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COMMUNICATIONS

Published on Web 09/06/2002

A Voltammetric Study of Interdomain Electron Transfer within Sulfite Oxidase

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Received July 19, 2002

We report a voltammetric investigation of the catalytic activity of sulfite oxidase (SO), a soluble mitochondrial enzyme that catalyzes the oxidation of sulfite to sulfate, a physiologically essential reaction in higher animals.[1] The crystal structure[2] of SO poses an interesting dilemma regarding intramolecular electron transfer (IET) that is of significance for other redox enzymes with multiple, mobile domains. Oxidation of sulfite occurs at the Mo active site that is ~30 Å away − a prohibitively large distance − from its proposed electron acceptor, the *b*-type heme; however, the region between the heme and Mo domains contains an unresolved flexible loop with no recognizable secondary structure.[2,3] Because the ability of the heme to engage in IET from the Mo active site to the natural redox partner of SO (cytochrome *c*) is well established,[4] it has been proposed that during catalysis, SO adopts an alternative conformation having a much shorter heme-to-Mo distance, thereby facilitating IET.[4,5] Conformational flexibility of the two domains in solution is supported by recent pulsed ELDOR studies[6] and by the dependence of IET upon viscosity.[5] In this report, we investigate the postulated relationship between variable SO conformations and catalytic activity using protein film voltammetry (PFV),[7−13] which probes electron transfer and catalysis in the "potential domain", thereby identifying rate-determining steps in terms of the characteristic potentials and number of electrons involved. We show that not only do electrons leave the active site via heme-*b*, but that the motion of the heme domain is a limitation for SO activity.

SO was immobilized on an electrode surface of either pyrolytic graphite edge (PGE) or modified polycrystalline gold (modified with a self-assembled monolayer of mercapto-6-hexanol[13]), and the electrochemical response was monitored by cyclic voltammetry (CV).[14] Whereas SO has been examined previously via mediated solution voltammetry[15,16] and microcoulometry,[17] PFV reports upon the direct (i.e., without mediators) redox properties of SO (including enzymatic activity). Figure 1 shows typical CV measurements of a film of SO on a PGE electrode, both in the absence (inset) and in the presence of sulfite (1 mM, main figure). Without substrate, a single stable signal (consisting of a pair of peaks) is observed, centered at a reduction potential of +90 mV (vs SHE). The signal is unchanged upon electrode rotation, showing that it arises from immobilized SO. The baseline-subtracted peaks (dotted line) are highly symmetrical, having a peak-width at half-height of 84 mV, consistent with a 1e$^-$ process at 0 °C,[18] while integration gives a surface coverage Γ_{tot} of 4.3 pmol/cm^2 based upon this 1e$^-$ assumption. PFV leads to facile interfacial electron transfer between SO and the electrode surface, as demonstrated both by the nernstian peak shape and by the small separation of the peaks (18 mV at 50 mV/s). Because SO has *two* cofactors per subunit, one of which

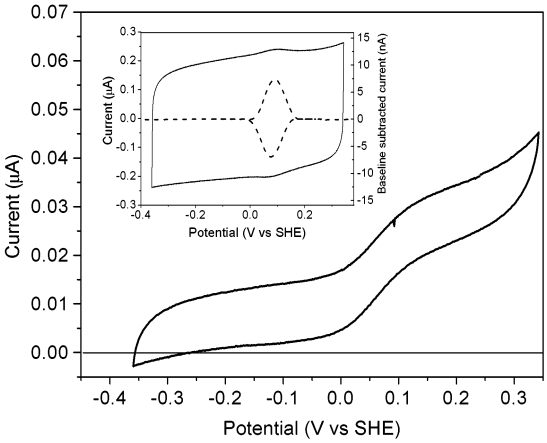

Figure 1. (Main) Catalytic CV of SO immobilized on a PGE electrode at 25 °C. [Na_2SO_3] = 1 mM, pH = 8.0, υ = 10 mV/s. (Inset) Raw and baseline-subtracted (dotted line) nonturnover CV of SO immobilized on PGE at 0 °C. No sulfite, pH = 8.0, υ = 50 mV/s.

