Biosensors and Bioelectronics 25 (2010) 2205–2211

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

Functionalised ZnO-nanorod-based selective electrochemical sensor for intracellular glucose

Muhammad H. Asif[a,*], Syed M. Usman Ali[a,*], Omer Nur[a], Magnus Willander[a], Cecilia Brännmark[b], Peter Strålfors[b], Ulrika H. Englund[b], Fredrik Elinder[b], Bengt Danielsson[c]

[a] Department of Science and Technology, Campus Norrköping, Linköping University, Bredgatan 34, SE-601 74 Norrköping, Sweden
[b] Department of Clinical and Experimental Medicine, Division of Cell Biology, Linköping University, SE-581 85 Linköping, Sweden
[c] Pure and Applied Biochemistry, Lund University, Box 124, SE-221 00 Lund, Sweden

ARTICLE INFO

Article history:
Received 13 November 2009
Received in revised form 22 February 2010
Accepted 23 February 2010
Available online 3 March 2010

PACS:
82.47.Rs
62.23.Hj
73.63.Bd

Keywords:
ZnO nanorods
Functionalisation
Intracellular glucose
Electrochemical sensor

ABSTRACT

In this article, we report a functionalised ZnO-nanorod-based selective electrochemical sensor for intracellular glucose. To adjust the sensor for intracellular glucose measurements, we grew hexagonal ZnO nanorods on the tip of a silver-covered borosilicate glass capillary (0.7 μm diameter) and coated them with the enzyme glucose oxidase. The enzyme-coated ZnO nanorods exhibited a glucose-dependent electrochemical potential difference versus an Ag/AgCl reference microelectrode. The potential difference was linear over the concentration range of interest (0.5–1000 μM). The measured glucose concentration in human adipocytes or frog oocytes using our ZnO-nanorod sensor was consistent with values of glucose concentration reported in the literature; furthermore, the sensor was able to show that insulin increased the intracellular glucose concentration. This nanoelectrode device demonstrates a simple technique to measure intracellular glucose concentration.

1. Introduction

Glucose, also known as grape sugar or corn sugar, is a fundamental carbohydrate in biology. Glucose is one of the main products of photosynthesis and serves as the human body's primary source of energy. Living cell uses it both as a source of energy and as a metabolic intermediate in the synthesis of more complex molecules such as fats. When glucose levels in the bloodstream are not properly regulated, diseases such as diabetes can develop. Because of the high demand for blood-glucose monitoring, significant research has been devoted to produce reliable methods for in vitro or in vivo glucose measurement, such as fluorescence spectroscopy (Ballerstadt and Schultz, 2000), diffraction spectroscopy (Asher et al., 2003), surface-enhanced Raman scattering (Shafer-Peltier et al., 2003), a wireless magnetoelastic sensor (Cai et al., 2004), an electrochemical transistor sensor (Forzani et al., 2004; Raffa et al., 2003), an enzyme-based amperometric sensor (Zen et al., 2003; Hrapovic et al., 2004; Lin et al., 2004; Yang et al., 2004; Zhou et al., 2005), a nanoenzymetric amperometric sensor (Park et al., 2003), nuclear magnetic resonance spectroscopy (Cline et al., 1998) and a potentiometric sensor (Shoji and Freund, 2001). Since the development of the first glucose biosensor, improvement of the response performance of enzyme electrodes has been the main focus of biosensor research (Raitman et al., 2002). In particular, searches for new materials and methods for immobilising enzymes are still very important subjects towards more active and stable biosensors (Yang et al., 2002; Tsai et al., 2005).

In general, a biosensor consists of a bio-sensitive layer that either contains biological recognition elements or consists of biological recognition elements covalently attached to the transducer. Different biosensor experimental setups have been used for the real-time detection, diagnosis, and classification of different forms of biochemical reactions within single cells in order to understand cellular behaviour. This work offers enormous potential to cellular biology research (Vo-Dinh et al., 2006; Koukin et al., 2005; Fasching et al., 2005; Firtel et al., 2004). In most of these biosensors, indirect methods or large experimental setups are required. A robust and simple technique that utilises direct intracellular measurement would be of great interest.

