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Short communication

Direct electron transfer between cytochrome P450scc and gold nanoparticles on screen-printed rhodium–graphite electrodes

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Abstract

This paper is concerned with an investigation of electron transfer between cytochrome P450scc (CYP11A1) and gold nanoparticles immobilised on rhodium–graphite electrodes. Thin films of gold nanoparticles were deposited onto the rhodium–graphite electrodes by drop casting. Cytochrome P450scc was deposited onto both gold nanoparticle modified and bare rhodium–graphite electrodes. Cyclic voltammetry indicated enhanced activity of the enzyme at the gold nanoparticle modified surface. The role of the nanoparticles in mediating electron transfer to the cytochrome P450scc was verified using ac impedance spectroscopy. Equivalent circuit analysis of the impedance spectra was performed and the values of the individual components estimated. On addition of aliquots of cholesterol to the electrolyte bioelectrocatalytic reduction currents were obtained. The sensitivity of the nanoparticle modified biosensor to cholesterol was 0.13 $\mu A\ \mu M^{-1}$ in a detection range between 10 and 70 μM of cholesterol. This confirms that gold nanoparticles enhance electron transfer to the P450scc when present on the rhodium–graphite electrodes.

Keywords: Electron transfer; Cytochrome; Gold nanoparticles; Cholesterol; Biosensors; Screen-printed

1. Introduction

Nanoparticles of metals have been the subject of intense investigation due to their novel material properties (Penn et al., 2003; Han et al., 2002). Gold nanoparticles display electronic, chemical and physical properties that may be employed in optical and electronic devices, catalysis and sensor technology. In the biological sciences the affinity of gold nanoparticles for proteins (Hu et al., 2003) has resulted in them being used as biomolecular labels (Penn et al., 2003; Ribrioux et al., 1996; Hainfeld and Furuya, 1992). In addition gold nanoparticles on electrode surfaces have been employed in bioelectroanalysis.

In an amperometric biosensor the enzyme activity is proportional to the measured current. A redox reaction between an enzyme and an analyte can be transduced to an electrical signal via subsequent direct electron transfer between the enzyme and the electrode. The direct electrochemistry of the bacterial cytochrome P450cam (CYP101), from *Pseudomonas putida*, has been reported (Zhang et al., 1997; Kazlauskaite et al., 1996; Lvov et al., 1998; Lo et al., 1999; Lei et al., 2000). There is also evidence of direct reduction of human cytochrome P450 3A4 (CYP3A4) embedded in a polyelectrolyte (Joseph et al., 2003). In addition cytochrome

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P450 2B4 (CYP 2B4) has been reduced on glassy carbon electrode modified with clay nanoparticles in the presence of detergent Tween 80 (Shumyantseva et al., 2004a). It has been suggested that the surface amino groups of cytochrome c favour interaction between the active heme moiety and the electrode surface (Liu et al., 2003). For the majority of enzymes the protein matrix of the enzyme insulates the active site, preventing the direct electron transfer. In such cases chemical mediators are commonly employed to transduce enzyme activity into an electrical signal (Armstrong and Wilson, 2000; Estabrooc et al., 1996); following the reaction between the enzyme and the analyte the mediator regenerates the enzyme and itself by exchanging electrons with the enzyme and with the electrode. An alternate strategy to using mediators is to employ roughened electrodes that can penetrate the insulting protein matrix (Geddes et al., 2004), thus permitting direct electron transfer. Electrodes modified with gold nanoparticles are suitably rough substrates for the fabrication of amperometric biosensors that transduce enzyme activity via direct electron transfer. It has been demonstrated that the hemoprotein horse heart cytochrome c may be reduced reversibly onto a tin oxide electrode modified with gold nanoparticles. The size of the gold nanoparticles used to modify the electrode was found to influence the rate of electron transfer (Brown et al., 1996). Direct electron transfer between hemoglobin and glassy carbon electrodes in the presence of lipid-protected gold nanoparticles has also been reported (Han et al., 2002).

