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Highly Sensitive and Stable Electrochemical Sulfite Biosensor Incorporating a Bacterial Sulfite Dehydrogenase

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This paper describes a highly sensitive electrochemical (voltammetric) determination of sulfite using a combination of *Starkeya novella* sulfite dehydrogenase (SDH), horse heart cytochrome *c* (cyt *c*), and a self-assembled monolayer of 11-mercaptoundecanol (MU) cast on a gold electrode. The biosensor was optimized in terms of pH and the ratio of cyt *c*/SDH. The electrocatalytic oxidation current of sulfite increased linearly from 1 to 6 μM at the enzyme-modified electrode with a correlation coefficient of 0.9995 and an apparent Michaelis constant ($K_{M,app}$) of 43 μM. Using an amperometric method, the low detection limit for sulfite at the enzyme-modified electrode was 44 pM (signal-to-noise ratio = 3). The modified electrode retained a stable response for 3 days while losing only ca. 4% of its initial sensitivity during a 2 week storage period in 50 mM Tris buffer solution at 4 °C. The enzyme electrode was successfully used for the determination of sulfite in beer and white wine samples. The results of these electrochemical analyses agreed well with an independent spectrophotometric method using Ellman's reagent, but the detection limit was far superior using the electrochemical method.

Sulfite is a widely used additive in food and beverages to prevent spoilage by oxidation and bacterial growth during production and storage.[1,2] However, sulfite is toxic in high doses and some people are extremely sensitive to even low levels of sulfite as it can induce mild to severe skin, respiratory, or gastrointestinal reactions.[3−6] Since 1986, the U.S. Food and Drug Administration (FDA) has required the labeling of products containing more than 10 ppm (125 μM) sulfite in foods or beverages. In quality control of manufactured food and beverage products a sensitive, easily applied, and accurate analytical method for the determination of sulfite is required to ensure compliance with these regulations.

Several methods have been proposed for sulfite determination in foods and beverages including high-performance liquid chromatography,[7] spectrophotometry,[8,9] and electrochemistry.[10−18] The Association of Official Analytical Chemists (AOAC) has recommended the long-standing wet chemical Monier−Williams method[19] for the determination of sulfite, which is based on the titration of sulfuric acid generated from SO_3^{2-} (liberated as SO_2, oxidized and hydrolyzed to H_2SO_4). Chromatographic methods offer better accuracy and sensitivity toward sulfite determination but require more expensive instrumentation and skilled operators.[20]

As an alternative, electrochemical methods[21] may be employed where the current generated by direct sulfite oxidation is measured at metallic or carbon/graphite electrodes. In order to minimize interference the electrodes are usually modified with films comprising Prussian blue analogues (copper−cobalt hexacyanoferrate,[16] nickel pentacyanonitrosylferrate,[13] cobalt pentacyanonitrosylferrate,[14] and nickel aquapentacyanoferrate[12]), ferrocene derivatives,[11,15] or poly[Ni−(protoporphyrin IX)][10] to mention a few examples. However, selectivity is an inherent

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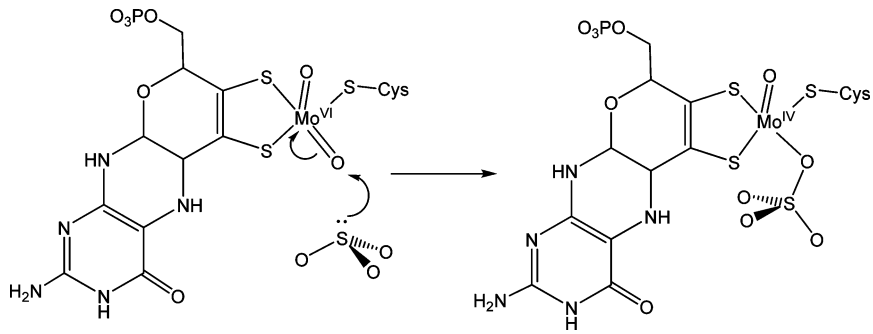
Scheme 1



problem with this direct approach as sulfite is oxidized at a rather high potential where other species such as ascorbate and polyphenols are also electroactive.

