analytical chemistry

Article

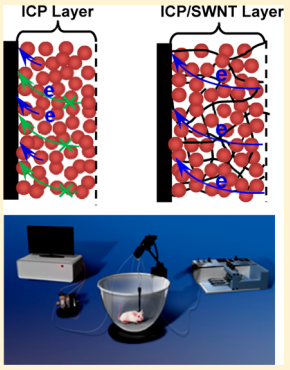
pubs.acs.org/ac

Hybridization of Bioelectrochemically Functional Infinite Coordination Polymer Nanoparticles with Carbon Nanotubes for Highly Sensitive and Selective In Vivo Electrochemical Monitoring

Xulin Lu, Hanjun Cheng, Pengcheng Huang, Lifen Yang, Ping Yu, and Lanqun Mao*

Beijing National Laboratory for Molecular Sciences, Key Laboratory of Analytical Chemistry for Living Biosystems, Institute of Chemistry, Chinese Academy of Sciences, Beijing 100190, P. R. China

ABSTRACT: This study demonstrates the formation of a three-dimensional conducting framework through hybridization of bioelectrochemically active infinite coordination polymer (ICP) nanoparticles with single-walled carbon nanotubes (SWNTs) for highly sensitive and selective in vivo electrochemical monitoring with combination with in vivo microdialysis. The bioelectrochemically active ICP nanoparticles are synthesized through the self-assembly process of NAD^+ and Tb^{3+}, in which all biosensing elements including an electrocatalyst (i.e., methylene green, MG), cofactor (i.e., β-nicotinamide adenine dinucleotide, NAD^+), and enzyme (i.e., glucose dehydrogenase, GDH) are adaptively encapsulated. The ICP/SWNT-based biosensors are simply prepared by drop-coating the as-formed ICP/SWNT nanocomposite onto a glassy carbon substrate. Electrochemical studies demonstrate that the simply prepared ICP/SWNT-based biosensors exhibit excellent biosensing properties with a higher sensitivity and stability than the ICP-based biosensors prepared only with ICP nanoparticles (i.e., without hybridization of SWNTs). By using a GDH-based electrochemical biosensor as an example, we demonstrate a technically simple yet effective online electroanalytical platform for continuously monitoring glucose in the brain of guinea pigs with the ICP/SWNT-based biosensor as an online detector in a continuous-flow system combined with in vivo microdialysis. Under the experimental conditions employed here, the dynamic linear range for glucose with the ICP/SWNT-biosensor is from 50 to 1000 μM. Moreover, in vivo selectivity investigations with the biosensors prepared by the GDH-free ICPs reveal that ICP/SWNT-based biosensors are very selective for the measurement of glucose in the cerebral system. The basal level of glucose in the microdialysates from the striatum of guinea pigs is determined to be 0.31 ± 0.03 mM (n = 3). The study offers a simple route to the preparation of electrochemical biosensors, which is envisaged to be particularly useful for probing the chemical events involved in some physiological and pathological processes.



Selective and sensitive in vivo monitoring of neurochemicals involved in brain functions has drawn much attention because the information on the chemical nature in the physiological and pathological events affords a platform for understanding of, for example, neurotransmission, and for the diagnosis and therapy of diseases.[1] As demonstrated by others and our group,[2] online detecting systems developed by efficiently integrating in vivo microdialysis with selective detection has emerged as one of the most promising approaches to accomplishing this purpose. This is because such integration substantially enables the in vivo neurochemical monitoring to be performed in a relatively simple way on living animals, even on freely moving animals, with excellent properties, including technical simplicity, near real-time nature, and easy adaptability by neurochemists. Moreover, the integrated systems can, in vivo, continuously track the neurochemical changes during different processes involved in a whole physiological event from one animal and, as a consequence, the number of animals employed in the experiments could largely be reduced.

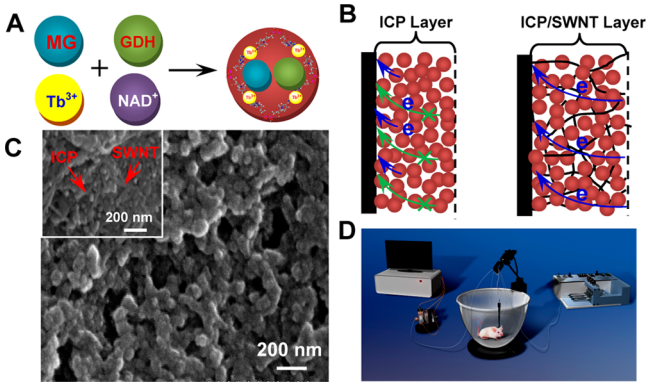
As reported previously, in the online detecting systems, selective detection remains very essential.[3] This is because, different from the separation-based analytical protocols employed for the neurochemical measurements, the online detecting systems do not employ sample separation and the microdialysates continuously sampled from the biological systems are detected directly at the online detector. While the uses of enzymes as the recognition units for neurochemical biosensing have successfully enabled the online detecting systems to be very selective for continuous in vivo neurochemical monitoring,[2−4] the involvement of multiple steps for surface confinement of all biosensing elements including biorecognition units (i.e., oxidases and dehydrogenases) and electronic transducers (i.e., enzyme cofactors, electron transfer mediators, or electrocatalysts) onto a conducting solid substrate inevitably leads to complicated and time-consuming processes for biosensor development with an unsatisfactory reproducibility.[5] These limitations unfortunately make it difficult for the nonelectrochemists to use these biosensor-based online detecting systems for the physiological and pathological investigations.

