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Mercaptoethylpyrazine promoted electrochemistry of redox protein and amperometric biosensing of uric acid

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Abstract

Electrochemistry of microperoxidase-11 (MPx-11) anchored on the mixed self-assembled monolayer (SAM) of 2-(2-mercaptoethylpyrazine) (PET) and 4,4′-dithiodibutyric acid (DTB) on gold (Au) electrode and the biosensing of uric acid (UA) is described. MPx-11 has been covalently anchored on the mixed SAM of PET and DTB on Au electrode. MPx-11 on the mixed self-assembly exhibits reversible redox response characteristic of a surface confined species. The heterocyclic ring of PET promotes the electron transfer between the electrode and the redox protein. The apparent standard rate constant k_s^{app} obtained for the redox reaction of MPx-11 on the mixed monolayer is ~2.15 times higher than that on the single monolayer of DTB modified electrode. MPx-11 efficiently mediates the electrocatalytic reduction of H_2O_2. MPx-11 electrode is highly sensitive to H_2O_2 and it shows linear response for a wide concentration range. The electrocatalytic activity of the MPx-11 electrode is combined with the enzymatic activity of uricase (UOx) to fabricate uric acid biosensor. The bienzyme assembly is highly sensitive towards UA and it could detect UA as low as 2 μM at the potential of −0.1 V. The biosensor shows linear response with a sensitivity of 3.4 ± 0.08 nA cm^{-2} μM^{-1}. Ascorbate (AA) and paracetamol (PA) do not significantly interfere in the amperometric sensing of UA.

Keywords: Microperoxidase-11; Mixed self-assembly; H_2O_2; Uricase; Uric acid biosensor

1. Introduction

Uric acid is the primary end product of purine metabolism, extreme level of UA in the body is symptoms of several diseases like gout, hyperuricaemia etc. (Fox, 1981; Ullman et al., 1982). The normal UA level in serum range from 240–520 μM and in urinary excretion is typically 1.4–4.4 mM (Kissinger et al., 1987). Various methodologies based on UV–vis spectrophotometry, chromatography, voltammetry, amperometry etc. have been developed for the accurate determination of UA (Thrasher and Abadie, 1978; Strochkova et al., 1997; Marquette et al., 2003; Perello et al., 2005; George et al., 2006; Popa et al., 2000). The electrochemical techniques are promising because of their fast response and high sensitivity. Unmodified and electrodes modified with polymers, sol–gels and SAMs with suitable functional groups have been employed for the detection of UA (Brajter-Toth et al., 2000; Ianniello et al., 1982; Zen and Chen, 1997; Khoo and Chen, 2002; Nakaminami et al., 1999; Raj and Ohsaka, 2003). The electrochemical methods based on enzyme UOx are highly selective and sensitive. The function of UA biosensors is based on the determination of the enzymatically generated CO_2 or H_2O_2 or the consumption of O_2 concentration during the enzymatic reaction (Nanjo and Guilbault, 1974; Kawashima and Rechnitz, 1976; Keedy and Vadgama, 1991; Tatsuma and Watanabe, 1991a; Miland et al., 1996). The method based on the determination of H_2O_2 has received considerable interest because H_2O_2 can be detected either by its reduction or oxidation. UA can be quantified conveniently by monitoring the concentration of enzymatically generated H_2O_2. Since electrochemical oxidation of H_2O_2 requires high overpotential, the easily oxidizable species such as AA would interfere in the measurement of UA. However, monitoring the concentration of H_2O_2 by its reduction does not invite the interference from these analytes. Hence the electrodes having excellent electrocatalytic activity towards reduction of H_2O_2 can be successfully utilized as a transducer for sensing UA.

MPx-11 is a small redox protein derived from proteolytic digestion of cytochrome c (Aron et al., 1986) and it contains

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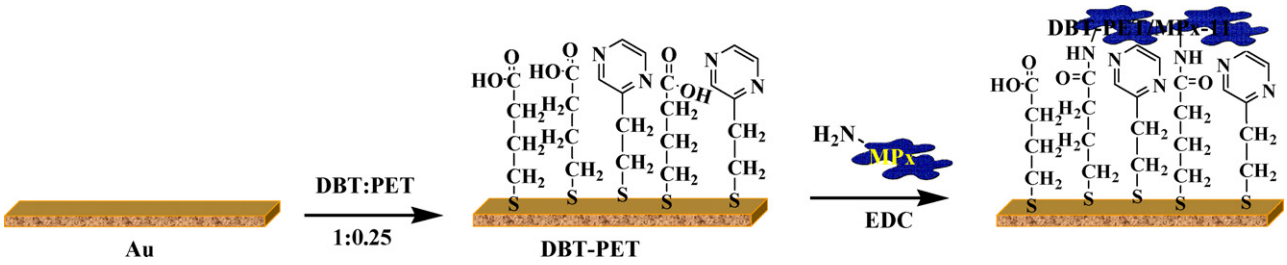

Scheme 1. Scheme illustrating the immobilization of MPx-11 on DTB electrode.

