Biosensors and Bioelectronics 24 (2008) 148–150

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

Short communication

Screen-printed electrodes based on carbon nanotubes and cytochrome P450scc for highly sensitive cholesterol biosensors

Sandro Carrara[a,*], Victoria V. Shumyantseva[b], Alexander I. Archakov[b], Bruno Samorì[a]

[a] Biochemistry Department, University of Bologna, Via Irnerio, 48-40126 Bologna, Italy
[b] Institute of Biomedical Chemistry, Pogodinskaya Street 10, Moscow 119121, Russia

ARTICLE INFO

Article history:
Received 24 January 2008
Received in revised form 6 March 2008
Accepted 11 March 2008
Available online 21 March 2008

Keywords:
Electron transfer
Cytochrome
Multi-walled carbon nanotubes
Cholesterol
Biosensors
Screen-printed electrodes

ABSTRACT

This paper is concerned with an investigation of electron transfer between cytochrome P450scc (CYP11A1) immobilized on nanostructured rhodium–graphite electrodes. Multi-walled carbon nanotubes (MWCNT) were deposited onto the rhodium–graphite electrodes by drop casting. Cytochrome P450scc was deposited onto MWCNT-modified rhodium–graphite electrodes. Cytochrome P450scc was also deposited onto both gold nanoparticle-modified and bare rhodium–graphite electrodes, in order to have a comparison with our previous works in this field. Cyclic voltammetry indicated largest enhanced activity of the enzyme at the MWCNT-modified surface. The role of the nanotubes in mediating electron transfer to the cytochrome P450scc was verified as further improved with respect to the case of rhodium–graphite electrodes modified by the use of gold nanoparticles. The sensitivity of our system in cholesterol sensing is higher by orders of magnitude with respect to other similar systems very recently published that are based on cholesterol oxidase and esterase. The electron transfer improvement attained by the use of MWCNT in P450-based cholesterol biosensors was demonstrated to be larger than 2.4 times with respect to the use of gold nanoparticles and 17.8 times larger with respect to the case of simple bare electrodes. The sensitivity was equal to 1.12 μA/(mM mm^2) and the linearity of the biosensor response was improved with respect to the use of gold nanoparticles.

1. Introduction

Starting from the first experiments on the catalytically active enzymes immobilized on carbon nanotubes (CNT) (Davis et al., 1998), many studies have been focused to characterize the proteins working on electrodes structured by CNT (Chen et al., 2003; Azamian et al., 2002; Sotiropoulou et al., 2003; Wang et al., 2002). CNT were also used for nanostructuring carbon paste electrodes (Rubianes and Rivas, 2003) as well as gold electrodes (Wang et al., 2003). In the field of cholesterol sensing, recently the cholesterol oxidase was immobilized by drop casting on a nanostructured glass carbon (Tan et al., 2005), and screen-printed electrodes (Li et al., 2005). However, the sensitivities of the proposed systems are limited to 0.123 μA/(mM mm^2) (Tan et al., 2005) and 0.037 μA/(mM mm^2) (Li et al., 2005). The electrode's areas were 12.56 mm^2 and 4 mm^2, respectively. In this short communication we propose a cholesterol sensing system with an improved sensitivity by orders of magnitude compared to that of very recently published systems. Our system is based on rhodium–graphite screen-printed electrodes modified by Multi-walled carbon nanotubes (MWCNT) and by cytochromes P450scc as catalytic enzyme. The P450scc is an isoform of the cytochrome P450 super-family. P450s are heme-proteins found in Bacteria, Archaea and Eukaryotes. Usually, they form a part of multi-component electron transfer chains, called P450-containing systems. P450s metabolize thousands of endogenous and exogenous compounds. This fact accounts for their central importance in metabolism. In this huge proteins family, the P450scc cytochrome catalyzes the side-chain cleavage of C27 cholesterol to C21 pregnenolone in the presence of molecular oxygen and NADPH-ferriHemoprotein reductase. This enzyme, encoded by the CYP11A1 gene, catalyzes the breakage between C20 and C22, which is the initial and rate-limiting step in the biosynthesis of various gonadal and adrenal steroid hormones. The P450scc is highly specific in cholesterol and cholesterol metabolites recognition. Therefore, it may be used to develop highly selective cholesterol biosensors (Shumyantseva et al., 2004), and its activity is highly enhanced when the electrodes are modified by gold nanoparticles (Shumyantseva et al., 2005). In particular, we obtained a sensitivity of 0.35 μA/(mM mm^2) by using riboflavin as molecular mediators between the cytochrome P450scc and

* Corresponding author at: Swiss Federal Institute of Technology-Lausanne (EPFL), Integrated Systems Laboratory, EPFL-IC-ISIM-LSI1-INF 333 (Bâtiment INF), Station 14, CH-1015 Lausanne, Switzerland. Tel.: +41 21 693 6782; fax: +41 21 693 4225.
E-mail address: sandro.carrara@epfl.ch (S. Carrara).