Received: December 22, 2012
Accepted: March 16, 2013
Published: March 17, 2013

   dx.doi.org/10.1021/ac303743a | *Anal. Chem.* 2013, 85, 4007−4013

To circumvent these problems, we have recently developed a straightforward approach to simplifying the biosensor development with infinite coordination polymer (ICP)-based technology.[6] In that case, all biosensing elements (i.e., electrocatalysts for the oxidation of NADH, NAD^+ cofactor, and dehydrogenase) are adaptively included and encapsulated into a single nanoparticle during the self-assembling process of NAD^+ and Tb^{3+} to form bioelectrochemically functional ICP nanoparticles (Scheme 1 A). By using this kind of integrative ICP nanoparticles as the biosensing unit, the dehydrogenase-based electrochemical biosensors could be simply and reproducibly prepared by one-step confinement of the ICP biosensing nanoparticles onto a substrate. Moreover, other kinds of dehydrogenases, rather than GDH, are also possibly encapsulated into the polymer nanoparticles together with the electrocatalysts and the cofactor. As a consequence, the ICP-based protocols could be explored to be general for the development of dehydrogenase-based electrochemical biosensors.

Scheme 1. (A) Schematic Illustration of Formation of Bioelectrochemically Active ICP Nanoparticles, (B) Structure of ICP- (Left Panel) and ICP/SWNT- (Right Panel) Based Biosensors, (C) SEM Images of ICP Nanoparticles and ICP/SWNT Nanocomposite (Inset), and (D) Online Electrochemical Detecting System for In Vivo Monitoring



Stimulated by the excellent properties of the protocols mentioned above, we wish to develop a new electroanalytical platform for the continuous in vivo monitoring of physiologically important species in the cerebral systems with the ICP-based biosensing technology. To achieve simple-in-preparation and robust-in-operation biosensors, providing efficient bioelectrocatalytic activity for biosensing applications, we previously drop-coated the ICP-based biosensing units onto a substrate electrode to form dehydrogenase-based electrochemical biosensors.[6] However, the poor electronic conductivity of the ICP nanoparticles themselves would not allow electron transport for most of the ICP nanoparticles at a flat, smooth electrode surface, as they are far away from the conducting substrate, as shown in Scheme 1 B (left panel). To validate well this kind of simply synthesized and bioelectrochemically active ICP nanoparticles for highly sensitive and selective in vivo monitoring, this study hybridizes the bioelectrochemically active ICP nanoparticles with carbon nanotubes to form a three-dimensional (3D) conducting framework, allowing an efficient electron transport for almost all ICPs since the ICPs can find a nearby conducting wire, regardless of the distance between the ICPs and the electrode (Scheme 1 B, right panel). Thus, the use of carbon nanotubes to conduct the ICP nanoparticles onto the electrode substantially increases the sensitivity of the as-prepared biosensors. Compared with other conductive biointerfaces such as those incorporating gold nanoparticles and/or conductive polymers,[7] the as-prepared ICP/SWNT biointerface contains all biosensing elements and efficiently transduces biorecognition events into an electric signal, which greatly simplifies the ICP/SWNT-based biosensors fabrication and, more remarkably, minimizes the biosensor-to-biosensor deviation. In addition, rather than glucose that is used as an example in this study, other kinds of physiologically important species such as glutamate and lactate are also potentially detectable with this ICP-based biosensing strategy. Thus, this study offers a new analytical platform for the understanding of chemical events involved in some physiological and pathological processes.

EXPERIMENTAL SECTION

Reagents and Solutions. D(+)-Glucose, β-nicotinamide adenine dinucleotide (NAD^+), 2-[4-(hydroxyethyl)-1-piperazinyl] ethanesulfonic acid (HEPES), and glucose dehydrogenase (GDH, EC 1.1.1.47, from *Pseudomonas* sp.) were all purchased from Sigma and used as supplied. $Tb(NO_3)_3{\cdot}6H_2O$ was purchased from Aladdin. Methylene green (MG) was purchased from Beijing Chemical Company (Beijing, China). Single-walled carbon nanotubes (SWNTs, <2 nm in diameter and 0.5−50 μm in length) were purchased from Shenzhen Nanoport Company, Ltd. (Shenzhen, China). Prior to use, SWNTs were purified by refluxing the as-received SWNTs in 2.6 M nitric acid for 5 h, followed by centrifugation, resuspension, filtration, and air-drying to evaporate the solvent. The purified SWNTs were further heated under vacuum at 500 °C for 2 h. Artificial cerebrospinal fluid (aCSF) used as the perfusion solution for in vitro experiments and in vivo microdialysis was prepared by mixing NaCl (126 mM), KCl (2.4 mM), KH_2PO_4 (0.5 mM), $MgCl_2$ (0.85 mM), $NaHCO_3$ (27.5 mM), Na_2SO_4 (0.5 mM), and $CaCl_2$ (1.1 mM) into Milli-Q water. Other chemicals were of at least analytical grade and were used as received. All aqueous solutions were prepared with Milli-Q water.

Synthesis of ICP Nanoparticles and Their Composites with SWNTs. Synthesis of bioelectrochemically active ICP nanoparticles was performed as reported in our earlier study.[6] Briefly, an aqueous solution of $Tb(NO_3)_3{\cdot}6H_2O$ was added into the HEPES buffer (pH 7.4) containing NAD^+, MG, and GDH to form a white precipitate. The obtained precipitate was then purified by centrifugation and washed several times with Milli-Q water to give the ICP nanoparticles, in which all biosensing elements including MG electrocatalysts, NAD^+ cofactor, and GDH were adaptively included and encapsulated during the self-assembly process of NAD^+ and Tb^{3+}. To prepare the nanocomposite of the ICP nanoparticles and SWNTs, aqueous dispersions of SWNTs (2 mg/mL) and ICPs (5 mg/mL) were mixed with a volume ratio of 1:2. The resulting mixture was then sonicated for 2 h to give a homogeneous ICP/SWNT dispersion that was used for the biosensor preparation.