The problems of nonspecific oxidation of interfering species can be avoided by integration of highly specific sulfite oxidizing enzymes into the transducing surface. The sulfite/acceptor oxidoreductase enzymes, comprising eukaryotic sulfite oxidase (SO) and bacterial sulfite dehydrogenase (SDH), are structurally similar members of the mononuclear molybdoenzyme family[22] and catalyze the oxidation of sulfite to sulfate with high selectivity. The bacterial sulfite dehydrogenases[23] donate electrons exclusively to cytochrome *c* (cyt *c*), whereas the oxidases, so far confined to eukarya,[24,25] can also use molecular oxygen as an electron acceptor, although this is a minor pathway.

The most thoroughly characterized bacterial sulfite oxidizing enzyme to date is SDH from the soil bacterium *Starkeya novella*.[23,26,27] SDH is a heterodimer comprising molybdenum and heme *c* binding subunits, and these separate subunits occupy fixed positions relative to one another during catalysis.[28] This is an interesting and important feature that contrasts with chicken liver SO (the most thoroughly studied sulfite oxidase), which undergoes conformational changes during catalysis that complicate and slow its reaction rate.[9] Furthermore, our investigations of SDH have shown it to be a robust enzyme and a high-yielding expression system has been developed allowing the enzyme to be obtained in good yields.[29]

During catalysis, the equatorial oxido ligand of hexavalent Mo is transferred to sulfite with concurrent reduction of the metal to its Mo^{IV} form (Scheme 1, charges omitted for clarity). The sulfate ligand is subsequently replaced by water, and the two electrons on the Mo^{IV} center are transferred in sequence to cyt *c* via the adjacent heme cofactor relay to restore the fully oxidized form.

The bioelectrochemical interrogation of sulfite oxidizing enzymes (mostly SO from chicken liver) has received much attention.[30−41] Some of these studies are based on monitoring the consumption of oxygen[42−44] (using an oxygen electrode) to report on the amount of sulfite present (eq 1).

$$SO_3^{2-} + O_2 + H_2O \xrightarrow{SO} SO_4^{2-} + H_2O_2 \qquad (1)$$

A more secure approach is to drive the bioelectrochemical reaction through either synthetic or natural electron acceptors (mediators) which are oxidized at much lower working potentials than either sulfite or other species in solution. The anodic current derived from oxidation of the mediator directly equates to the sulfite oxidation rate when the reaction is tightly coupled. This has been achieved in some cases with chicken liver SO through the use of small-molecule electron acceptors,[45−47] synthetic redox polymers,[40] or horse heart cyt *c*,[32,33,35] a promiscuous electron acceptor for a number of sulfite oxidizing enzymes[27,48] and a protein that exhibits well-understood electrochemistry.

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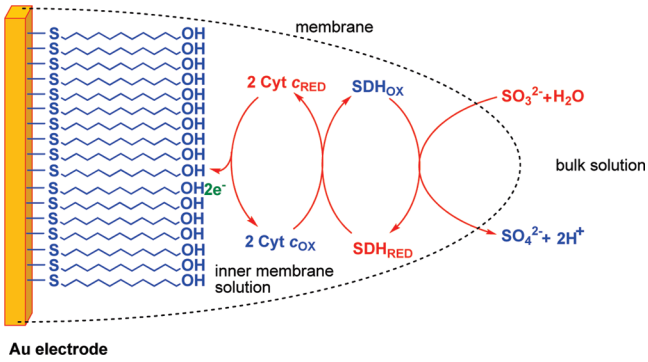
Scheme 2



