Journal of Chromatography A, 1469 (2016) 128–134

Contents lists available at ScienceDirect

Journal of Chromatography A

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

Determination of glucose in human stomach cancer cell extracts and single cells by capillary electrophoresis with a micro-biosensor

Xiaolei Wang*, Yanfang Ma, Man Zhao, Minfeng Zhou, Yan Xiao, Zifei Sun, Lili Tong*

College of Chemistry, Chemical Engineering and Materials Science, Shandong Normal University, Jinan 250014, PR China

ARTICLE INFO

Article history:
Received 15 June 2016
Received in revised form
21 September 2016
Accepted 23 September 2016
Available online 23 September 2016

Keywords:
Glucose
Capillary electrophoresis
Electrochemical biosensor
Single-cell analysis

ABSTRACT

Bioactive species in cells can provide information about signal transduction, cell function, and the effects of disease treatment. In this article, a novel micro-biosensor was fabricated to detect glucose in individual human stomach cancer cells (MGC80-3 cells) with capillary electrophoresis (CE). We fabricated the micro-biosensors by immobilizing a single-walled carbon nanotube-glucose oxidase (GOx)-glutaraldehyde (GA) bio-composite at the palladium nanoparticle (PdNPs) modified Pt electrode. The linear concentration of glucose ranged from 2.0 μM to 1.0 mM, with a detection limit of 0.5 μM. Using this method, the mean amount of glucose in MGC80-3 cell extracts and in single cells was 20.0 fmol and 20 ± 6 fmol (n = 10), respectively. The micro-biosensor exhibited high sensitivity, stability, and a long operating life, which are likely due to the biocompatible environment provided by BSA and GA, and the adsorption and faster electron transfer of SWNTs and PdNPs to GOx.

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1. Introduction

Glucose plays an indispensable part in cell growth, nutrition and viability. Optimum glucose concentrations are important to the growing of cells for cell growth [1]. In free cytosol, glucose concentrations are usually in the millimolar range. However, glucose transport inhibitors or reduced extracellular glucose can dramatically reduce cytosolic glucose levels [2]. Glucose deprivation can negatively impact signal transduction and induce oxidative stress in human cells [3]. Determining glucose levels in individual cells can help us understand the chemical and biological functions of the cells and aid in the diagnosis and treatment of diseases. Recently, fluorescent sensors have offered an attractive approach for determining glucose concentration at the cellular level. The glucose content in a single gastric cancer cell was detected by laser-induced fluorescence [4]. Fluorescence microscopy was used to determine glucose and Ca^{2+} levels in pancreatic beta cells in real time [5]. A highly sensitive apo-GOx-modified gold nanoprobe was developed for quantitative detection of glucose in living human hepatoma cells (HepG2) [1].

Following the fabrication of the first glucose biosensor, numerous glucose sensors, such as fluorescent and electrochemical sensors, have been developed. Although fluorometry is highly sensitive, the high cost of optical instruments and the need for chemical derivatization have led to a rise in electrochemical detection (especially amperometric detection) as the most common method. Electrochemical detection has several advantages over fluorometry, including higher sensitivity, faster response, and lower cost. However, to our knowledge, electrochemical biosensors have not been used to determine glucose levels in individual cells.

Capillary electrophoresis (CE) integrated with electrochemical detection (CE-ECD) can be used to detect analytes from femto- to atto-mole levels [6]. CE-ECD has been used to analyze ascorbic acid [7], glutathione [8], dopamine [9], histamine [10], amino acids [11,12], and glucose-6-phosphate dehydrogenase [13] in single cells. Almost all of the previously mentioned analytes were electroactive substances because carbon fiber microelectrodes (MEs) or modified MEs have typically been used in single-cell analysis. To our knowledge, CE-ECD has not been used to detect intracellular glucose. Enzymatic glucose electrochemical biosensors fabricated by the modification of glucose oxidase (GOx) on various substrates are particularly important because of their specific bioaffinity. How-

