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Low Potential Catalytic Voltammetry of Human Sulfite Oxidase

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ABSTRACT

Mediated electrocatalytic voltammetry of human sulfite oxidase (HSO) is demonstrated with synthetic one electron transfer iron complexes bis(1,4,7-triazacyclononane)iron(III) ($[Fe(tacn)_2]^{3+}$) and 1,2-bis (1,4,7-triaza-1-cyclononyl)ethane iron(III) ($[Fe(dtne)]^{3+}$) at a glassy carbon working electrode. The two synthetic electron acceptors for HSO, differing in redox potential by 270 mV, deliver different driving forces for electrocatalysis. Digital simulation of the catalytic voltammetry was achieved with single set of enzyme-dependent kinetic parameters that reproduced the experimental data across a range of sweep rates, and sulfite and mediator concentrations. Amperometry carried out in a stirred solution with the lower potential mediator $[Fe(tacn)_2]^{3+}$ was optimised and exhibited a linear increase in steady state current in the sulfite concentration range 5.0×10^{-6} to 8.0×10^{-4} M with a detection limit of 0.2 pM (S/N = 3). The HSO coupled electrode was successfully used for the determination of sulfite concentration in white wine and beer samples and the results validated with a standard spectrophotometric method.

1. Introduction

The molybdenum-dependent sulfite oxidizing enzymes comprise sulfite oxidase (SO) and sulfite dehydrogenase (SDH) [1,2]. SO is found in animals and plants whereas SDH is only found in bacteria [3]. Only the plant SO is a true oxidase while all other sulfite oxidizing enzymes donate electrons to cytochrome *c*. Vertebrate SOs can use either cytochrome *c* or dioxygen as an electron acceptor. Only one crystal structure is available for a vertebrate SO (from chicken liver) [4] revealing a 103 kDa homodimer in which each subunit contains a negatively charged small heme *b* domain at the N-terminus and positively charged larger molybdopterin domain at the C-terminus. The heme accepts electrons from the Mo ion following sulfite oxidation. A flexible connects the Mo and heme domains which are more than 30 Å apart in the crystal structure conformation; a distance too great for electron transfer. Spectroscopic and kinetic studies have demonstrated that the heme *b* domain swings around to be in proximity to the molybdenum active site where electron transfer (Mo to heme) can take place after sulfite oxidation [5–7].

$$SO_3^{2-} + H_2O + 2(cytc)_{ox} \xrightarrow{SO} SO_4^{2-} + 2(cytc)_{red} + 2H^+ \quad (1)$$

Human sulfite oxidase (HSO) shares a 68% sequence identity with chicken SO [4]. Among the eukaryotic SOs, HSO has been studied extensively because of its role in the potentially fatal disease SO deficiency [8,9]. The physiological role of SO is to remove toxic sulfite (a product of organo-sulfur compound metabolism) and covert it to chemically inert sulfate. Despite its name the physiological electron acceptor of SO is in fact cytochrome *c* (Eq. (1)). In the catalytic reaction, SO is active in its fully oxidized state (Mo^{VI}) in which molybdenum is coordinated by a cysteine thiolate, the dithiolene group of molybdopterin, and two terminal oxygen atoms as shown in Scheme 1 [7,10–12]. Upon reaction with sulfite, one oxido ligand is transferred to sulfite to give sulfate and the Mo ion is reduced its tetravalent state. Subsequently, hydroxide displaces sulfate, and the removal of this hydroxido ligand proton occurs spontaneously when the Mo ion is reoxidised to its hexavalent state by two cytochrome *c* molecules.

There have been a number of electrochemical investigations of SO and SDH enzymes from different organisms. In these cases the electrode is the ultimate electron acceptor resulting in an anodic catalytic current. Electrons may be transferred directly from the enzyme [2,13–17] or via a mediator which may be synthetic [18–20] or natural (cytochrome *c*) [18,21–25].

The dynamics HSO are potentially problematic for efficient electrocatalysis. While the Mo and heme cofactors are separated, the enzyme is unable to be reactivated through reoxidation. It is of interest whether confinement of HSO enzyme to a thin layer at the electrode surface suppresses this motion. Spectroelectrochemistry of HSO showed the $Fe^{III/II}$ redox potential to be +62 mV vs NHE (pH

