

A silicon nanowire-based electrochemical glucose biosensor with high electrocatalytic activity and sensitivity

Shao Su,^a Yao He,^{bc} Shiping Song,^a Di Li,^a Lihua Wang,^a Chunhai Fan^{*a} and Shuit-Tong Lee^{*c}

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An electrochemical glucose biosensor was developed by immobilizing glucose oxidase (GOx) on an electrode decorated with a novel nanostructure, silicon nanowires (SiNWs) with *in situ* grown gold nanoparticles (AuNPs). The immobilized GOx displayed a pair of well-defined and quasi-reversible redox peaks with a formal potential ($E^{\circ'}$) of -0.376 V in a phosphate buffer solution. The fabricated glucose biosensor has good electrocatalytic activity toward oxidation of glucose. In addition, such resultant SiNWs-based glucose biosensor possesses high biological affinity. Particularly, the apparent Michaelis–Menten constant was estimated to be 0.902 mM, which is much smaller than the reported values for GOx at a range of nanomaterials-incorporated electrodes. Consequently, this novel SiNWs-based biosensor is expected to be a promising tool for biological assays (*e.g.*, monitoring blood glucose).

Introduction

Nanomaterials have become popularly employed in biological applications such as biosensors, biocatalysis, biomedical diagnostics and therapy due to their unique physical and chemical properties, large surface area and good biocompatibility.¹ Particularly, nanomaterials-based biosensors lead to unprecedented advantages such as rapid response, high sensitivity, high mechanical, thermal and chemical stability, and low cost.² Gold nanoparticles (AuNPs),³ carbon nanotubes (CNTs),⁴ quantum dots (QDs),⁵ and silicon nanomaterials^{6–8} are among the most popularly employed nanomaterials in biosensor design. Silicon nanowires (SiNWs) are highly attractive for developing high-performance biosensors because of their unique optical, electronic, and mechanical properties, as well as their compatibility with the conventional silicon technology. Moreover, SiNWs of high quality can be mass produced since the pioneering work of Lieber's⁹ and Lee's groups.¹⁰ SiNWs have already been used as different sensors to detect a range of important chemical and biological targets, *e.g.* gas,¹¹ metal ions,¹² nucleic acids¹³ and proteins.^{14–16} Of our particular interest, the large surface of SiNWs can be conveniently decorated with silver,¹⁷ palladium¹⁸ and gold¹⁹ nanoparticles (NPs), leading to stable SiNWs@metal NPs nanocomplexes. Such nanocomplexes are expected to offer superior sensing properties, *e.g.* high sensitivity, high selectivity and rapid response.²⁰

In this study, we explore the use of SiNWs decorated with gold nanoparticles (SiNWs@AuNPs) to support direct electrochemistry of glucose oxidase (GOx) and develop an enzyme-based electrochemical sensor for sensitive glucose detection. Monitoring of blood glucose levels is of great importance for the

diagnosis and therapy of diabetes, a syndrome of abnormally high blood sugar levels (hyperglycemia) in the body that has become a chronic and worldwide public health problem.²¹ GOx is the most employed enzyme for the construction of enzyme-based glucose sensors, which catalytically converts glucose to gluconolactone *via* a redoxable flavin adenine dinucleotide (FAD) co-factor. Given that FAD is encapsulated within the insulating peptide shell, it is necessary to employ small-molecule redox mediators (*e.g.* ferrocene derivatives) to efficiently couple FAD to the electrode surface.^{22–26} More recently, various nanomaterials such as quantum dots,²⁷

which clearly showed that AuNPs had decorated the surface of SiNWs (Fig. 1, left). The high-resolution TEM (HRTEM, Fig. 1, right) clearly shows that AuNPs were steadily grown into the SiNWs, but not adsorbed on SiNWs.

It is interesting that the SiNWs@AuNPs can effectively accommodate GOx, leading to a stable film at the GC electrode surface. As shown in Fig. 2A, GOx exhibited a pair of well-defined and quasi-reversible redox peaks with the anodic and cathodic peak potentials at -0.360 V and -0.393 V, respectively (curve c), which were ascribed to the facilitated redox reaction of FAD at the electrode surface. As controls, the Nafion-SiNWs@AuNPs/GC electrode (curve a) did not show any electrochemical features in the cyclic voltammogram (CV), while Nafion-GOx/GC electrode only showed a pair of rather weak redox peaks (curve b). The stability of the Nafion-GOx-SiNWs@AuNPs/GC electrode was evaluated by continuous potential scan in the 0.1 M phosphate buffer of pH 6.0. We found that the peak currents showed minimal decrease even after 100 cycles ($\sim 95\%$ of the initial signal) (Fig. 2B), which suggested that this modified electrode possessed relatively high stability.

