Amperometric biosensor for xanthine with supramolecular architecture

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Xanthine oxidase modified with 1-adamantanyl residues was supramolecularly immobilized on Au electrodes coated with Au nanoparticles coated with a perthiolated β-cyclodextrin polymer; the analytical response of the electrode toward xanthine was evaluated.

Modifications of enzymes with cyclodextrin (CD) derivatives have been widely used for preparing neoglycoenzymes stabilized through supramolecular interactions.[1] CDs have also been employed as coating materials on metal electrodes and nanoparticles in order to immobilize adamantane-modified proteins *via* host–guest interactions.[2] CD-based polymers have been employed as three-dimensional matrices for adsorbing electrochemical mediator in enzyme biosensors.[3] In the present work we report a novel approach for constructing an enzyme biosensor device based on the ability of CDs to form stable inclusion complexes with hydrophobic compounds. The covalent transformation of xanthine oxidase (XO, EC 1.1.3.22, 2.0 U mg^{-1}) with 1-adamantanyl residues (XO-ADA) and its further immobilization on Au electrodes modified with Au nanoparticles coated with a perthiolated βCD-polymer (AuPolyCD) are described. The enzyme electrode prepared *via* supramolecular associations was further used for constructing an amperometric biosensor for xanthine (Fig. 1). Xanthine was selected as target compound for this study due to the importance of its biomedical quantification for the diagnosis of several diseases (*e.g.* xanthinuria).

βCD was polymerized with epichlorohydrin as described elsewhere,[4] and further modified with thiol groups for which to 2 g polymer, dissolved in 20 ml NaOH (20 mg), an excess of tosyl chloride in acetonitrile was added. After 24 h under continuous stirring, the mixture was neutralized with HCl (10%) to provoke the precipitation of the polymer which was washed with highly diluted HCl and dried under vacuum at 60 °C. The perthiolated polymer (10 mg) was mixed with a solution of Au nanoparticles, prepared according to Jiang *et al.*[5] (2 mg ml^{-1}, average diameter = 20 nm)[5] and the modified polymer was added over cool acetone and the capped nanoparticles (AuPolyCD) precipitated after stirring the solution for 2 h. The modified nanoparticles contained an average of 15 mmol of CD per mol of Au. A clean Au disk electrode (A = 7.07 mm^2) was dipped for 4 h in an aqueous solution of the AuPolyCD (10 mg ml^{-1}) and then exhaustively washed with distilled water.

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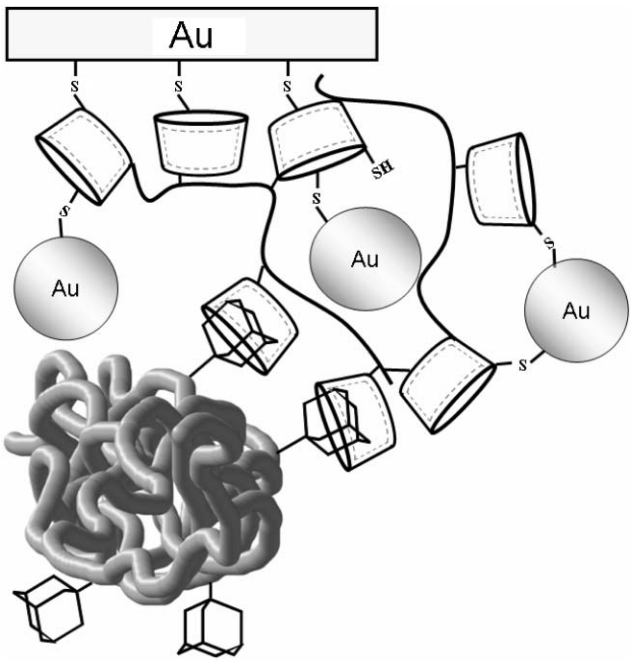

Fig. 1 Supramolecular-mediated immobilization of XO-ADA on AuPolyCD-modified Au electrode.

For preparing a XO form suitable to form inclusion complexes with the coated electrode, the enzyme was modified with 1-adamantane carboxylic acid as previously described for cytochrome c.[2a] The modified enzyme (XO-ADA) retained 82% of the

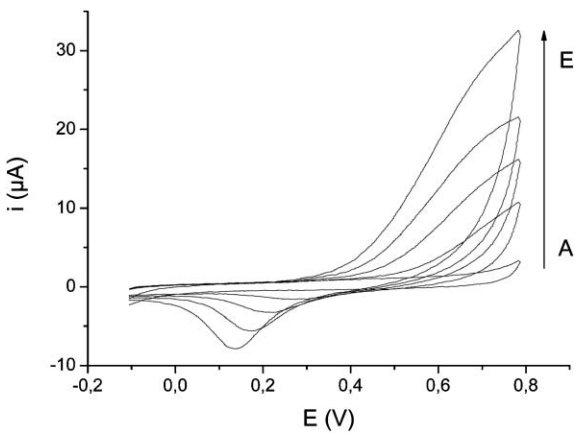

Fig. 2 Cyclic voltammograms of the enzyme electrode at 50 mV s^{-1} in 0.1 M sodium phosphate buffer, pH 7.0, in the absence (A) and the presence of xanthine at different concentrations: B) 0.96 mM; C) 1.85 mM; D) 2.68 mM; E) 3.45 mM.

