si/SiO$_2$/PEI substrates at different NaCl concentrations in GOx solutions.

Since fragments (a) and (b) in Figure 4 and Figure 5 differ in the surface charge sign (the SiO$_2$ surface is negatively charged, while PEI is a cationic polyelectrolyte), the influence of NaCl concentration on PSA must be explained by a change in the electrostatic interaction between GOx molecules and substrate surface.

The correct interpretation of the obtained results is possible when changes in the charge state of GOx and the semiconductor substrate, induced by illumination and NaCl concentration, are compared. Figure 6 illustrates change in the surface potential $\Delta \varphi$ after adsorption of GOx onto the surface of Si/SiO$_2$ and Si/SiO$_2$/PEI substrates in the dark and under illumination versus NaCl concentration of GOx solutions.

The value of $\Delta \varphi$ is determined using Eq. (1):

$$\Delta \varphi = \frac{\varphi_{GOx} - \varphi_{PEI}}{\varphi_{PEI}} \times 100\%$$  \hspace{1cm} (1)

It should be noted that $\Delta \varphi$ values are negative both at $C_{NaCl} = 0$ and in a case of GOx adsorption onto the Si/SiO$_2$/PEI surface. At a non-zero NaCl concentration, GOx adsorption onto Si/SiO$_2$ surface only leads to positive $\Delta \varphi$.

Figure 6. Change of surface potential after GOx adsorption on to the surface of (a) Si/SiO$_2$ and (b) Si/SiO$_2$/PEI substrates at different NaCl concentration.

To explain the results, we used the results of studies in the article [1], as well as information about the structure and composition of the GOx enzyme. Since GOx is a complex protein, despite the effective negative charge of the GOx molecule, there are positively charged regions on its surface grouped on the “back” side of the molecule [1]. On positively charged surfaces (for example, Si/SiO$_2$/PEI), electrostatic attraction of GOx molecules to the substrate prevails. When NaCl is added to the solution, Na$^+$ ions are attracted to GOx and screen its negatively charged groups. It was established that the positive charge of GOx is not screened even at high NaCl concentration (e.g., 0.5 M), since the potential of GOx as a whole remains negative (Fig. 2), which repels the Cl$^-$ anions from GOx. This fact explains the decrease of GOx adsorption onto the Si/SiO$_2$/PEI surface at $C_{NaCl} > 0.1$ M and the low GOx adsorption onto the negative Si/SiO$_2$ surface in the dark. Under illumination the surface charge of n-Si decreases. Therefore, the
electrostatic repulsion weakens and GOx molecules adsorbed onto Si surface by the “back” positively charged side. This orientation of GOx is preferred for biotechnology, because it facilitates the access of the substrate (glucose) to the active center (flavin-adenine dinucleotide).

4. Conclusion
Thus, an increase in the salt concentration in the solution leads to an increase in the number of adsorbed GOx molecules of more than 20 times compared to the salt-free solution. Moreover, salt addition increases the efficiency of PSA. The number of adsorbed GOx molecules onto Si/SiO₂ substrate due to illumination increases by 10–20% at C_{NaCl} = 0.1 M. Moreover, when NaCl is added to the solution, the GOx molecules are fixed by their positive sites to the negatively charged Si/SiO₂ surface. GOx molecules immobilized on the surface with the above orientation retain biocatalytic activity. Thus, the improvement of the sensitive layer parameters of enzyme-based biosensor occurs due to the choice of the mode of semiconductor substrate illumination and salt concentration in solution.

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