Spectroelectrochemistry of Redox Enzymes

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2006

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SCIENCE DIRECT®
Bioelectrochemistry 63 (2004) 49–53
Bioelectrochemistry
www.elsevier.com/locate/bioelechem

Spectroelectrochemical study of heme- and molybdopterin cofactor-containing chicken liver sulphite oxidase

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Received 23 June 2003; received in revised form 10 September 2003; accepted 16 September 2003

Abstract

Electron transfer (ET) in sulphite oxidase (SOx), a heme- and molybdopterin cofactor-containing enzyme, was studied spectroelectrochemically using capillary gold electrode modified with aldrithiol. Direct electron exchange between SOx and the surface of modified gold was observed, with a formal potential of −115 mV vs. Ag|AgCl, KCl_{sat} at pH 7.0. This value agreed well with that previously reported for redox transformation of the heme domain of SOx. However, no bioelectrocatalysis of sulphite oxidation was observed in phosphate buffer solutions. This fact evidently correlated with known inhibition of intramolecular ET in SOx by the presence of bivalent inorganic anions. After changing to a Tris buffer solution, spectra variations and cyclic voltammetry data designated direct ET-based bioelectrocatalysis of sulphite oxidation, upon addition of sulphite. Thus, the bioelectrocatalytic $2e^-$ oxidation of sulphite catalysed by SOx due to direct ET exchange with the electrode was attained at aldrithiol-modified gold electrodes and shown to depend essentially on the nature of the buffer solution.

Keywords: Sulphite oxidase; Direct electron transfer; Spectroelectrochemistry; Bioelectrocatalysis

1. Introduction

Sulphite oxidase (SOx), a Mo complex cofactor and a cyt b_5-type heme cofactor-containing intermembrane redox enzyme, catalyses a $2e^-$ oxidation of SO_3^{2-} to SO_4^{2-}, the terminal reaction in the degradation of sulphur-containing amino acids, with cytochrome *c* (cyt *c*) as its physiological electron acceptor [1 2]. Oxidation of SO_3^{2-} occurs at the Mo centre with concomitant reduction of Mo(VI) to Mo(IV), then the reducing equivalents are passed on from the Mo domain to the cyt b_5 domain of SOx and from there to cyt *c*:

$$SO_3^{2-} \rightarrow \text{Mo} - \text{cofactor} \rightarrow \text{cyt}b_5$$
$$\rightarrow \text{cyt } c \text{ or other electron acceptor}$$

Ideally, the electrode may replace cyt *c* and in this case, the electron transfer (ET) and catalysis of SOx can be studied electrochemically. Hitherto direct electrochemistry of SOx was a matter of question [3–5], several factors might be reasons for that. First, the available data on crystal structure of SOx demonstrate that significant reorganisation of the protein domains should occur for efficient internal ET between the Mo and heme domains [6]. Specifically, the long ET distance between the Mo and the heme Fe, 32 Å, and the orientation of the molybdopterine ring system away from the second cofactor imply that the cyt b_5 domain should adopt a proper conformation for internal ET to occur, with a reduced metal-to-metal distance providing an efficient ET pathway [6]. Second, the conformation-dependent SOx catalytic cycle is largely affected by the composition, ionic strength, viscosity and pH of the contacting solution [6–11]. Thus, to achieve efficient direct ET-based bioelectrocatalysis with SOx, very special conditions should be used, including a solution composition and a choice of the electrode material and its modification. Only recently, first works appeared reporting direct ET-based bioelectrocatalysis with SOx on pyrolytic graphite edge and alkanethiol-modified gold electrodes [12,13]. However, despite pronounced direct electrochemistry of SOx on these electrodes,

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doi:10.1016/j.bioelechem.2003.09.013

no undisputed spectroscopic evidence that it is the heme redox centre that undergoes redox transformations during SOx bioelectrocatalysis has been reported.