0956-5663/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.bios.2008.03.008

the rhodium–graphite screen-printed electrodes with an area of 20 mm^2 (Shumyantseva et al., 2004). By using same size electrodes that were modified by gold nanoparticles we reached a sensitivity improvement of one order of magnitude. The obtained current was one order of magnitude larger on sensing the same amount of cholesterol when using screen-printing electrodes modified by gold nanoparticles (Shumyantseva et al., 2005). This current increase was even higher than those achieved by the previously mentioned systems based on cholesterol oxidase (Tan et al., 2005; Li et al., 2005) even when the cholesterol oxidase electrodes are modified by using nanoparticles. In fact, a very recent publication demonstrates that a sensitivity of 8.49 nA/(mM mm^2) was reached by combining the effects of MWCNT and the platinum nanoparticles on gold electrodes with an area of 3.14 mm^2 (Yang et al., 2006). In the present short communication, we demonstrate further improvements of P450scc-based biosensors due to MWCNT.

2. Materials and methods

2.1. Rhodium–graphite electrodes preparation

We have prepared nanostructured rhodium–graphite electrodes modified by MWCNT and gold nanoparticles. Accordingly with our previous works (Shumyantseva et al., 2004, 2005), the electrodes were produced on flexible polyvinylchloride sheets from SKK (Denzlingen, Germany) using a DEK 249 screen printer (DEK Ltd., Weymouth, England) by using polyester screens from Steinmann GmbH (Stuttgart, Germany). Screen printing inks (Electrodag PF-410, Electrodag 6037 SS) were obtained from Acheson (Scheemda, Netherlands). Marastar SR 057, from Marabu (Tamm, Germany), was used as insulator ink.

2.2. Electrodes nanostructuring and functionalization

The fabricated screen-printed electrodes were modified by using MWCNT or gold nanoparticles. Gold nanoparticles with an average diameter of 12 nm were prepared by using a well-known procedure: the sodium borohydride reduction of $HAuCl_4$ in the presence of dodecanthiol. The prepared particles were deposited with drop casting (5 μl of mono-dispersed nanoparticles in chloroform solution at 35 mg/ml dried in 30 min) onto screen-printed electrodes. MWCNT were purchased from MER Corporation (Tucson, AZ). The purchased MWNTs had diameters ranging between 2 nm and 15 nm and lengths between 1 μm and 10 μm, with 5–20 graphitic layers. To prepare the MWCNT-modified screen-printed electrodes, 100 mg of accurately weighed MWNTs were dispersed in 100 ml of chloroform and ultra-sonicated for 1 min. Finally, the MWNTs were deposited by drop casting (5 μl of the so prepared solution) onto the screen-printed electrodes. The final preparation step was to add a 5-μl of a solution containing P450scc (196 μM in 50 mM phosphate buffer, pH 7.4, 10% glycerol, 1 M NaCl, 0.3% sodium cholate) dried overnight at +4 °C onto both kinds of nanostructured electrodes.

2.3. Electrochemical measurements

The functionalized working electrodes had an area of 20 mm^2 and they were used in horizontal regime within an electrochemical cell with a standard three-electrode configuration. Platinum wire was used as the counter electrode and screen-printed Ag/AgCl as the reference electrode. Electrochemical measurements were carried out using an EG&G (Princeton) potentiostat model 263A with M270 software. We have investigated the cyclic voltammograms of the nanostructured electrodes in comparison with the unmodified rhodium–graphite electrodes in order to demonstrate the enhanced activity of the cytochromes. Experiments were performed under aerobic conditions, in 100 mM phosphate buffer with 50 mM KCl, at pH 7.4, and the scan rate was 50 mV/s.

