Bioelectrochemistry 87 (2012) 33–41

Contents lists available at SciVerse ScienceDirect

Bioelectrochemistry

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

Human sulfite oxidase electrochemistry on gold nanoparticles modified electrode

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ARTICLE INFO

Article history:
Received 29 July 2011
Received in revised form 3 November 2011
Accepted 28 November 2011
Available online 8 December 2011

Keywords:
Direct electron transfer
Gold nanoparticle
Human sulfite oxidase
Ionic liquid
Sulfite biosensor

ABSTRACT

The present study reports a facile approach for sulfite biosensing, based on enhanced direct electron transfer of a *human* sulfite oxidase (*hSO*) immobilized on a gold nanoparticles modified electrode. The spherical core shell AuNPs were prepared via a new method by reduction of $HAuCl_4$ with branched poly(ethyleneimine) in an ionic liquids resulting particles with a diameter less than 10 nm. These nanoparticles were covalently attached to a mercaptoundecanoic acid modified Au-electrode where then *hSO* was adsorbed and an enhanced interfacial electron transfer and electrocatalysis was achieved. UV/Vis and resonance Raman spectroscopy, in combination with direct protein voltammetry, are employed for the characterization of the system and reveal no perturbation of the structural integrity of the redox protein. The proposed biosensor exhibited a quick steady-state current response, within 2 s, a linear detection range between 0.5 and 5.4 μM with a high sensitivity (1.85 nA μM^{-1}). The investigated system provides remarkable advantages in the possibility to work at low applied potential and at very high ionic strength. Therefore these properties could make the proposed system useful in the development of bioelectronic devices and its application in real samples.

1. Introduction

Over the last decades, electron exchange of redox enzymes with electrodes has gained interest for technological applications like in biosensors and other bioelectronic devices and biosynthesis [1–11]. Therefore, an efficient communication between protein and electrode continues being a challenge and the subject of extensive research. Several approaches have been developed including the addition of free and immobilized electron mediators [7–14], the modification of the enzyme [15–17], and the immobilization of enzymes on electroactive polymers [18–20].

The incorporation of gold nanoparticles (AuNPs) to assemble electrochemically active enzymes has been suggested [21–22]. For example, AuNPs have been successfully employed in a biocompatible matrix (chitosan) for the development of biosensors [23–26]. Based on the polyelectrolyte characteristics of this biopolymer, different enzymes were immobilized onto AuNPs by electrostatic interactions. Other approaches for the construction of biosensors have been reported using proteins adsorbed gold nanoparticles coated with polyelectrolytes like polyamidoamine and polypropyleneimine [27–28]. Alternatively, Willner and co-workers have reported the reconstruction of apo-glucose oxidase on 1.4 nm AuNPs functionalized with the cofactor flavin adenine dinucleotide into a conductive film to yield a highly efficient electrical contact with the electrode support [29–30]. Similar effect of AuNPs was observed by Jensen and Ulstrup who described an electrostatically conjugated system of cytochrome *c* and 3–4 nm size AuNPs coated with thiol ligands [31].

Animal sulfite oxidizing enzymes are molybdo- and heme-containing redox enzymes [32–33]. The enzyme catalyzes the conversion of sulfite to sulfate, the terminal reaction in the oxidative degradation of cysteine and methionine. The catalytic activity of eukaryotic sulfite oxidase (SO) is attributed to the functionality of distinct domains. The enzyme contains a N-terminal heme domain (HD) with a non-covalently bound heme *b5* cofactor connected by a flexible loops to large central domain, containing the Mo atom (molybdenum domain, MD) and a C-terminal dimerization domain [34–36]. Sulfite is oxidized to sulfate at the Moco center, and the reducing equivalents are transferred via intramolecular electron transfer (IET) to the heme *b5*, where the terminal electron carrier cytochrome *c* is reduced. It is generally accepted that conformational rearrangement between the MD and the HD occur before IET to reach a more effective orientation. Once the IET has taken place the HD moves away from the MD to interact with the positively charged cytochrome *c* [37].

This conformational change is thought to be induced by a cytochrome *c* binding event and can be largely affected by the ionic strength, viscosity and pH and the components of the solution [38–41].

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doi:10.1016/j.bioelechem.2011.11.012

Sulfite oxidizing enzyme interaction with different electrode surface has been previously investigated by electrochemical methods. The catalytic activity of eukaryotic SO [42–44] as well as the bacterial sulfite dehydrogenase [45–49] was retained when confined on electrode surface bearing amine groups. The investigations have been recently complemented by the formation of a multilayer assembly of *hSO* with cytochrome *c* [48–49] which was further used as an electrocatalytic biosensor for sulfite detection [50].

These systems however have problems due to the limited possibility to work in high ionic strength condition since the decrease in electrostatic interaction between the protein and the electrode lead to irreversible protein desorption.