(Mo) has two one-electron couples,[17] we should anticipate the observation of *three* distinct nonturnover signals at the potentials previously reported for the centers in a microcoulometric study.[17,19] The Fe(III/II) and Mo(VI/V) reduction potentials were found to overlap partially in the pH range studied;[6,17,19] thus, the peak observed here might have some contribution from a high-potential Mo signal. Yet, the nonturnover peaks should yield stoichiometric information, so that if the peaks observed in Figure 1 were due, in part, to the high-potential Mo couple, it is likely that a lower-potential couple of an equal signal intensity would be observed (as was detected in the microcoulometric study).[17] In our work, CV did not reveal multiple redox couples; there was no evidence for overlapping Fe(III/II)−Mo(VI/V) couples, nor was a low-potential Mo(V/IV) couple observed within the limits of detection.

Upon the introduction of 1 mM sulfite to the cell solution (well above the K_m value of 16 μM), the voltammogram of an SO film transforms into an oxidative, catalytic wave (Figure 1, main.) With such a high concentration of substrate, the shape and amplitude do not depend on electrode rotation rate, and the wave reflects the limiting enzymatic activity only. From the limiting current, i_{lim}, which is related to the turnover number, k_{cat}, by the relationship $k_{cat} = i_{lim}(\Gamma_{tot}\, AFn)^{-1}$,[20] we obtain $k_{cat} \approx 2-4\ s^{-1}$. Reported values of k_{cat} for SO in solution assays are approximately 100 s^{-1};[21] thus, we suggest that a large fraction of the SO molecules immobilized on the electrode surface do *not* engage in catalysis, but do undergo noncatalytic electron transfer. Importantly, the behavior we observe is similar regardless of the electrode. Therefore, the data do not seem to be caused by an artifactual, surface−protein interaction.

Important insight is provided by a detailed analysis of the shape and pH dependence of the catalytic wave,[11] which appears at a potential similar to that of the signal observed without substrate

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10.1021/ja027776f CCC: $22.00

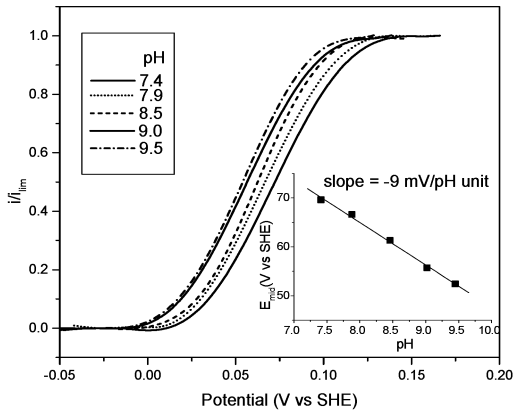

Figure 2. pH dependence of baseline-subtracted catalytic CV, for SO immobilized on a PGE electrode. All scans were taken at 10 mV/s, in 20 mM mixed buffer with 20 mM Na-acetate, at 20 °C. Inset shows the pH dependence of the midpoint potentials.

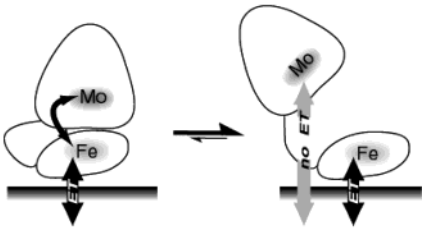

Figure 3. SO conformational motion upon an electrode. The cartoon shows one of the subunits of the dimer.

