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Short communication

Enzyme electrode for aromatic compounds exploiting the catalytic activities of microperoxidase-11

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ABSTRACT

Microperoxidase-11 (MP-11) which has been immobilised in a matrix of chitosan-embedded gold nanoparticles on the surface of a glassy carbon electrode catalyzes the conversion of aromatic substances. This peroxide-dependent catalysis of microperoxidase has been applied in an enzyme electrode for the first time to indicate aromatic compounds such as aniline, 4-fluoroaniline, catechol and *p*-aminophenol. The electrode signal is generated by the cathodic reduction of the quinone or quinoneimine which is formed in the presence of both MP-11 and peroxide from the substrate. The same sensor principle will be extended to aromatic drugs.

1. Introduction

Minienzymes, microperoxidases (MPs), which are produced by proteolytic digestion of cytochrome c, can catalyze the hydroxylation of aromatic compounds in a cytochrome P450 mode, oxidation of phenols in peroxidase-like reactions (Mohajerani et al., 2008), but also the dehalogenation of aromatic compounds (Cnubben et al., 1995). The catalytic activity as expressed by k_{cat}/K_M for the peroxidase-like reaction is around 100 times lower than that of horseadish peroxidase (HRP) and the respective value for the cytochrome P450-type catalysis is even almost 10 times smaller than that of the peroxidatic activity (Jeng et al., 2004).

In this study microperoxidase-11 (MP-11), which contains 11 amino acids in the peptide chain and heme c, was immobilised together with chitosan capped gold nanoparticles on the surface of a glassy carbon electrode. Gold nanoparticles have been applied in order to increase the surface area, but also mass transport, and to decrease the protein-metal distance (Campbell and Compton, 2010; Pingarrón et al., 2008; Willner and Katz, 2005).

This MP-11 modified electrode was applied for the indication of aromatic substances including aniline, 4-fluoroaniline, catechol, and *p*-aminophenol (pAP). Phenolic substances like aniline and its derivatives are used in the production of dyes, polymers, pesticides, pharmaceutical products, denaturing agents to vegetable oils etc. Due to their carcinogenic, nephrotoxic and teratogenic effects, quick, easy and sensitive determination methods are necessary (Yin et al., 2010; Wang et al., 2009; Dominguez-Sanchez et al., 1994; Trivedi and Patel, 2011).

2. Materials and methods

2.1. Chemicals

Microperoxidase-11 (MP-11) from horse heart cytochrome c was purchased from Sigma (Steinheim, Germany). A stock solution of MP-11 (0.5 mM) was prepared in 20% (v/v) methanol–water solution.

Hydrogen peroxide (H_2O_2, 30%), $HAuCl_4$, chitosan (CH) (85% deacetylated), anilinium chloride, 4-fluoroaniline, and catechol were purchased from Sigma (Steinheim, Germany) and

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p-aminophenol (pAP) from Fluka (Germany). All reagents were of analytical grade and used without further purification.

2.2. Preparation of electrodes

Gold nanoparticles (AuNP-CH) were prepared according to the method reported elsewhere (Liu et al., 2008; Peng et al., 2010a). The average size of the obtained AuNPs was determined to be 12.9 ± 3 nm by TEM (Yarman et al., 2011). The colloidal gold nanoparticle solution was stored at 4 °C until use. The glassy carbon electrodes (GCEs) were polished with 1.0, 0.3, and 0.05 μM Al_2O_3, respectively and cleaned with Milli-Q water by sonication immediately before each use.

Modification of the electrodes followed the method reported elsewhere (Peng et al., 2010a). MP-11-AuNP-CH modified electrodes were prepared by dropping 10 μL of a 1:1 mixture of MP-11 stock solution and AuNP-CH solution onto the freshly polished GCE surface (MP-11-AuNP-CH/GCE). For control experiments, electrodes without enzymes or without gold nanoparticles were prepared. All modified electrodes were dried at 4 °C overnight and rinsed thoroughly with Milli-Q water prior to use.

2.3. Apparatus and electrochemical measurements

Electrochemical measurements were performed in a stirred electrochemical cell with a three-electrode configuration. A glassy carbon disk electrode (3 mm in diameter) was used as the working electrode, an Ag/AgCl (in 1 M KCl solution) electrode was the reference electrode, and a platinum wire served as the counter electrode. Cyclic voltammetry (CV) was performed using an Autolab PGStat 30 potentiostat equipped with the GPES software (Eco chemie, Netherlands).

Amperometric measurements were performed under aerobic conditions in 2.5 mM phosphate buffer (PBS) at pH 7. A working potential of 20 mV and 0 mV for the reduction of quinoneimine and quinone, respectively, was applied. After baseline stabilization had occurred, 10 μM H_2O_2 was added. The current was recorded after substrate addition into the reaction chamber as a function of time. All the experiments were carried out at room temperature.