In recent years, we have reported direct (unmediated) electrocatalytic voltammetry of SDH (wild type and several variants) immobilized on an edge-plane pyrolytic graphite electrode where the enzyme retains its native function and activity.[49,50] Herein, we have extended our electrochemical studies to include cyt *c* as an electroactive relay between the enzyme and the electrode as illustrated Scheme 2. To this end we have employed a gold electrode chemically modified with 11-mercaptoundecanol which, among many other long-chain thiols, has been shown to provide an effective interface for direct electron transfer with cyt *c*.[33,51,52] Our results and biosensor optimization on standardized sulfite solutions have been extended to sulfite determination in beer and white wine samples, and these results are compared with an independent spectrophotometric method.

EXPERIMENTAL SECTION

Materials. Wild-type SDH (specific activity 246 units mg^{-1}) was purified from a heterologous expression system in *Rhodobacter capsulatus* as described previously.[29] Horse heart cytochrome *c* (molecular weight ca. 12 400 Da) was purchased from Sigma and was used as received. The reagents 5,5′-dithio-bis(2-nitrobenzoic acid) (DTNB, or Ellman's reagent) and 11-mercaptoundecan-1-ol (MU) were purchased from Aldrich Chemicals. All other reagents were of analytical grade purity and used without any further purification. The beer and wine samples were purchased from local retail outlets. All solutions were prepared in purified water (Millipore, resistivity 18.2 MΩ·cm). Tris acetate buffer (50 mM) was used for experiments at pH 8.0. For experiments conducted within the range $6 < pH < 10$, a buffer mixture containing both bis−tris propane and 2-amino-2-methylpropan-1-ol (50 mM) was used, titrated with acetic acid to give the desired pH. Standard stock sulfite solutions were prepared fresh daily with sodium sulfite.

Electrochemical Measurements and Electrode Cleaning. Cyclic voltammetry (CV) and chronoamperometry were carried out with a BAS 100B/W electrochemical workstation using a three-electrode system consisting of a gold working electrode (3 mm), a platinum wire counter electrode, and Ag/AgCl reference electrode. Experiments were carried out in Ar-purged solutions. The Au working electrode was mechanically, chemically, and electrochemically cleaned and polished according to a published procedure.[53] A monolayer of MU was prepared on the Au electrode by immersion in a 1 mM ethanolic solution of MU for 24 h. The electrode was subsequently washed with copious amounts of ethanol and water to remove any loosely bound MU molecules from the surface.

The electroactive surface area of the Au electrode (*A*) was determined by CV of 1 mM ferrocene methanol in 0.1 M KCl solution at different sweep rates using the Randles−Sevcik equation (eq 2).[54]

$$i_p = (2.69 \times 10^5) n^{3/2} A D_o^{1/2} C_o \nu^{1/2} \quad (2)$$

The standard diffusion coefficient (D_o) of ferrocene methanol is 6.7×10^{-6} cm^2 s^{-1},[55] i_p is the measured current maximum, *n* is the number of electrons, C_o is concentration of analyte (mol cm^{-3}), and ν is the sweep rate (V s^{-1}).

Enzyme Electrode Preparation. Depending on the experiment, different aliquots of SDH (~21 μM) and cyt *c* (~100 μM) were mixed in 50 mM Tris buffer and 7.5 μL of this combined solution was dispensed carefully onto the conducting surface of a freshly prepared, inverted Au/MU electrode and allowed to dry to a film at 4 °C. To prevent protein loss, the electrode surface was carefully covered with a perm-selective dialysis membrane (MW cutoff ca. 12 kDa), presoaked in water. The dialysis membrane was pressed onto the electrode using a Teflon cap and fastened with a rubber O-ring. The resulting modified electrode was stored at 4 °C in 50 mM Tris buffer solution (pH 8.0) while not in use.

