Electrochimica Acta 112 (2013) 500–504

Contents lists available at ScienceDirect

Electrochimica Acta

journal homepage: www.elsevier.com/locate/electacta

Electrochemistry and UV–vis spectroscopy of synthetic thiocholine: Revisiting the electro-oxidation mechanism

Livia F. Sgobbi, Claudia A. Razzino, Isac G. Rosset, Antonio C.B. Burtoloso, Sergio A.S. Machado*

Instituto de Química de São Carlos, Universidade de São Paulo, C.P. 780, 13560-970, São Carlos, SP, Brazil

ARTICLE INFO

Article history:
Received 13 May 2013
Received in revised form 21 August 2013
Accepted 22 August 2013
Available online 11 September 2013

Keywords:
Thiocholine
Spectroelectrochemistry in situ
Dimerisation
Biosensor
Acetylcholinesterase

ABSTRACT

Amperometric biosensors for pesticide detection based on acetylcholinesterase employ the enzymatically generated thiocholine as the electrochemical probe. The mechanism is generally assumed to be: 2 thiocholine $\rightarrow$ $2\,H^+ + 2e^- +$ dithio-bis-choline (dimer). However, this mechanism has never been confirmed experimentally, the detection of the dimer species. In the current work, anodic oxidation of synthetic thiocholine at a pyrolytic carbon electrode, modified with multiwalled carbon nanotubes, was studied for the first time using electrochemical, spectrophotometric and spectroelectrochemical techniques. Cyclic voltammetry was used to determine the linear relationship between I_p and thiocholine concentration. A detection limit of 5.96 ($\pm$0.22) μM (S/N = 3) and a detection sensitivity of 3.16×10^{-2} ($\pm 2.65 \times 10^{-3}$) μA μM^{-1} were observed with a linear range from 5.0 to 100.0 μM and correlation coefficient of 0.9954. Using controlled potential electrolysis coupled to a spectrophotometric probe in situ, the number of electrons involved in the electrochemical oxidation was confirmed to be 2. By the reaction with dithiothreitol through Cleland's method, in which the disulfide compound generated presents an absorption peak at 283 nm, the dimeric product of the assumed oxidation reaction was also confirmed.

1. Introduction

The enzyme acetylcholinesterase (AChE) is a serine protease that catalyses the hydrolysis of the neurotransmitter acetylcholine. It has been extensively studied as a biological component of amperometric biosensors for detection of carbamate or organophosphate pesticides, given that its enzymatic activity is strongly inhibited by such pesticide molecules [1–4]. As already extensively discussed in the literature, acetylcholine is enzymatically hydrolysed to choline and acetate [5].

Acetylcholine cannot be used as a substrate in amperometric electrochemical biosensing since the product of its hydrolysis, choline, is not an electroactive species, meaning that it cannot be detected by electrochemical techniques involving current measurements, being used only in potentiometric applications. However, acetylthiocholine (ATCh), an artificial analogue of acetylcholine, can be used as a substrate [6]. Specifically, ATCh is hydrolysed to thiocholine (TCh), an electroactive species, by AChE in an enzymatic reaction nearly identical to that of acetylcholine. Characterisation of the electrochemical behaviour of TCh is essential for development of sensors for pesticides. All existing literature on pesticide detection by AChE amperometric biosensors propose that TCh is anodically oxidised, resulting in the respective disulfide form. Regardless of the nature of the surface [1,7–12], as shown below (Scheme 1).

TCh is not commercially available, thus all reported experimental data refer to the enzymatically generated compound. To the best of our knowledge, the electrodic mechanism (Eq. (1)) has yet to be elucidated systematically. This paper reports the synthesis and characterisation of the electrochemical behaviour of synthetic TCh through the detection of products formed on the electrodic surface during the anodic oxidation process. A pyrolytic carbon electrode (PCE) modified with multiwall carbon nanotubes (MWCNTs) was employed for voltammetric experiments and electrolysis at constant potential, while the product of the reaction was analysed using spectrophotometric and electrochemical methods.

