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Development of amperometric biosensors using thiolated tetrathiafulvalene-derivatised self-assembled monolayer modified electrodes

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ABSTRACT

The usefulness of tetrathiafulvalene (TTF) derivative self-assembled monolayers (SAMs) modified electrodes as platforms for the construction of electrochemical biosensors is evaluated by using a 4-mercaptotetrathiafulvalene (HSTTF) modified gold electrode for the preparation of a fructose electrochemical biosensor. The electroactive SAM enhances the electron transfer between the immobilized FDH and the electrode surface, the electrochemical oxidation of HSTTF being used to monitor fructose concentration. The amount of HSTTF employed to form the electroactive SAM (2.2 μg), the FDH loading (20.4 U), and the applied potential to the biosensor (+0.40 V) were optimized. The stability of the FDH-HSTTF-AuE was evaluated under several aspects, a low reproducibility between different biosensors being observed. Calculation of the apparent Michaelis–Menten constant indicated that the affinity of the enzyme for the substrate is practically non-affected by the immobilization procedure. A detection limit of 2.7×10^{-7} mol L^{-1} fructose was achieved. The reproducibility of the responses obtained with different biosensors was largely improved by forming mixed monolayers consisting of HSTTF and mercaptohexanol (MCH). The limit of detection calculated for the FDH-MCH/HSTTF-AuE biosensor is 4.2×10^{-7} mol L^{-1} fructose. The performance of this biosensor for the determination of fructose in real samples is evaluated by the analysis of a Cola soft drink sample.

1. Introduction

Self-assembled monolayers (SAMs) of 3-mercaptopropionic acid (MPA) on gold disk electrodes have demonstrated to constitute excellent platforms for the construction of robust electrochemical biosensors suitable to work both under batch and flow injection conditions [1–3]. In these configurations, tetrathiafulvalene (TTF) was used as a mediator to connect enzymes with the electrode surface and it was coimmobilized together with the corresponding enzymes atop the SAM-modified electrode by cross-linking with glutaraldehyde. It is well known that TTF constitutes a reversible and thermodynamically very stable two-electron donor system due to the contribution from a 6π-electron heteroaromaticity of its both oxidized forms, the 1,3-dithiolium cation and the dication. The radical ion salt and charge transfer complex show a high conductivity that has been attributed to their electron donor ability and strong intermolecular interactions [4]. TTF water soluble complexes have been applied as mediators in the development of glucose oxidase and xanthine oxidase amperometric biosensors [5,6]. These enzyme electrodes were stable when stored in the dark at 4 °C but a run of a calibration curve reduced their sensitivity in a 15%, which was attributed to the mediator leaching from the electrode surface. Furthermore, Bartlett et al. [7] demonstrated the applicability of a glucose amperometric biosensor based on the covalent attachment of glucose oxidase to a TTF derivative (4-[3′-carbohydroxypropyl)thio]-5-(methylthio)tetrathiafulvalene) and its subsequent entrapment on a glassy carbon electrode by means of a dialysis membrane. It should be noted that although the attached TTF groups were stable for around 10 days when stored in glucose-free buffer, when the biosensor was operated continuously for the determination of glucose, the stability was significantly lower due to a reaction between traces of hydrogen peroxide generated during the enzymatic process and the attached TTF species leading to a loss in mediation efficiency of the enzyme electrode.

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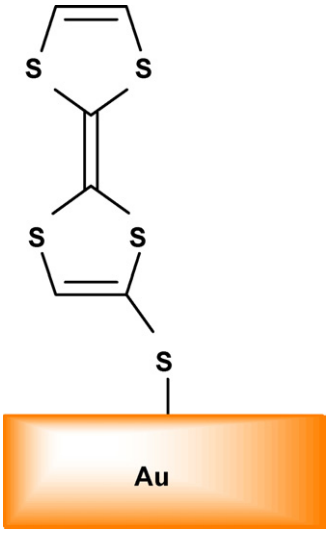

Fig. 1. Cartoon of the SAM structure of the 4-mercaptotetrathiafulvalene (HSTTF).

In order to avoid mediator leaching from the electrodic surface and to achieve high efficiencies in the mediation process with a control over the enzyme–mediator complex orientation atop the electrode surface, a novel strategy is the formation of electroactive SAMs by TTF modification substituting the central aromatic core with alkylthiols [8]. Some authors have studied the electrochemical properties of TTF-derivatives SAMs [4,8–10] but, in fact, the applicability of these SAMs for analytical purposes has only been demonstrated until now for the detection of a few metals such as K, Na, Li or Pb [11,12]. Our group suggested recently the possibility of using TTF-derivatised SAMs as platforms for the construction of electrochemical biosensors [13]. However, the performance of the biocatalytic integrated device with horseradish peroxidase as immobilized enzyme was shown to be very dependent on the molecular structure of the TTF derivative, and exhibited a moderate reproducibility and analytical usefulness.

In this work, the bioelectrocatalytic properties and applicability of a thiolated TTF-derivatised SAM has been demonstrated by constructing a fructose biosensor. A 4-mercaptotetrathiafulvalene (HSTTF) modified gold electrode was used as the platform for the construction of the electrochemical biosensor (Fig. 1). Fructose is frequently used as dietetic sweetener, and its determination is not only of interest in the food industry for quality control but also in clinical chemistry, given that high levels of fructose in blood and excretions are found in patients affected by essential fructosuria [14].