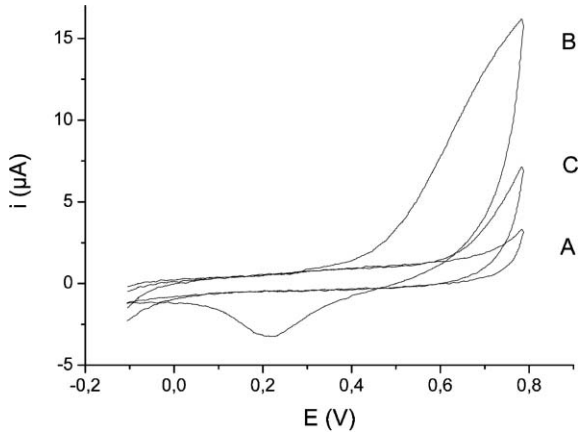

Fig. 3 Cyclic voltammograms of the enzyme electrode at 50 mV s^{-1} in 0.1 M sodium phosphate buffer, pH 7.0, in the absence (A) and the presence of 0.96 mM xanthine, before (B) and after (C) 24 h incubation in saturated 1-adamantane carboxylic acid solution.

initial enzyme activity and contained 8 mol 1-adamantane residues attached to each mol protein. XO-ADA was immobilized on the modified Au surface by dipping the electrode for 24 h at 4 °C in a 2 mg ml^{-1} enzyme solution in 20 mM sodium phosphate buffer, pH 7.0. The electrode was further washed with cool buffer solution and kept at 4 °C until use.

Fig. 2 shows the cyclic voltammograms of the enzyme electrode in the presence of xanthine at different concentrations.[6] An increase in the anodic response of the electrode, corresponding to the oxidation of the H_2O_2 produced by the enzymatic reaction, was observed when the concentration of xanthine was increased. The bioelectrochemical transformation of xanthine was 1.6-fold enhanced by the presence of Au nanoparticles, when comparing with the electrodes coated only with the perthiolated polymer.

In order to confirm our hypothesis about the supramolecular mechanism for the immobilization of the modified enzyme on the AuPolyCD-coated gold surface, the electrode was incubated at 4 °C in 0.1 M sodium phosphate buffer, pH 7.5, saturated with 1-adamantane carboxylic acid.

It is well known that 1-adamantane derivatives can form highly stable inclusion complexes with CDs,[7] so the presence of saturated 1-adamantane carboxylic acid in the incubation media should disrupt the possible host–guest interactions between the immobilized XO and the modified electrode.

Fig. 3 shows that the anodic current significantly decreased after 24 h incubation, suggesting that either the enzyme is released from

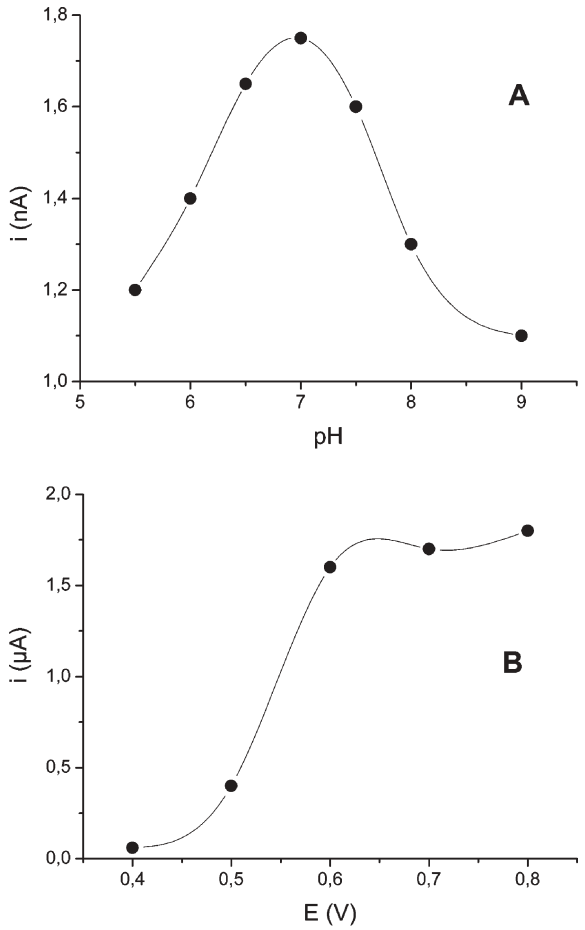

Fig. 4 Amperometric response of the electrode toward 0.96 mM xanthine at different pH (A) and applied potential (B).