In Vitro Electrochemical Measurements. All electrochemical experiments were performed with a computer-controlled electrochemical analyzer (CHI 832B, Chenhua, China) in a thin-layer radial flow cell with aCSF as the perfusion solution. Glassy carbon (GC, 6 mm diameter) electrodes and the thin-layer radial electrochemical flow cell

dx.doi.org/10.1021/ac303743a | *Anal. Chem.* 2013, 85, 4007−4013

used for the electrochemical measurements were both purchased from Bioanalytical Systems Inc. (BAS). The thin-layer radial electrochemical flow cell consists of a thin-layer radial flow block with a 50 μm gasket, a GC electrode as a working electrode, stainless steel as a counter electrode, and a Ag/AgCl electrode (3 M NaCl) as a reference electrode. Prior to the surface confinement of the ICP/SWNT nanocomposite onto GC electrodes, the electrodes were polished first with emery paper and then with aqueous slurries of fine alumina powder (0.3 and 0.05 μm) on a polishing cloth and were finally rinsed with ethanol and Milli-Q water under an ultrasonic bath, each for 5 min. After that, 10 μL of the ICP/SWNT dispersion was drop-coated onto GC electrodes to form the ICP/SWNT-based biosensors. For comparison, the same amount of pure ICP dispersion (i.e., without SWNTs) was also drop-coated onto GC electrodes to form the ICP-based biosensors. Moreover, the biosensors were also prepared by conventional procedures first through adsorption of MG and NAD^+ and then cross-linking GDH onto SWNTs, as reported in our early study.[8] In this case, a MG/NAD^+/SWNT composite was prepared by mixing 2 mg of SWNTs, 40 mg of MG, and 40 mg of NAD^+ into 4 mL of water while being stirred for 48 h at room temperature, and the resulting mixture was then filtered with a Millipore filter (0.45 μm, Millipore) and washed with water to remove NAD^+ and MG nonadsorbed onto SWNTs. The obtained sample was finally dried at 40 °C under vacuum overnight to obtain the MG/NAD^+/SWNT nanocomposite. The synthetic composite was dispersed into water with sonication to obtain a homogeneous dispersion (2 mg/mL). A 10 μL sample of the dispersion was drop-coated onto GC electrodes, and the electrodes (i.e., MG/NAD^+/SWNT-modified GC electrodes) were then air-dried. To prepare the electrochemical biosensors based on the MG/NAD^+/SWNT-modified GC electrodes, 2 μL of GDH (10 mg/mL) aqueous solution was mixed with 1 μL of 1% BSA aqueous solution and 1 μL of 1% glutaraldehyde and the resulting mixture was totally coated onto the MG/NAD^+/SWNT-modified GC electrodes to give the electrochemical biosensors (i.e., conventional integrative biosensors). All biosensors were stored in a dry state at +4 °C when not in use.

Animal Surgery and Online Monitoring of Glucose in Brain Microdialysates. To demonstrate the validity of the ICP/SWNT-based biosensors prepared in this study for continuous in vivo monitoring of glucose, in vivo microdialysis was directly coupled to a thin-layer radial electrochemical flow cell, in which the ICP/SWNT-based electrochemical biosensor was used as the detector to form an online detecting system for near real-time monitoring glucose in the brain microdialysates of guinea pigs. Brain microdialysates and external solutions were delivered from syringes and pumped through tetrafluoroethylene hexafluoropropene (FEP) tubing by a microinjection pump (CMA 100, CMA Microdialysis AB, Stockholm, Sweden) (Scheme 1 D). Brain microdialysates were sampled from the brain of guinea pigs with pure aCSF as the perfusion solution at 2 μL/min. The ICP/SWNT-based biosensors were polarized at +0.20 V for the continuous monitoring of glucose in the brain microdialysates.

Surgeries for in vivo microdialysis was performed with the methods reported previously.[9] Briefly, adult male guinea pigs (300−400 g) obtained from Health Science Center, Peking University, were housed on a 12:12 h light−dark schedule with food and water *ad libitum*. The animals were anaesthetized with pentobarbital (345 mg/kg, i.p.) and positioned onto a stereotaxic frame. The microdialysis guide cannulas (BAS) were implanted in the striatum (AP = 0 mm, L = 3 mm from bregma; V = 4.5 mm from dura) using standard stereotaxic procedures. The guide cannula was kept in place with three skull screws and dental cement. Stainless steel dummy blockers were inserted into the guide cannula and fixed until the insertion of the microdialysis probe (CMA, dialysis length, 2 mm; diameter, 0.24 mm). Throughout the surgery, the body temperature of the animals was maintained at 37 °C with a heating pad. Immediately after the surgery, the guinea pigs were placed into a warm incubator individually until they recovered from the anesthesia. The guinea pigs were allowed to recover for at least 24 h before in vivo microdialysis sampling. The microdialysates were continuously sampled and continuously monitored with the online detecting system after the microdialysis probe was implanted in the guinea pigs brain and equilibrated through perfusing aCSF for at least 90 min.