2. Material and methods

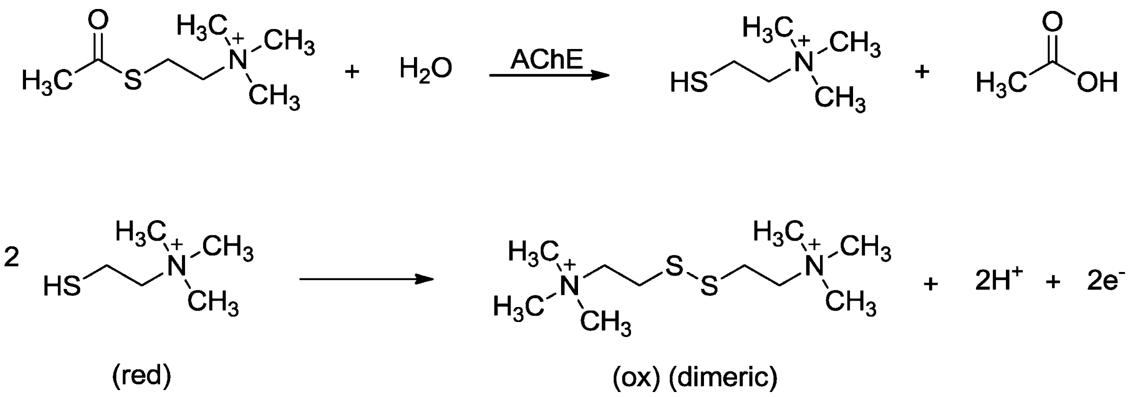
2.1. Reagents

(2-Chloroethyl)-trimethylammonium chloride, DL-dithiothreitol (DTT) and cystamine dihydrochloride were purchased from Sigma–Aldrich (St. Louis, MO, USA). Sodium thiosulfate pentahydrate was obtained from Merck (Darmstadt, Germany). MWCNTs 6–9 nm diameter and 5 μm were obtained from

---

* Corresponding author.
E-mail address: sasmach@iqsc.usp.br (S.A.S. Machado).

0013-4686/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.electacta.2013.08.143



Scheme 1. Pathway for the enzymatic generation and electrochemical oxidation of thiocholine.

Sigma–Aldrich (St. Louis, MO, USA), Product Number 724769, purity > 95%. All the reagents used were of analytical grade. High-purity deionised water (resistance, 18 MΩ cm) was obtained from a Nanopure Ultrapurification System (Barnstead Inc., Waltham, MA, USA). Phosphate-buffer solution (pH 7.0) was home prepared with Na_2HPO_4 and NaH_2PO_4 from Sigma–Aldrich (St. Louis, MO, USA) and then, employed as supporting electrolyte. All experiments were performed at room temperature, which was approximately 25 °C.

2.2. Apparatus

Proton nuclear magnetic resonance (^{1}H NMR) was performed using a 200 MHz equipment. For ^{1}H NMR spectra, chemical shifts (δ) were referenced from TMS (0.00 ppm) or CD_3OD proton residuals (3.31 and 4.87 ppm). Coupling constants (J) are reported in Hz. Carbon nuclear magnetic resonance (^{13}C NMR) was performed using a NMR spectrometer at 50 MHz. For ^{13}C NMR spectra, chemical shifts (δ) were reported relative to CD_3OD (49.0 ppm). Infrared spectra were obtained using a Bomem FT-IR MB-102 spectrometer (4.0 cm^{-1} resolution, 32 scans). UV–vis spectra were taken on a JASCO V-630 spectrophotometer with quartz cuvettes of 1 cm optical path. FT-Raman measurements were performed in a Bruker Optics spectrophotometer, model RFS10. Electrochemical measurements were carried out with an Autolab model PGSTAT40 (Eco Chemie) potentiostat/galvanostat coupled to a computer and controlled by a GPES 4.9 software. The electrochemical cell was assembled with a conventional three-electrode system: a pyrolytic carbon electrode (3 mm diameter) modified with MWCNTs as working electrode, a platinum foil (1 cm^2 geometric area) as the auxiliary electrode and the Ag/AgCl, 3 M KCl system as a reference. In situ UV–vis spectroelectrochemical measurements were obtained using an Avantes AvaSpec 2048 spectrometer, which was directly integrated with NOVA software to record spectra in the range of 200–1100 nm with a resolution of 2.4 nm. The potential of the working electrode in the cell was controlled by an Autolab Type PGSTAT40 (Eco Chemie) potentiostat/galvanostat with a computer running NOVA software (Eco Chemie), which delivered the trigger signals for the spectrometer.