In the present work, the ET reactions within SOx were studied spectroelectrochemically. This approach has a number of evident advantages over common electrochemical studies. The electrode displays universal oxidant–reductant properties upon continuously variable electrode potential, which enables the direct measurements of ET reactivity of a given redox enzyme over a continuous range of potentials. In parallel, the direct ET reaction from the electrode to and through the enzyme in close vicinity of the electrode surface can be detected spectrophotometrically, through the cofactor spectra variation. Therewith, the enzyme should be in the solution (not in the adsorbed state) to obtain reliable spectral data. To avoid any adsorption of SOx at the electrode surface, the modification of gold electrodes with an aldrithiol self-assembled monolayer (SAM) was used, thus offering possibility to study the "solution" electrochemistry of SOx.

2. Experimental

2.1. Chemicals and materials

Sulphite oxidase from chicken liver (SOx, EC 1.8.3.1) was from Sigma. The SOx activity assay was performed as previously described [11]; the specific catalytic activity of SOx towards cyt *c* determined spectrophotometrically was 24.3 U mg^{-1} at pH 7.4. Cyt *c* from bovine heart was from Sigma and used as received. Sodium sulphite, 98%, was from Fluka. Aldrithiol-4 (98%) was from Aldrich. 18.2 MΩ Millipore water was used throughout the work.

2.2. Instrumental procedure

All measurements were performed at 22 ± 1 °C in a specially designed spectroelectrochemical cell with a volume of less than 1 μl and an optical path length of about 1 cm. The cell design was described in detail elsewhere [14]. The cell itself was made of a 1-cm-long Au capillary with an inner diameter of 300 μm (Goodfellow), which served as the working electrode; optical fibres (∅ 50 μm) were directed into the Au capillary. An Ag|AgCl, KCl_{sat} (wire of ∅ 0.5 mm, Lawrence) was the reference and a Pt wire (∅ 0.5 mm, Goodfellow) was the counterelectrode. The potential was applied and controlled with LC-3E Petit Ampère potentiostat (BAS, USA). The absorbance spectra were obtained with a PC2000-UV-VIS miniature fibre optic spectrometer (Ocean Optics, Dunedin, USA) with an effective range between 200 and 1100 nm. The distance between the tips of the fibres in the Au capillary cell was about 1 cm.

Prior to the experiments, the Au capillary electrode was cleaned by filling it with Piranha mixture for a few minutes, followed by rinsing with water. After following electrochemical cleaning in 0.2 M NaOH, the electrode was modified with aldrithiol by 1.5 h adsorption from the aqueous aldrithiol-saturated solution. Then, the spectroelectrochemical cell was thoroughly assembled and filled either with buffer or with the 36 μM SOx solution in a corresponding buffer system. The enzyme solution was aspirated with a Hamilton syringe through the cell capillary and the absorbance spectrum of the enzyme in the solution was recorded. The electrode potential was slowly changed and held constant at discrete potentials between -0.3 and 0.15 V. In a complete set of data, about 32 data points at different potentials were recorded during 1.5 h. Common cyclic voltammetry (CV) was performed with polycrystalline Au disk electrodes (CHI, 0.0314 cm^2) at 22 ± 1 °C in a standard three-electrode cell connected to BAS CV-50W potentiostat (BAS, USA). An Ag|AgCl, KCl_{sat} and a Pt plate were used as the reference and auxiliary electrodes, respectively. The modification of the Au electrodes with aldrithiol was performed in the same manner as was described for the Au capillary electrode; the same solution concentration of SOx was used as well. The measurements were performed in deaerated 0.1 M Na-phosphate buffer (Na-PBS), pH 7.0 and pH 7.4 and 0.1 M Tris–HCl buffer solutions, pH 7.4. A 0.1-M aqueous solution of sulphite was prepared immediately before measurements and used within 40 min.

3. Results and discussion

The spectra of fully reduced (at -0.7 V) and fully oxidized (at $+0.2$ V) SOx, as well as the intermediate curves, are presented in Fig. 1a and b. The spectral features of reduced and oxidized SOx are practically identical to the spectra reported previously for spectral titration with mediators [15,16] and basically reflect the optical absorption properties of the heme. However, extra features can be followed from the obtained spectra at -0.7 V. In addition to absorbance of the heme moiety, an absorbance change occurs within 300–400 nm wavelengths, which could be presumably ascribed to the redox transformations of the Mo domain [2]. Although additional detailed studies of the Mo features of spectrum and parallel electrochemical studies are necessary, it may be seen that Mo redox changes can be followed spectroelectrochemically (Fig. 1a, region 300–400 nm).