Since the discovery of ZnO nanorods, they have been the target of numerous investigations due to their unique properties. The diameters of these nanostructures are comparable to the size of

* Corresponding authors. Tel.: +46 11363119; fax: +46 11363270.
E-mail addresses: muhas@itn.liu.se (M.H. Asif), syeal@itn.liu.se (S.M.U. Ali).

0956-5663/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.bios.2010.02.025

the biological and chemical species being sensed, which intuitively makes them excellent primary transducers for producing electrical signals. ZnO nanorods, nanowires, and nanotubes have recently attracted considerable attention for the detection of biological molecules (Kang et al., 2005; Batista and Mulato, 2005; Bashir et al., 2002; Wei et al., 2006; Kim et al., 2006; Kumar et al., 2006). These nanostructures have unique advantages, including high surface-to-volume ratio, non-toxicity, chemical stability, electrochemical activity, and high electron-communication features, which make them one of the most promising materials for biosensor application (Sun and Kwok, 1999). In addition, ZnO can be grown as vertical nanowires, is biosafe, has high ionic bonding (60%), and is not very soluble at biological pH-values. These properties make ZnO suitable for sensitive intracellular ion measurements. These advantages should allow for stable and reversible signals with respect to glucose concentration changes. Among a variety of nanosensor systems, our nanostructured electrochemical probe can offer high sensitivity and real-time detection. The detection sensitivity of the glucose sensor can be increased to the single-molecule level of detection by monitoring the very small changes in electrochemical potential caused by the binding of biomolecular species on the surface of the probe.

In a previous investigation, we measured concentrations of extracellular and intracellular Ca^{2+} using ZnO nanorods (Asif et al., 2008, 2009). Intracellular determination of glucose is of great interest, and ZnO-nanorod technology has potential for such measurements. The focus of the current study is the demonstration of a ZnO-nanorod-based sensor suitable for intracellular selective glucose detection. Our main effort has been directed towards the construction of tips that are selective for glucose and capable of penetrating the cell membrane, as well as the optimisation of electrochemical potential properties. Tips of borosilicate glass capillaries (0.7 μm in diameter) with grown ZnO nanorods have proven to be a convenient and practical choice, as we have demonstrated with our previously developed intracellular Ca^{2+} and pH nanosensors (Asif et al., 2009; Al-Hilli et al., 2007).

Various methods for immobilisation of glucose oxidase on different supporting materials have been proposed, including covalent binding (Piro et al., 2000), embedding methods (Cosnier et al., 1999), cross-linking methods (Muguruma et al., 2000; Yang et al., 1998; Wu et al., 2004) and physical adsorption (Sun et al., 2008; Topoglidis et al., 2001). In this study, electrostatic enzyme immobilisation has been used, drawing on the fact that there is a large difference in the isoelectric points of ZnO and glucose oxidase. The isoelectric point of ZnO is about 9.5, making it suitable to immobilise low-IEP proteins or enzymes such as glucose oxidase (IEP ~4.2) by electrostatic adsorption in proper buffer solutions around neutral pH (Usman Ali et al., 2009; Wink et al., 1997).

In the human body, the hormone insulin only stimulates glucose transport into muscle and fat cells. However, insulin has been found to affect glucose uptake in oocytes from frog *Xenopus laevis* (Simpson and Cushman, 1986: Janicot and Lane, 1989). The large size of these cells makes it possible to microinject specific reagents that interrupt or activate signal transmission to glucose. In this study, we used an intracellular electrochemical glucose sensor based on ZnO nanorods to measure intracellular glucose concentration in human adipocytes and *X. laevis* oocytes and to demonstrate a glucose transport system that is markedly activated by insulin in both cells.