In the present work, the direct electrochemistry of *hSO* immobilized by electrostatic interaction onto gold nanoparticles capped with cationic branched poly(ethyleneimine) (PEI) was investigated. Gold nanoparticle formation using PEI and derivates have been previously reported in diluted aqueous solution [51–53]; however, the use of ionic liquids in the synthesis provide a new solvent alternative that might induce the formation of well-defined and solvated gold nanocrystals [54]. The preparation of gold nanoparticles was carried out via a new method in the ionic liquid (IL) 1-ethyl-3-methylimidazolium ethylsulfate using PEI as a reducing and stabilizing agent.

The PEI forms a positive charged shell around the AuNPs preventing their aggregation in solution and providing a link to anchor the AuNPs onto the electrode surface.

2. Material and methods

2.1. Materials

The ionic liquid 1-ethyl-3-methylimidazolium n-ethylsulfate [emim $EtSO_4$] (99%), was purchased from Merck. The metal precursor $HAuCl_4$ was obtained from Aldrich. The branched poly(ethyleneimine) with a molecular weight of Mn = 5000 g/mol was obtained from BASF. 11-mercapto-1-undecanol (MU), 11-mercapto-1-undecanoic acid (MUA) and 1-[3-(dimethylamino]propyl]-3-ethylcarbodiimide methionine (EDC) were purchased from Sigma-Aldrich. The mesoporous indium tin oxide-coated borosilicate glass (mpITO) was a kind gift of T. von Graberg and Prof. B. M. Smarsly from Justus Liebig Universität Gießen, Gießen (Germany).

2.2. Human sulfite oxidase expression and purification

The *hSO* was expressed in *Escherichia coli* TP1000 cells containing plasmid pTG718 and purified as described by Temple et al. [54].

2.3. Synthesis of gold nanoparticles

Polyethyleneimine (0.5 wt.%) and the tetrachloroaurate of 2 mM were mixed with 1:1 ratio (wt/wt) at room temperature in an ionic liquid emim $EtSO_4$ solvent and heated up to 100 °C for 5 min. The change in color from yellow to dark red indicated the formation of gold nanoparticles. The AuNPs were dissolved in Tris buffer 0.5 mM pH 7.0 and the hydrodynamic diameter was determined by dynamic light scattering (DLS). Measurements were carried out at 25 °C at a fixed angle of 173° ("backscattering detection") by using a Nano Zetasizer (Malvern) equipped with a He–Ne laser (λ = 633 nm; 4 mW) and a digital autocorrelator. The refractive indices and viscosities of solvents were adapted to the respective measurements. Transmission electron microscopy (TEM) micrographs of the nanoparticles were recorded on an EM 902 microscope from Zeiss. The nanoparticle sample was dissolved in chloroform and prepared by dropping a small amount of the solution, on copper grids, dried and examined in the transmission electron microscope at an acceleration voltage of 90 kV.

The zeta potential was determined by means of the Nano Zetasizer based on the electrophoresis principle which considers the motion of charged particles by applying an electric field.

2.4. Electrode modification

A gold wire (Goodfellow, Bad Nauheim, Germany) with a diameter of 0.5 mm was cleaned by boiling 4 h in 2 M KOH and kept 10 min in concentrated HNO_3. A careful rinsing with Millipore water followed every successive step. The electrodes were stored in concentrated H_2SO_4 when not in use. The cleaned electrodes were incubated in an ethanol mixture of 5 mM MUA and 5 mM MU with a volume ratio of 1:3 for at least 24 h at 4 °C. After rinsing with water, the MU/MUA modified electrodes were incubated in 200 µl of a freshly prepared emim $EtSO_4$ solution of AuNPs for 2 h at room temperature. Further, 5 µl of EDC and 0.2 M in H_2O were then added into the solution in order to couple amino functions of the AuNP cap to the carboxylic acid functionalized SAM gold electrode. 20 min were used as coupling time. For the protein functionalization, the AuNPs modified gold wires were washed carefully with 0.5 mM Tris pH 7, dipped in a 2 µM *hSO* solution in the same buffer solution and kept for 10 min at 4 °C before a careful wash with 5 mM Tris solution pH 8.4 to remove not tightly bound enzyme. Modified electrodes were stored dry at 4 °C until use.

For preparation of the Ag ring electrodes mechanical polishing was applied as a first step. The electrode was then immersed in a 0.1 M KCl solution and electrochemically roughened by applying −2 V for 40 s followed by three oxidation–reduction cycles at potentials of +300 mV and −300 mV respectively. Finally the electrode was kept at +300 mV for additional 5 min. Further treatment before protein incubation was the same than for the Au electrode modification. In this last step the AuNPs modified Ag electrode was incubated in a 1.2 µM hSO solution for 45–90 min and immediately used for spectro-electrochemistry.

2.5. Electrochemistry

The electrochemical experiments were carried out in a home-made three-electrode cell with a total volume of 1 ml, employing a platinum wire as counter electrode, a Ag/AgCl, 1 M KCl reference electrode (Microelectrodes Inc., Bedford, USA) against which all potentials are reported, and the modified gold wire as working electrode. All the experiments were performed at room temperature with PalmSens potentiostat and analyzed with PSLite 1.8 software (Pamlsens, The Netherlands).