2. Experimental

2.1. Apparatus and electrodes

Amperometric measurements were carried out with an ECO Chemie Autolab PSTAT 10 potentiostat using the software package GPES 4.9 (General Purpose Electrochemical System). A P-Selecta ultrasonic bath and a P-Selecta agimatic magnetic stirrer were also used.

A BAS gold disk electrode (3-mm Ø, microscopic surface area of 0.12 cm^2) was used as electrode substrate for the biosensor preparation. A BAS MF-2063 Ag|AgCl|KCl 3 mol L^{-1} reference electrode and a Pt wire counter electrode were also employed. A 10-mL glass electrochemical cell was used in the experiments.

2.2. Reagents and solutions

Fructose (0.5 mol L^{-1}, Sigma) stock solutions were prepared in 0.05 mol L^{-1} phosphate buffer of pH 4.5. More dilute standards were prepared by suitable dilution with the same phosphate buffer solution, which was also used as the supporting electrolyte.

Monolithiation of TTF under the standard conditions reported by Bryce et al. [15], followed by the addition of elemental sulphur, affords the corresponding intermediate TTF-thiolate anion, which can be quenched with ammonium chloride to yield HSTTF [13] as a greenish solid that gradually decomposes to the TTF starting material. In fact, under high resolution matrix-assisted laser desorption ionization (HRMS-MALDI) analysis the addition of the HSTTF initially formed to TTF (generated from the degradation of HSTTF) is observed, yielding the molecular peak corresponding to bis(tetrathiafulvalenyl)sulphide [$(TTF)_2S$] (Fig. 2) [16]. The additional spectroscopic characterization of the compound (IR-TF, ^1H NMR, ^{13}C NMR and UV–vis: HSTTF (46% yield); mp 70–72 °C (from dichloromethane/hexane); FT-IR: 3068, 2925, 1602, 1529, 1495, 1454, 1409, 1260, 1091, 1030, 853, 794, 781, 734, 701, 644 cm^{-1}; ^1H NMR ($CDCl_3$) δ: 6.43 (s, 1H), 6.32 (s, 2H), 1.43 (s, 1H); ^{13}C NMR ($CDCl_3$) δ: 109.5 (TTF trace), 110.1, 119.0 (TTF trace), 125.6, 127.0, 128.2, 128.3, 142.5; UV–vis (CH_2Cl_2): λ_{max} (nm) 426, 371, 309, 231; HRMS (MALDI) m/z: calc. for $C_{12}H_6S_9$ 437.79505, found 437.79538) confirmed that the HSTTF structure is the one initially formed. In particular, the –SH proton of the molecule is observed at 1.43 ppm in the ^1H NMR spectrum, and six different carbons, corresponding to a monosubstituted TTF, were observed between 110.1 and 142.5 ppm for HSTTF.

A 3.75 mmol L^{-1} HSTTF solution, prepared in toluene (99.5%, Scharlau), was employed for the formation of the HSTTF monolayers. The solution used for the enzyme immobilization was a 5.1 U μL^{-1} solution of FDH (Sigma, EC 1.11.99.11 from Gluconobacter sp., 733 U mg^{-1} protein), prepared in the above-mentioned phosphate buffer solution, and a 25% glutaraldehyde (Aldrich) solution.

Other solutions employed were: a 1 mmol L^{-1} mercaptohexanol (MCH) (Fluka) solution and a 2 mol L^{-1} KOH (Panreac) solution, both of them prepared in deionised water; 0.01 mol L^{-1} stock solutions of caffeine, ascorbic acid, citric acid (all of them form Merck), D-glucose (Panreac), D-galactose (Sigma), L-arabinose (BDH), and D-sucrose (Fluka) were prepared in 0.05 mol L^{-1} phosphate buffer of pH 4.5 for the interference study. All chemicals were of analytical-reagent grade, and water was obtained from a Millipore Milli-Q purification system.

2.3. Procedures

Before carrying out the deposition of the HSTTF-SAM, the gold disk electrode (AuE) was pretreated as described previously [3]. HSTTF-SAMs were formed by depositing 2.5 μL of the 3.75 mmol L^{-1} HSTTF solution onto the pretreated-AuE and let to assemble at room temperature for at least 4 h under dry atmosphere in order to avoid a quick evaporation of the solvent. Then, 4 μL of the 5.1 U μL^{-1} FDH solution were deposited on the HSTTF-modified AuE. Once the electrode surface had dried up at ambient temperature, it was immersed in the 25% glutaraldehyde solution for 1 h at 4 °C.

When post-treatment with MCH was carried out after the formation of the HSTTF-SAM according to the procedure described above, 10 μL of the 1.0 mmol L^{-1} aqueous solution of MCH were placed onto the SAM-modified gold electrode surface for 1 h in a sealed vessel, and the modified electrode was rinsed with deionised water to remove any unbound thiol. The enzyme was subsequently immobilized following the same procedure as described above.