In this work, we studied in detail spectroelectrochemical features of the heme domain, which establishes the electronic contact with the electrode surface. This electronic contact provides conditions for bioelectrocatalytic oxidation of sulphite, being dependent on internal ET from the Mo centre to the heme centre. To determine the formal potential, $E^{0/}$, and the number of electrons, *n*, involved in the redox transformation of SOx on aldrithiol-modified Au, the absorbance, *A*, at 558 nm (Fig. 1b) was plotted as a function of

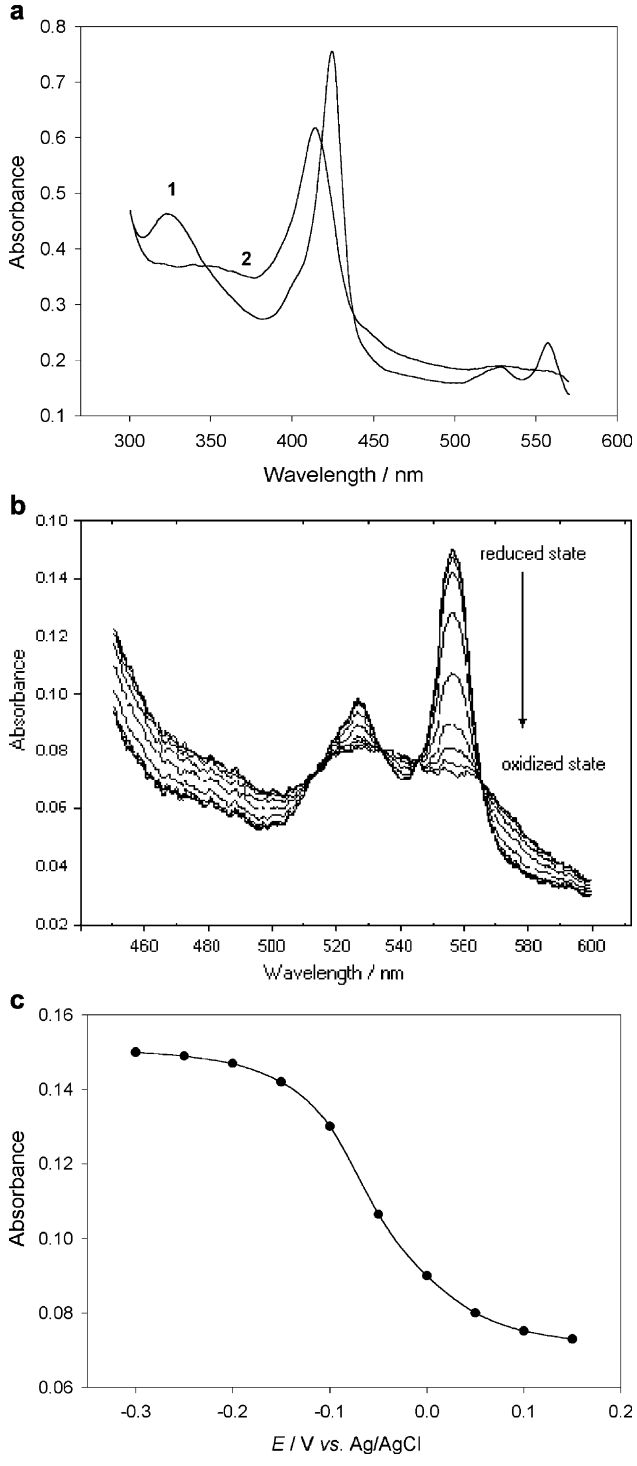

Fig. 1. (a)Absorption spectra of SOx in 0.1 M Na-PBS, pH 7.0, at (1) − 700 mV (reduced state) and (2) 200 mV (oxidised state). (b) Absorption spectra of SOx in 0.1 M Na-PBS, pH 7.0, at − 300, − 200, − 150, − 100, − 50, 0, 50, 100 and 150 mV. (c) Dependence of absorbance for SOx at $\lambda = 558$ nm on the applied potential. The solid line is a nonlinear least-squares fit of the data to Eq. (1). The measurements were performed in 0.1 M Na-PBS, pH 7.0.