2.6. Surface enhanced resonance Raman spectroelectrochemistry

For *surface enhanced resonance Raman* (SERR) spectroscopic measurements of HD the 413 nm excitation line of a Krypton ion laser and a confocal Raman spectrometer (Labram, HR-800, Jobin Yvon) were used. Spectro-electrochemistry was performed with a homemade electrochemical cell with a volume of ca. 10 ml. The cell contained an electrochemically roughened and functionalised Ag ring as working electrode, a 3 M KCl Ag/AgCl reference electrode and a platinum counter electrode.

The laser power on the sample was 1 mW. SERR spectra were obtained with an accumulation time of 30 s. The working electrode was constantly rotated to avoid laser induced protein degradation. More details about the setup are given elsewhere [55].

3. Results and discussion

3.1. Synthesis and characterization of gold nanoparticles

The synthesis of AuNPs using PEI as a reducing and stabilizing agent in ionic liquids is relatively simple and reproducible. The mass

ratio between the metal precursor and PEI was kept constant (1:1) during the synthesis. After heating up to 100 °C the solution turns to a deep red color showing an UV–vis absorption maximum at 529 nm, confirming the formation of gold particles of small dimension (S1). Fig. 1a shows a TEM micrograph of such spherical nanoparticles with an average core diameter of about 6 ± 2 nm as to be seen in the distribution histogram (Fig. 1b). It has to be mentioned that the hydrodynamic diameter determined by dynamic light scattering (DLS), 9 ± 1 nm, is larger (S2). As TEM images show only the gold core, one can conclude from the hydrodynamic diameter that the gold core is surrounded by a coating layer of around 2–3 nm. The nanoparticles show also partially solubility in chloroform. Therefore we suggest that the covering shell may be composed of a mixture of PEI and IL. That can be explained by electrostatic interactions between the positive charged polymer and the counter anion from the IL. In addition the zeta-potential (ξ) was measured. As expected, the AuNPs present a positive zeta potential of +18–+20 mV as a result of the cationic polymer coating.

The immobilization onto the mixed monolayer modified Au-exploits first the charge interaction and covalent coupling with EDC. This leads to disperse single gold particles with the core size order between 5 and 10 nm. No evidence of aggregation after solvent evaporation was found. The particles are in the same size order measured by dynamic light scattering and by TEM. The AuNPs are clearly separated from each other forming an accessible surface for protein immobilization.

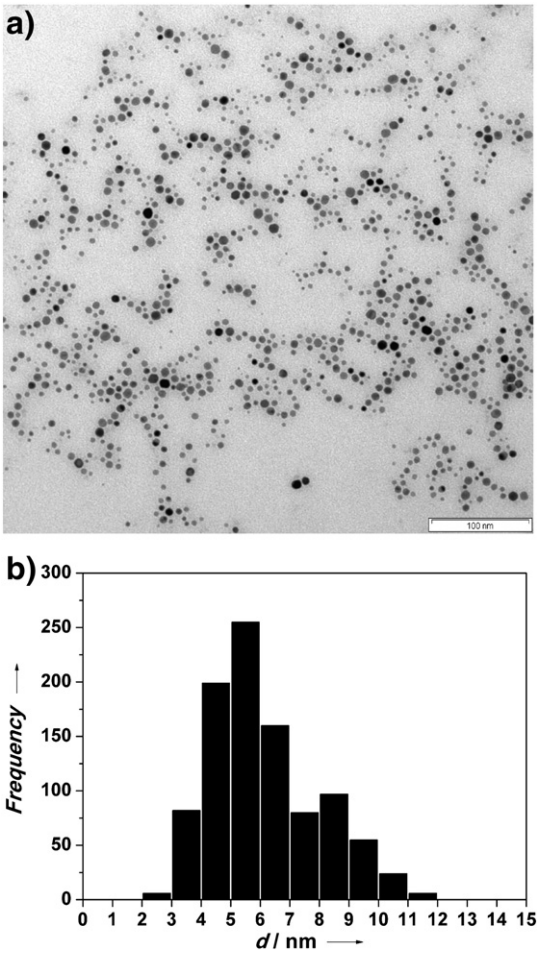

Fig. 1. a) Electron transmission micrograph of AuNPs synthesized in the ionic liquid 1-ethyl-3-methylimidazolium 1-ethylsulfate. b) Histogram of AuNP size distribution showing the frequency as a function of the particle diameter *d*/nm. The size interval *Δd* was 1 nm and the mean diameter was 6 ± 2 nm.