Abbreviations: CE, capillary electrophoresis; MGC80-3 cell, human stomach cancer cell; SWNTs, single-walled carbon nanotubes; GOx, glucose oxidase; GA, glutaraldehyde; PdNPs, palladium nanoparticle; HepG2, human hepatoma cells; CE, capillary electrophoresis; CE-ECD, CE with electrochemical detection; AA, ascorbic acid; GSH, glutathione; DA, dopamine; MEs, microelectrodes; CNTs, carbon nanotubes; BSA, bovine serum albumin; SCE, saturated calomel electrode; PtME, Pt micro-disk electrode; PdNPME, PdNPs modified PtME; A_p, peak area; SEM, scanning electron microscopy; $W_{1/2}$, peak half-height; i_p, peak current; t_m, migration time; N, theoretical plate number; C_b, buffer concentration; RSD, relative standard deviation.

* Corresponding author at: 88 WenHua Dong Road, Jinan 250014, PR China.
E-mail addresses: wangxl@sdnu.edu.cn (X. Wang), findrui@gmail.com (L. Tong).

http://dx.doi.org/10.1016/j.chroma.2016.09.054
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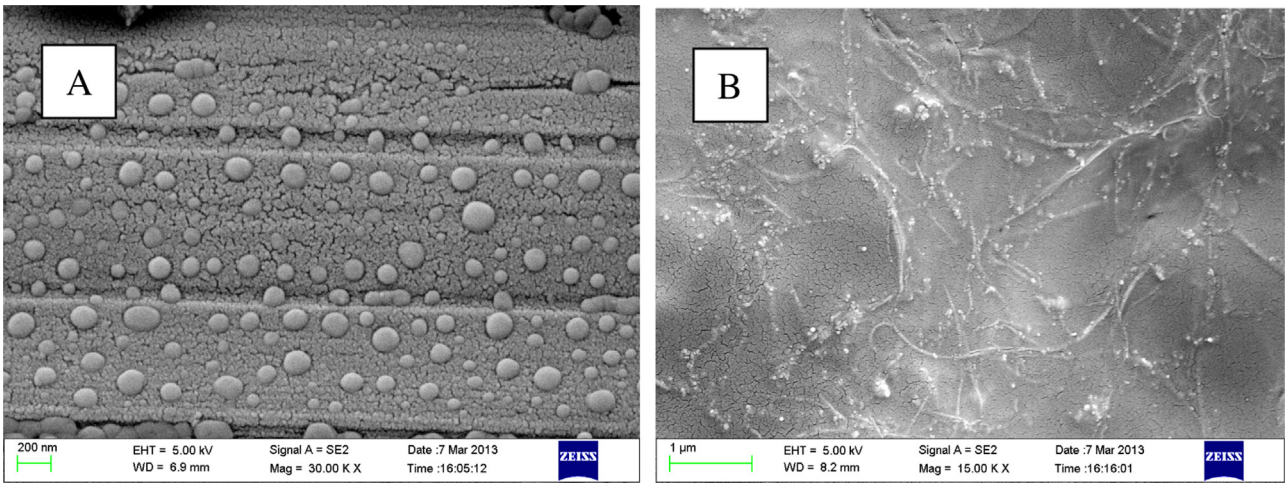

Fig. 1. SEM images of PdNPME (A) and the micro-biosensor (B); Accelerating voltage: 5000 V, Spray time: 30 s.

ever, endogenous electroactive substances such as DA, GSH, and AA always cause severe interference in single-cell measurements, which limits the application of enzymatic biosensors [14]. CE-based biosensors can provide accurate measurements of each individual component of a group of samples. Therefore, the development of improved micro-biosensor fabrication techniques and various modified materials will enhance sensitivity and selectivity when these novel micro-biosensors are used as the detectors of CE-ECD in single-cell assays.