Electrochemical Measurements and Data Processesing. Variation of the observed catalytic current (i_{lim}) as a function of substrate concentration followed Michaelis−Menten kinetics,[34] and the data were fit to eq 3.

$$i_{lim} = \frac{i_{max}[SO_3^{2-}]}{K_{M,app} + [SO_3^{2-}]} \quad (3)$$

where i_{max} is the saturation limiting current and $K_{M,app}$ is the apparent Michaelis constant.

The pH dependence of the catalytic current was modeled by eq 4,[56] which is applicable to an enzyme that is deactivated by either a deprotonation of an acidic functional group (pK_{a1}) or protonation of a basic functional group (pK_{a2}).

$$i_{lim}(pH) = \frac{i_{opt}}{1 + 10^{(pH-pK_{a1})} + 10^{(pK_{a2}-pH)}} \quad (4)$$

Chemical Sulfite Analysis. The sulfite concentrations in beer and wine samples were cross-checked using a variation of a published spectrophotometric method[8] employing the aromatic disulfide 5,5′-dithio-bis(2-nitrobenzoic acid) (DTNB, or Ellman's

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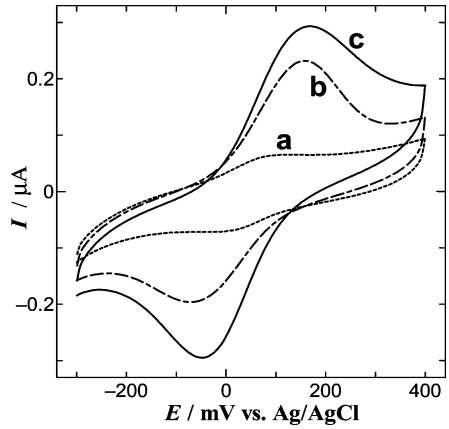

Figure 1. Cyclic voltammograms of different ratios of cyt *c*/SDH, (a) 2.5:1, (b) 5:1, and (c) 10:1, with a Au/MU electrode in 50 mM Tris buffer solution (pH 8.0) at a scan rate of 50 mV s^{-1}. In each case the concentration of SDH is ca. 20 μM.

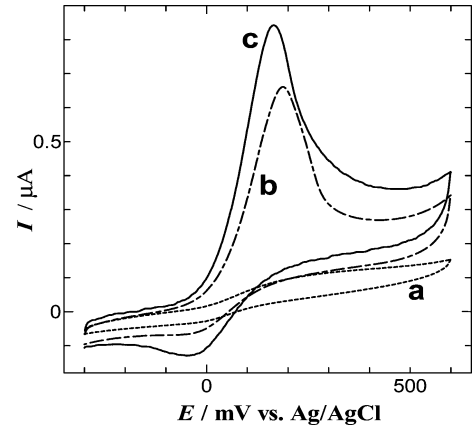

Figure 2. Cyclic voltammograms obtained for 100 μM sulfite at different ratios of cyt *c*/SDH with a MU/Au electrode, (a) 2.5:1, (b) 5:1, and (c) 10:1, in 50 mM Tris buffer solution (pH 8.0) at a scan rate of 50 mV s^{-1}.

reagent). DTNB forms an organic thiosulfate in reaction with sulfite liberating one molecule of 5-mercapto-2-nitrobenzoate (MNB) which is detected by its absorbance maximum at 410 nm (see the Supporting Information). Briefly, DTNB (1.0 mL, 1 mM) was added to 2.5 mL of each beer or wine sample. The pH of mixture was adjusted to pH 8.0 with dilute NaOH. Then the mixture was diluted to 10.0 mL with Tris buffer solution (50 mM, pH 8.0). Cleavage of the DTNB disulfide bond by sulfite is slow, and thus the mixture was allowed to equilibrate for 2 h during which the color of the mixture changed noticeably from pale yellow to dark yellow. The method of standard addition was employed by adding known amounts of sulfite to each sample before reaction with DTNB as above. A linear increase in the absorption of MNB (at 410 nm) was observed with increasing quantities of added sulfite which enabled the original sulfite concentration of the sample to be determined by back-extrapolation.