3.2. Direct electrochemistry of hSO

The incubation of an AuNP-modified electrode in an aqueous solution of *hSO* results in the formation of a protein film on the surface. After exchange of the protein solution by a protein-free buffer solution cyclic voltammetry was carried out, either in low and high ionic strength, to study the direct (unmediated) electron exchange between the protein and the electrode. An oxidation and a reduction peak were observed, centered at −145 ± 5 mV, demonstrating the interaction of *hSO* with the positive charged AuNPs (Fig. 2a and b) comparable with previous reported data [44]. From the peak area of the anodic wave an electroactive protein surface concentration, assuming a planar surface, of 30 fmol cm^{-2} was calculated. In absence of *hSO* no peak appeared. The peak separation and the peak current increase with increasing scan rate. The nonlinear dependency of the peak current from the scan rate indicates that weakly adsorbed species contribute to the redox process at the electrode surface (Fig. 2c). Presumably the mobility of the HD within the surface bound *h*SO is responsible for this behavior in contrast to the simple Laviron's model on diffusionless electrochemical system [56]. Furthermore, by increasing the buffer concentration also an increase of peak separation is observed, which indicates a slower heterogeneous electron transfer between protein and electrode. Also here an increased HD mobility, may be accounted for this phenomenon. The increased freedom of the HD promotes a better orientation for the intra molecular electron transfer as supported by the higher catalytic activity at elevated ionic strength which will be discussed below.

3.3. Surface enhanced resonance Raman spectroscopy

Surface enhanced resonance Raman (SERR) spectroscopy can be used to selectively monitor the HD structural state of immobilized hSO if the excitation line matches with the Soret band adsorption of the heme cofactor and the surface plasmon resonance of the metallic support. To achieve this two-fold resonance violet light excitation and nanostructured Ag supports are necessary. SERR spectroelectrochemical measurements of *hSO* were thus performed using electrochemically roughened Ag electrodes as bulk support. SERR spectra were recorded for *hSO* adsorbed on Ag-MUA/MU–AuNP-PEI surfaces and compared to the spectra in the absence of Au nanoparticles on Ag-MUA/MU-PEI surfaces (Fig. 3a). In both systems the spectra were identical to the RR spectra in solution and to the ones on Ag electrodes coated with amino-terminated SAMs, measured in a previous work [44]. Hence we can conclude that no alteration of the heme environment occurs upon enzyme adsorption.

The SERR intensities of HD on Ag-MUA/MU-PEI are 3 times lower than in the case of HD on Ag–C8(NH_2)(C6(OH) SAM coated electrodes, which can be rationalised by the larger distance of the heme from the Ag surface due to the different coating thickness (ca. 1.5 nm for C8(NH_2) and ~4 nm for MUA-PEI). Interestingly comparable SERR intensities were measured for Ag-MUA/MU-PEI and Ag-MUA/MU–AuNP-PEI systems although in the latter case the protein is distinctly further away from the electrode surface. We can explain this effect by a plasmon coupling of the AuNPs with the surface plasmon resonances of the Ag support observed previously for Au island films on rough Ag supports [57]. As a result a similar surface enhancement is achieved at the AuNP surface.

The molar fractions of reduced and oxidised HD were in the following determined with SERRS in the potential range from −0.4 to +0.2 V vs. Ag/AgCl, 3 M KCl. For the Ag-MUA/MU-PEI-*hSO* system at 750 mM Tris buffer concentration a sharp redox transition with a midpoint potential of −114 mV was determined (Fig. 4b), which is close to the value of −110 mV obtained for *hSO* on C8(NH_2)/C6(OH) SAMs at the same ionic strength [44]. The main fraction of the enzyme on the surface remained redox active as only 10% of HD could not be reduced at very negative potentials. The apparent

