Coulometric and Chronoamperometric Studies on Bisulfite Reduction at a Surfactant/Myoglobin Film on Glassy Carbon Electrode

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Abstract: *Background*. The redox-active protein, myoglobin (Mb), exchanges electrons very slowly with bare electrodes. Just like on a highly oriented pyrolytic graphite (HOPG) bare electrode, electron transfer between myoglobin (Mb) and a bare glassy carbon (GC) electrode was not observed. The myoglobin (Mb) in di-dodecyl dimethyl ammonium bromide (DDAB) film immobilized on glassy carbon electrode surface had a good charge transport, allowing Mb to be used as a redox catalyst for multi-electron transfer reactions. *Objective*. We report here the myoglobin on a glassy carbon (GC) electrode as a catalyst for the multi-electron reduction of bisulfite in aqueous buffered solutions. *Methods*. Coulometry and chronoamperometry are used as tools to probe the Mb/DDAB film on GC electrode as an effective electro-catalyst for the multi-electron reduction of bisulfite. *Results*. Variation in current with time of bisulfite reduction followed first-order reaction kinetics. The heterogeneous electron transfer rate constant of the film and catalytic rate constant for the reduction of bisulfite were determined. *Conclusion*. The study confirmed that bisulfite was the reactive species and the catalytic reduction reaction at Mb/DDAB film followed the EC' catalytic mechanism.

Keywords: Mb/DDAB Films, Glassy Carbon (GC) Electrode, Electro-catalysis, EC' Catalytic Mechanism

1. Introduction

The redox-active protein, myoglobin (Mb), exchanges electrons very slowly with bare electrodes. Just like on a highly oriented pyrolytic graphite (HOPG) bare electrode [1], electron transfer between myoglobin (Mb) and a bare glassy carbon (GC) electrode was not observed. This behavior was very similar to that reported by Rusling et al. for the tin-doped indium oxide (ITO) and pyrolytic graphite (PG) bare electrodes with myoglobin (Mb) in solution [2-4].

Surfactant films have been shown to be quite effective in enhancing the electron transfer rates between electrodes and myoglobin (Mb) as these films behave electrochemically as a thin layer within which Mb is oriented [5]. Myoglobin is a small hemo-protein which plays an important role in oxygen transport. Mb contains a single iron heme as the prosthetic group. Myoglobin gives slow electron transfer rate at bare electrode surfaces [6-8]. Mb in DDAB surfactant films can be reduced reversibly in two one-electron steps. The first step is the $MbFe^{III}/Fe^{II}$ reduction, while the second step involves the formation of $MbFe^{I}$ species [9].

Lin et used Mb/DDAB to catalyze the multi-electron reduction of nitrite. Catalytic bisulfite reduction was achieved using a surfactant/protein film, though the details of the reaction mechanism were not studied extensively [10-11]. The catalytic reduction of bisulfite by a water-soluble iron porphyrin was reported by Kline et al. [12]. Limited studies of model iron porphyrins complexes with oxysulfur species have been reported [13-16].

Electrochemistry is a good tool to study redox mediated electrocatalysis and electrochemical methods are straightforward and also utilized to study modified electrodes electro catalysis [17-26].

Investigation of the multi-electron reduction of bisulfite at the potential of the second reduction wave of the Mb/DDAB catalyst, $MbFe^{II}/Fe^{I}$, using cyclic voltammetry, coulometry and

chronoamperometry was undertaken and is reported in this work.

2. Experimental

2.1. Materials and Solutions

Horse skeletal muscle myoglobin *from* Sigma was dissolved in pH 7.3 Tris buffer solution containing 50 mM KBr. Didodecyl dimethyl ammonium bromide (*DDAB, 99%*) was obtained from ACROS. Sodium bisulfite anhydrous was purchased from Sigma (99%), and used as supplied. Potassium bromide was obtained from Aldrich Chemical Company. The supporting electrolytes were 50 mM KBr or 100 mM KCl. The buffers were Tris (pH 7.3), phthalate (pH 5), phosphate (pH 6), phosphate (pH 8) and borate (pH 9). The pH of the buffers was adjusted with HCl or NaOH solutions. All other chemicals were of analytical grade. The water was purified by Nano pure System to a specific resistance > 18 MΩ.cm.