3. Results

3.1. Peroxide dependent oxidation of aromatic compounds

The broad spectrum of catalytic activities as shown by microperoxidases has stimulated us to exploit them as biomimetic recognition elements for novel bioanalytical applications. We started this approach by applying the well documented procedures for the covalent coupling of microperoxidases to self assembled monolayers on gold electrodes (Ruzgas et al., 1999; Lötzbeyer et al., 1994). Whilst we could reproduce the reported fast heterogeneous electron transfer for the MP-11 monolayer, there was no indication for MP-catalyzed substrate conversion by the surface-immobilized heme peptide. We circumvented the restriction of the low enzymatic activity by immobilising the microperoxidase in an AuNPs containing matrix of chitosan.

We studied first the indication of phenolic substances, e.g. pAP and catechol at the MP-11 loaded electrode by addressing the peroxidatic activity. The peroxide-dependent oxidation of phenolic substrates like *p*-aminophenol and catechol is based on one-electron/hydrogen abstractions leading to reactive phenoxyl radicals that can form quinoneimines and benzoquinones, respectively by a disproportionation reaction, or polymeric products.

At concentrations above 10 μM of the co-substrate, H_2O_2, inhibition was found (Yarman et al., 2011) and MP-11 is deactivated by the destruction of heme (Jeng et al., 2004). As a compromise between stability and activity, 10 μM H_2O_2 were added to the measuring buffer solution (2.5 mM, pH 7) (Yarman et al., 2011).

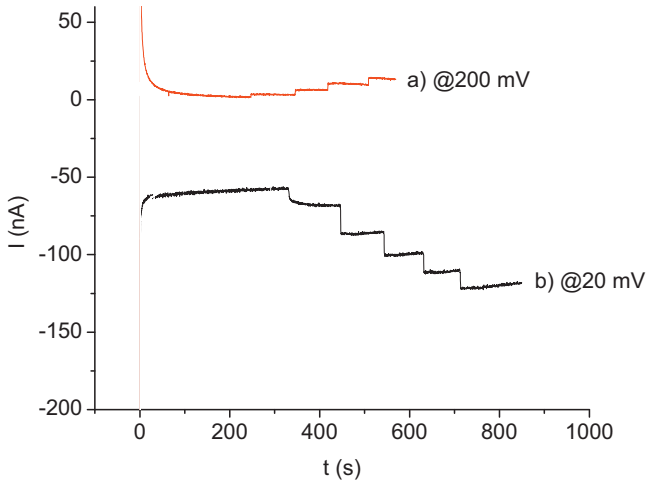

Fig. 1. Amperometric response of the GC electrode covered by MP-11, immobilized in a matrix of chitosan embedded AuNPs, at pH 7 on stepwise addition of pAP (a) at 200 mV in absence, (b) at 20 mV in presence of 10 μM H_2O_2.

As described in literature, the electrochemical oxidation of pAP to quinoneimine and reduction of this product is reflected in the CVs by a pair of redox peaks at 250 mV and 184 mV *vs*. Ag/AgCl, 1 M KCl, respectively, at the GC electrode. pAP was incubated in solution with peroxide in the presence of MP-11 in order to characterize the MP-11 catalyzed oxidation. The MP-11 catalyzed oxidation of pAP generated a product which can be reduced at 20 mV. The current decreases by only 15% during the reaction time of 20 min. The reduction current is attributed to the formation of quinoneimine. On the other hand, in the absence of microperoxidase only a negligible current change is generated on addition of pAP to the peroxide containing measuring solution. Therefore, the current increase is based on the microperoxidase catalyzed product formation.

Obviously, the MP-11 catalyzed reaction is distinct from the HRP-catalyzed reaction which generates a tetrameric final product which is reducible at −450 mV (Zhang et al., 1999; Sun et al., 2001). Fig. 1 compares the current responses of the MP-11-AuNP-CH/GCE for the direct anodic oxidation of pAP at 200 mV (curve a) with those for the reduction at 20 mV of the quinoneimine generated in the MP-catalyzed reaction. Curve b shows after addition of 10 μM H_2O_2, followed by four additions of 0.250 μM pAP, a step-wise increase in the reduction current reaching a steady state after 5 to 10 s. At the MP-11-AuNP-CH/GC electrode, the quinoneimine from the MP catalyzed reaction is reduced back to the original substrate (Fig. 2). This reaction competes with the formation of polymeric products. The electro-enzymatic recycling of the analyte results in an almost fivefold amplification of the current signal as compared with the anodic oxidation of pAP at 200 mV.

In the absence of AuNPs, the sensitivity is sixfold lower than that for the MP-11-AuNP-CH/GCE and the amplification factor is only 1.7. The higher efficiency of electro-enzymatic recycling is based on the increase of the electro-active surface by the “contacted” AuNPs (Yarman et al., 2011).

The catalytic current was linearly dependent on the pAP concentration from 0.1 μM to 2 μM with a linear correlation coefficient of 0.979. The limit of detection was determined to be 3.9 nM (S/N = 3). An almost identical calibration graph as for pAP was obtained for catechol (Fig. 3), showing a similar catalytic activity of MP-11 for both phenolic substances.

The conversion of aniline into aminophenol represents a two-electron oxidation associated with an oxygen-transfer from the peroxide to the product (P450-like reaction). The peroxygenatic