RESULTS AND DISCUSSION

Inner Membrane Protein Concentrations. The only electroactive species in this work, detected through CV, was cyt *c*. The enzyme SDH was electroinactive at the MU-modified Au electrode (data not shown), in contrast to its behavior on edge-plane pyrolytic graphite.[49,50] In the absence of sulfite, the electrochemical behavior of the cyt *c*/SDH-modified electrode was investigated in 50 mM Tris buffer solution (pH 8.0) at different concentrations of cyt *c* while keeping the amount of SDH constant. Figure 1 shows the CVs obtained for different ratios of cyt *c*/SDH at a sweep rate of 50 mV s^{-1} which are characteristic of a quasi-reversible single-electron redox couple. The averaged peak potentials in Figure 1 correspond to the ferrous/ferric cyt *c* redox potential of ca. +50 mV versus Ag/AgCl which is consistent with previous voltammetric studies of the protein.[52,57,58]

Diffusion-controlled CV was demonstrated by measuring the anodic peak currents as a function of sweep rate. It was found that the currents were directly proportional to the square root of sweep rate ($\nu^{1/2}$) as predicted by eq 2 (see Supporting Information Figure S1, parts a and b). Using eq 2, the concentration of cyt *c* under the membrane was estimated using the calibrated electroactive surface area (*A*) and diffusion coefficient of the protein ($D_o = 1 \times 10^{-6}$ cm^2 s^{-1}).[58,59] The concentration of cyt *c* in the 10:1 cyt *c*/SDH electrode (Figure 1c) was calculated to be ca. 100 μM yielding a volume under the membrane of ca. 7 μL. It is apparent that fluctuations of 1 or 2 μL in this volume will lead to variations of 10−20% in the absolute peak currents from one experiment to the next (all other things being equal such as electrode area and amount of protein added). Most importantly, in the absence of sulfite, the electrochemistry of cyt *c* is unaffected by SDH.

Catalytic Voltammetry. In the absence of either cyt *c* or SDH, no catalytic current is observed in the presence of sulfite within the potential range studied ($-300 < E < +600$ mV vs Ag/AgCl). Nonspecific oxidation of sulfite at the electrode occurs at very high potentials (>+600 mV vs Ag/AgCl at pH 8, data not shown). Upon addition of sulfite to the cyt *c*/SDH electrode, the voltammetric wave centered at +50 mV changes from a simple one-electron quasi-reversible process as shown in Figure 1 (E mechanism) to that of a coupled catalytic chemical reaction (EC_{cat} mechanism, Figure 2). This is associated with an amplification of anodic current due to regeneration of ferrous cyt *c* at the electrode by reduced SDH (see Scheme 2). Diminution of the cathodic peak is also a characteristic feature of this behavior.

The cyt *c*/SDH ratio has an influence on the catalytic voltammetric waveform. When the mediator/enzyme ratio is low (Figure 2a), the voltammogram approximates a sigmoid which is consistent with a steady-state process.[60] Under these conditions, the concentration of the ferric cyt *c* at any point follows a Nernstian profile and the half-wave potential corresponds to that of the cyt *c* redox potential (+50 mV vs Ag/AgCl, Figure 2a). As the amount of cyt *c* under the membrane increases, a point is reached where the SDH/sulfite reaction is unable to supply electrons at a rate sufficient to maintain a steady state of cyt *c* and the voltammetry becomes characteristic of transient (peak-shaped) behavior. This is apparent in Figure 2, parts b and c. The tailing off is due to accumulation of oxidized cyt *c* in the diffusion layer.