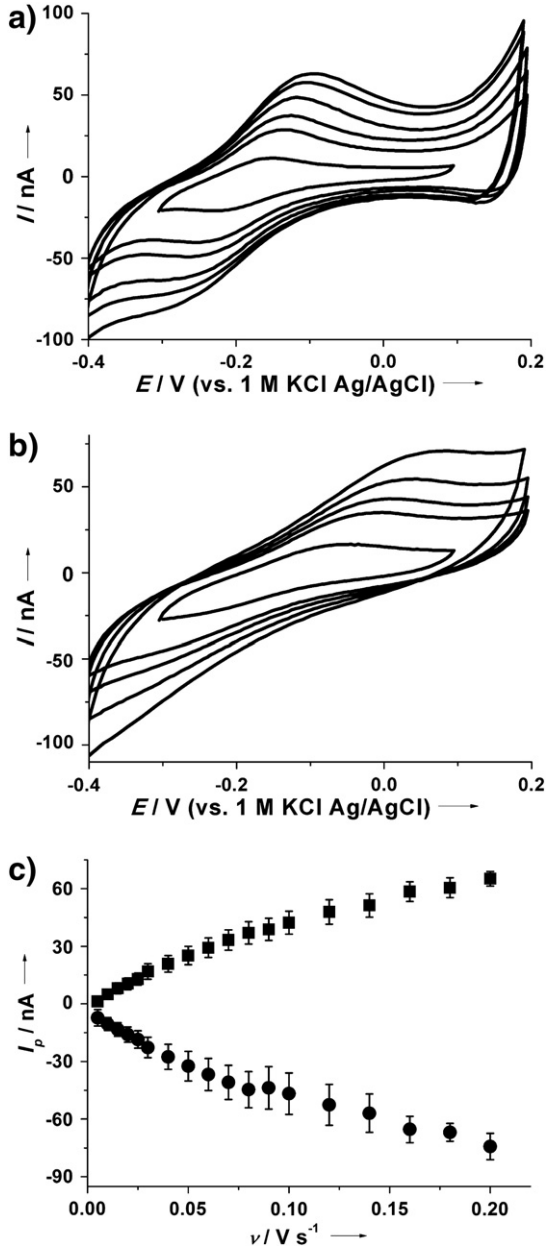

Fig. 2. Cyclic voltammogram of *hSO* on an AuNP-modified gold electrode in a a) 5 mM and b) 750 mM Tris buffer pH 8.4 for various scan rates (25, 60, 80, 120, 200 mV s^{-1}). In c) is showed the dependency of the oxidation (square) and reduction (circle) peak current I_p/nA on the scan rate ν/V s^{-1}.

number of electrons *n*, derived from a Nernstian analysis, was determined to be 0.65, which is lower than in the case of SAM coated electrodes where under these conditions $n = 0.9$ was measured [44]. However, this lower value is in agreement with previous measurements of redox proteins on polyelectrolyte coated electrodes [58] and can be explained by the much more heterogeneous arrangement of NH_2 groups in the case of PEI in comparison to the highly ordered SAM coatings.

For the Ag-MUA/MU–AuNP-PEI system the same midpoint potential (−115 mV) with a slightly lower *n*-value (n = 0.6) was observed (Fig. 4b). Also the amount of redox active HD remains the same indicating a good electrical communication between the AuNPs and the electrode.

These results support the experimental data from the electrochemical study of the adsorbed *hSO*.

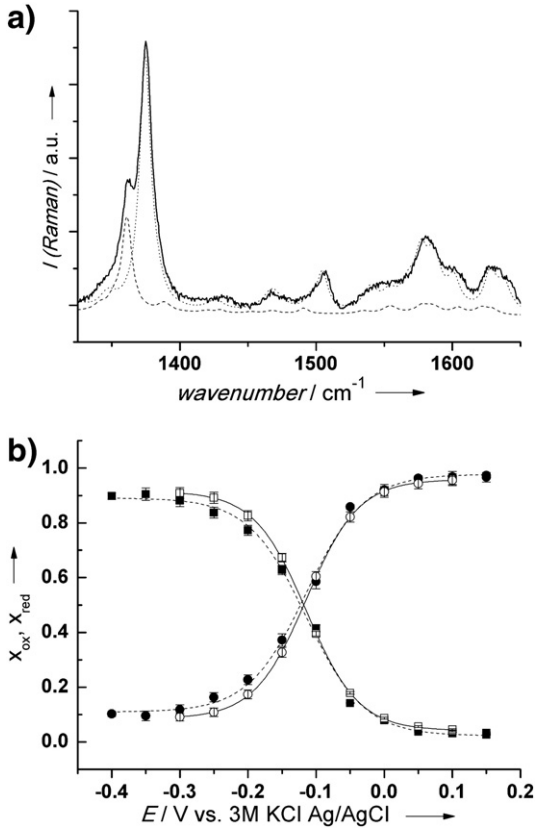

Fig. 3. a) SERR spectrum of hSO at open circuit. The dashed and dotted lines represent the component spectra of the reduced and oxidized species respectively. b) Molar fraction of reduced X_{red} (circles) and oxidized X_{ox} (squares) HD adsorbed on Ag-MUA/MU-PEI (open symbols) and Ag-MUA/MU–AuNP-PEI (solid symbols) electrodes, with an experimental error in the order of 0.01, as a function of potential *E*/V.

3.4. Catalytic activity of hSO

When the AuNP-PEI/*hSO* modified gold electrode was immersed in a sulfite solution the peaks of the CV disappeared and a drastic increase in the oxidation current is observed for potentials higher than about −150 mV (Fig. 4). Since sulfite does not directly oxidize at bare and AuNP-modified gold electrode in this potential region the observed electrocatalytic oxidation can be attributed to the presence of *hSO*. For comparison the gold electrode modified with PEI, but without NPs has also been loaded with *hSO*. As for SERR experiment an electroactive amount comparable to the AuNPs modified electrode

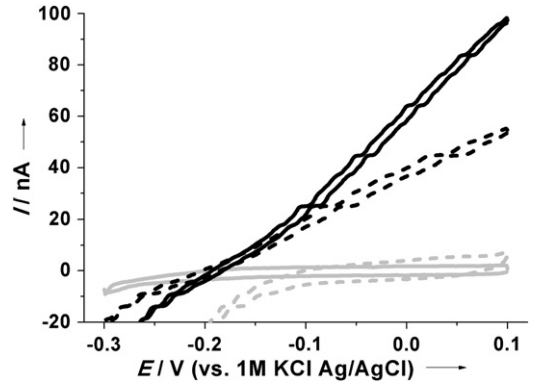

Fig. 4. Cyclic voltammogram of a *hSO*/AuNP-functionalized (solid black line), *hSO*/PEI-functionalized (dashed black line), AuNP-functionalized (dashed gray line) and bare (solid gray line) SAM-modified gold electrode in a 750 mM Tris buffer pH 8.4, 200 μM SO_3^{2-} solution. Scan rate: 2 mV s^{-1}.

is obtained. Nevertheless the electrocatalytic sulfite oxidation current was significantly smaller without AuNPs (dashed solid curve in Fig. 4).

