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Preparation and characterisation of a quinone-functionalised polythiophene film on a modified electrode. Application to the potentiometric determination of glutathione and cysteine concentrations

Stéphanie Étienne [a,†], Muriel Matt [b], Thierry Oster [c,*,‡], Mohammad Samadi [a,b], Marc Beley [b,d,*,‡]

[a] LIMBP, IPEM, Université Paul Verlaine-Metz, 1 Boulevard Arago, 57070 Metz, France
[b] LCME, Université Paul Verlaine-Metz, 1 Boulevard Arago, 57070 Metz, France
[c] Laboratoire Lipidomix ENSAIA-INPL, Nancy-Université, 15 rue de la Champelle, 54500 Vandœuvre-lès-Nancy, France
[d] Synthèse Organique et Réactivité, UMR CNRS/UHP7565, Nancy-Université, Boulevard des Aiguillettes, 54506 Vandœuvre-lès-Nancy, France

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ABSTRACT

The new compound 3-((2,5-dimethoxyphenyl)ethynyl)thiophene has been synthesised by Sonogashira coupling. A modified electrode coated with a polythiophene film bearing a quinone moiety was obtained by electropolymerisation of the thienyl group followed by anodic oxidation of *para*-dimethoxyphenyl group. The cyclovoltammetric response resulting from the reaction of glutathione with the benzoquinone moiety was investigated. The responses of the modified electrode as a new potentiometric sensor of reduced thiols are proposed.

1. Introduction

Reduced thiols are essential in biological systems, on account of their crucial role in the maintenance of redox homeostasis. Their concentration and bioavailability are of major importance to balance the accumulation of reactive oxygen and nitrogen species and to prevent subsequent deleterious oxidative stress associated with the pathogenesis of cancer, cardiovascular diseases, atherosclerosis, hypertension, ischemia/reperfusion injury, diabetes mellitus, neurodegenerative diseases, rheumatoid arthritis and ageing.[1]

Over the past three decades, intensive studies have been devoted to the detection of biological thiols as cysteine, homocysteine and more particularly glutathione.[2] Ellman's test is the classical method used for quantification of thiols based on a spectrophotometric method laying an exchange reaction between the thiol and the disulfide DTNB (5,5′-dithiobis-(2-nitrobenzoic acid)).[3] The sulfide produced shows a characteristic yellow colour. However, this test and more generally spectrophotometric methods require a pre-treatment when thiols have to be assayed in coloured samples and their results may be influenced by variable levels of specific enzyme activities such as glutathione *S*-transferase or γ-glutamyltransferase.

High-performance liquid chromatography and capillary electrophoresis are used as separative techniques to determine glutathione and congeners in several bio-matrices.[4] Besides, fluorescence methods have been recently developed for the measurements of glutathione in yeast.[5]

In contrast with most of these techniques, the electrochemical methods provide the possibility of being used with coloured samples, such as blood, without tedious pre-treatment. Two types of electrochemical sensors for the determination of thiol concentrations are commonly studied.

- With an amperometric sensor, the thiol detection is generally based on chemically-modified electrodes that induce an electrocatalytic oxidation of another thiol obtained after an exchange reaction as in the Ellman's test.[6–9] Another amperometric approach is the use of organic redox cofactors like pyrroloquinoline quinone, glutathione peroxidase or inorganic redox mediators as transition metal complexes immobilised in a matrix.[10–13] In these systems, intermediate

* Corresponding authors. Tel.: +33 3383678211 (T.O); +33 3383684783 (M.B).
E-mail addresses: thierry.oster@mtm.nancy.inserm.fr (T. Oster), marc.beley@sor.uhp-nancy.fr (M. Beley).
† Present address: Centre de Recherche Public Henri Tudor, Laboratoire de Technologies Industrielles, Unité des Matériaux, 66, Rue de Luxembourg, BP 144, L-4002 Esch-sur-Alzette, Luxembourg.
‡ T.O. and M.B. are Associate Professor and Professor, respectively, at the University Paul Verlaine-Metz.

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steps are necessary because the direct oxidation of thiols is slow at conventional electrodes and consequently require an important overpotential due to electrode passivation.

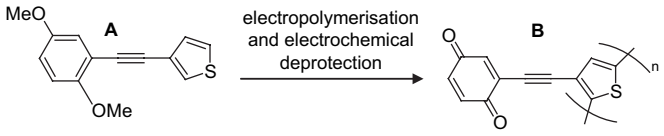
- In potentiometric methods, the sensors are usually based on the reaction between thiols and quinone indicators, as described by Davis et al.[14]Among the electrochemical methods, the potentiometric method can be implemented very easily.

Lau et al. have shown that the reaction of 1,4 benzoquinone (BQ) with the reduced form of glutathione (GSH) results in the formation of adducts that exhibit increasing degrees of glutathione substitution.[15] Moreover, as preliminary studies, the interaction of GSH with BQ by cyclic voltammetry on a vitreous carbon electrode was investigated (data not shown). The cyclic voltammogram of BQ was significantly modified when this compound was added to a solution of GSH. The principal changes consisted in the oxidation process that became irreversible and in the significant decrease in intensity of the oxidation peak. The latter modification characterised the diffusion of a bigger species than BQ. Consequently, in agreement with Lau's work, we postulated that the modification of the electrochemical behaviour of the couple BQ/BHQ (BHQ=1,4 hydroquinone) was due to the formation of adducts between GSH and BQ.