It is known that SDH exhibits a high affinity for both sulfite and the electron acceptor cyt *c* and the reaction follows a ping-pong mecha-

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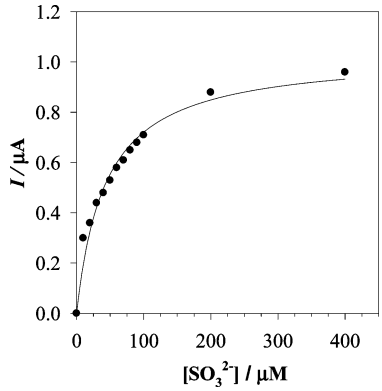

Figure 3. Plot of the electrocatalytic oxidation current at +0.20 V vs Ag/AgCl at the Au/MU/cyt *c*/SDH electrode as a function of sulfite concentration (50 mM Tris buffer solution (pH 8.0), scan rate 50 mV s^{-1}).

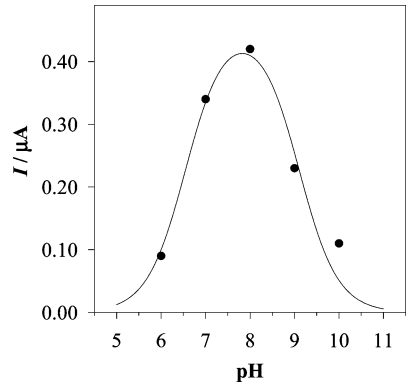

Figure 4. Plot of the pH dependence of the maximum oxidation current in the presence of 100 μM sulfite at the Au/MU/cyt *c*/SDH electrode at a scan rate of 50 mV s^{-1}. The solid curve is obtained from a fit to the experimental points using eq 4.

nism,[27] like other vertebrate sulfite oxidizing enzymes,[61,62] so reaction of the enzyme with either the substrate or electron acceptor may be rate-limiting depending on their concentrations.

We also explored the use of 11-mercaptoundecanoic acid as the Au electrode surface modifier. The negatively charged carboxylate headgroups (at pH 8) are strongly attracted toward the positively charged cyt *c*, and well-defined electrochemistry at Au electrodes treated with this thiol was observed (data not shown) as reported.[52] However, under the same conditions, no sulfite oxidation catalysis was observed. We believe that, as reported by Haas et al.,[51] the orientation of the heme domain of cyt *c* (adjacent to a highly positively charged region of its surface) is oriented toward the electrode by salt bridges formed with the carboxylate groups of the self-assembled monolayer (SAM) rather than toward the solution interface where SDH must approach. Thus, although heterogeneous electron transfer is facile, homogeneous electron transfer (between cyt *c* and SDH) is disabled. The weaker interactions between the MU-modified Au electrode and cyt *c* evidently permit reorientation of the protein as it switches between hetero- and homogeneous electron transfer at the surface in contrast to the stronger electrostatic attractions between the positively charged protein and the negatively charged carboxylate-functionalized electrode.

Sulfite Concentration Dependence. The response of the enzyme-modified electrode as a function of sulfite concentration (at pH 8.0) was studied, and the data are summarized in Figure 3. The catalytic oxidation current at +0.20 V versus Ag/AgCl depends linearly on sulfite concentrations in the range of 1−6 μM (correlation coefficient 0.9995), and saturation was reached at 400 μM. This value is comparable with values reported for human sulfite oxidase coimmobilized with cyt *c* in a polyelectrolyte-containing multilayer.[35] Further, we have calculated the apparent Michaelis constant ($K_{M,app}$ eq 3) to be 43 (±5) μM. This value is somewhat higher than determined from direct electrochemistry (K_M = 26 μM) at an edge-plane pyrolytic graphite electrode,[50] but the mechanism is more complicated in this case with the current being limited either by the sulfite or cyt *c* concentrations (Scheme 2).[60] The higher $K_{M,app}$ value reflects this.