3. Results and discussion

Fig. 1 reports our results. The data shows how the oxidation of P450 is varying in comparison to the different electron transfer mediators. The voltammograms show the oxidation peak corresponding to the electrochemical reduction of the cytochrome with the oxygen. There is a huge increase of the current peak in the case of the P450 working on electrodes modified with gold nanoparticles with respect to the case of the bare electrodes, and it confirms our previous experiments (Shumyantseva et al., 2005). Moreover, a further enhancement of the oxidation current is clearly observed in the case of the electrodes modified with MWCNT. This is a direct proof that the MWCNT are able to mediate the electron transfer between the rhodium–graphite electrodes and the heme groups of the cytochromes in a more efficient way with respect to the nanoparticles. In the case of MWCNT, the peak is shifted in the positive direction of the voltage axis. This means that the MWCNT are more suitable for P450 with respect to the metallic nanoparticles since the P450 is easier to reduce in the presence of CNT. A positive shift indicates that it is easier to reduce the heme iron incorporated in the protein core. The peak shoulder is due to the fact that MWCNT used in the experiments are a mix of metallic and semi-conducting carbon nanotubes. The metallic MWCNT provide a better electron transfer and thus they are responsible for the peak located at −327 mV, while semi-conducting CNTs are responsible for the peak located at −400 mV. Fig. 1 also shows that the current enhancement in the current peak corresponding to the redox reaction of the cytochrome is larger than 2.4 times if the electron transfer is mediated by the MWCNT with respect to the case of the gold nanoparticles achieving a peak current of 196 μA. Moreover, the peak current in equivalent redox reactions achieved by the P450scc onto bare electrodes is equal to 11 μA (Shumyantseva et al., 2005). Therefore, the MWCNT improve the electron transfer between the bare electrode and the cytochrome P450scc 17.8 times. On the other hand, recent works on the use of MWCNT to improve the electron transfer rate in the cholesterol oxidase have shown an improvement of 3.7 times with respect to the non-nanostructured sol/gel chitosan electrodes (Tan et al., 2005) or an improvement of 1.6 times

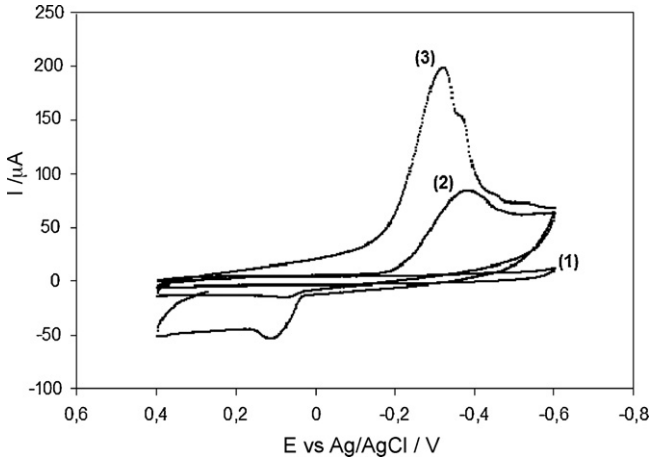

Fig. 1. Cyclic voltammograms of screen-printed bare rhodium–graphite electrode (1), on electrodes modified with Au nanoparticles and P450scc (2), or with multi-walled carbon nanotubes and P450scc (3). Experiments were performed under aerobic conditions, 100 mM phosphate buffer, 50 mM KCl, pH 7.4, and the scan rate was 50 mV s^{-1}.

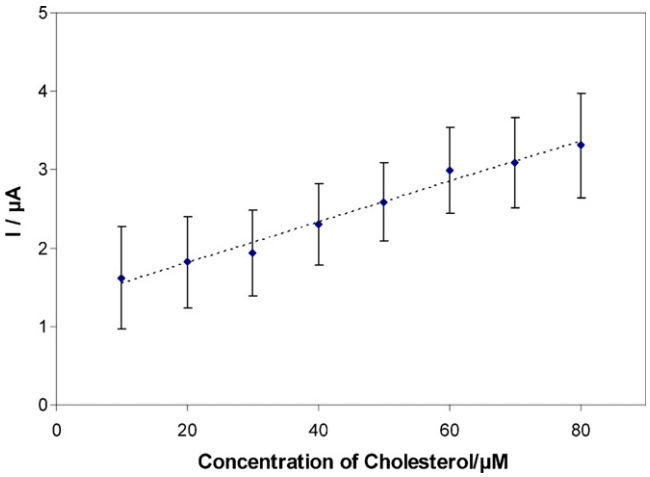

Fig. 2. The ampérometric response of screen-printed rhodium–graphite Au–P450scc electrode to increasing cholesterol concentration. About 100 μM stock solution in 30% sodium cholate, measured when fractions of 10 μM of cholesterol has been repeatedly added. The total volume of electrolyte was 1 ml. The current was measured at the potential −400 mV (vs. Ag/AgCl).