In this work, we describe a new potentiometric sensor for the detection of glutathione (GSH) and cysteine (CSH). This system is based on the reaction between the BQ moiety borne by a polymeric film and the thiol.

2. Results and discussion

The indicating modified electrode was obtained by anodic electropolymerisation (Scheme 1) of the thiophene group of 3-((2,5-dimethoxyphenyl)ethynyl)thiophene **A** where the 1,4-dimethoxyphenyl moiety is the precursor of quinone **B**.



Scheme 1. Electropolymerisation of 1-(3-(1,4-dimethoxyphenyl)-2-(3-thienyl))ethyne and deprotection of methoxy groups.

2.1. Synthesis of 3-((2,5-dimethoxyphenyl)ethynyl)thiophene monomer A

After different attempts, the more efficient route to obtain **A** is described below (Scheme 2).

By bromination of *para*-dimethoxybenzene with *N*-bromosuccinimide in acetonitrile, 2-bromo-1,4-dimethoxybenzene (**I**) was obtained with a yield of 90%. Two routes were tested (step 1, Scheme 2). In the first one, we used a water/acetonitrile mixture (1:10) as solvent, room temperature, without copper(I) in presence of a quaternary ammonium salt to improve the efficiency.[16] However, the yield was no better than 34%. In the second route, with CuI in triethylamine at 80 °C after refluxing for one night, compound **II**, 2-methyl-4-(2,5-dimethoxyphenyl)-3-butyn-2-ol, was isolated with a yield of 40%. By a retro-Favorskii reaction, the protective group R_1 was removed by heating compound **II** in a solution of sodium hydroxide in toluene.[17] The expected product **A** was prepared by a second coupling between **III** and 3-bromothiophene. To try to improve the yield in this last step, we also used the conditions described by Thorand and Krause,[18] but the efficiency was no better than 38%. The quinonic product corresponding to the chemical oxidation of **A** by cerium(IV) ammonium nitrate (CAN) appeared to be very unstable. Consequently, we attempted to obtain the quinone group after electropolymerisation of monomer **A**.

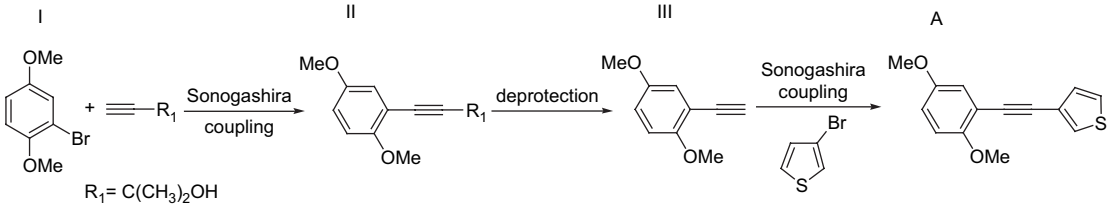
2.2. Electrochemical preparation of a polythiophene film bearing a quinone moiety

The electrodeposition of the polythiophene film was performed by successive scans in the positive potential part (0.5–1.5 V). The coverage of deposited polymer can be adjusted by varying the number of cyclical potential scans. Typically, after 20 scans corresponding to the exchange of 34 mC, the thickness of the dark green polymeric film was estimated at 0.2 μm.[19]

By chronoamperometry at 1.6 V/SCE for 5 min, the methoxy groups were oxidised to the corresponding quinonic forms. The presence of the quinone moiety was confirmed by the cyclic voltammetry experiments performed in the monomer-free buffer electrolyte (pH 7), the resulting yellow-brown film being washed with the solvent and water before this experiment. The voltammogram of the corresponding coated electrode (Fig. 1A) indeed clearly shows a reversible peak at 0.0 V/SCE (ΔE_p=40 mV) corresponding to the BQ/BHQ couple immobilised at the modified electrode. Moreover, the formation of the quinone after electrochemical oxidation of the methoxy group was confirmed by FTIR in diffuse reflectance mode (DRIFT). The DRIFT analysis of the film after oxidation shows a strong band at 1659 cm^{-1} corresponding to the presence of the quinone group. The efficiency of the electrochemical oxidation is confirmed by a large decrease of the 1221 cm^{-1} band attributed to the methoxy function observed on the film before the oxidation step.

2.3. Cyclic voltammetric response in the presence of glutathione

The voltammograms of the modified electrode bearing the quinone moiety are modified in presence of GSH in pH 7 buffer. Indeed, with 1 mM of GSH, the intensity of the current peak corresponding to the BQ/BHQ couple is time-dependent (Fig. 1B, C and D) and decreases over time. The signal corresponding to the redox couple completely disappeared after about 1 h. There are two possible different pathways through which quinone moieties could interact with GSH. In the first one, in agreement with the apparent redox potential ($E^{0'}_{GSSG/GSH}$=−0.47 V/SCE at pH 7), the quinone form could oxidise GSH to its corresponding disulfide form (GSSG) and be reduced in hydroquinone.[20] In the second pathway, the modification of the electrochemical behaviour could be due to



Scheme 2. Synthesis of 1-(3-(1,4-dimethoxyphenyl)-2-(3-thienyl))ethyne.