RESULTS AND DISCUSSION

Formation of 3D Conducting Framework. SWNTs were used to construct the 3D conducting framework because of their good electronic conductivity and structural properties, of which the former actually enables the nanostructures to be used for electron exchanging between the ICP nanoparticles and the electrode, while the latter makes it possible to transfer electrons from the frameworks to the conducting substrate (Scheme 1 B, right panel). In addition, the uses of SWNTs could efficiently enlarge the effective surface area of the electrode and thus enhance the response sensitivity. As demonstrated in our previous study,[6] the bioelectrochemically active ICP nanoparticles were synthesized based on the coordination chemistry between the N atoms in the imidazole ring and phosphate groups of NAD^+ with Tb^{3+} to form the Tb-NAD^+ network, in which MG and GDH were adaptively coencapsulated in the Tb-NAD^+ framework during the self-assembly process (Scheme 1 A).[6] The ICP nanoparticles synthesized with our method were colloidal spheres with diameters ranging from about 50 to 100 nm (Scheme 1 C). The hybridization of SWNTs with the ICP nanoparticles eventually forms an ICP/SWNT nanocomposite, in which the ICP nanoparticles are either adsorbed on the surface of SWNTs or entrapped in the SWNT network (Scheme 1 C, inset). This structural property of SWNTS substantially makes it possible to form 3D conducting ICP/SWNT framework, in which SWNTs serve as wires to electrochemically conduct ICP nanoparticles to the conducting substrate, enabling almost all ICP nanoparticles bioelectrochemically active for catalyzing the oxidation of glucose with a higher efficiency than that with the pure ICP nanoparticles (i.e., without hybridization with SWNTs). This feature largely improves the sensitivity of the ICP-based biosensors for the glucose measurements, as typically depicted in Figure 1. In the continuous-flow system (Scheme 1 D), the ICP-based biosensor produces a very small current response (about 1.5 nA) toward 1.0 mM glucose. Moreover, the current response decreases gradually as a function of time, mainly because of the poor stability of the ICP nanoparticles confined onto the electrode, while the ICP/SWNT-based biosensor exhibits a largely enhanced current response toward glucose (i.e., 298 nA) under the same conditions as those employed for the ICP-based biosensor. Furthermore, the current response remains almost unchanged in the experimental timescale employed in this study (Figure 1 B), indicating that the hybridization of SWNTs with the ICP nanoparticles also remarkably increases

dx.doi.org/10.1021/ac303743a | *Anal. Chem.* 2013, 85, 4007−4013

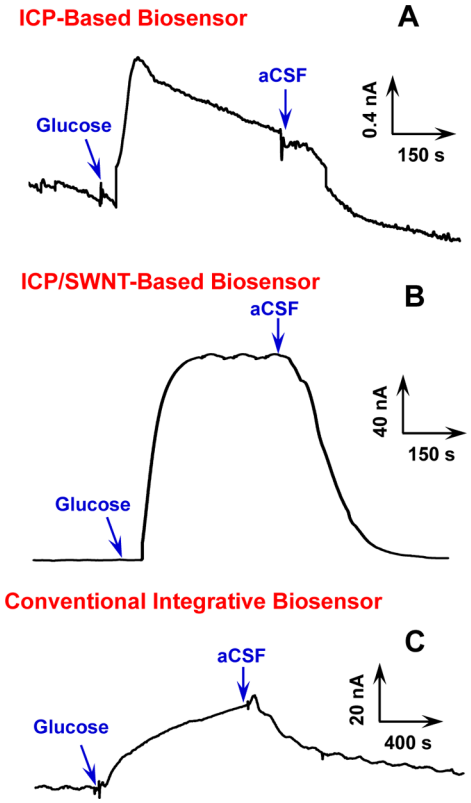


Figure 1. Current responses recorded with the online detecting system, with the (A) ICP-based, (B) ICP/SWNT-based, and (C) conventional integrative biosensors as the detector, toward 1.0 mM glucose. The (A) ICP-based, (B) ICP/SWNT-based, and (C) conventional integrative biosensors were prepared by drop-coating 10 μL aqueous dispersion of ICP nanoparticles, ICP/SWNT nanocomposite, and MG/NAD$^+$/SWNT nanocomposite onto GC electrodes, respectively. Glucose standard in the aCSF was perfused into the system at a perfusion rate of 2 μL/min. The biosensors were polarized at +0.20 V vs Ag/AgCl electrode.

the stability of the as-prepared biosensors. This comparison strongly demonstrates that the use of SWNTs to hybridize with the ICP nanoparticles eventually forms a 3D conducting framework that could actually "wire" more ICP nanoparticles onto the electrode, providing efficient bioelectrocatalysis toward glucose oxidation with a largely enhanced current output and operation stability in the continuous-flow system.

We should note that, as demonstrated in our previous studies,[8] the special electronic and structural properties of SWNTs well-enabled their ability to serve as the support for NAD$^+$ cofactor and MG electrocatalyst through the hydrophobic and/or charge-transfer interaction(s), providing a simple approach to the preparation of integrative dehydrogenase-based electrochemical biosensors (i.e., conventional integrative biosensors). Nevertheless, the advantages of the ICP/SWNT-based biosensors prepared in this study are still remarkable in terms of the improved sensitivity and stability and simplified fabrication procedures of the ICP/SWNT-based electrochemical biosensors. For example, as displayed in Figure 1 C, the current response obtained at the conventional integrative biosensor toward glucose was lower and less stable, as compared with that at the ICP/SWNT-based biosensor, suggesting that the strategy demonstrated here greatly improves the sensitivity and stability of the biosensors and simplifies the biosensor fabrication as well.