The reduction current visible, when the AuNPs were present in the more negative potential region is to be assigned to the higher catalytic activity of the AuNPs confined on the electrode surface towards reduction of oxygen [59–61] in respect to the gold electrode [61–62] which is in our case additionally blocked by the SAM layer.

To avoid the influence of oxygen the following hydrodynamic voltammograms were recorded in inert atmosphere. The hydrodynamic voltammograms were recorded in order to obtain a substrate diffusion limited experiment at different applied potential (Fig. 5). A high response to sulfite was detected starting from −0.1 V vs. Ag/AgCl, 1 M KCl corresponding to the potential range where roughly all the electroactive HD of the protein are oxidized by the electrodes (see Fig. 2a). The stationary oxidation current increases with increasing applied potential. Due to the considerable low electron transfer rate no leveling-off is obtained, but rather a continuous increase with the increasing potential. Above 0.1 V however a direct sulfite oxidation may also occur (Fig. 5, open square curve). Therefore in order to avoid direct sulfite oxidation, as side reaction, a potential of 0 V was used for the following amperometric experiments. This potential further ensures minimization of unspecific oxidization from other interfering compounds at high potentials.

The pH plays an important role in the overall mechanism and interactions of this system. The formal potential of the molybdenum center has a strong pH dependence since two protons are involved in the catalytic cycle whereas the heme has no virtually pH dependence [38,64]. At the same time the interaction of the MD and the HD depending on the respective charge is expected to be pH influenced. Likely the interaction of negative charged *hSO* on the countercharged NPs surface is also affected by pH influencing the final heterogeneous electron transfer.

Variation of the solution pH results in drastic changes of the catalytic response of AuNPs-PEI/*hSO* on sulfite addition. A basic pH is the most favorable for a high response (see Supporting information S3). This is in agreement with the pH optimum of the protein in solution [65] and confined on an electrode surface [20,46–47].

The role of the ionic strength was investigated by means of different buffer concentrations. As shown in Fig. 6, with all other conditions being equal, the catalytic current strongly depends on the buffer concentration. From a Tris concentration of 25 mM the current begins to be detectable, reaching an increase of 2000% at 750 mM. Interestingly at such an elevated ionic strength no detectable loss of electroactive protein occurred. The last data supports the mechanism reported by Sezer et al. [44] for immobilized *hSO* on amino functionalized silver electrodes. From molecular modeling it has been suggested that a high ionic strength increases the mobility of the HD while the enzyme

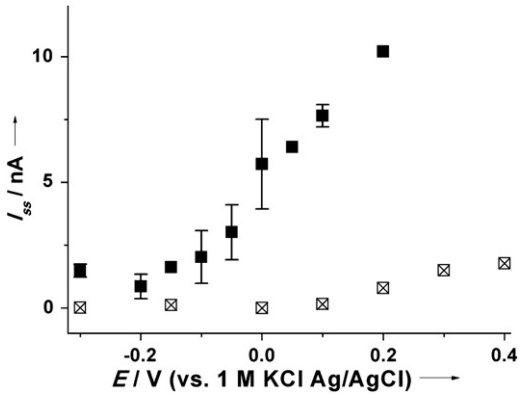

Fig. 5. Stationary oxidation current response I_{ss}/nA of a AuNPs modified electrode with (solid square) and without (open square) *hSO* functionalization at different applied potential *E*/V in an oxygen-free 750 mM Tris buffer pH 8.4 solution containing 200 μM SO_3^{2-}.

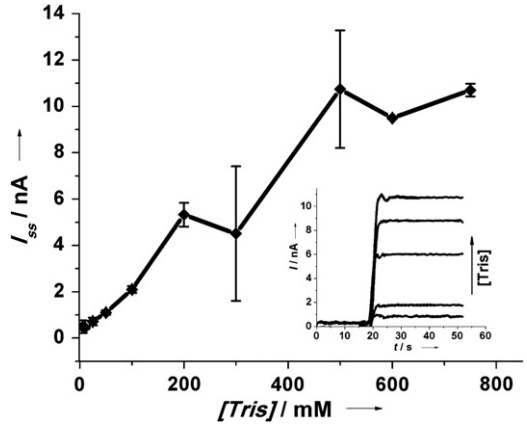

Fig. 6. Stationary current response I_{ss}/nA at different Tris buffer pH 8.4 concentrations *[Tris]*/mM. The inset shows amperometric curves obtained at 0 V in a 200 μM SO_3^{2-} oxygen-free solution containing 25, 100, 200, 500 and 750 mM Tris.

is immobilized via the dimerization domain to the SAM surface. The flexible loop connecting the HD allows alternating contact with the MD and the electrode surface, thereby promoting the intramolecular and heterogeneous electron transfer [37].