2. Experimental details

2.1. Materials

Glucose oxidase (E.C. 1.1.3.4) from *Aspergillus niger*, type GO3A360 U/mg was purchased from BBI Enzymes (UK) Ltd., D-(+)-glucose (99.5%), zinc nitrate hexahydrate $Zn(NO_3)_2{\cdot}6H_2O$ and hexamethylenetetramine (HMT) were purchased from Sigma–Aldrich. Borosilicate glass capillaries (sterile Femtotip® II with tip inner diameter of 0.5 μm, tip outer diameter of 0.7 μm, and length of 49 mm) were purchased from Eppendorf AG, Hamburg, Germany. Phosphate-buffered saline 10 mM solution (PBS) was prepared from Na_2HPO_4 and KH_2PO_4 with 0.138 M NaCl, and the pH was adjusted to 7.40. Glucose stock solution was kept at least 24 h after preparation for mutarotation. All chemicals used (Sigma–Aldrich) were of analytical reagent grade.

2.2. Fabrication of sensor and reference electrodes

To prepare the sensor and reference electrodes, we affixed the aforementioned borosilicate glass capillaries inside a flat support of the vacuum chamber of an evaporation system (Evaporator Satis CR725) to uniformly deposit chromium and silver films (with thicknesses of 10 and 125 nm, respectively) at the outer surface of the capillary tips. After some optimisation, the reference electrode Ag/AgCl tip was electrochemically prepared by dipping the coated end of a capillary in 0.2 M HCl solution and then by electrolysing the silver film to form AgCl by polarising it at 1.0 V for 1 min. A 3-cm-long Ag/AgCl layer was coated on the tip of the capillary and covered with insulating material, leaving 3 mm of Ag/AgCl exposed at the tip to serve as a reference electrode. The outer end of the Ag/AgCl layer was connected to a copper wire (0.5 mm in diameter and 15 cm in length) and fixed by means of high-purity-silver conductive paint. To prepare the working electrode, we grew hexagonal single crystals of ZnO nanorods on another silver-coated capillary glass tip using a low-temperature method (Greene et al., 2003; Vayssieres et al., 2001; Li et al., 2005). The ZnO-nanorod layer covered a small part of the silver-coated film. The part of the capillaries covered with ZnO nanorods varied from 3 mm down to 10 μm. The nanostructure had a rod-like shape with a hexagonal cross-section and primarily aligned along the perpendicular direction, as shown in Fig. 1. The nanorods are uniform in size with a diameter of 100–120 nm and a length of 900–1000 nm. The electrical contact was made on the other end of the Ag film for obtaining an electrical signal during measurements.

Careful efforts were taken to ensure sufficiently small tip geometry. Intracellular electrodes must have extremely sharp tips (sub-micrometer dimensions) and must be >10 μm in length. These characteristics are necessary for effective bending and gentle penetration of the flexible cell membrane.

2.3. Immobilisation of the enzyme

Glucose oxidase solution, 5 mg/mL, was prepared in 10 mM PBS containing 1.5 mM Na_2HPO_4, 8 mM KH_2PO_4, 0.138 M NaCl, and 2.7 mM KCl pH 7.40. Glucose oxidase was electrostatically immobilised by dipping the tip of a borosilicate glass capillary with well-aligned ZnO nanorods into 2 mL of the enzyme solution for 15 min at room temperature and then drying it in air for more than 20 min. Fig. 1(c) shows ZnO nanorod with immobilised GOD. All enzyme electrodes were stored in dry condition at 4 °C when not in use.

2.4. Electrochemical measurements

The selective intracellular glucose measurements were performed by a potentiometric method utilising two electrodes. A ZnO-nanorod-decorated electrode coated with enzyme served as the intracellular working electrode, and an Ag/AgCl electrode was used as the intracellular reference microelectrode. The electrochemical response of the glucose probe was measured with a Metrohm pH meter model 827 versus the Ag/AgCl reference