To further demonstrate the formation of the 3D conducting framework by hybridizing SWNTs with the ICP-based biosensing units, we changed the amounts of pure ICP nanoparticles or ICP/SWNT nanocomposites drop-coated onto the electrode surface and compared the current responses of the as-prepared biosensors toward glucose in the continuous-flow system. As depicted in Figure 2, in the same continuous-flow system, both the ICP- and ICP/SWNT-based biosensors were responsive toward glucose with current responses increasing with increasing amounts of ICP nanoparticles or

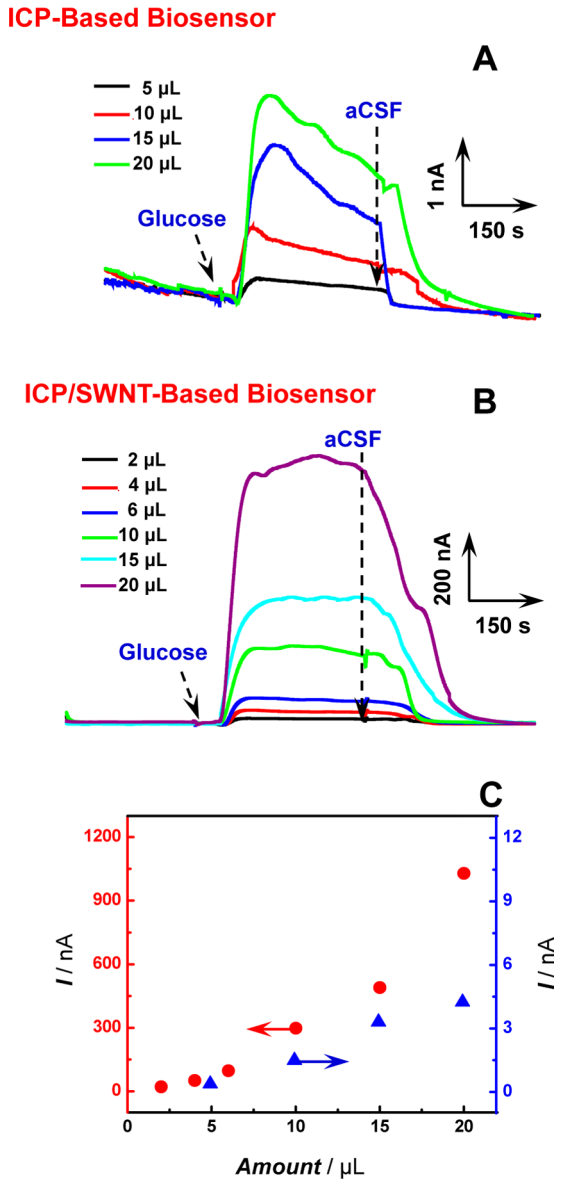


Figure 2. Current responses recorded with the online detecting systems, with the (A) ICP- and (B) ICP/SWNT-based biosensors as the detectors, toward 1.0 mM glucose. The (A) ICP-based and the (B) ICP/SWNT-based biosensors were prepared by drop-coating different amounts (indicated in the figure) of ICP nanoparticles and ICP/SWNT nanocomposites onto GC electrodes, respectively. (C) Plots of the current responses obtained at the ICP-based (▲) and the ICP/SWNT-based (●) biosensors in the continuous-flow system toward 1.0 mM glucose against the amounts of ICP nanoparticles and ICP/SWNT nanocomposites one-time drop-coated onto GC electrodes. Other conditions were the same as Figure 1.

dx.doi.org/10.1021/ac303743a | *Anal. Chem.* 2013, 85, 4007−4013

ICP/SWNT nanocomposites drop-coated onto GC electrodes. However, a comparison of the current responses obtained with the (A) ICP-based and (B) ICP/SWNT-based biosensors reveals several striking differences: (1) under the same conditions employed here, the current responses obtained at the ICP/SWNT-based biosensors were much larger than those at the ICP-based biosensors. For example, the ICP-based biosensors produce very small current responses toward glucose (typically several nanoampere) regardless of the amount of ICP nanoparticles applied onto the electrodes, while the ICP/SWNT-based biosensors produce largely enhanced current responses toward the same concentration of glucose (almost at a microampere level), as can be seen from Figure 2 B. (2) Compared with those at the ICP-based biosensors, the current responses at the ICP/SWNT-based biosensors were relatively stable. For instance, within the timescale employed in this study, the current responses recorded at the ICP-based biosensors were decreased by about 15% relative to the initial values, while those at the ICP/SWNT-based biosensors remains almost unchanged. (3) More strikingly, as depicted in Figure 2 C, the current responses achieved at the ICP-based biosensors tend to level off with increasing the amount of the ICP nanoparticles applied onto the electrodes (▲). In contrast, the current responses obtained at the ICP/SWNT-based biosensors dramatically increase with increasing amounts of the ICP/SWNT nanocomposite drop-coated onto the electrodes (●). These comparisons virtually indicate that the hybridization of SWNTs with the ICP-based biosensing units greatly enhances the sensitivity and stability of the as-prepared biosensors toward glucose.

The sensitivity enhancement was elucidated by the formation of a 3D conducting framework through the hybridization of SWNTs and the ICP nanoparticles. As mentioned above, in the as-formed ICP/SWNT nanocomposite, SWNTs act as "wires" for electrochemically conducting almost all ICP biosensing nanoparticles onto the conducting substrate, successfully transducing biorecognition events occurring inside single ICP nanoparticle into the current readout. The largely enhanced sensitivity, along with the remarkably improved stability and the simple-in-preparation feature and the inherent selectivity of biosensors, substantially makes the biosensors prepared in this study very attractive for in vivo measurements, as demonstrated below.