the electrode potential, E (Fig. 1c). This dependence was fitted with the following equation using nonlinear least squares procedure:

$$A = \varepsilon_{\mathrm{red}} \cdot l \cdot Ct + l(\varepsilon_{\mathrm{ox}} - \varepsilon_{\mathrm{red}}) \frac{C_t \cdot \exp\left[\frac{(E - E_{1/2})nF}{RT}\right]}{1 + \exp\left[\frac{(E - E_{1/2})nF}{RT}\right]} \quad (1)$$

Here, l is the length of the optical path of the cell; F, R and T are the Faraday constant, the gas constant and the temperature. All the other parameters were not important to estimate $E^{0/}$ and n. As can be seen from Fig. 1c, the experimental data are well fitted to the curve described by Eq. (1) with the parameters: the number of electrons n equal to 0.98 ± 0.05, the formal potential − 115 mV. These values correlate well with previously reported potentials for redox transformations of the heme domain of SOx [15,16]. Additionally, the absence of a histeresis of the titration curve might designate that it is the heme domain that is in the direct ET contact with the electrode surface; that is, no other redox centre acts as a mediator between the heme and the electrode. However, considering the shown possibility of fast internal ET between the Mo and heme active sites [6–9] and solution electrochemistry of SOx in the studied system, certain probability for the electrode—heme ET mediated by Mo centre, also exists. The last can be possible if the redox potentials of heme and the Mo-cofactor overlap. To decide which redox centre, heme or Mo-cofactor, communicates with the electrode, the catalysis of sulphite oxidation based on this direct ET reaction was studied.

For this purpose, slow scan CV with aldrithiol-modified Au disk electrodes was performed in Na-PBS, pH 7.4, containing SOx, in the absence and in the presence of a saturating concentration of sulphite (well above K_m [10]). However, despite the pronounced electronic communication

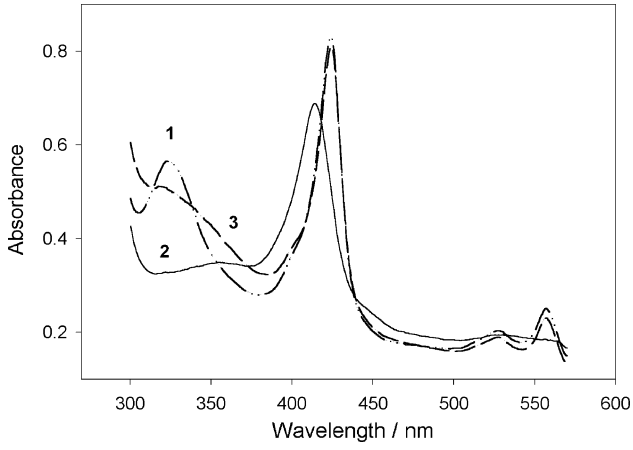

Fig. 2. Absorption spectra of SOx in 0.1 M Tris–HCl, pH 7.4, (1) at − 600 mV (reduced state), (2) 0 mV (oxidised state) and (3) 200 mV upon addition of 3.3 mM Na_2SO_3.

