Applied Surface Science xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Applied Surface Science

journal homepage: www.elsevier.com/locate/apsusc

Nano-structured carbon materials for improved biosensing applications

J. Razumiene[a,*], I. Sakinyte[a], J. Barkauskas[b], R. Baronas[c]

[a] Institute of Biochemistry, Vilnius University, Mokslininku 12, Vilnius 08662, Lithuania
[b] Faculty of Chemistry, Vilnius University, Naugarduko 24, Vilnius 03225, Lithuania
[c] Faculty of Mathematics and Informatics, Vilnius University, Naugarduko 24, Vilnius 03225, Lithuania

ARTICLE INFO

Article history:
Received 11 June 2014
Received in revised form
10 September 2014
Accepted 11 September 2014
Available online xxx

Keywords:
Graphite oxidation products
Surface function groups
Bioelectrocatalysis
Direct electron transfer
PQQ-GDH

ABSTRACT

A set of oxidized graphite samples have been newly synthesized using different protocols. Atomic force microscopy, Raman spectroscopy, thermal gravimetric analysis and Brunauer–Emmett–Teller analysis revealed the changes in structure and functionalities of obtained graphite oxidation products (GOPs) compared to pristine graphite. The substances have been tested as electrode materials applicable for bioelectrocatalytic systems using pyrroloquinoline quinone-dependent glucose dehydrogenase (PQQ-GDH). The application of GOPs allowed achieving the direct electron transfer (DET) from active site of PQQ-GDH to the electrode surface. Needless of additional electron transfer (ET) mediating compounds highly improved features of the biosensors. The efficiency of the biosensors has been evaluated for all types of biosensors varied from 32 μA/cm^2 to 64 μA/cm^2 using as electrode materials GOP1 and thermally reduced graphite oxide (TRGO), respectively. TRGO containing function groups (according TGA, ~6% of the weight loss) and smallest particles (average diameter was ~11 nm and the average height was ~0.5 nm) exhibited the higher efficiency for ET acceleration in the biosensor acting on principle of DET.

1. Introduction

Bioelectrocatalysis is an acceleration of the electrochemical reaction by using of biological catalysts which usually are enzymes or whole cell systems [1]. The integration of them into industrial processes for effecting the conversion of readily available, inexpensive starting materials to high value products is strongly demanded nowadays. Enzymes are fully recyclable catalytic proteins that frequently display exquisite chemo-, enantio- and regioselectivity and operate under mild conditions of pH and temperature. These characteristics make them cost-effective and sustainable catalysts for a wide range of chemical transformations. However, for functioning of bioelectrocatalytic process it is necessary to achieve an effective electron transfer (ET) between active sites of biocatalysts and electrode surface. The implementation of industrially promising biocatalysts especially oxidoreductases is confronted with difficulties concerning availability of an efficient ET. Owing to overcome these problems redox mediators, which promotes regeneration of cofactors by improving of ET between active site of enzyme and electrode surface usually are used. A great number of electron donors or acceptors are known and used in chemical technologies, however, many of them suffer of low efficiency or are expensive. Thus, there still is a lack of cheap but efficient ET systems applicable for enzyme catalyzed technologies.

As alternative for mediating ET, direct ET (DET) in a bioelectrocatalytic system can be achieved. For biosensors based on DET the absence of mediators is the main advantage, providing them with superior selectivity, both because they should operate in a potential window closer to the redox potential of the enzyme itself, and therefore, less prone to interfering reactions and also because of the lack of yet another reagent in the reaction sequence [2]. It can be obtained by choosing the suitable enzyme, which could perform DET, coupled with the appropriate electrode material, on which this enzyme can function [3]. Obviously, carbon nanomaterials are promising in this field due to their electroconductivity, biocompatibility, and tunable structural properties. Each sort of carbon nanomaterial has a complex of specific properties, which depends on the structure of the certain nanoform. Carbon nanoparticles are highly attractive due to the big variety of forms, which can be tuned to the different enzyme-containing systems. One of the methods to obtain new carbonaceous materials is chemical oxidation of graphite. Oxidation creates oxygen containing functional groups at the graphene surface such as hydroxyl, epoxide, carbonyl and

* Corresponding author. Tel.: +370 67061893.
E-mail addresses: julija.razumiene@bchi.vu.lt, julija.razumiene@gmail.com (J. Razumiene).

http://dx.doi.org/10.1016/j.apsusc.2014.09.063
0169-4332/

carboxyl resulting in polar surface properties. Therefore, graphite oxide (GO) behaves strongly hydrophilic, and is easily exfoliated in water forming stable colloidal dispersions. The complete exfoliation into sheets of atomic thickness can also be achieved either by thermal or mechanical treatments. As synthesized, graphite oxide typically possesses a carbon to oxygen ratio of about 2, and the material is non-conducting. Each fundamental layer of GO consists of a dense two-dimensional carbonaceous skeleton containing a larger number of sp^3 hybridized carbon atoms and a smaller number of sp^2 carbons. GO can be reduced either by heating or by reducing agents, and changes into a graphite-like structure with turbostratic tendency containing few oxygen groups [4].

Nowadays also graphene has been widely studied [5–7] and applied for the development of optoelectronic devices, super capacitor and various types of high performance sensors due to its high surface-area-to-volume ratio, excellent electrical conductivity and high electron mobility [8–10].

