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Direct electrochemically driven catalysis of bovine milk xanthine oxidase

Paul V. Bernhardt [a,*], Michael J. Honeychurch [a], Alastair G. McEwan [b]

[a] Centre for Metals in Biology, Department of Chemistry, University of Queensland, Brisbane 4072, Australia
[b] Centre for Metals in Biology, Department of Microbiology and Parasitology, University of Queensland, Brisbane 4072, Australia

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Abstract

The complex molybdoenzyme xanthine oxidase (XO) catalyses the oxidation of xanthine to uric acid. Here we report the first direct (unmediated) catalytic electrochemistry of the enzyme in the presence of xanthine. The only non-turnover response (without substrate present) is a sharp two-electron wave from the FAD cofactor at −242 mV vs. NHE (pH 8.0). Upon addition of xanthine to the electrochemical cell a pronounced electrocatalytic anodic current appears at ca. +300 mV vs. NHE, but the FAD peak remains. This is unusual as the onset of catalysis should occur at the potential of the FAD cofactor (the site at which oxygen or NAD^+ binds to the enzyme in solution). The observed electrochemical catalysis is prevented by the addition of known XO inhibitors allopurinol or cyanide.

Keywords: Xanthine oxidase; Enzyme; Catalysis; Voltammetry

1. Introduction

The molybdoenzyme xanthine oxidase (XO) has been studied intensively over the last 50 years [1]. It is a complex enzyme that catalyses the hydroxylation of a range of heterocycles including purines, pyrimidines and pterins. XO comprises four distinct redox active centers: the Mo active site, two spectroscopically distinct [2Fe–2S] clusters and a flavin adenine dinucleotide (FAD) cofactor. The Mo ion is the site at which xanthine binds and is subsequently oxidized to uric acid in a two electron, O-atom transfer reaction (effectively a hydroxylation). A simplified catalytic cycle is represented in Scheme 1, where the XO redox cofactors are enclosed within the broken line.

The electrons from this oxidation reaction are relayed via the two 2Fe–2S clusters to an FAD cofactor. It is now established [2–4] that the well studied XO from bovine milk is actually a dehydrogenase in its native form (with NAD^+ as the ultimate electron acceptor) and that xanthine dehydrogenase (XDH) must undergo a structural change [5] to XO where dioxygen may then act as the electron acceptor. XO is linked with medical conditions such as gout (associated with high circulating levels of uric acid, the product of xanthine oxidation) and inhibitors of XO such as allopurinol have been used successfully to treat this condition [6]. Also serum levels of XO rise in pathological states such as hepatitis, inflammation, ischemia-reperfusion, carcinogenesis and aging [6].

R. capsulatus XDH is highly homologous to bovine milk XO, but does not undergo an XDH-to-XO conversion [7–9]. Furthermore, it is ca. ten times more active than the extensively studied bovine milk XO. Recently we reported the direct electrochemical study of *R. capsulatus* XDH [10]. where the redox potentials of all cofactors were determined using a combination of

* Corresponding author. Fax: +61 7 3365 4299.
E-mail address: p.bernhardt@uq.edu.au (P.V. Bernhardt).

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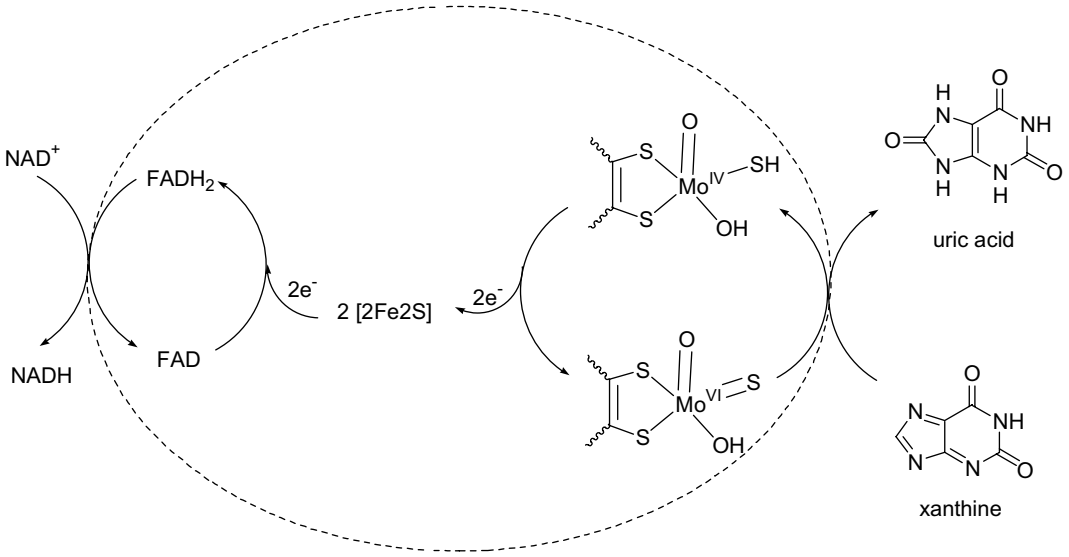