11–21 protein segments. The heme is attached to the peptide via thioether linkages involving the side chains of Cys 14 and Cys 17. The axial position of the heme centre is coordinated with the imidazole ring from His 18 and a water molecule. MPx-11 shows peroxidase activity and it can catalytically reduce H_2O_2 to H_2O (Adams, 1991). The electrochemical and electrocatalytic properties of MPx-11 have been studied using different electrodes (Santucci et al., 1988; Narvaez et al., 1997; Lotzbeyer et al., 1994, 1997; Katz et al., 2005; Goldston et al., 2002; Huang et al., 2001, 2003; Kulys et al., 1998; Gobi and Mizutani, 2001; Xu et al., 2005; Liu et al., 2005). Wiring of redox enzymes on the electrode surface by covalent coupling is of great interest, as fast electron transfer between the electrode surface and the redox centre of the protein can be easily achieved (Willner and Katz, 2000). MPx-11 can be conveniently wired on the electrode surface using suitable coupling agents. Herein we describe the electrochemistry of MPx-11 anchored on a mixed self-assembly of PET and DTB and the fabrication of UA biosensor. The enzymatically generated H_2O_2 has been successfully detected at −0.1 V by MPx-11.

2. Experimental

2.1. Chemicals

DTB, PET, UA, AA, PA, chitosan (Chit), MPx-11, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), uricase from *candida* sp. 1.7.3.3 were obtained from Sigma–Aldrich. All other chemicals used in this investigation were of analytical grade. Sodium phosphate buffer solution (PBS) was used as supporting electrolyte in all the cyclic voltammetric and amperometric measurements.

2.2. Instrumentation

All the electrochemical measurements were performed with CHI643B electrochemical analyzer attached with a Faraday cage picoampere booster. A two-compartment three-electrode cell with a polycrystalline Au working electrode (1.6 mm diameter), a platinum wire auxiliary electrode and a Ag/AgCl reference electrode (3 M KCl) was used in the measurements. All the results described here were carried out at least three times and reproducible results were obtained in all the cases.

2.3. Procedure

Polycrystalline Au electrodes were polished repeatedly with alumina powder (0.06 μm) and sonicated in water for 5–10 min. The polished electrodes were then electrochemically cleaned by cycling the potential between −0.2 and 1.5 V at the scan rate of 10 V/s in 0.25 M H_2SO_4 until the characteristic cyclic voltammogram for a clean Au electrode was obtained. The single monolayer assemblies were made by soaking the pretreated electrode in ethanolic solution of DTB or PET (1 mM) for a period of 2 h at room temperature. Mixed self-assemblies of DTB and PET were made by immersing the cleaned Au electrode in ethanol solutions containing DTB and PET in the molar ratio of 1:0.25 (DTB:PET) for 2 h. These SAM modified electrodes were rinsed extensively with ethanol and water and subjected to further modification. The electrode modified with the self-assembly of DTB and the mixed self-assemblies of DTB and PET will be referred as DTB and DTB–PET electrodes, respectively.

MPx-11 was covalently anchored on the SAM electrodes according to Scheme 1. Typically, the DTB and DTB–PET electrodes were incubated in 0.05 mM MPx-11 in 5 mM PBS of pH 7.2 containing EDC (2.5 mM) for 3 h at 4 °C. This electrode was rinsed well with PBS to remove the physically adsorbed MPx-11 on the electrode surface and used in the voltammetric and amperometric experiments. The MPx-11 anchored DTB and DTB–PET electrodes will be referred as DTB/MPx-11 and DTB–PET/MPx-11 electrodes, respectively. For the fabrication of UA biosensor, the enzyme UOx was immobilized on the MPx-11 anchored electrode using the biopolymer Chit. The MPx-11 electrode was first modified with a thin layer of the Chit (0.05 wt%) by drop casting 10 μl on the electrode surface and allowed to dry at 4 °C. An aliquot of 10 μl of the UOx solution (132.5 U/ml) was loaded on the electrode surface and allowed to dry at 4 °C. All the voltammetric and amperometric experiments for the sensing of UA were performed in PBS of pH 8.5.

3. Results and discussion

3.1. Characterization of the self-assembled monolayer

The self-assemblies of DTB, PET and DTB–PET on Au electrode were electrochemically characterized by measuring the interfacial capacitance, surface coverage (Γ) and voltammetric response towards the redox probe $Fe(CN)_6^{3-/4-}$. Capacitance of DTB, PET and DTB–PET electrodes were measured in neutral pH at −0.1 V taking the charging current into account (Bard and Faulkner, 2000). The capacitance values were calculated to be 14 ± 1, 8 ± 1.5 and 18 ± 2 μF/cm^2 for DTB, PET and DTB–PET electrodes, respectively. The capacitance data suggests that dilution of the DTB monolayer with PET causes