The working electrode was a DDAB/Mb modified glassy carbon electrode (GC disk, 3 mm in diameter, A = 0.071 cm^2, Cypress Systems Co.). Before use, the electrode was polished in order to remove any previous adsorbed monolayer and to regenerate the bare surface. Just before modification, the electrode was rinsed with distilled water in a sonicator and treated with 1:3:4 (v/v/v) solution of concentrated HNO$_3$-HCl-H$_2$O for 10-15 minutes in order to roughen the electrode so as to increase the amount of the immobilized biocatalyst. A 46.0 mg sample of DDAB was dispersed in 10 mL distilled water and ultrasonicated for at least 6 hours to obtain a clear solution. An 80.0 mg sample myoglobin, 9.7 mg of trizma base and 66.1 mg of trizma. HCl (Sigma) were dissolved in 10 mL distilled water (0.5 mM in myoglobin and pH 7.3 in Tris buffer). A 2μL DDAB aliquot and 2μL myoglobin were spread onto the glassy carbon electrode. The modified electrode was dried in air at room temperature for at least 2 hours. After use, it could be stored in air or buffer at room temperature.

2.2. Equipment

All cyclic voltammetry experiments were performed using a CS-1200 Cypress Systems potentiostat. Controlled potential electrolysis (Coulometry) and double-potential step (cyclic) chronoamperometry data were collected with a CS-1200 Cypress Systems software.

A three electrode electrochemical cell was used for the electrochemical experiments. The working electrode was a DDAB/Mb modified glassy carbon electrode (GC disk, d = 3 mm, Cypress Systems Co.). A semi-micro Ag/AgCl (saturated KCl solution) and a platinum disk (Cypress Systems Co.) were used as reference and auxiliary electrodes, respectively. All solutions were purged with dinitrogen gas, which was also used for stirring the electrolysis solutions.

3. Results and Discussion

3.1. Coulometry

Using cyclic voltammetric technique, the mechanism for

bisulfite reduction by Mb/DDAB/GC modified electrode has been reported [27] and the catalytic reduction of bisulfite is diffusion - controlled process.
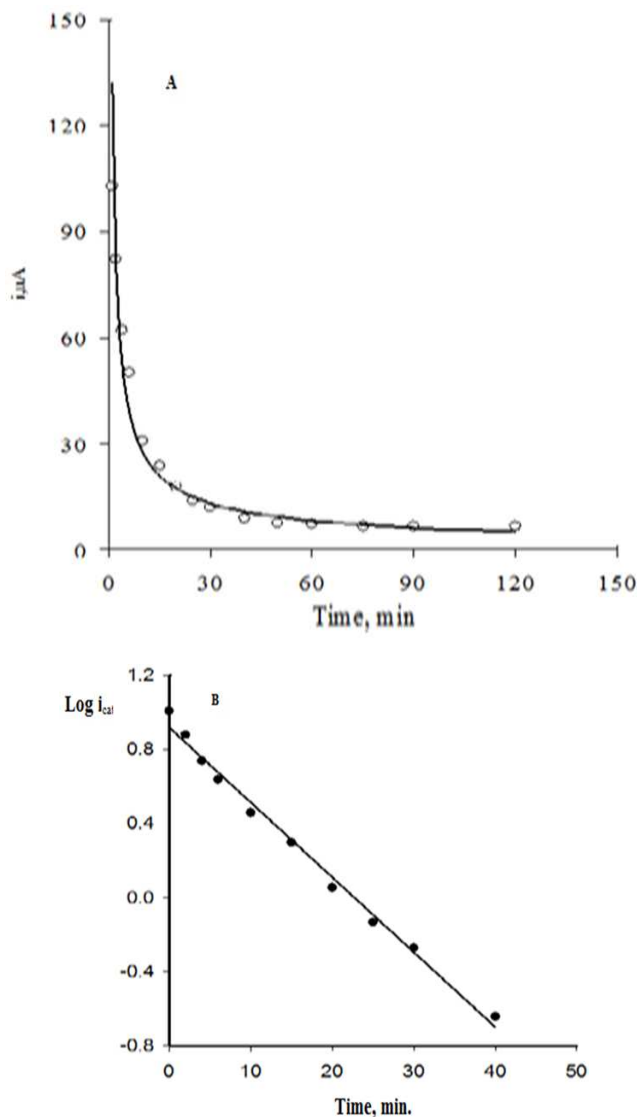
Figure 1. *(A) The current as a function of time for coulometry of 20 mM bisulfite in pH 7.3 Tris buffer solution containing 100 mM KCl using Mb/DDAB modified GC electrode at -1.300 V vs. Ag/AgCl at room temperature, and (B) plot of log catalytic current vs. Time.*