Graphene, with a large surface area, enhances the loading of biomolecules by passive adsorption or covalent crosslinking, while its excellent conductivity and small band gap are beneficial for the conduction of electrons between the biomolecule and the electrode surface. It has been claimed that graphene may not be beneficial as an electrode material, due to its lower edge surface area, leading to slow heterogeneous electron transfer. The surface coverage and orientation of graphene on the electrode may also significantly affect its electrochemical performance [11]. Graphene is an inexpensive material with good mechanical, electrical and thermal properties. The large surface to volume ratio and biocompatibility are attractive parameters for its potential application in the fabrication of electrochemical biosensors. However, it is difficult to prepare a uniform dispersion of graphene in aqueous solution due to its hydrophobic character. While GO is highly soluble in water because of the presence of remarkable amount of oxygen containing functional groups on its edge plains and surface [12]. Also, the functional groups are useful for anchoring enzymes and it was demonstrated successful immobilization of enzyme HRP on electrochemically reduced graphene oxide [13].

Biosensing arrays, based on bioelectrocatalytic reactions, are not very common in regular clinical use yet, however they may enable real time monitoring of blood sugar level in future and thus direct control of insulin dosing for diabetes patients. So far, enzymatic bioelectrocatalysis of glucose oxidation is only exploited in test-strips [14] and here the interference of other blood constituents plays a crucial role. However, the establishment of these exemplary devices may open the door for a multitude of different electrochemical biosensors for medical applications. For environmental monitoring, however, the main concerns for the application of electrochemical biosensors are the number of possible side-reactions and the diversity and concentration range of denaturing agents that far exceed the conditions existing in the medical sphere. As discussed above, this will not only limit the selectivity of the sensor, but also its lifetime [15].

The aim of this research was to discover carbon materials prospective to be employed in the reagentless biosensing systems, which are able to operate on the principle of the DET. For this reason a few types of graphite nanoparticles have been manufactured by oxidation of graphite using different protocols and further such products (GOPs) have been tested as the electrode material for the amperometric biosensors working with pyrroloquinoline quinone dependent glucose dehydrogenase from *Acinetobacter calcoaceticus*. While various oxidoreductases are used for bioelectrocatalysis [16–18], however, due to insensitivity to oxygen and tightly bound cofactor PQQ-dependent enzymes have clear advantages when comes to development of various electrocatalytic processes [19–21]. The PQQ dependent enzymes can be as alternative biocatalysts for wide range of reactions. In our previous report it was shown possibility to design glucose biosensor working on a principle of DET [22]. DET was achieved by synthesized a few appropriate electrode materials and using these in bioelectrocatalytic system based on PQQ-GDH. Since, the implementation of biocatalysts acting on principle of DET allows highly improve the industrially technologies it is very important to study all steps of biosensors design. Thus, in this paper the similar approach for synthesis of some more carbonaceous materials has been applied and the detail characterization of products has been performed.

2. Materials and methods

2.1. Materials

PQQ-GDH from *Acinetobacter calcoaceticus* L.M.D. 79.41 was purified according to the known protocol [23] and was kindly provided by the Department of Molecular microbiology and biotechnology (Institute of Biochemistry). The PQQ-GDH solution of 18000 U/ml was used as a solution in 5 mM Tris–HCl buffer (pH 8.5). D-Glucose solution (100 mM) was used as a default substrate. Laccase from *Coriolopsis byrsina* was separated according to the protocol [24] and was kindly provided by the Department of Molecular microbiology and biotechnology (Institute of Biochemistry).

Sodium acetate, acetic acid, $CaCl_2$ and D-glucose were obtained from J.T. Baker (Holland, NL). 95–97% H_2SO_4, Penta, USA. Ethanol, KCl were purchased from Riedel-de Haen (DE). Graphite was obtained from Merck (extra pure grade). Other chemical reagents were obtained from Sigma–Aldrich and were of analytical grade unless otherwise mentioned.

2.2. Preparation of electrode materials

2.2.1. Graphite oxide (GO)

GO was synthesized using the protocol proposed by Hummers and Offeman ($KMnO_4$ and $NaNO_3$ in concentrated H_2SO_4) [25]. According to this protocol 2.5 g of $NaNO_3$ and 5 g of graphite powder was gradually put into 115 ml of concentrated H_2SO_4 by keeping temperature of 273 K. After that 15 g of $KMnO_4$ was added gradually under stirring and the temperature of the mixture was kept below 295 K. After all operations, the mixture was stirred at 308 K for 30 min and then carefully diluted with 230 ml of distilled H_2O. After that concentrated H_2O_2 was added into the mixture until the total volume of 700 ml. The slurry was filtered and further decanted with H_2O aiming to remove the acidity of the filtrate until the neutral pH. Further GO particles were dried for one day under atmospheric conditions and successively for one weak in a vacuum.

2.2.2. GOP1 and GOP2

GOP1 and GOP2 samples have been prepared by carrying out the synthesis in the alkaline media. Two types of pristine graphite powder: as-purchased, and sonified have been treated with concentrated H_2O_2 under temperature not exceeding 273 K during 3 weeks. Firstly, 50 ml of saturated KOH solution in H_2O_2 was prepared under stirring and cooling and 5 g of graphite was added into this solution gradually under stirring and the temperature of the mixture was kept below 278 K. Secondly, the mixture was kept at 273 K for 3 weeks. Further the reaction mixture was decanted and rewashed with deionized water until the pH revived neutral. Then the powder was dried under atmospheric conditions. Two types of the graphite GOP1 and GOP2 have been obtained by proposed protocol accordingly to the pristine graphite powder: as-purchased or sonified.

2.2.3. GOP3 and GOP4

GOP3 and GOP4 samples have been prepared by treating two types of pristine graphite powder: as-purchased and sonified with

Please cite this article in press as: J. Razumiene, et al., Nano-structured carbon materials for improved biosensing applications, Appl. Surf. Sci. (2014), http://dx.doi.org/10.1016/j.apsusc.2014.09.063