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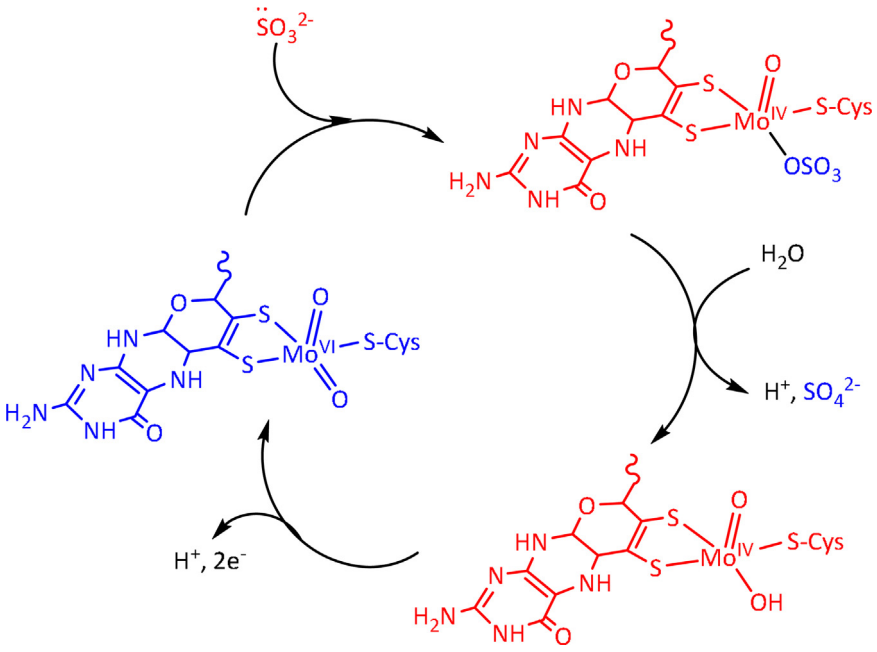

Scheme 1. Simplified catalytic cycle of SO reduced forms of enzyme and substrate in red and oridised forms of enzyme and product in blue.

7.5) [6]. At applied electrochemical potentials above this value, the enzyme will be continually reoxidsed and reactivated for sulfite oxidation. To achieve this we employed two artificial electron acceptors; the hexa-amine complexes $[Fe(tacn)_2]^{3+}$ and $[Fe(dtne)]^{3+}$ (Fig. 1) with redox potentials of +144 and +415 mV vs NHE, respectively which present significantly different overpotentials but are structurally almost the same. The higher $Fe^{III/II}$ redox potential of $[Fe(dtne)]^{3+}$ is due to the presence of two tertiary amines compared to the all-secondary amine $[Fe(tacn)_2]^{3+}$. It is notable that nonspecific oxidation of sulfite at an electrode (without any enzyme present) is inevitable above ca. +550 mV vs NHE [26] and this places an upper bound on the redox potential of any mediator in a sulfite oxidizing electrochemical system.

An additional feature of this study is electrochemical simulation of the experimental voltammetry. Given that the catalytic cycle involves several steps, some chemical reactions between HSO and sulfite/sulfate and some being outer sphere electron transfer reactions between HSO and the mediators, a set of rate constants can be defined (Scheme 2). These rate constants must be able to reproduce the catalytic voltammetry under a variety of conditions including sweep rate, mediator concentration and sulfite concentration. Finally, amperometry is employed to estimate the lowest detection limit and linear current response for the determination of sulfite in aqueous solution and in the quantification of sulfite in beer and wine samples where it is a commonly found as an additive to combat spoilage from oxidation and microbial activity [27,28].

2. Experimental

2.1. Materials

Human sulfite oxidase (HSO) was purified in *E. coli* TP1000 as previously described [29]. The iron complexes bis(1,4,7-triazacyclononane) iron(III) bromide ($[Fe(tacn)_2]Br_3$) [30] and 1,2-bis (1,4,7-triaza-1-cyclononyl) ethane iron(III) bromide ($[Fe(dtne)]Br_3.3H_2O$) [31] were synthesized according to the previous procedures. Sodium sulfite and 5,5′-dithio-bis(2-nitrobenzoic acid) (Ellman's reagent) were purchased from Aldrich and were

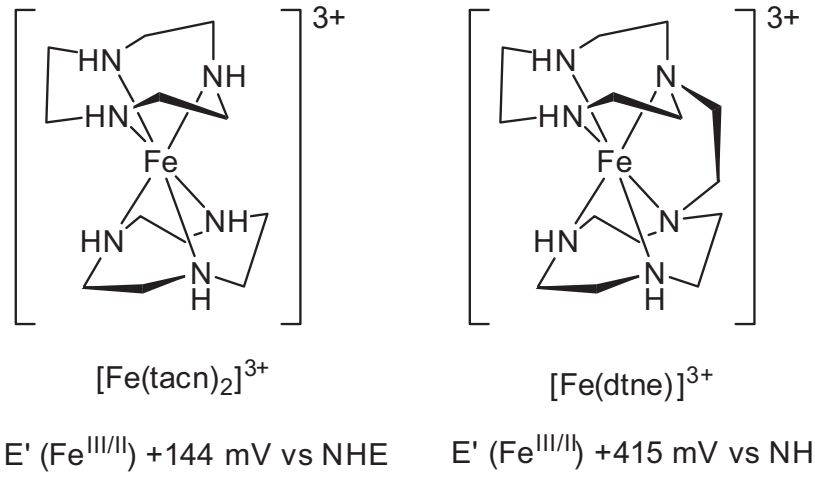

Fig. 1. Molecular structures and redox potentials of the mediators used in this study.