Available online at www.sciencedirect.com
ScienceDirect
Biosensors and Bioelectronics 22 (2007) 2508–2515
BIOSENSORS & BIOELECTRONICS
www.elsevier.com/locate/bios

Bioelectrocatalytic detection of theophylline at theophylline oxidase electrodes

Elena E. Ferapontova [a,*], Stepan Shipovskov [b], Lo Gorton [c]

[a] *School of Chemistry, The University of Edinburgh, West Mains Road, Edinburgh EH9 3JJ, UK*
[b] *Manchester Interdisciplinary Biocentre, The University of Manchester, 131 Princess Street, Manchester M1 7DN, UK*
[c] *Department of Analytical Chemistry, Lund University, P.O. Box 124, SE-221 00 Lund, Sweden*

Received 20 June 2006; received in revised form 17 September 2006; accepted 29 September 2006
Available online 1 November 2006

Abstract

Bioelectrocatalytic oxidation of theophylline was studied at gold and graphite electrodes modified with microbial theophylline oxidase (ThOx), a multi-cofactor redox enzyme capable of selective oxidation of theophylline. Gold electrodes were additionally modified with self-assembled monolayers (SAMs) of (–OH)- and (–NH_2)-terminated alkanethiols of different chain lengths, to achieve compatibility between ThOx and the electrode surface. On graphite, ThOx was either physically co-adsorbed with a surfactant didodecyldimethylammonium bromide (DDAB), or entrapped within an Os-redox-polymer film. At all electrodes, ThOx was bioelectrocatalytically active; direct electrochemistry of ThOx in the absence of theophylline was followed only at the SAM-modified gold electrodes. Direct electrochemistry of ThOx correlated with redox transformations of the heme domain of ThOx, with a $E^{o\prime}$ of -110 ± 2 mV versus Ag|AgCl, at pH 7. Bioelectrocatalytic oxidation of theophylline was optimal at mixed (–OH)/(–NH_2)-terminated SAMs; co-adsorption of ThOx with DDAB improved the bioelectrocatalytic performance of the ThOx-electrode. In both cases, the response to theophylline was within the mM range. Alternatively, a reagentless ThOx-electrode based on ThOx cross-linked within the Os-redox-polymer matrix demonstrated a linear response to theophylline within the physiologically important 0.02–0.6 mM (3.6–72 mg l^{-1}) concentration range with a sensitivity of 52.1 ± 7.8 mA cm^{-2} M^{-1}.
© 2006 Elsevier B.V. All rights reserved.

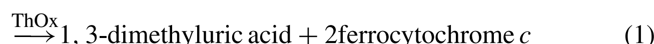
Keywords: Theophylline oxidase; Bioelectrocatalysis; Alkanethiols; Gold; Graphite; Osmium-complex redox polymer

1. Introduction

Theophylline is a methyl-derivative of xanthine most commonly used in the treatment of the symptoms of obstructive pulmonary disease and bronchial asthma (Kawai and Kato, 2000; Rowe et al., 1988). The therapeutic use of theophylline is complicated by its high toxicity at concentration levels in plasma exceeding 20 mg L^{-1}; the therapeutically useful blood levels in adults are within a narrow 10–20 mg L^{-1} concentration range, making the determination of theophylline a key factor in drug administering (Jackson et al., 1973; Kawai and Kato, 2000). The isolation of microbial theophylline oxidase (ThOx), a redox enzyme involved in metabolic transformation of theophylline, promoted the development of highly selective enzymatic assays for theophylline as alternative to the existing ones, most of which require either cumbersome extractions or depend on theophylline-recognising antibodies (deCastro et al., 1989; Gupta et al., 1988).

The catalytic mechanism of ThOx involves oxidation of theophylline by ThOx to form 1,3-dimethyluric acid; the reduced enzyme can then in turn be re-oxidised by cytochrome *c* (cyt *c*) or by other electron acceptors thus completing the biocatalytic cycle:

Theophylline + 2ferricytochrome *c*



$$\xrightarrow{\text{ThOx}} 1,3\text{-dimethyluric acid} + 2\text{ferrocytochrome } c \quad (1)$$

The majority of the developed ThOx-based commercial assays are based on spectrophotometrical detection of theophylline oxidation catalysed by ThOx in the presence of cyt *c* (Fe^{3+}) or other electron acceptors (deCastro et al., 1989; Gupta et al., 1988; Vaughan and Gottehrer, 1992). Electrochemical detection of theophylline oxidation catalysed by ThOx in the

* Corresponding author. Tel.: +44 131 650 4752.
E-mail addresses: elena.ferapontova@ed.ac.uk, ferapontova@nsk.fio.ru (E.E. Ferapontova).

0956-5663/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.bios.2006.09.034

presence of both its redox partner cyt *c* and ferricyanide (Wang et al., 1991) and such mediators as ferrocene monocarboxylic acid (FMCA) or conducting organic salt NMP·TCNQ (McNeil et al., 1992) was also reported.