Scheme 1.

EPR potentiometry and voltammetry (cyclic and square wave). In addition, a pronounced anodic catalytic current was seen in the presence of xanthine.

Despite the fact that bovine milk XO has been one of the most intensively studied Mo enzymes, previous attempts at direct electrochemically driven catalysis of this enzyme have been ineffective. The electrode is required to play the role of the oxidant and thus, in an electrochemically driven system, catalysis should be accompanied by an increased anodic current appearing in the vicinity of the FAD/$FADH_2$ couple (the natural site of electron egress). Rodrigues et al. [11] reported direct electrochemistry of bovine milk XO at glassy carbon and Hg working electrodes, but these studies were hampered by protein denaturation and interactions between cysteine residues and the Hg surface. No catalysis was reported. Recently, it has been claimed that bio-electrochemical catalytic oxidation of hypoxanthine (a reduced form of xanthine) can be achieved by co-immobilizing XO and DNA on an electrode. However, a mass transport limited *cathodic* current was reported in the region where one would expect to see dioxygen reduction [12]. Additionally these experiments were carried out at pH 5.0 which is at odds with the known pH optimum of bovine milk XO activity of pH 7–8. There is a recent report of direct electrochemistry of *Desulfovibrio gigas* aldehyde oxidoreductase, a closely related member of the xanthine oxidase family [13], but again only a *cathodic* (reducing) catalytic current was seen in the presence of aldehyde substrates.

In an effort to resolve some of these questions, we have turned our attention to the direct electrochemistry of bovine milk XO. For the first time we shall demonstrate genuine anodic catalytic voltammetry of this enzyme. We have been able to switch off catalysis by the addition of known inhibitors of XO to the electrochemical cell.

2. Materials and methods

Cyclic voltammetry was performed with a BAS 100B/W electrochemical workstation with a modified edge plane pyrolytic graphite (EPG) working electrode, an Ag/AgCl reference electrode (+196 mV vs. NHE), and a Pt counter electrode. Measurements were performed in an argon-purged 2-amino-2-methylpropan-1-ol/bis-tris propane buffer (each 20 mM) at 4 °C. The electrochemical cell was blanketed with argon during the experiment and purged continually with argon at all other times. The modified EPG electrode was prepared by initially cleaving several 1 μm layers from the face of the electrode with a microtome followed by sonication in MilliQ water. No abrasives were used. A 2 μL aliquot of 1 mM didodecyldimethylammonium bromide (DDAB) (Aldrich) solution was then pipetted on the EPG surface and allowed to dry at room temperature. Xanthine oxidase was isolated from cow's buttermilk according to the procedure of Massey et al. [14]. The enzyme was assayed as 30% active as described [14]. A 2 μL aliquot of a 500 μM solution of enzyme was pipetted onto the electrode and allowed to dry in a refrigerator at 4 °C. Another 2 μL aliquot of DDAB solution was added onto the electrode surface and the electrode allowed to dry in a refrigerator at 4 °C.

3. Results and discussion

To aid both reproducibility and protein film stability bovine milk XO was confined within a DDAB surfactant film cast on the surface of an edge-oriented pyrolytic graphite working electrode [15]. We have successfully used this technique with other Mo enzymes including the *R. capsulatus* DMSO reductase [16] and *S. novella* sulfite dehydrogenase [17].