NOVEL PLATFORM FOR DIRECT ELECTRON TRANSFER TO/FROM HEMOPROTEINS BASED ON A SINGLE LAYER GRAPHITE ELECTRODE

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Importance of electrode material choice could be compared to that of solvent in chemical reaction. Since electron transfer rate and effectiveness of electrochemical conversion depends on electrode substrate material [1–3].

There is a scope for development of electrochemical device, with an improved sensitivity to biological samples. Direct electron transfer to/from redox proteins was observed on highly ordered pyrolitic graphite electrodes, [4–6; 7–9]. Pyrolitic graphite is a material with a highly ordered crystal structure and anisotropic properties, which electrochemical properties depend on it orientation. However, highly ordered carbon materials are expensive, which limits its area of its application in bioelectrocatalysis, where most of the samples are short living and therefore demands frequent surface regeneration.

Current study suggests design of multiple carbon layer electrodes with similar surface properties from a single piece of highly order pyrolitic graphite (HOPG).

Surface layer of the (HOPG) was stripped off by a carbon conductive selotape [10], which was connected via the silver nanowire into the standart three electrode cell as a working electrode. Resulting carbon layer electrodes (CLEs) were evaluated by cyclic voltammetry so that to evaluate its electrochemical intertness. The obtained CVs of CLEs in buffered solution showed no peaks, which indicates that there was no internal electrochemical reaction occurring within the structure of CLEs (data not shown). The next step we tested the area electro active surface of CLEs, which we compared with total surface area of CLE, obtained by another method.

There were two peaks observed on CV of 0.01 M $K_3[Fe(CN)_6]$ in buffered solution measured with CLEs (fig. 1,*a*). The observed peaks had similar shape, which indicates a reversible reduction/oxidation process of the $K_3[Fe(CN)_6]$ (fig. 1,*a*). Peak separation values was 93 mV at the scan rate 20 mV, which corresponds to the quasi- reversible system and could be due to the low concentration of the supporting electrolyte in the system. The peak current values were linearly proportional to square root of the scan rate, indicating lack of side electrochemical processes in the system (fig. 1,*a*, inset). Electrode surface area was estimated in therms of Eq. (1) and was found 0.53 cm^2 respectively.

$$i_p = 0{,}4463nFA\sqrt{\frac{nF\nu D}{RT}} \qquad (1)$$

where, n is the number of electrons appearing in half-reaction for the redox couple, v is the rate of potential sweeping (v / s^{-1}), F is Faraday's constant (96485 Q/ mol), A is the electrode area (cm^2), R is the universal gas constant (8.314 J / mol K), T is the absolute temperature (K), and D is the $K_3[Fe(CN)_6]$ diffusion coefficient (cm^2/sec). Alternatively the surface area was estimated by weighting the backpaper of the selotape and was 0.55 cm^2. Determined values were in a good agreement showing that all CLE's surface was electro-active.

The next step we tested the obtained CLEs in a model bioelectrocatalytic process, such as electrochemical regeneration of hemoproteins. The model proteins shocs were myoglobin (Mb) and hemoglobin (Hb). As it was previously reported, Mb and Hb could be electrochemically regenerated on basal plane pyrrolitic graphite electrodes while casted in didodecyldimethylammonium bromide (DDAB) film, so we used DDAB film so that immobilise Mb and Hb on CLEs [11, 12].

The cyclic voltamperogramm of obtained CLE/Mb/DDAB is shown at the fig. 1B. As it could be seen, immersion of CLE/DDAB in Mb solution was accompanied by appearance of two peaks on CV, which corresponds to oxidative and reductive process of Mb. The ΔE_p was 73 mV for Mb and 150 mV for Hb, which could be due to the low ionic strength of the tested solution. However it clearly indicates the ability of a Mb and Hb to transfer electrons directly on the CLEs surface on, without any need for a mediator.