Metal nanoparticles have emerged as a novel type of compound in chemistry, physics, biology, medicine, and material sciences due to their unique optical, electrical, magnetic, and catalytic properties [15]. Current research on metal nanoparticles focuses on the synthesis and application of noble metal nanoparticles such as gold, platinum, silver, palladium, and their alloys. Palladium nanoparticles (PdNPs), with their relatively high catalytic activity, have been used successfully for the measurement of nitrite [16], oxygen [17], microRNA [18], H_2O_2 [19], and glucose [20]. Meanwhile, carbon nanotubes (CNTs), which have excellent electrical conductivity and biocompatibility, are suitable candidates for improving the rate of heterogeneous electron transfer. CNTs and enzymes such as GOx are similar in size, which can facilitate their interaction [21,22]. Furthermore, CNTs functionalization in acidic solutions creates surface binding sites such as carboxylic and hydroxyl groups, which may be favorable for biosensor applications [23,24]. Therefore, fabrication of glucose micro-biosensors with PdNPs and CNTs may improve the detection sensitivity and reduce the response time in CE-ECD.

In this paper, a novel strategy was employed to fabricate a micro-biosensor as an electrochemical detector in CE-ECD. We fabricated the micro-biosensor by immobilizing an SWNT–GOx–glutaraldehyde biocomposite on palladium nanoparticles on a modified Pt microelectrode. The presence of glutaraldehyde (GA) and bovine serum albumin (BSA) provided a favorable biocompatible microenvironment for GOx and also played a role in reducing GOx loss. Single-wall carbon nanotubes (SWNTs) can not only interact with GOx through its binding sites but also promote electron transfer, improve the surface area of the biosensors, and enhance catalytic activity in conjunction with PdNPs. Compared with other CE electrochemical detectors of glucose, our results demonstrated that the novel micro-biosensor exhibited better sensitivity, stability, reproducibility, and anti-interference. Glucose concentrations in both single MGC80-3 cells and cell extracts were successfully determined without complex pretreatment.

2. Experimental

2.1. Reagents and apparatus

The CE apparatus was assembled in the laboratory. The applied high voltage was provided by a 0.0 kV-30.0 kV power supply (Instrument Company of Shandong Normal University, Shandong, China). The schemes of the CE-ECD system and detection cell (Fig. 1S) were explicitly detailed in our previous work [8,9]. Briefly, a 60-cm fused-silica capillary (25 μm ID, 375 μm OD) was set between the electrophoresis buffer reservoir and the detection buffer reservoir. The separation voltage was fixed at 18.0 kV. A CHI 832 B electrochemical workstation (Shanghai, China) was used to detect glucose with CE-ECD. A glucose biosensor, a saturated calomel electrode (SCE), and a platinum wire were utilized as the working (WE), reference (RE), and auxiliary electrodes (AE), respectively.

D-glucose (>99.8%), GOx (109 U/mg) and cysteine were purchased from Amresco (USA). SWNTs (>90%, 5–15 mm in length, <2 nm in diameter) were purified with nitric acid before use [25]. DA, AA, BSA and GA (25%) were purchased from Sigma-Aldrich Chemical Company (USA). K_2PdCl_4 was purchased from Shanghai BaoMan Biotechnology Limited Company. A phosphate buffer solution (PB, 25 mM, pH 7.4) was chosen as the running buffer for the capillary. Human stomach cancer cells (MGC80-3 cells) were purchased from the Chinese Academy of Sciences (Shanghai, China). The running buffer was used to dilute glucose stock solution (0.1 M) to the desired concentration weekly. Balanced phosphate-buffered saline (PBS, 0.2 M Na_2HPO_4 and 0.162 M NaH_2PO_4, pH 7.4) was used to suspend and clean cells. All solutions were filtered through a 0.22-μm film. Double-distilled water was used in all experimental preparations.

2.2. Fabrication of Pt micro-disk electrode and glucose micro-biosensor

The Pt micro-disk electrode (PtME) was fabricated based on our previous work [9]. A Pt wire (200 μm in diameter, 5.5 cm in length) was used. The surface of the PtME was carefully polished to a mirror-like surface on metallographical sand paper and then sonicated in ethanol and double-distilled water for 5 min. PdNPs were electrodeposited on the surface of PtME by applying a constant potential of −0.2 V (*vs.* SCE) for 15 s in a solution containing 1 mM K_2PdCl_4 and 0.5 M H_2SO_4 [26]. The elec-

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