The cathodic and anodic peak currents for the Nafion-GOx-SiNWs@AuNPs/GC electrode increased linearly along with the scan rate in the range of 10 – 500 mV s^{-1} (Fig. 3), suggesting that the electroactive species was confined at the electrode surface. Based on the dependence of the peak separation (ΔE_p) on scan

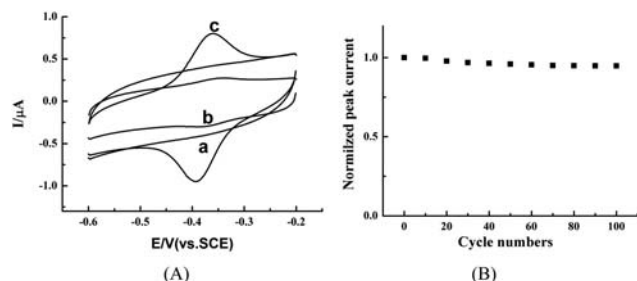


Fig. 2 (A) Cyclic voltammograms of (a) Nafion-SiNWs@AuNPs/GC, (b) Nafion-GOx/GC and (c) Nafion-GOx-SiNWs@AuNPs/GC electrodes in 0.1 M PBS (pH 6.0) with N_2 -saturated at a scan rate of 50 mV s^{-1} . (B) Stability of the Nafion-GOx-SiNWs@AuNPs/GC electrode on continuous cyclic voltammetric response. The normalized peak current was calculated by comparing the response of the electrode with that of the first cycle.

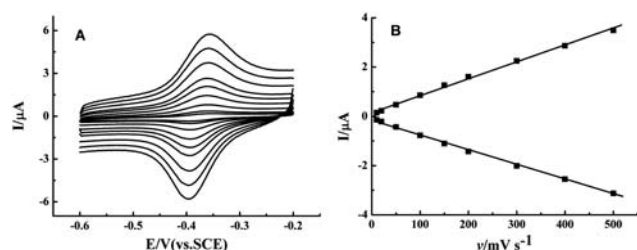


Fig. 3 (A) Cyclic voltammograms of the Nafion-GOx-SiNWs@AuNPs/GC electrode in 0.1 M pH 6.0 PBS at various scan rates. The scan rate is 10 , 20 , 50 , 100 , 150 , 200 , 300 , 400 and 500 mV s^{-1} (from inner to outer). (B) Relationship between scan rate and the cathodic and anodic peak currents.

rates, we estimated that the apparent electron transfer rate constant (k_s) of GOx was 1.11 s^{-1} .³³

We also evaluated the pH-dependent response of the Nafion-GOx-SiNWs@AuNPs/GC electrodes. Significantly, elevation of the solution pH led to negative shift of both anodic and cathodic peaks,

Nafion-GOx-SiNWs@AuNPs/GC electrode in a 0.1 M PBS solution (pH 6.0) with various concentrations of glucose. No redox peaks were observed at the Nafion-GOx-SiNWs@AuNPs/GC electrode in the measured potential range (curve a). When FMCA was added to the solution, a couple of well-defined redox peaks were observed, corresponding to the redox reaction of FMCA (curve b). With the addition of glucose, the anodic peak current increased and the cathodic peak current decreased (curve c and d), characteristic of a typical electrocatalytic reaction. Further addition of glucose could increase the anodic peak. These data clearly showed that the immobilized GOx retained its biocatalytic activity for glucose oxidation. This mediated biocatalytic reaction can be employed to detect glucose; however we found that a relatively poor detection limit of 500 μM was reached.

We then explored the possibility of using direct electron transfer of GOx with the assistance of SiNWs@AuNPs to increase the sensitivity. When the Nafion-GOx-SiNWs@AuNPs/GC electrode was immersed in a solution saturated with oxygen, we found a marked increase (~ 9 fold) of the cathodic peak along with the diminishment of the anodic peak, implying electrocatalytic reduction of O_2 in the O_2 -saturated buffer (Fig. 5B). Significantly, the anodic peak decreased along with the addition of glucose, which was due to the oxygen consumption during the glucose oxidation that competitively reduced the supply of oxygen for electrocatalysis. The anodic peak was then expected to provide a quantitative measure of glucose concentrations.