(Figure 2).[22] Although oxidation of sulfite is a two-electron reaction, the shape conforms to that of a simple one-electron process; that is, n_{app} is 1. This implicates a single electrochemically active species, undergoing one-electron transfers, as being crucial to the enzymatic activity. This is likely to be either (a) the catalytically significant Mo(VI/V) couple or (b) the catalytically limiting ET of the Fe(III/II) couple. As a low-potential Mo(V/IV) couple is not detectable in the nonturnover experiments, possibility (a) is unlikely. From the microcoulometric study, the pH dependences of the Fe(III/II) and Mo(VI/V) couples are significantly different; thus, whereas the reduction potentials are similar at neutral pH, the Fe(III/II) heme-*b* displays virtually no pH dependence (as expected), while the Mo(VI/V) *and* Mo(V/IV) couples have strong pH dependences (−60 mV/pH unit each).[17] The midpoints of the catalytic waves (E_{mid}) show a pH dependence of approximately −9 mV/pH unit (Figure 2, inset); this is only consistent with the wave arising from action at the Fe(III/II) center and identifies it as the central distributive site for catalytic ET.

The cumulative evidence leads to the model shown in Figure 3, in which the proposal of Pacheco and co-workers[4] for the conformational rearrangement of SO allowing IET is extended to an enzyme bound at an electrode surface. As shown, the majority of SO molecules do not engage in ET from the Mo active site because of a prohibitively large distance between the electrode and the active site (Figure 3, right), even though the heme domain is in an electroactive conformation. On the electrode, only a small fraction of SO molecules adopt a conformation in which the heme can act as a relay and catalysis may proceed. Further, interconversion between these conformations must be slow. The existence of this smaller population (∼ 4%) yields the low calculated value of k_{cat}, and the Mo center of such a small population would escape detection in nonturnover experiments. Functional SO is a tight α_2 dimer that keeps the Mo sites ∼39 Å apart, and the heme sites are separated even further.[2] Thus, intersubunit ET is unlikely, and IET kinetics are adequately described by considering the interdomain motions of a single subunit (Figure 3). This limiting conformational reorganization gives rise to the apparent $n = 1$ nature of the observed signals because the passage of electrons, one by one, from the catalytic center is limiting at steady state. A similar finding has been observed for the electrochemical characterization of cytochrome *c* oxidase in the presence of cytochrome *c*.[23]

Thus, catalytic turnover of SO is mediated and limited by electron transfer between the heme domain and the Mo domain. Only when the correct conformation is attained can the Mo center be "wired" to the electrode, with the heme acting as a relay; otherwise, the Mo is "disconnected".

Acknowledgment. This work was supported by NIH (Grant GM-37773 to J.H.E.) and by HFSO and BBSRC (F.A.A.). S.J.E. acknowledges support by an EMBO Postdoctoral Fellowship. We thank Dr. C. Léger for helpful comments.

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(14) Chicken liver sulfite oxidase was purified as described previously.[2] Protein film voltammetry was performed as in earlier papers.[7−13] Freshly polished PGE electrodes were coated with enzyme by painting a 0.1 mM solution of SO directly upon the surface. Films of SO on mercapto-6-hexanol-modified gold electrodes were prepared either as for PGE or by incubating the electrode in a 10 μM SO solution at 4 °C, while cycling between +0.3V and −0.3V (vs SCE) for 5 min, after which the cell was refilled with a protein-free solution. All electrochemical experiments were carried out using an Autolab Electrochemical Analyzer (Eco-Chemie, Utrecht, Netherlands) with Fourier smoothing and background-subtraction as described before.[13] All buffers were 20 mM in each of MES, HEPES, and TAPS and contained 10−20 mM sodium acetate as additional supporting electrolyte.
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(19) Specifically, the midpoint potentials for the Fe(III/II), Mo(VI/V), and Mo(V/IV) couples for the low pH Cl, inhibited form (and the high pH, uninhibited form) were reported as 90 mV (51 mV), 131 mV (−57 mV), and 86 mV (−233 mV), respectively, all versus the hydrogen electrode (ref 17).
(20) Where i_{lim} is the limiting current, Γ_{tot} is the calculated surface coverage, *A* is the area of the electrode surface, and *n* is the stoichiometric number of electrons passed.
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JA027776F