2.3. Synthesis of thiocholine chloride

The synthesis of thiocholine chloride was carried out employing a modified protocol previously described by Bernardes et al. [13]. ((2-Chloroethyl)-trimethylammonium chloride (3.00 g, 18.98 mmol) and sodium thiosulfate pentahydrate (4.71 g, 18.98 mmol) were mixed in a 100 mL round bottom flask containing a polytetrafluoroethylene-coated stir bar and dissolved in water (30 mL, ultrapure water). The solution was heated to reflux (oil bath temp set to 120 °C) and stirred for 13 h. After this period, the reaction solution was cooled to room temperature, and the solvent was removed under reduced pressure to give the crude Bunte salt as a white residue. This white residue was dissolved in hydrochloric acid (30 mL of 6 M aqueous solution) and stirred at 85 °C for 2 h. Next, the solution was cooled, and the solvent was removed under reduced pressure. Dichloromethane (150 mL) was then added, and the suspension was heated with stirring under reflux for 30 min. Next, a 3:1 mixture of ethanol and chloroform was added slowly to this solution until it was no longer cloudy. Sodium chloride and sulfate salts were removed by filtration, and the concentrated filtrate to give a white solid. The solid was then recrystallised in a mixture of methanol and chloroform by first dissolving the solid in 20 mL of methanol, cooling it in an ice bath and adding chloroform until the product precipitated. The white crystal was isolated by filtration, washed with chloroform and dried under vacuum to furnish 1.77 g (60%) of thiocholine chloride. ν_{max} (KBr disc) 3428, 3157, 3010, 2510, 1638, 1486, 1402, 1221, 1051, 899, 595, 449; ^{1}H NMR (200 MHz, CD_3OD) δ 2.94 (m, 2H), 3.18 (s, 9H), 3.55 (m, 2H); ^{13}C NMR (50 MHz, CD_3OD) δ 17.5, 53.3, 69.3; ^{1}H NMR (200 MHz, D_2O) δ 2.93 (m, 2H), 3.13 (s, 9H), 3.52 (m, 2H).

2.4. Preparation of modified electrode

Prior to modification, the PCE was polished with a 1.0, 0.3 and 0.05 μm alumina slurry until a mirrored finish was obtained. Subsequently, the electrode was sonicated for 5 min in deionised water, ethanol then water again in order to remove any alumina residue. After sonication, the electrode was rinsed with deionised water and inserted in the electrochemical cell containing 0.5 M H_2SO_4. It was initially polarised at −2 V for 30 s then voltammetrically cycled (from 0 to 1 V) to obtain a stable cyclic voltammogram profile. Finally, it was cycled in phosphate buffer (pH 8, 0.1 M) for surface activation (−0.3 to 0.8 V, 50 mV/s) [14].

The MWCNTs were used without further functionalisation process and present only the hydroxyl and carbonyl original groups in its surface. To modify the electrode, a 15 μL aliquot of a solution containing 1 mg MWCNTs dissolved in 1 mL DMF was dropped and allowed to dry at room temperature [12]. After drying, the surface was rinsed thoroughly with deionised water, and the electrode was stored in phosphate buffer (pH 7). After preparation, a coated electrode was highly stable and could be used for several days.

2.5. Measurements

Cyclic voltammograms were obtained by scanning the electrode potential from −50 to 500 mV, employing scan rates between 10 and 100 mV/s. Sample solutions of TCh were stirred using a