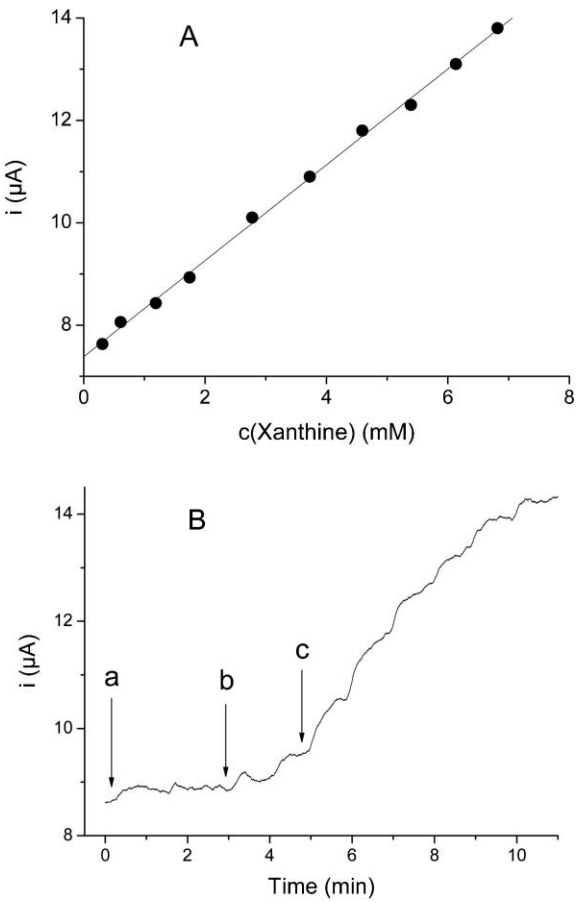

Fig. 5 A) Calibration curve for the electrode. B) Amperometric response of the electrode to successive addition of 25 mM xanthine solution: a) 250 μl, b) 500 μl, c) 1.0 ml. Applied potential: +0.7 V; stirring rate: 300 rpm; temperature: 25 °C; initial working volume: 20 ml; supporting electrolyte: 0.1 M sodium phosphate buffer, pH 7.0.

the electrode surface in the presence of 1-adamantane carboxylic acid or that the enzyme is inhibited by this compound. The latter possibility was discarded by testing the enzymatic activity of XO and its modified form after the same time of incubation in saturated 1-adamantane carboxylic acid solution. According to these facts, it is evident that high concentrations of 1-adamantane carboxylic acid can disrupt the supramolecular association between XO-ADA, through the 1-adamantanyl residues, and the CD moieties covering the surface of the electrode.

The enzyme electrode showed maximum analytical response at +0.7 V and pH 7.0 (Fig. 4), and these conditions were used as optimum for further experiments. The amperometric response of the modified electrode to successive additions of xanthine was evaluated. The electrode showed an excellent and fast bioelectrocatalytic response, with 95% of the steady-state current being achieved in about 14 s for xanthine (Fig. 5). A linear relation between the steady-state current and xanthine concentration in the range of 310 μM–6.8 mM, with a correlation coefficient of 0.998 (n = 5) was observed. The sensitivity of this sensor was estimated to be 13.3 mA/M cm^{-2}. On the other hand, a detection limit of 150 μM was estimated for this biosensor at a signal-to-noise ratio of 3. The enzyme electrode showed K_M and I_{MAX} values of 2.1 mM and 17 μA, respectively.

The sensor showed good storage stability since its response decreased only 7% after 21 days of storage at 4 °C. The high stability shown by the biosensor should be associated with the improved resistance conferred on enzymes by immobilization in CD-coated Au nanoparticles.[2b,c] However, it was confirmed that the use of high anodic potential affected the selectivity of the electrode, reducing by 22–47% the electroanalytical signal of the biosensor toward xanthine in the presence of equimolar concentration of L-ascorbic acid and uric acid, respectively.

In conclusion we have described for the first time the preparation of an enzyme electrode *via* supramolecular associations. Experiments are in progress to generalize this approach.

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6 All the electrochemical measurements were performed using an AMEL 7050 potentiostat/galvanostat and the data were acquired using JuniorAssist software (Amel, Milan, Italy). A conventional three-electrode system was employed in all electrochemical studies. The working electrode was the Au disk covered with the enzyme. Ag/AgCl/KCl (3 M) and a Pt wire were used as reference and counter electrodes, respectively. Bioelectrode measurements were carried out at 25 °C in 0.1 M sodium phosphate buffer, pH 7.0 (working volume 20 ml). The solution was exhaustively aereated before each electrochemical experiment, and stirred at 300 rpm during amperometric studies.

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