3.5. Biosensor

The AuNP-PEI/*hSO* electrode was applied for sulfite detection at high ionic strength condition applying a constant low potential. Fig. 7 shows the amperometric response to subsequent addition

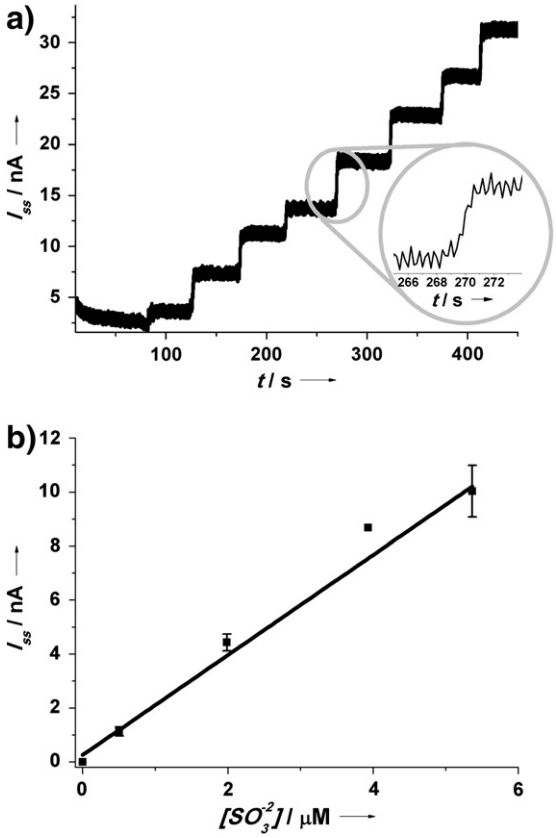

Fig. 7. a) Current response *I*/nA after subsequent additions of SO_3^{2-} in stirring 750 mM Tris buffer solution at pH 8.4 and applied potential 0 V. One of the current steps is zoomed to emphasize the quick increase of current. b) Linear dependency of the stationary current signal I_{ss}/nA on the sulfite concentration $[SO_3^{2-}]$/μM defined by the equation $I_p = a + b\,[SO_3^{2-}]$ where *a* is 0.2 ± 0.2 μM and *b* is 1.8 ± 0.1 μA/μM.

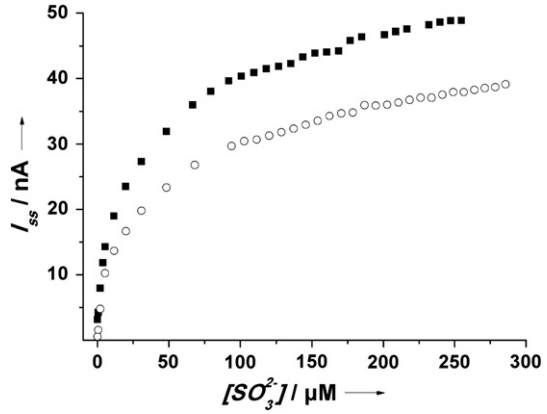

Fig. 8. Stationary oxidation current I_{ss}/nA at different sulfite concentrations $[SO_3^{2-}]$/µM in presence (open circles) and absence (solid square) of oxygen in stirred 750 mM Tris buffer solution at pH 8.4 and applied potential 0.

of sulfite to the constantly stirred 750 mM Tris pH 8.4 buffer solution at an applied potential of 0 V versus Ag/AgCl, 1 M KCl. After each sulfite injection a rapid increase of current is observed that reached a steady state within 2 s (zoom in Fig. 7). The current increased linearly with sulfite concentration. The slope of the linear regression indicates a sensitivity of 1.85 nA μM^{-1}. This is in a similar order of magnitude of other enzyme-based sulfite biosensors, such as the cytochrome c-mediated *hSO* [50] and bacterial sulfite dehydrogenase [47] or chicken SO — polytyramine-modified glassy carbon electrode [67], but higher than other mediated SO-based biosensors [20,68].

The high sensitivity allowed measurement of down to 0.5 µM sulfite. A linear dependence of the current as a function of sulfite concentration could be seen in a range between 0.5 and 5.4 µM (Fig. **7**b).

The short response time of the AuNPs/*hSO* system enabled nearly immediate detection. The sensitivity together with the possibility to work at elevated ionic strength is very advantageous for biotechnological application, such as a sulfite biosensor or as component of a biofuel cell or more complex bioelectronic systems.

