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ScienceDirect
Biosensors and Bioelectronics 22 (2007) 1820–1824
BIOSENSORS & BIOELECTRONICS
www.elsevier.com/locate/bios

Short communication

The use of single walled carbon nanotubes dispersed in a chitosan matrix for preparation of a galactose biosensor

Jan Tkac [a,b,*], James W. Whittaker [c], Tautgirdas Ruzgas [d]

[a] *Department of Analytical Chemistry, Lund University, P.O. Box 124, SE-221 00 Lund, Sweden*
[b] *Institute of Chemistry, Slovak Academy of Sciences, Dubravska cesta 9, 812 37 Bratislava, Slovak Republic*
[c] *Department of Environmental and Biomolecular Systems, Oregon Health and Science University, Beaverton, OR 97006, USA*
[d] *Faculty of Health and Society, Malmö University, SE-2005 06 Malmö, Sweden*

Received 4 April 2006; received in revised form 9 August 2006; accepted 14 August 2006
Available online 12 September 2006

Abstract

Chitosan was chosen as a natural polymer for dispersion of single walled carbon nanotubes (SWNT) based on its ability to efficiently solubilize SWNTs to form a stable dispersion. Moreover, chitosan films deposited on a surface of a glassy carbon (GC) electrode are mechanically stable. Further stabilisation of the chitosan film containing SWNT (CHIT–SWNT) was done by chemical crosslinking with glutaraldehyde and free aldehyde groups produced a substrate used for covalent immobilisation of galactose oxidase (GalOD). Different galactose biosensor configurations were tested with optimisation of composition of inner and outer membrane; and enzyme immobilisation procedure, as well. Detection of oxygen uptake by GalOD on CHIT–SWNT layer at −400 mV is robust and, when flow injection analysis (FIA) was applied for assays, a low detection limit (25 μM) and very high assay throughput rate (150 h^{-1}) was achieved. This new galactose biosensor offers highly reliable detection of galactose with R.S.D. well below 2% and it has been successfully applied to assaying galactose in a blood sample with recovery index between 101.2 and 102.7%.

Keywords: Carbon nanotube; Nafion; Chitosan; Galactose oxidase; Interferents; Blood plasma

1. Introduction

Carbon nanotubes have been recognized as one of the most promising electrode materials in the field of electroanalysis because many electroactive species can be detected at lower overvoltage compared to ordinary electrodes resulting in increased selectivity of detection in a complex matrix (Davis et al., 2003; Katz and Willner, 2004; Gooding, 2005; Wang, 2005; Wildgoose et al., 2006). However, unmodified carbon nanotubes are extremely hydrophobic and assemble into bundles and ropes of individual nanotubes. For applications in electrochemistry, it is often necessary to make them soluble. Different approaches to solubilizing carbon nanotubes have appeared in literature, but frequently dimethylformamide (Gooding et al., 2003; Landi et al., 2004; Tsai et al., 2004; Kim et al., 2005), Nafion (Wang et al., 2003; Lin et al., 2005; Tsai et al., 2005; Viswanathan et al., 2006) and recently chitosan (Zhang and Gorski, 2005a,b; Jiang et al., 2005; Luo et al., 2005; Kandimalla and Ju, 2006) have been used for dispersion.

We have previously shown that the choice of dispersing agent is an important factor in the preparation of high performance biosensors exhibiting high detection sensitivity (Tkac and Ruzgas, 2006). Chitosan fulfils many of the requirements for preparing a robust, electrochemically active film composed of nanotubes (Tkac and Ruzgas, 2006). Moreover, chitosan is a natural polyelectrolyte containing free amino groups ($pK_a \approx 6.5$) and is suitable for the convenient preparation of membranes and films (Krajewska, 2004; Yi et al., 2005; Kumar et al., 2004). Due to its biocompatibility, chitosan has been extensively used for the immobilisation of biomolecules (Krajewska, 2004).

There are many strategies for designing an immobilised-oxidase biosensor based on carbon nanotube films. Most commonly, the release of hydrogen peroxide or a consumption of oxygen is monitored during oxidase turnover (Tsai et al., 2005; Lim et al., 2005). Alternatively, the use of artificial electron acceptors may permit higher sensitivity of detection

* Corresponding author. Fax: +46 46 222 45 44.
E-mail address: jantkac@hotmail.com (J. Tkac).

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doi:10.1016/j.bios.2006.08.014

and eliminate the dependence on oxygen (Guan et al., 2005; Joshi et al., 2005). Detection of hydrogen peroxide can be improved by co-immobilisation of horseradish peroxidase (Ruzgas et al., 1996; Gorton et al., 1999).

Galactose oxidase (GalOD) has been a subject of extensive fundamental research (Shleev et al., 2005; Whittaker, 2003) and has been successfully used for preparation of a variety of biosensors (Tkac et al., 2000, 2001; Manowitz et al., 1995). The aim of the present study was to optimise the performance of a GalOD biosensor based on nanotube films by using different immobilisation protocols and biosensor configurations. The objective was to develop a biosensor capable of detecting very low galactose concentrations with high selectivity in complex biological mixtures. We report here for the first time an extremely robust, sensitive and selective galactose biosensor suitable for high throughput detection. The biosensor is based on GalOD immobilised on a CHIT–SWNT modified electrode with detection of oxygen depletion upon action of the enzyme. The biosensor has been successfully applied in the analysis of galactose in a blood plasma.

