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Gold nanoparticles and polypyrrole for glucose biosensor design

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Abstract

In this study electrochemistry of glucose oxidase (GOx) immobilized on a carbon rod electrode modified by gold nanoparticles (Au-NPs) and π-π conjugated polymer - polypyrrole (Ppy) was studied. Au-NPs facilitated indirect electron transfer via red-ox mediator and showed a positive effect on the amperometric signals of such type electrodes. Several types of electrodes modified by GOx and Au-NPs of different sizes and additionally covered by Ppy layer were investigated. The sensitivity and stability of developed biosensors were evaluated and compared.

Keywords: Gold nanoparticles, biosensors, polypyrrole, conjugated polymers;

1. Introduction

Immobilization of enzymes is the most challenging and important step in the development of biosensors. Immobilized enzymes have many operational advantages over the free enzymes including reusability, continuous operational mode, easy separation from the reaction mixture, and possible modulation of the catalytic properties. Fast, simple and low-cost detection of biologically active analytes is the major advantage of biosensors [1,2]. In some cases a combination of nanomaterials and nanotechnological approaches resolve challenging bioanalytical problems, including specificity, stability and sensitivity. Conjugated polymers provide an effective immobilization patterning for biomolecules on different substrates and in some cases facilitate electron transfer from enzymes to electronically conductive electrodes and improve biosensor sensitivity [3].

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The main aim of this research was to investigate the influence of π-π conjugated polymer, polypyrrole, formed over GOx/CR and GOx/Au-NPs/CR electrodes modified with 3.5, 6.0 and 13 nm gold nanoparticles on sensitivity and stability of biosensors. The influence of the formed Ppy layer on the Michaelis-Menten kinetics of designed electrochemical biosensors was investigated.

2. Experimental

Three different types of electrodes were prepared: carbon rod modified with GOx (GOx/CR); Au-NPs and GOx (GOx/Au-NPs/graphite); with GOx and Au-NPs (Au NPs/GOx/graphite). During preparation of GOx/CR electrode 3 µl of 40 mg mL^{-1} GOx solution were deposited on the electrode and water was evaporated at room temperature by intensive ventilation. For the preparation of Au-NP/GOx/CR electrode additionally 3 µl of gold nanoparticles colloid solution were deposited on the GOx/CR electrode. For the preparation of GOx/Au-NPs/CR electrodes 3 µl of gold nanoparticles colloid solution were deposited on the working electrode and after evaporation of water additionally 3 µl of 40 mg mL^{-1} GOx solution were deposited. After water evaporation all electrodes were stored for 15 min in a closed vessel over 25% solution of glutaraldehyde at room temperature [4].

Chemical polymerisation of pyrrole over GOx/CR and GOx/Au-NPs/CR electrodes was performed in 0.05 mol L^{-1} sodium acetate buffer (SA), pH 6.0, containing 0.05 mol L^{-1} glucose and 0.5 mol L^{-1} pyrrole (polymerisation solution) for a defined period at +4°C. For the evaluation of Au-NP size (3.5, 6 and 13 nm) effect on the performance of the electrodes before and after the polypyrrole layer formation, electrodes (GOx/3.5Au-NPs/CR; GOx/6Au-NPs/CR; GOx/13Au-NPs/CR and GOx/CR) were immersed in the polymerisation solution for appropriate time. Prior to electrochemical measurements the electrodes were thoroughly washed with distilled water [5].

All electrochemical measurements were performed using a computerized potentiostat PGSTAT 302N/Autolab EcoChemie, Netherlands) with GPES 4.9 software in amperometry mode at working electrode potential of +0.3 V vs Ag/AgCl. A conventional three-electrode system comprising a working carbon rod electrode (modified as it was described above), 2 cm^2 platinum as an auxiliary electrode and Ag/AgCl with 3 mol L^{-1} KCl Metrhom (Herisau, Switzerland) as a reference electrode was employed for electrochemical experiments. All experiments were performed at room temperature in stirred 0.05 mol L^{-1} SA, pH 6.0, with 0.1 mol L^{-1} KCl. Electrochemical detection of the analytical signal was performed at different concentrations of glucose in the presence of Au-NPs without and with 2 mmol L^{-1} of PMS. The activity of GOx was estimated by measuring the oxidation current of PMSH2 and/or H_2O_2 at +0.3 V potential vs Ag/AgCl [6].

The kinetic parameters: the maximal current during enzymatic reaction and the apparent Michaelis constant are correspondingly *a* and *b* parameters of hyperbolic function $y=ax/(b+x)$ used for approximation of results.

