Sensors and Actuators B 222 (2016) 638–644

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

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

Preparation of photoluminescent enzymatic nanosensors for glucose sensing

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ARTICLE INFO

Article history:
Received 27 June 2015
Received in revised form 11 August 2015
Accepted 20 August 2015
Available online 22 August 2015

Keywords:
Glucose
Enzymatic nanosensor
Oxygen
Time-resolved fluorescence
Ratiometric photoluminescence

ABSTRACT

A novel photoluminescent glucose nanosensor was facilely prepared by coupling glucose oxidase (GOx) with poly-L-lysine coated oxygen nanosensors via a glutaraldehyde-mediated Schiff-base reaction. The GOx molecules residing on particle surface catalyzed glucose with the expense of oxygen, which was detected by the sensing particle core incorporated with the reference dye coumarin 6 and oxygen probe Pt(II)-*meso*-tetra(pentafluorophenyl)porphine. The proposed glucose nanosensors (~150 nm in hydrodynamic diameter) had a quick response time varied from less than 2 min to 4 min. Glucose calibration was performed with ratiometric photoluminescence and time-resolved fluorescence (TRF) respectively, and a series of calibration plots were constructed according to determination time. In comparison, the ratiometric method resulted in wide dynamic range (e.g. 2–10 mM) and high limit of detection (~1–2 mM), while the TRF mode gave narrow dynamic range (e.g. 1–6 mM) with low detection limit (~0.1–0.2 mM). Finally the enzymatic glucose nanosensors were tested in human serum samples with a TRF microplate reader.

1. Introduction

As a primary energy source in most organisms and an important metabolic intermediate, glucose is indispensable to all living creatures. Determination of glucose concentration in fluids plays a big role in biochemistry, clinical chemistry and diagnosis of diabetes particularly [1,2]. Tremendous amount of biosensor for glucose has been developed to this end [3–6]. More recently, a great number of nano-systems have emerged as bioprobes for glucose sensing with extra bonus of improved sensitivity due to the increasing surface area of nanoparticles (NPs) and nanoscale sensibility [7]. Based on electrochemical mechanism, for instance, nanoporous materials (e.g. Pt thin film [8] and PtPb networks [9]), nanotubes (ZnO arrays [10] and carbon nanotube) [11] and hybrid nanostructures (Pd NPs-carbon nanotube [12] and Cu NPs-graphene [13]) have been fabricated to assay glucose. Unfortunately, these sensing nanosystems may suffer from interference from electroactive species like urea, ascorbate and urate, or absorptive species, and also are difficult to realize non-invasive detection which is much desirable for in vivo measurement.

Optical glucose nanosensors, in contrast, are capable of non-intrusive measuring while immune from the common interfering species. Several types of nanoprobe have been constructed by using of noble metal NPs, semiconductor quantum dots (QDs) or carbon dots, which are further conjugated with bioactive molecules such as concanavalin A [14], boronic acid [15–18] and glucose oxidases (GOx) [19–22]. With the presence of glucose, the sensing nano-systems either aggregate to give rise to shifted plasmonic absorption or weakened fluorescence, or biocatalytically generate H_2O_2 to quench fluorescence of QDs. It is noticed, however, that the widely used detection approach in bulk enzymatic sensor – oxygen-sensitive fluorescence (from oxygen probe) cooperated with GOx – is rarely adopted to build glucose nanosensors [23]. As far as we are concerned, only one such glucose nanosensor has been reported so far, which was constructed by incorporating GOx and a phosphorescent oxygen indicator sulfo-Ru(dpp) into polyacrylamide nanoparticles [24]. Although the phosphorescent nanosensors are sensitive to glucose, the encapsulation of GOx might retard the catalytic oxidation of glucose.

Previously we had prepared poly-L-lysine (PLL) coated oxygen nanosensors that based on oxygen-sensitive phosphorescence

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http://dx.doi.org/10.1016/j.snb.2015.08.092
0925-4005/

[25–27]. The nanosensors can facilely conjugate with functional groups such as (3-carboxypropyl) triphenylphosphonium through surface amino groups on PLL shell [28]. It naturally occurs to us that coupling GOx molecules with PLL-coated oxygen nanosensors would result in a fluorescent glucose nanosensor. Herein, a novel glucose nanosensor is proposed in this way, which consists of (i) a PLL-coated sensing particle core doped with the reference dye coumarin 6 and oxygen probe Pt(II)-*meso*-tetra(pentafluorophenyl)porphine and (ii) an outer layer of GOx for glucose. The resultant nanosensors, with a hydrodynamic diameter of ~150 nm, are highly sensitive to glucose in both photoluminescence intensity and lifetime, and respective calibration curves are plotted with different determination times. The functionality of glucose nanosensors are tested in human serum samples by using of a time-resolved fluorescence based plate reader, and reasonable results are derived.

2. Materials and methods

2.1. Materials

Polystyrene (PS), poly-L-lysine (PLL), coumarin 6 (C6) and Pt(II)-*meso*-tetra(pentafluorophenyl)porphine (PtTFPP) were purchased from Sigma–Aldrich. Dodecyltrimethoxysilane (DTS), glucose oxidase (GOx from Aspergillus niger, 100 units/mg) and glutaraldehyde (50%, GA) were from Jiaxing Sicheng Chemicals Co. Ltd (China), Aladdin (China) and Sinopharm (China), respectively. THF, D-glucose anhydrous (99%) and mineral oil (pure) were obtained from J&K (China). All reagents were used as received without further purification. Deionized water (DI) was used in all experiments.