pH Optimum. Given that pH is a critical determinant of enzymatic activity, the pH dependence of the maximum voltammetric current at the Au/MU/cyt *c*/SDH electrode was measured. The bell-shaped pH profile (Figure 4) was modeled with eq 4[56] where protonation (low pH) and deprotonation (high pH) events at the active site lead to a loss of maximum activity (i_{opt}). Approximate protonation constants of 9.1 and 6.6 were obtained corresponding to pK_{a1} and pK_{a2}, respectively, in eq 4. The optimal value of pH ~ 8 mirrors behavior seen in solution assays[27] and direct (unmediated) electrochemistry of SDH adsorbed on a pyrolytic graphite electrode.[49,50] Optimal activity in the range of pH 8−9 is a standard feature of sulfite oxidizing enzymes.[27,56,61] The loss of activity at higher pH is often attributed to deprotonation of a catalytically important tyrosine (pK_a ~ 10) residue near the active site, whereas loss of activity at low pH is less well-understood. It was observed that solution pH 8 and a ratio of 10:1 cyt *c*/SDH on the MU/Au electrode were ideal in terms of the sensitivity toward sulfite detection, so this combination was chosen for all electrochemical measurements reported below.

Amperometric Determination of Sulfite. The sensitivity of enzyme-modified electrode to sulfite was examined through constant potential amperometry under steady-state conditions. Figure 5 depicts the amperometric $i-t$ curve for the catalytic sulfite oxidation reaction at the Au/MU/cyt *c*/SDH electrode in a constantly stirred 50 mM Tris buffer solution at an applied potential of +0.40 V versus Ag/AgCl. An initial steady-state current response was observed in the presence of 0.5 μM sulfite, and further 0.5 μM sulfite increments at intervals of 50 s led to a step in the current, with a steady state being reached within 3 s. The current increased linearly with sulfite concentration from 0.5 to 5.5 μM, and the detection limit calculated from the standard deviation of the baseline current as described[63] is found to be 44 pM (S/N = 3) (inset of Figure 5).

The sensitivity of the Au/MU/cyt *c*/SDH electrode under amperometric conditions is very high by comparison with the detection limits of other enzyme-based sulfite sensors such as chicken SO−polytyramine-modified glassy carbon (GC) electrode (1 μM),[34] chicken SO/cyt *c* coimmobilized on screen-printed

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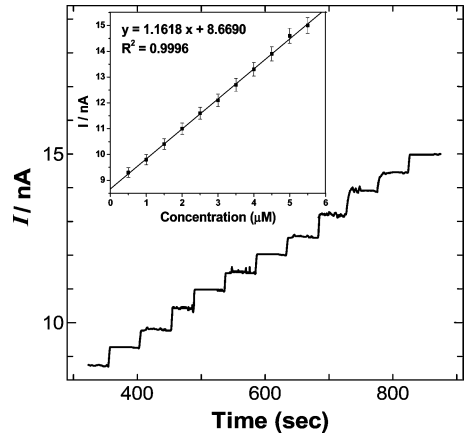

Figure 5. Amperometric *i*−*t* curve (at a constant working potential of +0.40 V vs Ag/AgCl) obtained for the determination of sulfite at the Au/MU/cyt *c*/SDH electrode in stirred 50 mM Tris buffer solution (pH 8.0). Each 50 s step coincides with an addition of 0.5 μM sulfite. The error bars in the inset indicate the drift in current over the 50 s plateau.

transducer (50 μM),[30] and human SO coupled with an Os-based redox hydrogel (0.5 μM).[40]

Reproducibility and Stability. The stability of the sulfite biosensor was assessed in two ways, first for repeated use and second with regards to long-term storage. The CVs of 100 μM sulfite solutions in 50 mM Tris buffer were recorded every 3 min to evaluate the stability of the biosensor. It was found that the anodic peak current of sulfite remained the same with a relative standard deviation of 1% for 10 repeat measurements. Very recently, Dinckaya et al. have reported a relative standard deviation (RSD) of 4% for sulfite determination at a chicken SO based CG electrode coated with thin mercury film;[31] the higher RSD value was due to loss of enzyme activity and deterioration of the mercury film.