Recently, the development of mediatorless ThOx-based electrodes for quick and continuous monitoring of plasma levels of theophylline has attracted attention (Christenson et al., 2004; Ferapontova and Gorton, 2005). In mediatorless enzyme electrodes, the electronic coupling between the enzyme and the electrode is achieved by direct electron transfer (ET), i.e. in the absence of any mediators (Gorton et al., 1999). That is the main advantage providing their superior selectivity due to an operational potential window corresponding to the inherent redox potentials of the enzyme, lesser contribution from interfering reactions and the lack of yet another reagent in the reaction sequence (Gorton et al., 1999). In mediatorless enzyme electrodes, the compatibility between the electrode and the enzyme is important and may be modulated by modifying either the protein or electrode surface (Gilardi et al., 2001). Alternatively, the enzyme may be entrapped within the redox-polymer matrix serving as an electron-shuttling medium and thus mediating ET between the electrode and the redox-active sites of the enzyme (Karan, 2005). These "wired" enzyme electrodes may be also referred to as the "reagentless" ones due to the non-leaching enzyme–electrode system employing a non-diffusional type of mediators. Relevant choice of the redox-polymer medium, e.g. Os-redox polymer complexes of appropriate redox potentials and structural flexibility (Degani and Heller, 1989; Heller, 1992; Ohara et al., 1993), may provide both the optimal operational potential window and the increased sensitivity of the analyte detection resulting from the increased loading of the electrode with a catalytically active enzyme and advanced ET properties of the media. In this work, both concepts of the enzyme electrodes were exploited to develop reagentless ThOx-electrodes for detection of theophylline.

Up to date, the available data on the structure and electron transfer (ET) reactions of ThOx have been restricted predominantly to its spectral characterisation (deCastro et al., 1989). Specifically, the adsorption features of heme dominate in the UV/vis spectrum of ThOx, and characteristic spectral changes from the oxidised to the reduced state of heme are observed upon reaction with theophylline (deCastro et al., 1989; Ferapontova and Gorton, 2005). Reduced ThOx is able to react with cyt *c* (Fe^{3+}), which re-oxidises the heme of ThOx (Eq. (1)) (deCastro et al., 1989; Gupta et al., 1988; Vaughan and Gottehrer, 1992). Presumably, the communication between ThOx and cyt *c* occurs directly through the heme domain of ThOx, in the same manner as known for other heme-containing multiple-redox-centre enzymes (Hille, 1996; Igarashi et al., 2002; Kisker et al., 1997; Rogers et al., 1994). Direct spectroelectrochemical titration of microbial ThOx in an aldrithiol-modified gold capillary electrode enabled determination of at least two redox active centres present in ThOx (Christenson et al., 2004). One of them is the heme which serves as a "built-in mediator" wiring the electrons between the electron accepting site and the electrode, and another one is the electron accepting site, where theophylline oxidation takes place. No direct electrochemical response from the individual redox centres of ThOx was detected at bare electrodes: the ET reactions of ThOx appeared to be kinetically sluggish in the case when the electrode surface did not resemble the natural redox partner.

In this work, we electrochemically characterised the redox properties of microbial ThOx and bioelectrocatalysis of theophylline oxidation in the heterogeneous gold|ThOx and graphite|ThOx systems. To provide immobilisation of ThOx favouring ET between the electrode and ThOx, we chemically designed the electrode surface. For modification of gold electrodes we used self-assembled monolayers (SAM) of (–OH)- and (–NH_2)-terminated alkanethiols of different chain lengths (Finklea, 1996). Previously, natural electron acceptors/donors of a number of complex redox enzymes, e.g. cellobiose dehydrogenase (CDH) (Larsson et al., 2001; Lindgren et al., 2001, 2000) and sulphite oxidase (SOx) (Elliott et al., 2002; Ferapontova et al., 2004, 2003), were successfully replaced by properly chosen SAM-modified electrodes. On graphite electrodes, ThOx was either co-adsorbed with a surfactant didodecyldimethylammonium bromide (DDAB), stabilising the bioelectrocatalytic function of the enzyme, or it was incorporated in a cross-linked, three-dimensional Os-redox-polymer matrix immobilised at the graphite surface. In the latter case, the electronic communication between the electrode and ThOx was achieved through the "electron-hopping" between the Os redox-centres attached to the polymer matrix, thus wiring the active sites of ThOx to the electrode (Degani and Heller, 1989; Heller, 1992; Ohara et al., 1993). Bioelectrocatalytic detection of theophylline was performed with all studied ThOx-electrode systems and compared in relation to the sensitivity and concentration range of theophylline determination.

2. Experimental

2.1. Materials

The kit containing a solution of microbial ThOx (14 U/ml) was obtained from STANBIO Laboratory (Boerne, TX, USA). Cytochrome *c* from horse heart, theophylline, 99%, and didodecyldimethylammonium bromide were from Sigma (St. Louis, MO, USA) and used as received. The buffer salts were from Merck (Darmstadt, Germany). 2-Mercaptoethanol (98%, MC_2-OH), 6-mercapto-1-hexanol (97%, MC_6-OH) and cysteamine were from Sigma–Aldrich (UK); 4-mercapto-1-butanol (97%, MC_4-OH) was from Fluka. 6-Amino-1-hexanethiol hydrochloride was from Dojindo Laboratories (Japan). Poly(ethylene glycol) (400) diglycidyl ether (PEGDGE) was from Polysciences (Warrington, PA, USA). Chloride of poly(4-vinylpyridine)-$[Os(N,N'\text{-dialkylated-}2,2'\text{-biimidazole})_3]^{2+/3+}$ complex (Mao et al., 2003) (Os-redox-polymer) was a generous gift of TheraSense, Almeida, CA, USA. 18.2 MΩ Millipore water (Millipore, Bedford, MA, USA) was used throughout the work.

2.2. Electrode modification with alkanethiols

Thiol films were prepared by 8 h adsorption from 5 mM solutions of alkanethiols in absolute ethanol and by 2 h of adsorption

Download English Version:

https://daneshyari.com/en/article/869618

Download Persian Version:

https://daneshyari.com/article/869618

Daneshyari.com