Controlled potential electrolyzes at potentials more negative than the second Mb wave potential (Mb/e^I) in different pH buffer solutions containing 100 mM KCl as a constant ionic strength at room temperature was carried out as in Figure 1A. The current decayed exponentially with time as expected for a diffusion/convection controlled electrolysis. The solid line was the best-fit line for a first order reaction with electrolysis parameter, p = 0.040 s^{-1}, where p = m$_{HSO3^-}$ A/V; m$_{HSO3^-}$ is the mass diffusion coefficient, A is the area of the electrode and V is the volume of the solution electrolyzed for bisulfite. The variation in current with time showed first-order reaction

kinetics (Figure 1B) typical for controlled - potential electrolysis.

3.2. Chronoamperometry

Bisulfite reduction at Mb/DDAB modified films on carbon electrodes in different buffer solutions were performed. The potential was stepped from 0.0 V to -1.300 V vs. Ag/AgCl and current-time curves were obtained. The potential-step chronoamperogramm at $\tau = 10$ s for 20 mM bisulfite in pH 5 phthalate buffer solution is shown in Figure 2A. Chronoamperometric catalytic currents with modified electrodes were much larger than those with bare carbon electrodes (data not shown). When the electron transfer rate to the film was fast compared to the bisulfite reduction kinetics (which was assured by choosing a potential well negative of the second wave potential of the Mb film), the catalytic current of bisulfite reduction has been shown to follow the following current-time equation [28-32]:

$$i_{cat} = n\,FA\,k_s\,C^* \exp[k_s\,t^{1/2}/D^{1/2}]^2\,\mathrm{erfc}[k_s\,t^{1/2}/D^{1/2}] \quad (1)$$

where k_s is the heterogeneous rate constant of the film. 20 mM bisulfite solutions are inspected with Cottrell plot. The Cottrell i- $t^{-1/2}$ plot for bisulfite reduction at pH 5 is shown in Figure 2B.

At longer experimental times, equation (4) reduces to Cottrell equation for bisulfite diffusion to Mb/DDAB./GC modified electrode [13, 14]:

$$i_{cat} = n\,FA\,D_{HSO3-}^{1/2}\,C_{HSO3-}^*/\pi^{1/2}\,t^{1/2} \quad (2)$$

At shorter experimental times, the electrode reaction is dominated by Mb reduction according to the Cottrell equation:

$$i = n\,FA\,D_{Mb}^{1/2}\,C_{Mb}^*/\pi^{1/2}\,t^{1/2} \quad (3)$$

The slope of the shortest experimental times linear segment (Figure 2B, $t^{-1/2} > 0.2$) gives D_{Mb} value of 1.85×10^{-6} cm.s^{-1} for the 2-electron reduction of Mb in DDAB film.

At longer experimental times, (Figure 2B, $t^{-1/2} < 0.1$) another linear segment appears. At such times, Mb reduction is complete and the rate of the electrolyzed bisulfite exceeds that of bisulfite diffusion from solution to the Mb/DDAB//GC modified electrode and equation (5) applies. Using Eq. (5), the slope of the diffusion controlled longer times segment gives n = 6 electrons for the catalytic reduction reaction of bisulfite assuming the diffusion coefficient of bisulfite is 10-fold greater than that of Mb in the DDAB film.