2. Experimental procedures

2.1. Reagents and material

Single walled carbon nanotubes ($d = 1.1$ nm, $L = 0.5$–100 μm, >90% purity) were obtained from Fluka. Nafion (20% solution in low molecular weight alcohols) was purchased from Aldrich. Chitosan (degree of deacetylation of 85%) was provided from Sigma. A dialysis membrane (cut-off 6–8 kDa) was purchased from Serva. Recombinant galactose oxidase expressed in a *Pichia pastoris* was prepared according to published procedure (Whittaker and Whittaker, 2000). The specific activity of enzyme of 500 U mg^{-1} (20 g l^{-1}) was detected by oxygen electrode in the presence of 2 mM ferricyanide. All other chemicals used were of high purity and were used without any purification/pretreatment. All aqueous solutions were prepared in highly pure water (Millipore system). Glassy carbon (GC) disk electrodes (CH Instruments, $d = 3$ mm) were used for casting of a dispersion of carbon nanotubes. For batch electrochemical measurements a BAS equipment (CV-50W, Bioanalytical systems, West Lafayette, IN, USA) was used with GC as a working electrode, saturated calomel electrode (SCE) as a reference electrode and platinum plate as a counter electrode. In flow injection experiments an Ag/AgCl electrode was used as reference one.

2.2. Dispersion of SWNT and electrode modification

A dispersion of SWNT in chitosan (CHIT–SWNT) was prepared by sonication of 1 mg of SWNT in 1 ml of a 0.1% chitosan in 1% acetic acid. Sonication was performed in an ultrasonication bath (Branson 5510, Branson Ultrasonic Corp., CT, USA) for 170 min (if not specified otherwise). GC electrodes were polished using 1 and 0.1 μm alumina/diamond slurry (Struers A/S) and sonicated in distilled water for 3 min. In all cases, GC electrode was modified by casting of 5 μl of dispersion on a polished GC electrode and allowed to dry at ambient temperature. To optimise the performance of the biosensor the CHIT–SWNT layer was further modified by application of the following films/layers in different order (e.g. CHIT–SWNT/NAF(E)/GalOD/NAF(P)): 10 μl of 0.5% Nafion in 50 mM phosphate buffer pH 7.4, NAF(P); 10 μl of 0.5% Nafion in absolute ethanol, NAF(E); 10 μl of 0.1% chitosan in 1% acetic acid, CHIT; a dialysis membrane; 5 μl of a 10 g l^{-1} GalOD solution in 20 mM phosphate buffer pH 7.4, GalOD. CHIT–SWNT layer was in some cases stabilised by dipping of the electrode into 2.5% glutaraldehyde solution for 5 min and extensively washed by distilled water. The films/layers were cast on the electrode in a liquid form and left to dry before application of a next layer. In some cases, a GalOD solution stored in the fridge for couple of days was used with lower specific activity during optimisation steps, but the final characterisation of the biosensor and assay of a plasma sample were done with a fresh portion of a GalOD solution.

2.3. Flow injection experiments

In case of flow injection analysis, the electrodes were mounted into a flow-through amperometric cell of wall-jet type (Appelqvist et al., 1985) containing the modified GC electrode, a platinum wire counter electrode and an Ag|AgCl (0.1 M KCl) reference electrode. Samples were injected with an injector (type 7125 LabPRO, Rheodyne, Cotati, CA, USA) supplied with an injection loop of 50 μl. A 50 mM phosphate buffer pH 7.4 containing 0.1 M KCl was pumped at a flow rate of 0.55 ml min^{-1} (Minipuls 2, Gilson, Villier-le Bel, France), if not mentioned otherwise. Connections between the various parts were made with Teflon tubing; i.d. 0.5 mm and Altex screw couplings. The potential of the working electrode versus the reference electrode was kept at the required value using a potentiostat (Zäta Electronics, Lund, Sweden) the current registered with a recorder (model BD 112, Kipp & Zonen, Delft, The Netherlands).

3. Results and discussion

3.1. The effect of sonication time

Preliminary work has shown that the dispersion of long SWNT in chitosan matrix is more efficient and faster compared to Nafion and dimethylformamide dispersions (Tkac and Ruzgas, 2006). Sonication times up to 120 min were previously tested and longer sonication times (up to 300 min) were examined in the present study. The sensitivity towards galactose increased 2.6-fold as the sonication time increased from 60 to 170 min. However, further sonication time had a negative influence on the sensitivity of the biosensor (data not shown). The same effect was observed for Nafion dispersion and most likely reflects a decrease in sensitivity as a result of polymer degradation upon prolonged sonication (Tkac and Ruzgas, 2006). Moreover, the electrode noise increased with longer sonication time and for further study a sonication time of 170 min was used for preparation of dispersion of SWNT in chitosan.