2.2. Synthesis of PLL-coated photoluminescent oxygen nanosensors (PLL-NPs)

In a typical synthesis, C6, PtTFPP, PS and DTS were dissolved in THF, in a 2:1:47:50 weight ratio, and at a total concentration of 200 ppm. Then, 500 μL of mixed solution was rapidly injected into 8 mL water containing 0.16 mg PLL (pH 9, adjusted by ammonium hydroxide) under ultrasonication. The solution was then left standing for 2 h, and afterwards dialyzed against water for 24 h to remove the organic solvent. Finally, the NPs suspension was centrifugated (16,000 r/min, 30 min) to get rid of surplus PLL and other small molecules, and redispersed into DI water at a concentration of ~29 mg L^{-1}. The resultant PLL-NPs suspension was stored at 4 °C for further applications.

2.3. Synthesis of enzymatic photoluminescent glucose nanosensors (GOx-NPs)

GOx molecules were conjugated to PLL-NPs through GA-assisted crosslinking. Specifically, 2.4 mg GOx molecules were dissolved in 8 mL of as-prepared PLL-NPs solution. After thoroughly mixing, 14.4 μL of GA solution (diluted by 100 times with water) was added into the mixture, followed by shaking with table concentrator for 2 h at room temperature to complete the reaction. The solution was centrifugated at 16,000 r/min for 30 min, and GOx-NPs were collected and subsequently redispersed into 8 mL water with a concentration of ~232 mg L^{-1}. The resultant GOx-NPs suspension was stored at 4 °C for further application.

2.4. Characterization

TEM images were obtained with an electron microscope (type Hitachi H-800) at an acceleration voltage of 120 kV, using NPs aqueous dispersion placed on the TEM specimen support (cuprum). Zeta potentials and hydrodynamic sizes were determined by photon correlation spectroscopy and by dynamic light scattering (DLS), respectively, using a Zetasizer Nano instrument (Malvern Instruments; www.malvern.com). UV–vis absorption and steady-state photoluminescent spectra were recorded on a UV-3101PC spectrophotometer (Shimadzu) and an F-4600 fluorescence spectrophotometer (Hitachi), respectively. Time-resolved fluorescence (TRF) measurements were performed on a commercial plate reader (Victor×4; from Perkin-Elmer).

2.5. Ratiometric photoluminescence measurements and sensor calibration

The fluorescence intensity-based calibration was carried out in a standard spectrophotometric cuvette, in which 500 μL of air-saturated NPs-GOx solution was placed and sealed by 500 μL of mineral oil. To the cuvette bottom below oil layer, 125 μL of air-saturated glucose solution (in phosphate buffer saline buffer) with different concentrations were injected, respectively, to reach a final concentration of 0, 2, 4, 6, 8, 10 and 12 mM. Immediately after the addition of glucose, fluorescence spectra were recorded every 0.5 min with a scanning speed of 1200 nm/min under a 393-nm excitation. Because the photoluminescence intensity ratio was time-evolved, 8 s was further added to the registration time of each intensity ratio which considered the scanning speed and spectral range (490–650 nm).

2.6. Time-resolved fluorescence measurements and sensor calibration

The lifetime-based calibration was performed in a black 96-well microplate. Normally, 8–12 wells were used for one measurement, each filled with 100 μL of GOx-NPs solution containing bovine serum albumin (BSA, 0.07 g/mL). The microplate was initially conditioned for 20 min to equilibrate with air and at constant temperature (37 °C). Then 25 μL of air-saturated glucose solution (in phosphate buffer saline buffer) with different concentrations were quickly injected, respectively, to reach a final concentration of 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5 and 6 mM. After addition of 200 μL of mineral oil to prevent ingress of air, fluorescence signal was immediately measured on a commercial plate reader (Victor×4; from Perkin-Elmer). Rapid lifetime determination of phosphorescent nanosensors was performed based on time-resolved fluorescence (TRF) mode [27,29]. Specifically, two TRF intensities were recorded after delay times of 30 μs (t_1) and 70 μs (t_2), each with a gating time of 100 μs and using 340 nm excitation and 642 nm emission filters. Measured TRF intensity signals were converted into lifetime (τ) values according to the following equation: $\tau = (t_1 - t_2)/\ln(F_1/F_2)$, where F_1 and F_2 correspond to the integrated signals over 100 μs as obtained after delay times of t_1 and t_2, respectively.

2.7. Human serum test

GOx-NPs were utilized to detect glucose concentration of human serum samples by means of lifetime-based approach. Before measurements, all samples (obtained from Zhongguancun Hospital, China) were diluted 3 times by phosphorate buffer at pH 7.0. In a black 96-well microplate, 100 μL of GOx-NPs solution was placed inside each well. The microplate was initially conditioned for 20 min to equilibrate with air and at constant temperature (37 °C). Then 25 μL of diluted serum sample was quickly injected into the well, followed by addition of 200 μL of mineral oil. After 2 min, phosphorescence signals were measured on a commercial plate reader according to the procedures of TRF method, and the intensity signals were converted into to lifetime consequently. Finally