The intermediate experimental times, (Figure 2B, $0.1 < t^{-1/2} < 0.2$), corresponded to a transition from control of current by charge transport in the Mb/DDAB film to control of cuurent by diffusion of bisulfite from solution to Mb/DDAB/GC modified electrode and is non linear. The current-time form of the equation (4) can be inspected in several ways; we elected to use plots of $it^{1/2}$ vs. t, as in Eq. (4):

$$i\,t^{1/2}/nFAD^{1/2}\,C^* = \lambda\,\exp(\lambda)^2\,\mathrm{erfc}(\lambda) \quad (4)$$

Where $\lambda = (k_s\,t^{1/2}/D^{1/2})$ and the current is considered over the entire experimental time and the heterogeneous electron

transfer kinetics of the film is easily separated from the catalytic reaction kinetics. Using the known values of the electrode area, concentration and diffusion of bisulfite, the left-hand side of equation (7) can be calculated. The values of k_s can be determined by the best-fit between the right-hand side of equation (7) and its experimental left-hand side.
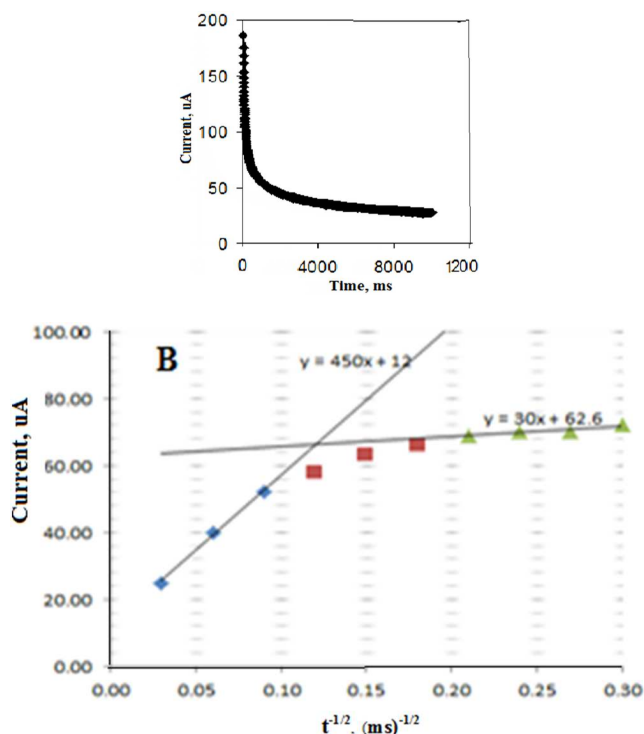
Figure 2. (A) Chronoamperogram of Mb/DDAB/GC modified electrode in the presence of 20 mM bisulfite at $\tau = 10$ seconds when potential was stepped from 0.0 V to - 1.300 V vs. Ag/AgCl in pH 5 phthalate buffer solution containing 100 mM KCl at room temperature. and (B) Cottrell plot: i vs. $t^{1/2}$, $(s)^{-1/2}$.
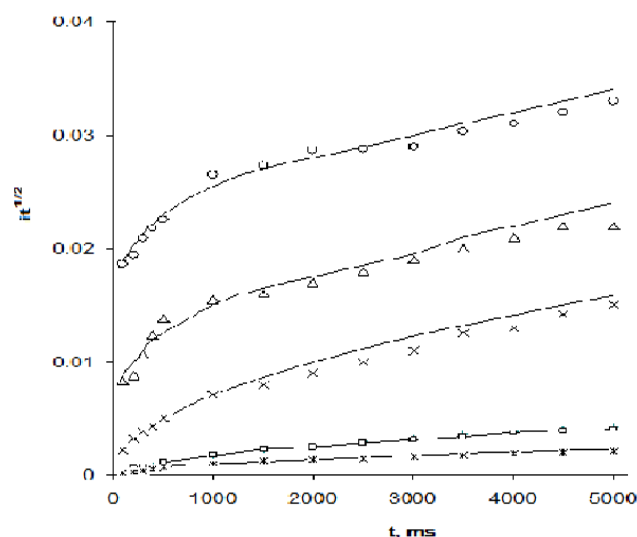
Figure 3. The $it^{1/2}$ vs. time (solid lines) from the experimental chronoamperometric data for the reduction of 20 mM bisulfite at a Mb/DDAB film modified on a pyro lytic graphite electrode for a potential step from 0 to -1.3 V vs.Ag/AgCl reference in different buffers (from top pH5, pH6, pH7, pH8, and pH9 respectively). The points are the best-fit between the right-hand side of Eq. 10 and its experimental left-hand side.