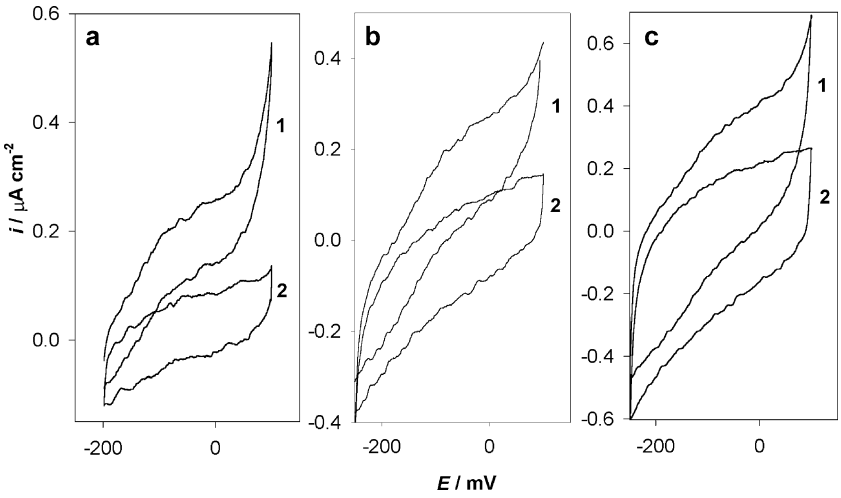

Fig. 3. Catalytic CVs of SOx on aldrithiol-modified Au in the presence (1) of 3.3 mM Na_2SO_3 in 0.1 M Tris–HCl, pH 7.4; (2) corresponding noncatalytic CVs in the absence of sulphite; scan rates v (a) 1 mV s^{-1}, (b) 2 mV s^{-1}, (c) 5 mV s^{-1}.

between the electrode and the heme domain of SOx (Fig. 1a), no catalytic oxidation of sulphite was observed upon addition of sulphite. The absence of the catalysis was attributed to the inhibition effect of bivalent anions being components of the buffer solution, i.e., phosphate anions. Previously, phosphate and sulphate anions have been shown to reduce the catalytic activity of SOx towards sulphite oxidation through competitive binding in the Mo active site [7,9,11,16]. This binding was also shown to affect internal ET from Mo-cofactor to heme active site by providing SOx conformation with an increased ET distance. The absence of the catalysis under these conditions allows us to conclude that most probably, it is the heme redox centre that electronically communicates with the electrode, but not the Mo-cofactor. That is, no internal ET is needed (which is inhibited by phosphate ions) to reduce or oxidise heme in spectroelectrochemical cell (Fig. 1a). If the electrons would enter the heme redox centre through the Mo site, no spectral changes of heme could be expected in phosphate buffer solutions, under conditions when internal ET is hampered. To attain bioelectrocatalysis with SOx, 0.1 M Na-PBS was changed for a 0.1 M Tris–HCl buffer solution, known to be optimal for studies of SOx catalysis [11].

No principal changes in spectra of SOx were observed upon changing the buffer media from PBS to the Tris buffer solution (Fig. 2, curves 1, 2). However, upon addition of 3.3 mM sulphite to a SOx solution in 0.1 M Tris, the spectrum characteristic to oxidized heme changed to a spectrum of reduced heme, even at potentials when it should be fully oxidized in the absence of the substrate (Fig. 2, curve 3). Thus, reduction of Mo centre by sulphite and fast internal ET of the reducing equivalents to the heme active site of SOx (accompanied by a relatively slow heterogeneous ET) can explain permanent reduced state of heme under the conditions when it is usually oxidized in the absence of the substrate. Upon these conditions, CVs of SOx at aldrithiol-modified electrodes transformed into oxidative catalytic waves (Fig. 3). The catalysis of sulphite oxidation started at potentials similar to those of the heme redox transformations of SOx recorded by spectroelectrochemistry. The waves were pronounced only at low sweep rates, because the solution electrochemistry of the enzyme contributed to the observed catalysis through the slow diffusion of SOx to the electrode surface. Further addition of sulphate anions suppressed the observed catalytic waves as well. It is worth to mention, that in the absence of SOx, the oxidation of sulphite started at potentials close to +0.1 V.

Thus, spectroelectrochemical results demonstrated that SOx could communicate electronically with the modified Au electrode through the heme-containing domain of SOx, which undergoes redox transformations. The $E^{0/}$ calculated from the absorbance-potential dependencies corresponded to −115 mV vs. Ag|AgCl, KCl_{sat}, correlating fairly well with the previously reported $1e^-$ redox potential for the heme Fe(III/II) couple in SOx, specifically, −113 mV at pH 7.0 [15]. However, the efficiency of direct ET-based SOx catalysis of sulphite oxidation was principally dependent on the nature of the supporting electrolyte.

Acknowledgements

E.E.F. acknowledges the Wenner-Gren Foundations for financial support. Authors thank Prof. Fraser Armstrong for fruitful discussions.

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