We investigated in addition if oxygen acts as competitive electron acceptor to the electrode since this enzyme is also capable to transmit electrons from sulfite to different artificial mediators (2,6-dichlorophenol indophenol, ferricyanide and methylene blue) besides the natural electron acceptor cytochrome *c* [63–64]. As shown in Fig. 8, for different sulfite concentrations, a decrease of catalytic current is visible for sulfite. A $K_{m,\ app.}$ of 101 ± 13 µM and 72 ± 14 µM with a I_{max} of 60 ± 2 nA and 73 ± 3 nA were estimated for measurement in air and in nitrogen atmosphere, respectively.

The proposed biosensor may operate even against high solution ionic strength, which facilitates also the catalytic activity and results in a higher electric output. Since the assembly of *hSO* does not involve cross-linking or protecting membranes, the stability of the described sensor is restricted to a few days. Further effort will be focused towards the improvement of the long term stability. The case of oxygen presence in solution has been evaluated to elucidate its interference on the system. Further optimization of the immobilization to a more efficient and flexible electrode coupling should minimize the effect of oxygen.

4. Conclusion and summary

We have developed an approach for sulfite biosensing, based on direct electron transfer of an immobilized *human* sulfite oxidase on a gold nanoparticles modified electrode.

Gold nanoparticles were synthesized in ionic liquid using PEI as reducing agent for the Au(III) and as stabilizer for the final particles. Basic characterization of the AuNPs by electron microscopy techniques and dynamic light scattering indicate a gold core particle diameter of about 6 nm capped by a PEI/IL shell of around 2–3 nm.

The *hSO* was expressed in *E. coli* and the properties of the purified protein were studied on AuNPs-modified electrodes employing direct electrochemistry. The small mobile protein domain containing the *b5*-type heme cofactor was spectroelectrochemically found to be responsible for the electron shuttle between the catalytic active molybdenum center and the electrode.

The structural integrity of the heme pocket was demonstrated by surface enhanced resonance Raman spectroscopy, which further displayed that approximately all the immobilized enzyme molecules are reachable for a direct electron transfer with the electrode.

The *hSO* direct electron transfer was possible even at a very high ionic strength, whereas other systems based on mediated electron transfer or on layer-by-layer technique need a membrane protection.

Basic pH condition, elevated ionic strength, oxygen-free solution and very low applied potential (0 V) were the optimum condition for sulfite biosensing. At the current status we are able to detect sulfite in a range between 0.5 and 5.4 µM with a high sensitivity (1.85 nA μM^{-1}). This system based on *hSO* and gold nanoparticles, shows relevant properties. On the one hand it provides the possibility to work at low applied potential, avoiding sulfite direct oxidation, and on the other hand allows working at very high ionic strength without leaking of protein. These functionalities are of high interest also for other biotechnological application, such as a sulfite biosensor or as component of a biofuel cell or more complex bioelectronic systems. The short response time of the proposed biosensor is of remarkable importance because enable nearly an immediate detection.

Noteworthy, considering that finely dispersed single gold particles in the size order between 5 and 10 nm have an enhancing influence on the catalytic activity of the protein, further understanding the role of the nanoparticles is of high interest.

Although the effect of the AuNPs on electron transfer efficiency is still an open question, it has been claimed that the small size of the particles provide a suitable microenvironment for a favorable protein orientation and act as nanoscale electrodes that electrically communicate between redox proteins and bulk electrode materials. The latter makes the distance for electron transfer between the protein and the metal particles shorter, and therefore facilitating the interfacial electron transfer process and the electrocatalytic activity [22,69–70].

The possibility to increase the packing density as well as to vary the particles size and shape in the current system has to be explored more deeply. Further investigation should also be extended to the usage of ILs as an electrolyte material due to their high conductivity and electrochemical window [66].

Supplementary materials related to this article can be found online at doi:10.1016/j.bioelechem.2011.11.012.

Acknowledgments

This work was supported by the Cluster of Excellence "Unifying Concepts in Catalysis" funded by the Deutsche Forschungsgemeinschaft (EXC 314, DFG, SM 199/7-1), ILB (TERAsens) and Fonds der Chemischen Industrie (I.W.). We acknowledge also the National University of Costa Rica, the National Science Bureaus of Costa Rica (MICIT and CONICIT) and the DAAD for the financial support.

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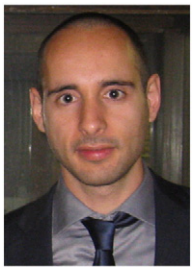
Abbreviations

AuNP: Gold nanoparticle
DLS: Dynamic light scattering
EDC: 1-[3-(dimethylamino]propyl]-3-ethylcarbodiimide methionine
emim EtSO4: 1-ethyl-3-methylimidazolium n-ethylsulfate
HD: Sulfite oxidase heme b5 domain
(h)SO: (Human) Sulfite oxidase
IET: Intramolecular electron transfer
IL: Ionic liquid
MD: Sulfite oxidase molybdenum cofactor domain
mpITO: Mesoporous indium tin oxide
MU: 11-mercapto-1-undecanol
MUA: 11-mercapto-1-undecanoic acid
PEI: Poly(ethyleneimine)
SAM: Self assembled monolayer
SERR: Surface enhanced resonance Raman
TEM: Transmission electron microscopy



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