Figure 3 shows the chronoamperometric experimental currents plotted according to equation (7) together with the best-fit data points for electrocatalytic reduction of 20 mM bisulfite at Mb/DDAB/GC modified electrode in the different buffer solutions (pH 5-9). The values of k_s are calculated by the best-fit method and are tabulated in Table 1.

The catalytic rate constant for bisulfite reduction, k_{cat}, is related to the heterogeneous rate constant of the film, k_s, for Mb/DDAB film by the relation:

$$k_{cat} = 10^{-3} \times k_s / \Gamma_{Mb} \qquad (5)$$

Where: Γ_{Mb} is the surface coverage for the myoglobin in Mb/DDAB film on GC electrode in mol/cm^2 (Γ_{Mb} = Q / nAF). Based on a value of 2×10^{-11} mol Mb/cm^2 for surface coverage of Mb in the film calculated from the charge under the peak, (Q = 30×10^{-5} C) of the film, by cyclic voltammetry at 100 mVs^{-1} in the absence of bisulfite in solutions of buffers, values of k_{cat} in M^{-1} s^{-1} for the catalytic reduction of bisulfite in different buffers (pH 5-9) are calculated and are tabulated (Table 1). The variation in the catalytic rate constant values with pH confirms the only reactive oxysulfur species was bisulfite. Where: Γ_{Mb} is the surface coverage for the myoglobin in Mb/DDAB film on GC electrode in mol/cm^2 (Γ_{Mb} = Q / nAF). Based on a value of 2×10^{-11} mol Mb/cm^2 for surface coverage of Mb in the film calculated from the charge under the peak, (Q = 30×10^{-5} C) of the film, by cyclic voltammetry at 100 mVs^{-1} in the absence of bisulfite in solutions of buffers, values fur species.

Table 1. *The values of k_s calculated by the best-fit method of equation (10) and values of k_{cat} calculated by equation (11) for bisulfite reduction at Mb/DDAB films in different buffer solutions (pH 5-9).*

pH	k_s (s^{-1})	k_{cat} (M^{-1}s^{-1})
5.00	42 ± 0.3	(2.1 ± 0.02) x10^3
6.00	38 ± 0.1	(1.9 ± 0.01) x10^3
7.3	30 ± 0.2	(1.5 ± 0.02) x10^3
8.00	10 ±0.2	(0.5 ± 0.02) x10^3
9.00	4 ± 0.4	(0.2 ± 0.04) x10^3

4. Conclusion

Myoglobin in DDAB surfactant film formed on the surface of a glassy carbon electrode. The film was reduced reversibly in two one-electron steps. Moreover, the film electrode revealed a good catalytic behavior towards the multi-electron reduction of bisulfite. The kinetics of the reduction of bisulfite by Mb/DDAB surfactant film was examined using voltammetry techniques and values of k_s of the film and k_{cat} of the catalytic reaction were obtained at different pH values. The catalytic bisulfite reduction is an irreversible, diffusion-controlled, and pH dependent process. In addition, catalytic bisulfite reduction reaction by Mb/DDAB film followed EC' catalytic mechanism.

Future Work

Further electrochemical and spectroelectrochemical work are needed in order to identify intermediates in the reduction of bisulfite, and to further clarify the reduction mechanism.

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