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Photoelectrochemical signal chain based on quantum dots on gold—Sensitive to superoxide radicals in solution

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ABSTRACT

A photoelectrochemical signal chain sensitive to the presence of superoxide radicals was developed on the basis of CdSe/ZnS quantum dots which were immobilized on gold electrodes using a dithiol compound. The conditions of photo current generation under illumination have been characterized with respect to the dependence on the applied electrode potential, the wavelength of the light beam and the stability of the measurement. Because of photoexcitation electron–hole pair generation is enforced in the nanoparticles enhancing the conductivity of the quantum dot layer. This was independently verified by impedance measurements.

In order to observe direct electron transfer with the redox protein cytochrome *c* different surface modifications of the quantum dots were investigated—mercaptopropionic acid, mercaptosuccinic acid and mercaptopyridine. Varying superoxide concentrations in solution can be detected by an enhanced conversion of superoxide-reduced cytochrome *c* and thus by an enhanced photo current at the quantum dot modified electrode. The electrode was found to be sensitive to higher nanomolar concentrations of the radical.

1. Introduction

The incorporation of nanoparticles in analytical detection systems or sensors is an actual and intensively investigated area (Lin et al., 2007; Katz et al., 2004; Baughman et al., 2002). Besides metal nanoparticles and carbon nanotubes, semiconductor nanoparticles – or quantum dots – have gained considerable interest. This is because of their unique photophysical properties such as size-controlled fluorescence and stability against photobleaching. Particularly the first allows a highly parallel, multiplexed analysis. Binding molecules can be linked to this kind of label with the same coupling strategy since the different labels (nanoparticles) differ only in size but not in surface chemistry. The progress in chemical synthesis of these particles but also in surface modification strategies provides the basis for an increasing use of quantum dots also in bioanalysis (Medintz et al., 2005; Alivisatos, 2004; Chan et al., 2002). Thus, the particles have been applied in immunoassays (Goldman et al., 2004; Hoshino et al., 2005) and the detection of nucleic acids (Gerion et al., 2003; Pathak et al., 2001; Xiao and Barker, 2004; Kim et al., 2007). Another important feature of quantum dots is going along with their electronic structure and consists in the generation of electron–hole pairs during photoexcitation. This provides the basis for the application of these nanoparticles on electrodes (Bakkers et al., 2000; Sharma et al., 2003). When sufficiently long-lived electron–hole pairs are generated this allows the ejection of conduction band electrons to the electrode or the injection of electrons from the electrode into the valence band of the particle. Thus, a photo current can be detected which is much enhanced when electron donors or acceptors are present in solution. The use of quantum dots on electrodes may thus lead to the development of light-switchable devices (such as light-addressable potentiometric sensors LAPS: Parak et al., 1997), energy conversion systems (such as solar cells: Huynh et al., 2002) or sensors where the recognition event controls the photo current. Sensorial applications for the combination of quantum dots with DNA have been shown with several systems (Willner et al., 2001; Gill et al., 2005; Freeman et al., 2007). However, only few examples are reported in the combination with proteins. In accordance with the concept of biosensors of the first generation (i.e. detection of a product of an enzymatic conversion) actylcholine esterase was coupled to the quantum dot surface which was immobilized on an electrode. The biocatalytic conversion of the substrate acetylthiocholine was detected by an electron exchange of the produced thiocholine with the illuminated quantum dots (Pardo-Yissar et al.,

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2003). Following the concept of biosensors of the 3rd generation (direct electrochemical communication of the redox protein with an electrode) proteins can also be coupled to quantum dots in such a way that a direct electron exchange is feasible.

Surface modification is necessary here and has been shown first with mercaptopropionic acid on CdSe/ZnS QD and cytochrome *c* (Stoll et al., 2006). Oxidized cytochrome *c* can act as electron acceptor for the photoexcited quantum dots which are subsequently reduced by the electrode. Such a system can be further developed by combining cytochrome *c* with enzymes such as lactate dehydrogenase (Katz et al., 2006). This results in a signal chain starting from lactate in solution via the enzyme, cytochrome *c* and finally via the quantum dots towards the electrode. Here an anodic photo current was generated in the presence of lactate.

In this study we present results coupling quantum dots and cytochrome *c* to gold electrodes in order to construct a photoelectrochemical signal chain which is sensitive to superoxide radicals in solution. This is based on the use of cytochrome *c* as a recognition element in superoxide sensors (Cooper et al., 1993; Lisdat et al., 1999; Gobi and Mizutani, 2000; Ge and Lisdat, 2002). These electrodes also rely on direct electron transfer from cytochrome *c* to a promoter-modified gold electrode and allow a concentration dependent radical analysis and have also been used for the in vivo detection of the radical (Büttemeyer et al., 2003; Stoffels et al., 2007) or the detection of antioxidants (Krylov et al., 2006; Ignatov et al., 2002). The use of a quantum dot layer on the electrode provides a photo-switchable interlayer allowing the spatial read-out of a sensor surface. In this study the basic features of a signal chain from the short-lived radical in solution via the protein and the quantum dot towards the electrode will be demonstrated.