The long-term aim of our research program is to develop a cholesterol sensor based on cytochrome P450scc (CYP11A1). This heme enzyme catalyses the cholesterol side chain cleavage reaction, the key initial step in steroid hormone biosynthesis (Lewis, 2001). The mechanism of the reaction is complex (Lepesheva et al., 1999). Initially the enzyme binds cholesterol; during this process the Fe^{3+} ion of the heme group undergoes a low spin to high spin transition. The Fe^{3+} is then reduced to Fe^{2+} by $NADPH^{+}$, via andrenodoxin reductase. Next the reduced complex binds molecular oxygen. A second electron is then transferred to the complex and the molecular oxygen reacts enabling the cholesterol to be hydroxylated and the second oxygen atom is released. In most P450 catalyzed reactions the hydroxylated product is released and the enzyme returns to its high spin Fe^{3+} state. However, in the case of the P450scc the product is rebound and at least further four electrons are transferred to convert the cholesterol to pregnenolone. Previous studies of P450scc electrochemistry have employed screen-printed rhodium–graphite electrodes and riboflavin as a mediator of electron transfer (Shumyantseva et al., 2004b). In this paper we report direct electron transfer between cytochrome P450scc and Au colloid modified screen-printed rhodium–graphite electrodes. It is shown that modification of screen-printed rhodium–graphite electrodes with gold nanoparticles integrated with cytochrome P450scc yields a bioelectrocatalytic system for cholesterol measurements. The amperometric response to the addition of cholesterol is demonstrated. The combination of bioelectrochemistry and nanobiotechnology permits the construction of a highly sensitive amperometric biosensor for cholesterol measurements.

2. Materials and methods

2.1. Materials

Wild-type cytochrome P450scc (CYP11A1) was over-expressed and purified using published procedures (Lepesheva and Usanov, 1998). Cholesterol, hydrogen tetrachloroaurate ($HAuCl_4{\cdot}3H_2O$), tetraoctylammonium bromide, sodium borohydride and dodecanethiol were purchased from Aldrich and sodium cholate was obtained from Sigma. All chemicals were used as supplied. Gold nanoparticles of diameter average size of 12 nm (measured by AFM, data not shown) were prepared by the sodium borohydride reduction of $HAuCl_4$ in the presence of dodecanthiol (Berthell et al., 1996). The prepared particles were precipitated by addition of ethanol, washed, dried and then re-suspended in chloroform at a concentration of 35 mg ml^{-1}. The concentration of cytochrome P450scc in the solutions was determined from the carbon monoxide difference spectra using a molar extinction coefficient of 91 mM^{-1} cm^{-1} at 450 nm (Omura and Sato, 1964).

2.2. Preparation of enzyme electrode

The amperometric biosensors were of area 2 mm × 10 mm. They were prepared in a two-step procedure. First gold nanoparticles were drop cast onto the surface of screen-printed working electrodes, typically 5 μl of the suspension of gold nanoparticles in chloroform was used and allowed to dry for 30 min. Then 5 μl of cytochrome P450scc (196 μM in 50 mM phosphate buffer, pH 7.4, 10% glycerol, 1 M NaCl, 0.3% sodium cholate) was added and the electrode was dried at +4 °C overnight.

2.3. Electrochemical measurements

Electrochemical measurements were carried out using an EG&G (Princeton) potentiostat model 263A with M270 software. The impedance measurements were performed using an EG&G (Princeton) model 1025 frequency response detector. Modified screen-printed rhodium–graphite electrodes were used as working electrodes, screen-printed Ag/AgCl as reference electrodes (Bachmann et al., 2000; Bachmann and Schmid, 1999) and a platinum wire as counter electrode.

The electrochemical response and the stability of the electrodes with immobilized cytochrome-P450scc were investigated by cyclic voltammetry and impedance spectroscopy. The total volume of electrolyte, 100 mM potassium phosphate buffer (pH 7.4), was 20 μl. In the cyclic voltammetric investigations scan rates from 5 to 100 mV s^{-1} were employed and the potential was swept between −600 and