Linearity, Reproducibility, and Stability. Figure 3 displays typical current responses obtained with the online detecting system with the ICP/SWNT-biosensor as the detector toward glucose. With aCSF as the perfusion solution and at a perfusion rate of 2 μL/min, the system produces well-defined current responses toward glucose (Figure 3 A). The dynamic linear range for glucose with the ICP/SWNT-biosensor was from 50 to 1000 μM [I (nA) = $0.35C_{glucose}$ (μM) − 3.61, γ = 0.98]. The reproducibility and stability of the online detecting system with the ICP/SWNT-based biosensor as the detector were also studied with standard solutions of glucose. With aCSF as the perfusion solution and at a perfusion rate of 2 μL/min, the relative standard deviation (RSD) of the repeated measurements of glucose standard (300 μM) was 2.0% (n = 8) (Figure 3 B). In addition, the system developed here was quite stable for the continuous measurements of glucose; the current responses for glucose remain unchanged for the continuous monitoring of glucose (300 μM) for at least 2 h (Figure 3 C). In addition, the online system was also stable for the discontinuous measurements of glucose; the current

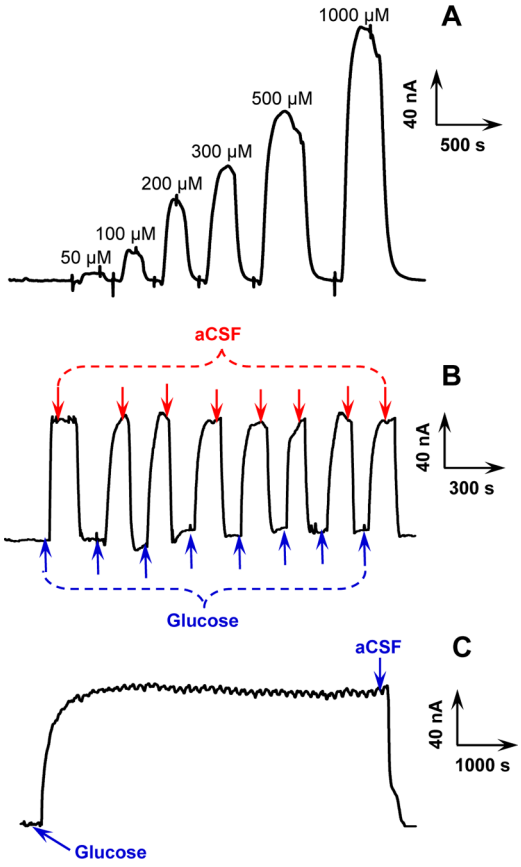


Figure 3. (A) Current responses obtained with the online detecting system with ICP/SWNT-based biosensor as the detector toward glucose with different concentrations indicated in the figure. Current responses (B) repeatedly and (C) continuously recorded with the online detecting system toward the glucose standard (300 μM). The ICP/SWNT-based biosensor was prepared by drop-coating 10 μL of ICP/SWNT nanocomposites onto GC electrodes. Other conditions were the same as Figure 1.

responses for glucose were not changed obviously for the measurements of glucose (300 μM) for at least 10 days with continuous monitoring for at least 4 h each day (data not shown). The high sensitivity, good stability, and reproducibility and the ease-in-preparation feature of biosensors substantially enable the as-established online detecting system for reliable and durable in vivo monitoring of glucose in the cerebral systems, as demonstrated below.

Selectivity and Online Monitoring of Glucose in the Brain Microdialysates. As described previously,[10] the online electrochemical methods bear advantages over other kinds of electrochemical methods employed for in vivo analysis in terms of their simplicity both in the operation mechanism and in the experimental procedures, sample freshness maintenance, largely reduced animal numbers, and as such, physiological and pathological usefulness. However, the abrogation of sample collection and separation procedures actually requires the online electrochemical detecting systems to have a high selectivity. As documented in the previous studies,[2a] a variety of electrochemically active species, such as dopamine, 3,4-dihydroxyphenylacetic acid, uric acid, and 5-hydroxytryptamine endogenously coexisting in the brain could be readily oxidized electrochemically at the detectors and may thus potentially interfere with the online monitoring of glucose in the brain microdialysates in this study. In our in vitro experiments, we

dx.doi.org/10.1021/ac303743a | Anal. Chem. 2013, 85, 4007−4013

found that the perfusion of dopamine (2 μM), 3,4-dihydroxyphenylacetic acid (5 μM), 5-hydroxyltrypatamine (2 μM), or uric acid (10 μM) did not produce obvious current response with the online detecting system (data not shown), demonstrating that these species do not interfere with glucose measurements. However, such in vitro selectivity tests may not be very essential for in vivo analysis of neurochemicals in the cerebral systems because of the chemical complexity of the cerebral systems. The chemical complexity in the cerebral systems substantially necessitates in vivo selectivity studies, as demonstrated in our early studies.[3e,4a,10e] To accomplish such a purpose in this study, we synthesized infinite coordination polymer nanoparticles containing no GDH (i.e., MG/Tb-NAD^+ ICP) with the procedures reported in our early study[6] and hybridized the GDH-free ICP nanoparticles with SWNTs to form the nanocomposite. The nanocomposite was then confined onto GC electrode to form a GDH-free ICP/SWNT-based sensor that was thus used as the detector for the online detecting system. As depicted in Figure 4 (red curve), the

