Reduction of Bisulfite by Protein Films
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The reduction of bisulfite was studied at two types of protein films: surfactant film with myoglobin and a conjugate bilayer with *E. coli* sulfite reductase hemoprotein (SiR-HP). The *E. coli* SiR-HP was cross-linked with goat anti-rabbit IgG. The bilayer film was made by first deposit ing a solution of rabbit anti-goat IgG on the surface of a glassy carbon electrode. After the solution was allowed to sit for 2 hr in a water saturated atmosphere, it was immersed in a solution of gelatin, and then a solution of goat anti-rabbit IgG-SiR-HP. The surface coverage was verified by the use of ^{35}S analysis. The cyclic voltammetry of an electrode with one (curve a) and two (curve b) layers are shown in Figure 1. These results clearly show that the SiR-HP species can be directly reduced in the electrode film.

In the presence of bisulfite (20 mM), additional current was observed (curve c). It is interesting to note that two waves are observed in the presence of bisulfite, while only one was observed without substrate. This is consistent with the known redox potentials for the siroheme and 4Fe-4S clusters. In the absense of substrate, the two redox po tentials are close together ($\Delta E \approx 60$ mV), while the ΔE increases significantly in the presence of ligands. In addi tion, the additional catalytic current is mostly associated with the first wave (Fe^{III}/Fe^{II}), rather than the cluster wave. The catalytic current depended strongly on scan rate (Figure 2) and concentration of bisulfite. From the kinetic current, the rate of the catalytic reaction can be calculated.

The reduction of bisulfite was also examined using myoglobin in a surfactant film. A typical set of voltammo grams are shown in Figure 3 for 9 mM bisulfite at pH 7.4. No catalysis was observed for the first wave (Fe^{III}/Fe^{II}) but a rapid reaction was obtained for the second wave (Fe^{II}/Fe^{I}). A maximum rate for the catalytic reaction was observed between pH 6-7, with significantly lower rates at different pH values. The apparent rate constants for the reduction of bisulfite was measured over a pH range of 4-10.

Coulometric reduction of bisulfite in the presence of the surfactant myoglobin film was carried out. The con centration of bisulfite and possible products (e.g., sulfate, H_2S) were monitored during the electrolysis. From the overall stoichiometry and the voltammetric data, the rate and mechanism of the reduction of bisulfite by myoglobin was determined. It is interesting to observed that the electro catalytic reduction of bisulfite was much more efficient for the *E. coli* SiR-HP than for the myoglobin film. In the latter case, reduction only occurred for the Fe(I) species, while electrocatalysis was observed for the ferrous species in SiR-HP.

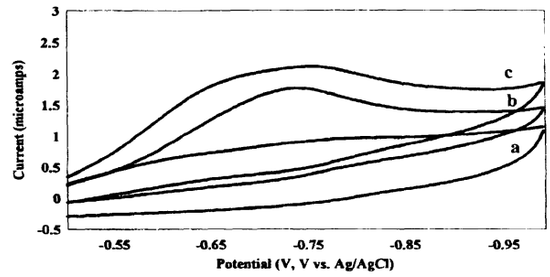

Figure 1. Cyclic voltammetry at a glassy carbon electrode. A) bilayer of IgG. B) bilayer of IgG-SiR-HP, C) curve b in the presence of 20 mM bisulfite. pH 7.7, scan rate = 60 mV/s

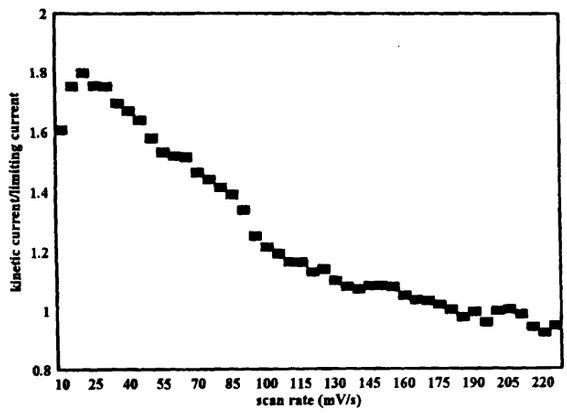

Figure 2. Ratio of the kinetic current to the limiting current as a function of scan rate. IgG-SiRHP bilayer. pH 7.7. 20 mM bisulfite. Glassy carbon electrode.

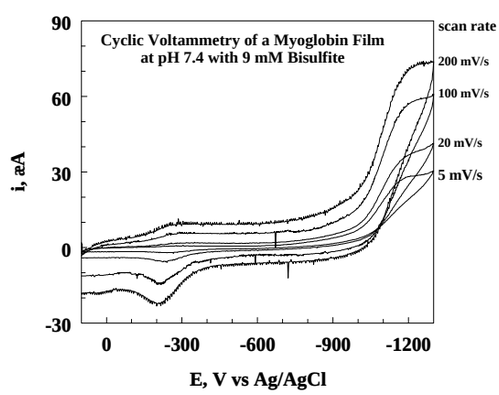

Figure 3