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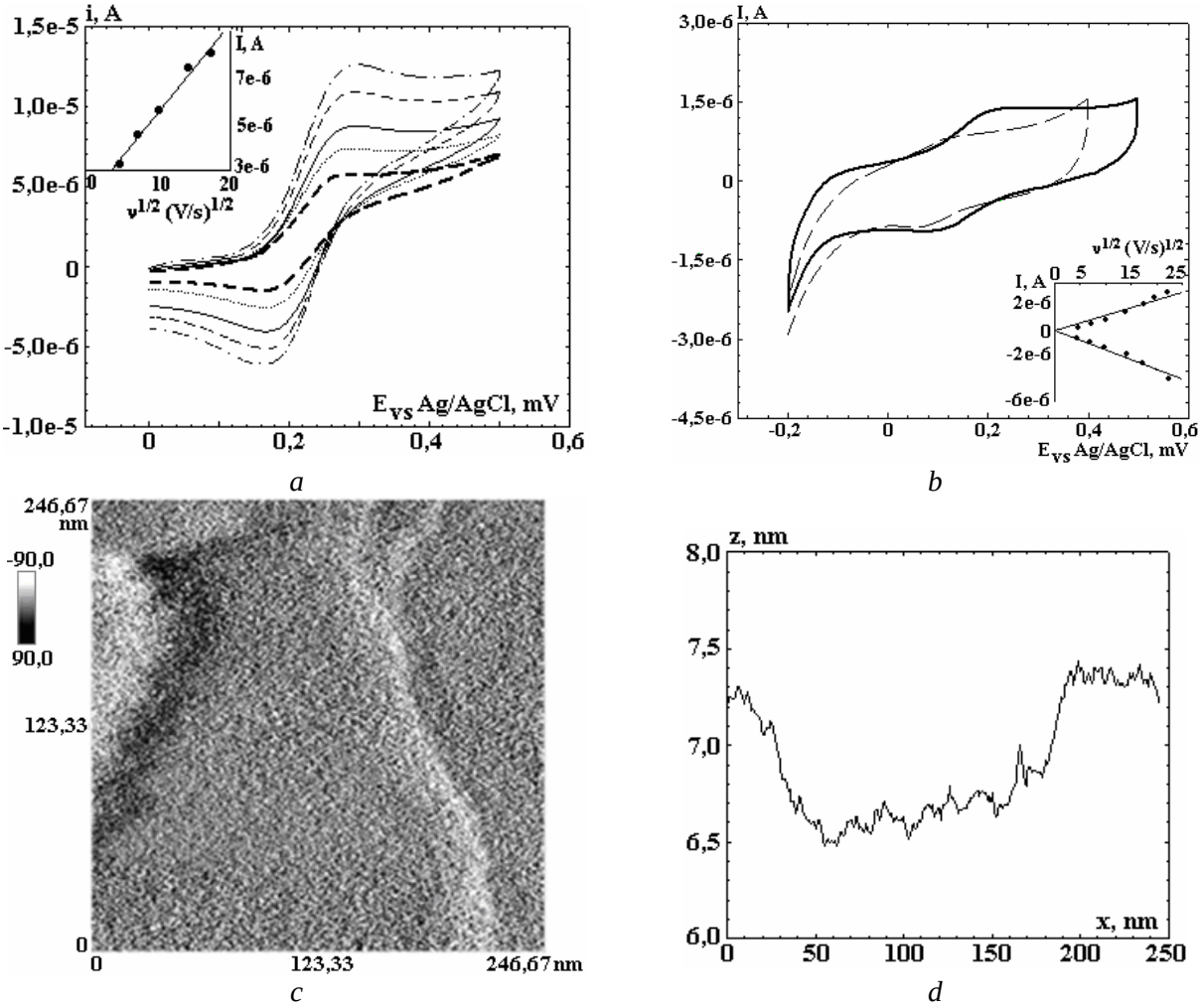

Fig. 1,a. Cyclic votammogramms of the HOPG electrode in the 0.1 KCl, 50 mM tris-HCl, 0.01 M $K_3[Fe(CN)_6]$ at the scan rates 20, 50 100, 200, 300 mV/s. b. Cyclic votammogramms of the HOPG electrode immobilized of Hb-DDAB (black line) /Mb-DDAB (dashed line) scan rates 20 mV/s, 50 mM tris-HCl. c. AFM image of surface of CLE. d. Line analysis of CLE surface

Next step, we tested morphology of electrode surface by the atomic force microscopy (AFM), which is shown at (fig. 1,*c*,*d*). Overage of the surface roughness was 1 nm as it could be seen on the AFM image. Observed step was 1 nm, which matches the size of a single carbon layer. The distance between the steps were reproducible for all studied CLEs and well match quality of the original HOPG material, which suggest that defects are inherited from the precursor HOPG.

Materials and Methods

HOPG was ordered from SP and was SPI-3 grade. The carbon double layered selotape was from Nishin em. Co. ltd. The silver wire was from Goodfellow Cambridge and has 0.025 mm diameter. Myoglobin (horse skeletal muscle), haemoglobin (human), and tris[hydroymethyl]aminomethane were obtained from Sigma. (DDAB) and potassium ferrocyanide and potassium chloride were from Aldrich. The surface of the HOPG was cleaved with the carbon conductive selotape. The carbon selotape was pressed into the flat surface and then gently pulled off. Tape invariable takes off the thin layer of HOPG. The area covered with HOPG was cut off with scissors and stick onto the non-conductive glass. The electrical contact was established via silver wire connected to the carbon selotape on the other side. The square of the electrode surface was estimated by weighting the backpaper of the carbon selotape. The electrical connection was established via the silver wire.

Immobilisation of Hb and Mb was performed as it was described [12] Didodecyldimethylammonium bromide (DDAB) vesicles were prepared by sonication of the 1 mM solution of the DDAB film in water. Formed vesicles were misxied with 1 μM solution of protein (Hb or Mb) and filtered throught the 0.22 μ so that the reformed vesicles would occlude protein molecules. ClE's surface was immersed in to the solution and let dry on air.

The AFM images reported in this study were obtained using an Explorer™, Scanning Probe Microscope, (TopoMetrix-ThermoMicroscope-VEECO). Non-contact mode was used throughout. The tips used were high resonance frequency silicon tips (frequency range 354–409 KHz), with a 120 micron long cantilever and tip of 3–6 micron base, 10–20 micron in length and with a 20 nm tip radius. The raw data collected were processed by the TopoMetrix SPMLab NT Version 5.0, using left shadowing.

Therefore, current study is suggesting novel cheap and easy way of producing highly ordered CLEs, which could be used for bioelectrochemical application. Low production cost allows to generate multiple disposable CLE, which highly ordered structure maintain the properties of original piece of HOPG.

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Summary

A novel cheap and easy way of producing carbon electrodes from single layer graphite is proposed. Low production cost allows to generate multiple disposable carbon layer electrodes. The electrodes were tested in a model bioelectrocatalytic process, such as electrochemical regeneration of hemoproteins, and positive results were obtained.