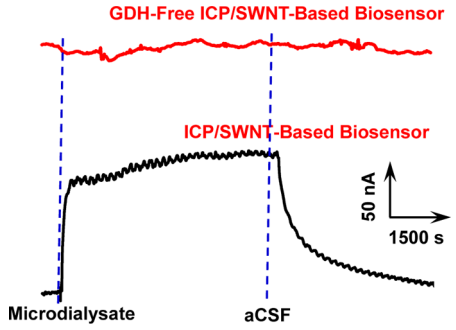


Figure 4. Current responses continuously recorded with the online electrochemical detecting systems with the GDH-free ICP/SWNT-based (red curve) and ICP/SWNT-based (black curve) biosensors, as the detectors for the brain microdialysate of the guinea pigs. Other conditions were the same as Figure 1.

switching of the perfusion solution from aCSF to the brain microdialysates continuously sampled from the striatum of guinea pigs did not produce an obvious current response, relative to the background current level, suggesting the other neurochemicals endogenously existing in the brain were not electrochemically oxidized or reduced at the GDH-free ICP/SWNT-based sensor. This result substantially demonstrates that the as-established online electrochemical detecting system with the ICP/SWNT-based biosensors as the detector was very selective toward glucose monitoring in the striatum of guinea pigs. As typically depicted in Figure 4 (black curve), the online system produces a well-defined current response toward glucose in the microdialysate, continuously sampled from the striatum of guinea pigs. Moreover, the current response was relatively stable as the function of time was not changed upon the continuous monitoring of the brain microdialysates for more than 1.5 h. The basal level of glucose was determined to be 0.31 $\pm$ 0.03 mM (n = 3), which was almost consistent with those reported in the literature.[11]

CONCLUSIONS

By efficiently hybridizing SWNTs with bioelectrochemically active ICP biosensing units, we have successfully demonstrated a new and simple route to the preparation of highly sensitive electrochemical biosensors. The sensitivity enhancement of the biosensors has been elucidated through the formation of a 3D conducting framework consisting of SWNTs and ICP nanoparticles, in which the presence of SWNTs largely facilitate efficient electron transport for almost all ICPs confined onto the electrode since the ICPs can easily find a nearby conducting wire, even though they are far from the electrode. The ICP/SWNT-based biosensors prepared by simply drop-coating the ICP/SWNT nanocomposite onto a conducting substrate exhibit highly selective, stable, and reproducible responses toward glucose. By using the simply prepared ICP/SWNT-based biosensors as the detector, we have successfully established an online electrochemical detecting system for in vivo electrochemical monitoring of glucose in the brain of guinea pigs. This study essentially offers a technically simple yet effective electrochemical approach to in vivo measurements of neurochemicals in the brain of living animals, which is believed to find wide applications in the understanding of chemical events involved in some physiological and pathological processes.

AUTHOR INFORMATION

Corresponding Author

*E-mail: lqmao@iccas.ac.cn. Fax: +86-10-62559373.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This research was financially supported by the NSF of China (Grants 20975104, 20935005, 21127901, 21210007, and 91213305 for L. M. and 91132708 for P. Y.), the National Basic Research Program of China (Grants 973 programs, 2010CB33502, 2013CB933704), and The Chinese Academy of Sciences (Grants KJCX2-YW-W25 and Y2010015).

REFERENCES

(1) (a) Miller, G. *Science* **2009**, *325*, 24−26. (b) Trouillon, R.; Passarelli, M. K.; Wang, J.; Kurczy, M. E.; Ewing, A. G. *Anal. Chem.* **2013**, *85*, 522−542. (c) Lin, Y.; Trouillon, R.; Safina, G.; Ewing, A. G. *Anal. Chem.* **2011**, *83*, 4369−4392. (d) Erecinska, M.; Cherian, S.; Silver, I. *Prog. Neurobiol. (Oxford, U.K.)* **2004**, *73*, 397−445. (e) Fillenz, M. *Neurochem. Int.* **2005**, *47*, 413−417. (f) Parkin, M. C.; Hopwood, S. E.; Jones, D. A.; Hashemi, P.; Landolt, H.; Fabricius, M.; Lauritzen, M.; Boutelle, M. G.; Strong, A. J. *J. Cereb. Blood Flow Metab.* **2005**, *25*, 402−413. (g) Burdette, S. C.; Lippard, S. J. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 3605−3610. (h) Giddings, J. C.; Yang, F. J.; Myers, M. N. *Anal. Chem.* **1976**, *48*, 1126−1132. (i) Stamfold, J. A.; Justice, J. B. *Anal. Chem.* **1996**, *68*, 359A−363A. (j) Stuart, J. V.; Hummon, A. B.; Sweedler, J. V. *Anal. Chem.* **2004**, *76*, 120 A−128 A. (k) Liu, K.; Lin, Y.; Yu, P.; Mao, L. *Brain Res.* **2009**, *1253*, 161−168. (l) Liu, J.; Yu, P.; Lin, Y.; Zhou, N.; Li, T.; Ma, F.; Mao, L. *Anal. Chem.* **2012**, *84*, 5433−5438.

(2) (a) Zhang, M.; Yu, P.; Mao, L. *Acc. Chem. Res.* **2012**, *45*, 533−543. (b) Lin, Y.; Zhang, Z.; Mao, L. Combination of In Vivo Microdialysis with Selective Electrochemical Detection for Online Continuous Monitoring of Brain Chemistry. In *Microdialysis Techniques in Neuroscience*; Giovanni, G. D.; Matteo, V. D., Eds. Springer: New York, 2013. (c) Robinson, D. L.; Hermans, A.; Seipel, A. T.; Wightman, R. M. *Chem. Rev.* **2008**, *108*, 2554−2584. (d) Khan, A. S.; Michael, A. C. *Trends Anal. Chem.* **2003**, *22*, 503−508. (e) Watson, C. J.; Venton, B. J.; Kennedy, R. T. *Anal. Chem.* **2006**, *78*, 1391−1399. (f) Zhang, M.; Mao, L. *Front. Biosci.* **2005**, *10*, 345−352. (g) Pravda, M.; Marvin, C. A.; Sarre, S.; Michotte, Y.; Kauffmann, J.-M. *Anal. Chem.* **1996**, *68*, 2447−2450. (h) Osborne, P.; Niwa, O.; Kato, T.; Yamamoto, K. *J. Neurosci. Methods* **1997**, *77*, 143−150. (i) Niwa,