We then employed an amperometric method (i - t curves) to perform glucose detection with the Nafion-GOx-SiNWs@AuNPs/GC electrode. Fig. 6A depicted the amperometric responses for Nafion-GOx-SiNWs@AuNPs/GC electrode in solutions of various concentration of glucose. The steady-state currents were found to be reversely linearly proportional to the glucose concentrations (Fig. 6B). Importantly, the detection limit was found to be 50 μM , a ten-fold improvement as compared to the mediated electrochemistry. This sensitivity would be sufficient to support blood glucose monitoring since the blood glucose level is typically in the range of 4.4–6.6 mM.²⁶ We also calculated the apparent Michaelis–Menten constant (K_M^{app}) of GOx confined at the electrode surface *via* the Lineweaver–Burk plot, which was found to be 0.902 mM. This value is smaller than the reported value for GOx for a range of nanomaterials-incorporated electrodes (see Table 1), which suggests the synergistic effect of hybrid nanocomplex that improves the affinity of GOx to glucose. In order to evaluate the selectivity of this glucose

Table 1 The comparison of the performance of present sensor and others reported in the literatures for glucose detection. The apparent Michaelis–Menten constant (K_M^{app}) of GOx in solution is 33–100 mM (see the reference to GOx at www.sigmaaldrich.com)^a

Glucose biosensor	Linear range/mM	Detection limit/ μM	K_M^{app} /mM	Ref.
Nafion-GOx-SiNWs@AuNPs/GC	0.1–0.8, 1–16	50	0.902	This work
GNPs-Pb NWs	0.005–2.2	2	4.58	36
Nafion/GOx-GNPs/GC	<6	34	4.6	37
GOx/NdPO ₄ NPs/CHIT/GCE	0.05–10	80	2.5	38
GOx-nanoPANi/Pt	0.01–5.5	~ 0.3	~ 2.37	39
GOx/Au _{nanop} /Pt _{nanop} /CNT/Au	0.5–16.5	400	10.73	40
GOx/MWNTs-Fc/CS	0.			

Instrumentation

All electrochemical experiments were carried out with a CHI 630B (CHI Instruments Inc., Austin, USA). A conventional three-electrode system was used in this work. Glassy carbon (GC) electrodes were used as the working electrodes, and a platinum wire and an Ag/AgCl (3 M KCl) electrode were used as the counter and the reference electrodes, respectively. Phosphate buffer of 0.1 M (pH 6.0) was employed as the supporting electrolyte unless specifically indicated. Electrolyte solutions were deoxygenated with nitrogen bubbling for at least 30 min and a nitrogen atmosphere was kept over the solution in electrochemical measurements.

Preparation of SiNWs and AuNPs@SiNWs

SiNWs were synthesized as previously reported. Briefly, they were prepared by oxide-assisted growth *via* simple thermal evaporation of silicon monoxide powder as the single source.^{34,35} The oxide sheath was removed by immersing the as-prepared SiNWs into a 0.1 M NaOH solution for 10 min. Then 15 μL of 1% HAuCl₄ was added to the solution, which was readily reduced by SiNWs to AuNPs, leading to the formation of the SiNWs@AuNPs nanocomplex.

Preparation of the Nafion-GOx-SiNWs@AuNPs/GC electrode

GC electrodes of 3 mm in diameter was mechanically polished with 0.3 and 0.05 μm alumina slurry and then sequentially sonicated in water, ethanol and water for several min. Cleaned GC electrodes were dried with nitrogen stream. The SiNWs@AuNPs nanocomplex was added to 1 mL of Nafion solution (0.5 wt%), which was ultrasonicated for several min to form a stable suspension. Then this suspension was thoroughly mixed with a GOx solution (5 mg mL⁻¹) at 1 : 1 ratio (v/v). The Nafion-GOx-SiNWs@AuNPs modified GC electrodes were obtained by casting the mixed solution of 5 μL onto GC electrodes, which was then covered to allow slow evaporation and the film formation at the electrode surface. The enzyme modified electrodes were stored in refrigerator when not in use.

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