To check the long-term storage stability of the biosensor, the SDH/cyt *c*-modified electrode was kept in 50 mM Tris buffer solution (pH 8.0) in a refrigerator (4 °C). No apparent decrease in the catalytic current response of sulfite was observed over initial 3 days, and the current decreased by only 4% after 2 weeks.

To ascertain the reproducibility of the results, three different Au electrodes were modified with the enzyme in the same way and each electrode's response toward 100 μM sulfite was tested by 10 repeat measurements. The peak current obtained in the 10 repeat measurements of three independent electrodes showed an RSD of 1%, confirming that the results are highly reproducible.

Determination of Sulfite in Wine and Beer Samples. The optimized electrochemical sulfite biosensor was applied to the determination of sulfite in three commercial beer samples and one white wine sample. The results were validated by a standard spectroscopic method using Ellman's reagent.[8] The beers comprised two aged beer samples, past their recommended use-by date, and a freshly purchased sample. The aged samples were chosen in anticipation of lower than normal sulfite concentrations due to oxidation.

The normal pH values of beer and wine are around 4, and at this pH the enzyme is inactive (see Figure 4). Therefore, the beer and wine samples were neutralized to pH 8 with alkali, diluted with Tris buffer solution, and analyzed without any other pretreatment (see the Experimental Section). The method of standard additions was employed by injecting known amounts of sulfite to each beer or wine sample within the linear range and measuring the catalytic current at +0.3 V versus Ag/AgCl, enabling the original sulfite concentration to be determined by back-extrapolation to zero current. Moreover, no interference from other electroactive species in the beer and wine samples was found. Table 1 shows the results of sulfite determination in each beer and wine sample using the present electrochemical biosensor. The results were compared with the standard spectroscopic method[8] and are in excellent agreement. Relative errors between the results obtained with the present electrochemical method and the spectroscopic method were within acceptable limits. The advantages of the electrochemical method are time of the analysis (a few minutes as opposed to hours for the spectrophotometric method) and also that the color or turbidity of the sample has no influence on the analysis. The results obtained illustrate that the present electrochemical biosensor is suitable for the determination of sulfite in beer and wine samples.

Table 1. Determination of Sulfite in Wine and Beer Samples Using the SDH/cyt *c* Electrochemical Method Compared with the Standard Spectroscopic Method (All Results Are a Mean of Three Determinations)

sample	electrochemical method (μM)	RSD (%)	spectrophotometric method (μM)	RSD (%)
aged white wine	135	2	140	2
fresh standard beer sample	133	2	132	2
aged standard beer sample (1)	26	2	27	2
aged midstrength beer sample (2)	27	3	26	2

CONCLUSIONS

We have developed a highly sensitive electrochemical biosensor for sulfite using SDH coupled with cyt *c* and a MU SAM-modified Au electrode. It was found that the enzyme-modified electrode showed optimal electrocatalytic activity toward sulfite oxidation at pH 8 and at a ratio of 10:1 of cyt *c*/SDH. The electrocatalytic oxidation current of sulfite increased linearly from 1 to 6 μM at the enzyme-modified electrode with a correlation coefficient of 0.9995 and an apparent Michaelis constant (K_M) of 43 μM. The lowest detection limit of 44 pM (S/N = 3) was achieved at this enzyme-modified electrode, which exhibited a fast response time (~3 s) and good stability toward electrocatalytic oxidation of sulfite. The practical application of the present electrochemical biosensor was successfully demonstrated by measuring the concentration of sulfite in beer and wine samples.

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SUPPORTING INFORMATION AVAILABLE

Sweep rate dependent cyclic voltammograms of cyt *c* and typical cyclic voltammetry and spectrophotometric analysis of sulfite in beer samples (method of standard additions). This material is available free of charge via the Internet at http://pubs.acs.org.

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