3. Results and discussion

13 nm gold nanoparticles showed a positive effect on the amperometric signals of GOx/Au-NPs modified electrodes. This might be explained by a significantly increased electron transfer rate from GOx to graphite electrode, where Au-NPs increased effective surface area of electrodes and/or played a role of a red-ox mediator as it was reported in other studies. The best amperometric signals were obtained when gold nanoparticles were immobilized between the electrode surface and GOx (GOx/Au-NPs/CR). This type of electrodes showed higher sensitivity, lower limit of detection and good reproducibility [4] and was used in our further study.

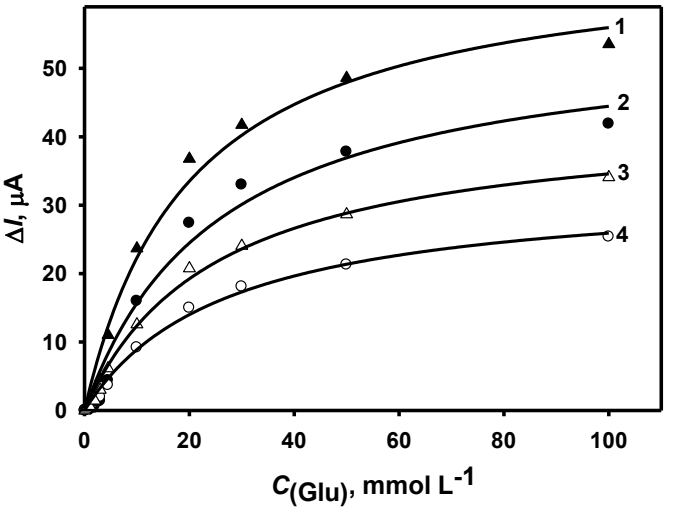

Fig. 1. Calibration plots of GOx/Au-NPs/CR (1, 3 curves) and GOx/CR (2, 4 curves) electrodes before (1, 2 curves) and after (3, 4 curves) chemical polymerisation of pyrrole. The size of Au-NPs – 13 nm. Polymerization time – 5 h.

Gold nanoparticles present on the electrode surface allow a more favourable configuration of the immobilized enzyme molecules. Based on the obtained amperometric results it could be predicted that some Au-NPs were close to the red-ox site of GOx. This, in turn, reduced the insulating properties of the enzyme shell, thereby enabling the active sites of the enzyme to get closer to the electrically conducting surface of graphite/gold, this way decreasing formal resistance of the electrochemical system, reducing diffusion distance for the oxidized/reduced forms of the electron transfer mediator and facilitating electron transfer between the enzyme and the electrode [7].

Hyperbolic dependences of amperometric signals on the concentration of glucose in the range from 0.1 to 100 mmol L^{-1} were observed for two types (GOx/CR and GOx/Au-NPs/CR) of electrodes before and after the chemical polymerisation (5 hours) of pyrrole (Fig. 1). All presented dependences were in agreement with Michaelis-Menten kinetics. Additionally, Au-NPs in a combination with the enzymatically formed Ppy layer offered some advantages for the design of electrochemical biosensors. The sensitivity of analytical systems with Ppy depended on the sizes of Au-NPs. Systems based on smaller Au-NPs showed a higher amperometric response at the same substrate concentration before and after the formation of Ppy layer [5].

The stability of GOx/Au-NPs/CR electrodes with Ppy layer after 4, 12 and 40 days was investigated. Electrodes modified with polymer (after 13 hours of chemical polymerization) were stored at +4°C in a

closed vessel hanging over the solution of 0.05 mol L^{-1} SA, pH 6.0. During the investigations it was found that after 40 days the GOx/Au-NPs/CR electrode modified with 3.5 nm Au-NPs was less stable than the electrode modified with 13 nm Au-NPs, so the stability of developed electrodes depended on the size of Au-NPs. Analytical signal of the investigated biosensors decreased faster using electrodes modified with smaller nanoparticles. After the registration of analytical signal by GOx/Au-NPs/CR electrode modified with 13 nm Au-NPs, the 50 % of maximal current was obtained after 12 days, 6 nm Au-NPs – after 7 days, 3.5 nm – after 3 days, respectively. It can be concluded that after 40 days the sensitivity of the developed analytical systems based on 3.5 nm, 6 nm and 13 nm Au-NPs and modified by polypyrrole decreased to 9.02 %, 16.1 % and 21.8 %, respectively.

4. Conclusions

Enzyme glucose oxidase in a combination with Au-NPs of different sizes and enzymatically formed polypyrrole layer offered some advantages for the design of electrochemical biosensors. The sensitivity of analytical systems with Ppy depends on sizes of Au-NPs. Smaller Au-NPs based systems showed higher amperometric response at the same substrate concentration before and after the formation of Ppy layer. However, the best stability was observed for electrodes modified with 13 nm Au-NPs.

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