Journal of Electroanalytical Chemistry 775 (2016) 235–242

Contents lists available at ScienceDirect

Journal of Electroanalytical Chemistry

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

Three-dimensional graphene/carbon nanotubes hybrid composites for exploring interaction between glucose oxidase and carbon based electrodes

Vinicius R. Gonçales [a,1], Rafael N.P. Colombo [a], Marco A.O.S. Minadeo [a], Elaine Y. Matsubara [b], J. Maurício Rosolen [b], Susana I. Córdoba de Torresi [a]

[a] Instituto de Química, Universidade de São Paulo, POBox:26077 – Cidade Universitária, São Paulo, SP, Brazil
[b] Departamento de Química-FFCLRP, Universidade de São Paulo, 14040-901 Ribeirão Preto, SP, Brazil

ARTICLE INFO

Article history:
Received 13 January 2016
Received in revised form 2 June 2016
Accepted 7 June 2016
Available online 9 June 2016

Keywords:
Glucose oxidase
Graphene
Carbon nanotube
Felt
Three-dimensional

ABSTRACT

This work presents a 3D carbon composite electrode matrix, consisting of reduced graphene oxide (RGO), carbon nanotubes, and carbon fiber felt. In this matrix, the edges of the RGO sheets are bounded to cup-stacked carbon nanotubes (CSCNTs) while the micrometer scale basal planes of the RGO sheets are suspended between the fibers. The presence of free-standing RGO sheets in which π orbitals of the basal planes do not interact with the electrode surface offers advantageous conditions to explore demanding bioelectrochemical reactions, such as the $FADH_2$/FAD redox transition often claimed in the literature as originated from a direct electron communication between micro/nano carbon based electrodes and glucose oxidase (GOx). At least, three important questions rise from this system: (i) how the junction between the carbon nanostructures and the carbon microfibers affects the monitored electron transfer? (ii) are the monitored Faradaic peaks originated from native or denaturated GOx? (iii) is GOx still active for glucose sensing after suffering conformational changes due to the adsorption process on the surface of the electrode? It is intended here to contribute with the investigation of these questions. According to our results, the electronic interactions between CSCNTs and RGO sheets seem to modulate the performance of the aforementioned reaction, as improved kinetic parameters were observed on the 3D composite matrix rather than the obtained with 3D electrodes modified with CSCNTs or RGO sheets themselves. However, spectroscopic experiments suggested that GOx suffers significant conformational changes when adsorbed on the surface of the carbon nanostructures, leading to the denaturation of the protein. Even though the electrochemical experiments indicate that the denaturation of the enzyme does not seem to completely release the FAD moiety, the operational capability of the resulting glucose biosensor was greatly compromised.

1. Introduction

The development of third-generation glucose electrochemical biosensors is based on an attempt to achieve direct electronic communication between glucose oxidase (GOx) and the electrode itself. [1–4] Such biosensors would be able to work at potentials closer to the redox potential of the cofactor, which would improve biosensor selectivity and should minimize the severity of issues related to the instability of mediators [5]. This configuration would also allow for the calculation of the kinetic parameters involved in the hypothesized direct electron transfer process, which is interesting for mechanistic and relevant fundamental studies. However, the flavin adenine dinucleotide (FAD) moiety of GOx that is fundamental to direct electron transfer is embedded rather deeply within the protein, [6] so achieving efficient direct electron communication between the enzyme and the electrode remains a controversial subject.

Reduction of the electron tunnelling distance is an attempt to achieve communication between the enzyme and the electrode. In one example, Degani and Heller [7] attached electron relays to the protein backbone of the enzyme, suggesting an enhancement of the electron transfer between the electrode and the protein redox site by providing a series of smaller jumps. In another approach, Xiao et al. [8] and Katz and Willner [9] incorporated gold nanoparticles into the redox enzymes to suggest the preparation of a biomaterial in which gold acted as a nanoscale current collector and relayed electrons to the macroelectrode. Another strategy claimed by Guiseppi-Elie et al. [10] did not shorten the distance of electron tunnelling. Instead, it was

E-mail addresses: vromero@iq.usp.br (V.R. Gonçales), rosolen@ffclrp.usp.br (J.M. Rosolen), storresi@iq.usp.br (S.I. Córdoba de Torresi).
[1] Present Address: School of Chemistry, The University of New South Wales, Sydney, NSW 2052, Australia.

http://dx.doi.org/10.1016/j.jelechem.2016.06.002
1572-6657/

suggested the creation of a full conductive pathway that transported the electron between GOx and the electrode. The use of small (diameters of approximately 1 nm) and mechanically rigid single-walled carbon nanotubes (SWCNTs) were claimed for enabling this connection [11, 12].

Another recent approach is the use of graphene-based materials with the aim of mediating electron transfer between the enzyme and the electrode [13–18]. However, graphene consists of a two-dimensional carbon sheet obtained with areas on the micron or centimetre scale, which arise questions about how the carbon structure mediates electrochemical communication between the active redox site and the electrode. [15] Indeed, one should note that the active site of GOx is only 4 Å and funnels down 13 Å to the actual FAD. Shao et al. [19] demonstrated that GOx undergoes substantial conformational changes after assembly onto the free-standing graphene oxide that serve to expose the FAD moiety of the enzyme. Zhao et al. [20] reported that this same phenomenon also occurred in the case of GOx immobilized onto multi-walled carbon nanotubes (MWCNTs) with diameters between 10 and 20 nm, albeit to a lesser extent. Therefore, interactions between supported carbon nanomaterials and GOx could be responsible for exposing the FAD moiety to the electrode. Here, it is intended to explore the utilization of carbon based micro/nanomaterials to investigate if GOx would be still active for glucose biosensing after enzyme adsorption and exposition of the FAD moiety.