2. Experimental

2.1. Materials

The CdSe/ZnS nanoparticles were prepared according to a procedure described previously (Reiss et al., 2002; Dabbousi et al., 1997). The hard-core diameter of the inorganic CdSe/ZnS core/shell particles was ~5 nm with an absorbance peak at 527 nm. The concentration was determined by UV–vis spectroscopy (extinction coefficient 78000 M^{-1} cm^{-1}, Yu et al., 2003).

4-Dithiane and 1,4-benzene dithiol were purchased from Lancaster (Frankfurt/Main, Germany). Mercaptopropionic and mercaptosuccinic acid, propane dithiol, hexane dithiol, cytochrome *c* (from horse heart), 4-mercaptopyridine, hypoxanthine and all buffer salts were from Sigma (Taufkirchen, Germany). Sodium dithionite (80%) and ethanol (99%) were obtained from Merck (Darmstadt, Germany). Xanthine oxidase (XOD) *from cow milk* was provided by Roche diagnostics. All aqueous solutions were prepared using 18 MΩ ultra purified water (SG Wasseraufbereitung und Regenerierstation Ltd., Germany).

2.2. Electrode modification

The Au electrodes (from BASi, UK) were polished with Al_2O_3 powder of decreasing grain size (1 μm, 0.05 μm), voltammetrically cycled in 1 M NaOH (−800 mV to +200 mV, scan rate 300 mV/s), rinsed with water, cycled in 0.5 M H_2SO_4 (−250 mV to +1.75 V, scan rate 300 mV/s) and were again rinsed with ultra pure water.

Electrodes were coated with either dithiane or benzene dithiol. In the first case the cleaned electrodes were incubated in a saturated dithiane solution in ethanol at 65 °C for 5 days with an intense ethanol rinsing afterwards. To immobilize the nanoparticles the dithiane modified electrodes were immersed into a 14 μM particle suspension (CdSe/ZnS) in chloroform for 3 days at 50 °C. In the second case, for the benzene dithiol modification the cleaned electrodes were incubated in a 10 mM dithiol solution in chloroform for 40 h at room temperature. The dithiol modified electrodes were intensively rinsed with chloroform, immersed into the nanoparticle solution (14 μM CdSe/ZnS) and incubated "upside down" for 2 days at room temperature.

In a final step the surface of the nanoparticles immobilized to the gold electrode was alternatively modified with mercaptopropionic acid, mercaptosuccinic acid, or mercaptopyridine. The modification of the nanoparticle surface fixed at the gold electrode with mercaptopropionic and mercaptosuccinic acid was done by incubation of the modified electrode in a 10% solution of the latter compounds in water for 3 days at 50 °C. The modification with mercaptopyridine was achieved by incubation of the particle modified electrode in a 20 mM aqueous solution of the compound for 30 h at room temperature.

2.3. Cytochrome c reduction

A stock solution of 1–5 mM oxidized cytochrome *c* was incubated with sodium dithionite for 5 min at room temperature (1 g of the salt per mmol protein). The excess salt was removed using a NAP 25 column (Amersham Biociences). The concentration of reduced cytochrome *c* was determined by UV–vis spectroscopy evaluating the absorption at 550 nm prior to use (Van Gelder and Slater, 1962). The solution was stored for a maximum of 24 h at 4 °C.

2.4. Electrode characterization

All electrochemical measurements were performed in a home made cell using a three-electrode arrangement. The nanoparticle modified electrode was the working electrode, the reference electrodes was an Ag/AgCl, 1 M KCl electrode from Biometra (Germany) and a Pt wire served as counter electrode. The volume of the measuring cell was 1 ml. Opposite to the working electrode a wave guide was fixed allowing the illumination of the full working electrode area from a defined distance of 1 cm.

For the amperometric and voltammetric measurements an Autolab PGSTAT 12 (Methrom, Germany) with the GEPS 4.9 electrochemical data evaluation software was used. Impedimetric measurements were performed using an IM6e-potentiostat (Zahner-Elektrik, Germany). Here a 10 mV ac perturbation voltage was used and the electrodes were characterized at open circuit potential in the frequency range: 50 kHz–50 mHz.

Within the transparent cell, the electrode surface was exposed to light for a period of 4–20 s and the current response was recorded under the condition of a fixed dc potential. The light was emitted from a 150 W Xe lamp purchased from L.O.T. ORIEL (Darmstadt, Germany) and the light pulses were generated by manually opening/closing an aperture.

For wavelength dependent measurements a monochromator, model 77250 (L.O.T., Germany) was coupled to the Xe lamp. The grating was 1200 l/mm and the entry slit was fully opened (3.2 mm). These settings cause a bandpass of +/−10 nm.

As supporting electrolyte 0.1 M sodium phosphate buffer pH 7.4 was used. Hexacyanoferrate (II/III) was used in concentrations of 5 mM each. The cytochrome *c* concentration was in the range of 1–12 μM.

For the generation of superoxide radicals the xanthine oxidase catalysed conversion of hypoxanthine to uric acid was used. During this enzymatic conversion oxygen is reduced and thus hydrogen peroxide and superoxide are liberated simultaneously. The hypoxanthine concentration was 100 μM and the xanthine oxidase activity was varied from 9 mU/ml to 100 mU/ml in order to estab-