with respect to the carbon electrodes (Li et al., 2005). Therefore, as seen before, systems based on the cytochromes P450s integrated with nanostructures present improvements of orders of magnitude in the sensitivity of cholesterol biosensors, while systems based on oxidases do not show such an improvement. The improvement is due to the fact that cytochrome P450 is a metallo-protein providing electrons directly from its heme, while oxidase provide electrons not directly but from peroxidase. In conclusion, the development of a cholesterol biosensor based on screen-printed electrodes modified with MWCNT and with the cytochromes P450scc will ensure a high sensitivity as demonstrated by the calibration curve shown in Fig. 2. The figure shows an increased sensitivity that compare quite well with those achieved by the extremely high sensitive systems based on nano-wires recently developed with FET technology (1 μA for 388 μM of oxidized LDL-cholesterol) (Rouhanizadesh et al., 2006). In our case, the reached sensitivity was 1.12 μA/(mM mm^2). This sensitivity is of the same magnitude order of that obtained by using the gold nanoparticles (Shumyantseva et al., 2005), but the presence of the carbon nanotubes highly improve the linearity of the detection response. In fact, data reported in Fig. 2 are very close to that of a linear regression while those reported previously for gold nanoparticles clearly present non-linearity (Shumyantseva et al., 2005). The linearity coefficients *R* were estimated equal to 0.99; 0.60; 0.99 in the case of carbon nanotubes (Fig. 2), gold nanoparticles [elaborated from (Shumyantseva et al., 2005)], and riboflavin (Shumyantseva et al., 2004) mediators, respectively. This is a further confirmation that MWCNT may play a crucial role in biosensor development (Carrara et al., 2005).

4. Conclusions

For cholesterol biosensors, a further improvement in sensitivity due to carbon nanotubes is demonstrated in this paper. Multi-walled carbon nanotubes were deposited onto the rhodium–graphite electrodes by drop casting. The cytochrome P450scc was also deposited to detect the cholesterol. Cyclic voltammetry indicated largest enhanced activity of the enzyme at the MWCNT modified surface. Systems based on the cytochromes P450s integrated with nanostructures present improvements of orders of magnitude in the sensitivity of cholesterol biosensors, while systems based on oxidases do not show such an improvement. In case of cytochrome P450scc, the role of the nanotubes in mediating electron transfer was observed to be further improved with respect to the case of rhodium–graphite electrodes modified by the use of gold nanoparticles. The presence of carbon nanotubes highly improve the linearity of the detection response. The current peak related to cholesterol redox reactions is split into a shoulder. This phenomena may be further investigated by using nanotubes that are mainly metallic or semi-conducting. However, working with only metallic or semi-conducting nanotubes fixing all the other characteristics is still a challenging task. Therefore, this will be the object of further investigations, along with the quantum nature of improved sensitivity.

Acknowledgments

S.C. thanks Uwe Fuhr, at Köln University, for his kind approach and for highly useful discussions on the role of the heme-proteins in Electron Transfer Enhancement due to nanostructures in biosensing. Anil Leblebici is also acknowledged for her help in revising the manuscript.

References

Azamian, B.R., Davis, J.J., Coleman, K.S., Baghaw, C.B., Green, M.L.H., 2002. J. Am. Chem. Soc. 124, 12664–12665.
Carrara, S., Bavastrello, V., Ricci, D., Stura, E., Nicolini, C., 2005. Sens. Actuators B 109, 221–226.
Chen, R.J., Bansaruntip, S., Drouvalakis, K.A., Kam, N.W.S., Shim, M., Li, Y., Kim, W., Utz, P.J., Dai, H., 2003. Proc. Natl. Acad. Sci. 100, 4984–4989.
Davis, J.J., Green, M.L.H., Hill, H.A.O., Leung, Y.C., Sadler, P.J., Sloan, J., Xavier, A.V., Tsang, S.C., 1998. Inorg. Chim. Acta 272, 261–266.
Li, G., Liao, J.M., Hu, G.Q., Ma, N.Z., Wu, P.J., 2005. Biosens. Bioelectron. 20, 2140–2144.
Rouhanizadesh, M., Tang, T., Li, C., Hwang, J., Zhou, C., Hsiai, T.K., 2006. Sens. Actuators B 114, 788–798.
Rubianes, M.D., Rivas, G.A., 2003. Electrochem. Commun. 5, 689–694.
Shumyantseva, V., De Luca, G., Bulko, T., Carrara, S., Nicolini, C., Usanov, S.A., Archakov, A., 2004. Biosens. Bioelectron. 19, 971–976.
Shumyantseva, V.V., Carrara, S., Bavastrello, V., Riley, J.D., Bulko, T.V., Usanov, S.A., Nicolini, C., Archakov, A.I., 2005. Biosens. Bioelectron. 21, 217–222.
Sotiropoulou, S., Gavalas, V., Vamvakaki, V., Chaniotakis, N.A., 2003. Biosens. Bioelectron. 18, 211–215.
Tan, X., Li, M., Cai, P., Luo, L., Zou, X., 2005. Anal. Biochem. 337, 111–120.
Wang, G., Xu, J.-J., Chen, H.-Y., 2002. Electrochem. Commun. 4, 506–509.
Wang, S.G., Zhang, Q., Wang, R., Yoon, S.F., Ahn, J., Yang, D.J., Tian, J.Z., Li, J.Q., Zhou, Q., 2003. Electrochem. Commun. 5, 800–803.
Yang, M., Yang, Y., Yang, H., Shen, G., Yu, R., 2006. Biomaterials 27, 246–255.