It is known though that the electronic properties of graphene depend heavily on the substrate upon which it is deposited. The presence of corrugation on the substrate surface, the number and orientation of graphene layers deposited, and the nature of the graphene edges, for example, all affect the electronic properties of the graphene. Consequently, the deposition of dispersed graphene oxide or graphene sheets on a substrate is a key process to reproduce the electronic properties of the graphene-based materials. [21,22] In this sense, compact three-dimensional felt consisting of several micrometric conductive carbon fibers is attractive for use in the construction of electrodes for demanding bioelectrochemical reactions [23,24]; Fig. 1A represents a scheme indicating that it is possible to decorate each of the carbon microfibers in the felt with cup-stacked carbon nanotubes (felt/CSCNTs) grown directly onto the surface of the felt, resulting in a hybrid micro/nanostructured composite that exhibits excellent mechanical, thermal, and electronic properties [25]. CSCNTs contain a particularly interesting electronic structure with an unique π orbital distribution [26,27].

Upon another three-dimensional felt, one can alternatively immobilize graphene oxide sheets derived from either natural graphite flakes or crystals consisting of graphene sheets hundreds of microns in size. The modification of carbon felt with graphene oxide, meanwhile, furnishes an even more versatile and compact three-dimensional material with a larger surface area (felt/graphene oxide). The graphene oxide sheets can directly adhere to neighbouring microfibers within the felt, to form a graphene oxide network (Fig. 1B). Finally, the carbon fiber felt can also be covered with various types of aligned or nonaligned CNTs before the deposition of graphene oxide (felt/CSCNTs/graphene oxide) to yield an interesting system that allows one to study the behavior of CNTs as an electrode material as well as the interactions between CNTs and uni- or bidimensional nanomaterials, such as graphene (Fig. 1C). Because the microfibers of the felt are conductive, electrochemistry can be used to reduce the graphene oxide sheets, to give felt/reduced graphene oxide (RGO) or felt/CSCNTs/reduced graphene oxide (felt/CSCNTs/RGO) and partially restore the structure and electronic properties of the graphene sheets.

The three procedures depicted in Fig. 1 generated platforms that can be potentially employed to the observation of demanding bioelectrochemical reactions. Indeed, Grosse et al. [28] and Mani et al. [29] have stressed that the biological applications of composite graphene-CNT electrodes have been still barely explored. The work presented here compares results achieved through GOx immobilization to the electrode in the cases of the felt/CSCNTs, felt/RGO, and felt/CSCNTs/RGO platforms and aims to contribute with the discussion of this issue. For that, measurements of Raman spectroscopy and circular dichroism were evaluated as well as the glucose oxidation in the absence of any electron mediator, such as O_2.

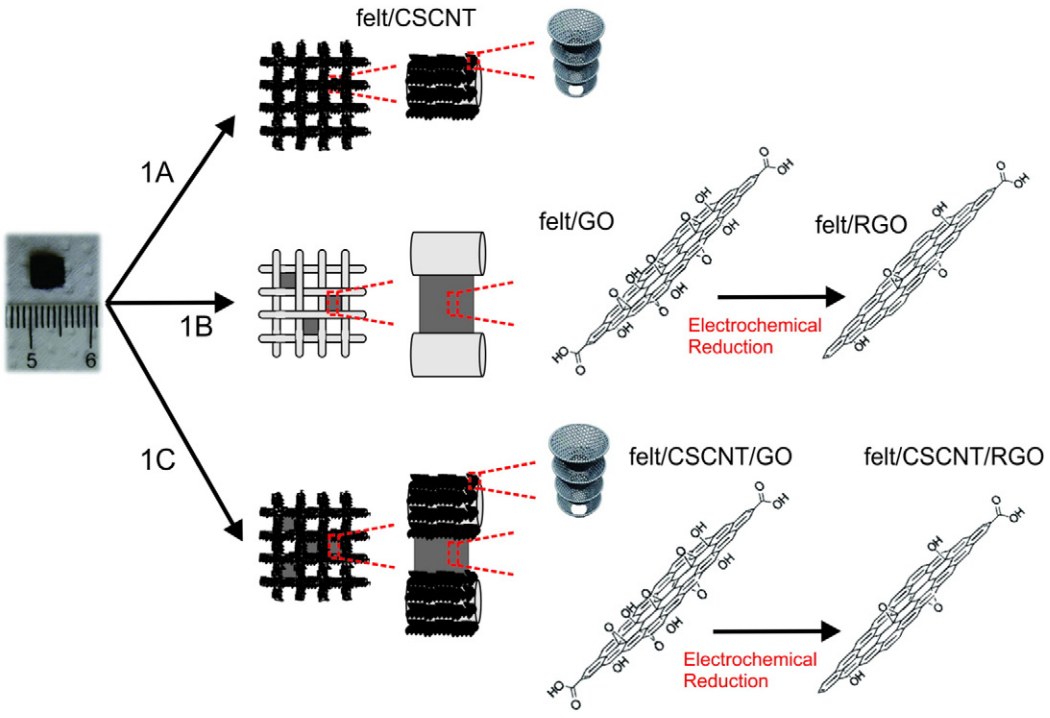

Fig. 1. Schematic representation of (A) felt/cup-stacked carbon nanotubes (felt/CSCNTs), (B) felt/reduced graphene oxide (felt/RGO), and (C) felt/cup-stacked carbon nanotubes/reduced graphene oxide (felt/CSCNTs/RGO).