O.; Horiuchi, T.; Kurita, R.; Torimitsu, K. *Anal. Chem.* **1998**, *70*, 1126−1132.
(3) (a) Hayashi, K.; Kurita, R.; Horiuchi, T.; Niwa, O. *Electroanalysis* **2002**, *5*, 333−338. (b) Kurita, R.; Hayashi, K.; Fan, X.; Yamamoto, K.; Kato, T.; Niwa, O. *Sens. Actuators, B* **2002**, *87*, 296−303. (c) Berners, M. O. M.; Boutelle, M. G.; Fillenz, M. *Anal. Chem.* **1994**, *66*, 2017−2021. (d) Mao, L.; Osborne, P. G.; Yamamoto, K.; Kato, T. *Anal. Chem.* **2002**, *74*, 3684−3689. (e) Zhang, M.; Liu, K.; Gong, K.; Su, L.; Chen, Y.; Mao, L. *Anal. Chem.* **2005**, *77*, 6234−6242. (f) Liu, K.; Lin, Y.; Xiang, L.; Yu, P.; Su, L.; Mao, L. *Neurochem. Int.* **2008**, *52*, 1247−1255.
(4) (a) Lin, Y.; Liu, K.; Yu, P.; Xiang, L.; Li, X.; Mao, L. *Anal. Chem.* **2007**, *79*, 9577−9583. (b) Osborne, P. G.; Niwa, O.; Yamamoto, K. *Anal. Chem.* **1998**, *70*, 1701−1706. (c) Kaptein, W. A.; Zwaagstra, J. J.; Venema, K.; Korf, J. *Anal. Chem.* **1998**, *70*, 4696−4700.
(5) (a) Wang, J. *Chem. Rev.* **2008**, *108*, 814−825. (b) Heller, A.; Feldman, B. *Chem. Rev.* **2008**, *108*, 2482−2505. (c) Turner, A. P. F.; Karube, I.; Wilson, G. S. *Biosensors: Fundamentals and Applications*; Oxford University Press: Oxford, NY, 1997. (d) Raitman, O. A.; Katz, E.; Bückmann, A. F.; Willner, I. *J. Am. Chem. Soc.* **2002**, *124*, 6487−6496.
(6) Huang, P.; Mao, J.; Yang, L.; Yu, P.; Mao, L. *Chem.—Eur. J.* **2011**, *17*, 11390−11393.
(7) (a) Höök, F.; Kasemo, B.; Grunze, M.; Zauscher, S. *ACS Nano* **2008**, *2*, 2428−2436. (b) Higgins, M. J.; Grosse, W.; Wagner, K.; Molino, P. J.; Wallace, G. G. *J. Phys. Chem. B* **2011**, *115*, 3371−3378. (c) Zhang, W.; Xie, G.; Li, S.; Lu, L.; Liu, B. *Appl. Surf. Sci.* **2012**, *258*, 8222−8227. (d) Yagatia, A. K.; Leeb, T.; Minc, J.; Choi, J.-W. *Colloids Surf., B* **2012**, *92*, 161−167. (e) Bhallaa, V.; Carrarab, S.; Sharmaa, P.; Nangiaa, Y.; Suri, C. R. *Sens. Actuators, B* **2012**, *162*, 761−768. (f) Higgins, M. J.; Grosse, W.; Wagner, K.; Molino, P. J.; Wallace, G. G. *J. Phys. Chem. B* **2011**, *115*, 3371−3378.
(8) Zhou, H.; Zhang, Z.; Yu, P.; Su, L.; Ohsaka, T.; Mao, L. *Langmuir* **2010**, *26*, 6028−6032.
(9) Zhuang, X.; Wang, D.; Lin, Y.; Yang, L.; Yu, P.; Jiang, W.; Mao, L. *Anal. Chem.* **2012**, *84*, 1900−1906.
(10) (a) Hayashi, K.; Kurita, R.; Horiuchi, T.; Niwa, O. *Biosens. Bioelectron.* **2003**, *18*, 1249−1255. (b) Niwa, O.; Kurita, R.; Horiuchi, T.; Torimitsu, K. *Anal. Chem.* **1998**, *70*, 89−93. (c) Pravda, M.; Bogaert, L.; Sarre, S.; Ebinger, G.; Kauffmann, J.-M.; Michotte, Y. *Anal. Chem.* **1997**, *69*, 2354−2361. (d) Lin, Y.; Zhang, Z.; Zhao, L.; Wang, X.; Yu, P.; Su, L.; Mao, L. *Biosens. Bioelectron.* **2010**, *25*, 1350−1355. (e) Zhang, Z.; Zhao, L.; Lin, Y.; Yu, P.; Mao, L. *Anal. Chem.* **2010**, *82*, 9885−9891.
(11) (a) Gramsbergena, J.; Kjøth-Rasmussenb, J.; Rasmussenc, S. C.; Lambertsena, K. L. *J. Neurosci. Methods* **2004**, *140*, 93−101. (b) Lin, Y.; Zhu, N.; Yu, P.; Su, L.; Mao, L. *Anal. Chem.* **2009**, *81*, 2067−2074.