612bis—THIONINE AND FERRIC CHELATE COMPOUNDS AS COUPLED MEDIATORS IN MICROBIAL FUEL CELLS

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SUMMARY

The mediating effects of mixed mediator systems consisting of a series of ferric chelate compounds and thionine were studied in bio-fuel cells containing Escherichia coli. Significant increases in the coulombic output from the bio-fuel cells containing mixed mediators were observed compared with those obtained from the fuel cells containing single mediators. A correlation was observed between the cell voltage of a bio-fuel cell containing Fe(III)EDTA and thionine and the concentration of Fe(III)EDTA in the anode compartment, indicating that the concentration of the reduced form of ferric chelate was dominant in maintaining the cell voltage. The reduction of thionine by E. coli in the presence of Fe(III)EDTA, and that of Fe(III)EDTA by E. coli in the presence of thionine were measured. The results support a proposed mechanism involving a redox coupling reaction between Fe(III)EDTA and reduced thionine.

INTRODUCTION

In microbial fuel cells, electrons obtained from the oxidation of a substrate in the presence of micro-organisms are transferred to an electrode with the aid of an electron carrier, a so-called mediator [1]. The effectiveness of the mediator directly reflects the capabilities of microbial fuel cells. Our earlier work [2] showed that thionine and several other organic dyes were effective mediators, although they have several disadvantages such as limited solubilities in buffer solutions, strong adsorption on an electrode surface, etc.

In a previous paper [3], the requirements for a mediator were discussed, and several kinds of ferric chelate compounds were shown to be effective mediators. Although the rates of reduction of ferric chelate compounds by micro-organisms were much lower than that of thionine, the electrode reaction rates of these ferric chelate compounds were rather fast, and brought about comparable coulombic outputs from bio-fuel cells containing thionine or ferric chelate compounds as
mediators. Therefore, a combination of mediators consisting of thionine and ferric chelate compounds having coupled redox reactions for both efficient scavenging and delivering of electrons might have many advantages.

In this paper we describe experiments on the mediating effects of coupled mediator systems of thionine with a series of chelate compounds in fuel cells containing *Escherichia coli*. The chelating agents used were ethylenediaminetetraacetic acid (EDTA), 1,2-cyclohexanediamine-N,N′,N″,N‴-tetraacetic acid (CyDTA), diethylenetriamine-N,N′,N″,N‴-pentaacetic acid (DTPA), triethylenetetramine-N,N′,N″,N‴-pentaaacetic acid (TTHA), ethylenediamine-N,N′-diacetic acid-N,N′-dipropionic acid (EDAPDA), glycolethlenediaminetetraacetic acid (GEDTA) and nitrilotriacetic acid (NTA). Coulombic outputs from fuel cells containing mixed mediators were determined and compared with those obtained from fuel cells containing single mediators. The changes in concentration of Fe(III)EDTA in an anode compartment containing thionine and Fe(III)EDTA as mediators were measured polarographically as a function of time in a working cell, and correlated with the changes in the cell voltage. The reduction of Fe(III)EDTA by *E. coli* in the presence of thionine, together with that of thionine by *E. coli* in the presence of Fe(III)EDTA, was studied by means of polarography and spectrophotometry, respectively. In the light of these results, a mechanism for the coupled redox reactions in the bio-fuel cell is proposed.

**EXPERIMENTAL**

**Materials**

The procedures for growing and harvesting *Escherichia coli* K12 were as described previously [3]. Freshly prepared *E. coli* was used for all experiments. The chelating reagents (Dojin Kagaku) were complexed by mixing equivalent amounts with ferric ammonium sulphate in aqueous solutions. The structures of the chelating agents have been given in the previous paper [3]. Thionine (Eastman Kodak) was used without further purification.

**Microbial fuel cells**

The anode compartment of the bio-fuel cells contained *E. coli* (ca. 0.05 g dry weight), thionine (0.05–0.5 mM), a ferric chelate compound (1 or 10 mM) and glucose (10 μmol) in 0.1 M phosphate buffer solution of pH 7.0; the cathode compartment contained potassium ferricyanide (0.1 M) in the same buffer solution. Details of the construction and operating procedures for the bio-fuel cells were as described previously [3]. The bio-fuel cells were discharged through a known resistor for 24 h, and coulombic outputs were calculated from the integration of the current versus time plots.
**Polarography**

Reduction rates of Fe(III)EDTA by *E. coli* in 0.1 M phosphate buffer solutions of pH 7.0 at 30 °C in the presence of thionine were determined by means of polarography under anaerobic conditions. The apparatus and procedures for these measurements were as in the previous study [3]. The polarographic limiting current of Fe(III)EDTA and that of thionine were additive; the changes in concentration of Fe(III)EDTA in the bio-fuel cell under working conditions were monitored polarographically using a dropping mercury electrode directly inserted into the anode compartment containing *E. coli* (0.1 g dry weight), Fe(III)EDTA (1 mM), thionine (0.05 mM) and glucose (10 μmol).

**Spectrophotometry**

The reduction of thionine by *E. coli* in the presence of Fe(III)EDTA in 0.1 M phosphate buffer solutions of pH 7.0 at 30 °C was followed spectrophotometrically. The measurements were made in 3 cm³ cuvettes of 1 cm path length in a recording spectrophotometer (Shimazu UV-210A) at 30 ± 0.1 °C. The sample solution in the cuvette containing glucose (1 μmol), thionine (5–60 μM) and Fe(III)EDTA (0.1–2.0 mM) was purged with N₂ gas for 15 min through a rubber seal using syringe needles as gas inlets and outlets. A deaerated suspension of *E. coli* (0.5–5 mg dry weight in 0.1 cm³ of pH 7.0 buffer) was injected into the sample solution in the cuvette, and the decrease in the thionine absorbance at 600 nm with time was immediately recorded.

**RESULTS AND DISCUSSION**

*Microbial fuel cells containing Fe(III)EDTA and thionine as mediators*

Figure 1 shows the typical voltage versus time curves for microbial fuel cells in the presence of single or mixed mediators of thionine and Fe(III)EDTA. Bio-fuel cells with a single mediator failed to hold a high voltage under load, although the thionine mediator cell showed a high open-cell voltage (curves A and B). The presence of both thionine and Fe(III)EDTA in the anode solution, however, gave a significant improvement in performance as shown in curve C, where the cell voltage stayed around 0.4 V for several hours under load. Figure 2 shows the dependence of coulombic outputs on thionine concentration for the first 24 h delivered by fuel cells containing 10 μmol of glucose, thionine (0–1.0 mM) and *E. coli* in the presence or absence of 1 mM Fe(III)EDTA. The coulombic outputs at zero concentration of thionine were obtained from fuel cells containing no mediator (curve B) and 1 mM Fe(III)EDTA as a single mediator (curve A). It can be seen that the addition of 1 mM Fe(III)EDTA to the anode compartment of a cell containing only thionine doubled the coulombic outputs, demonstrating the effectiveness of the combination of thionine with Fe(III)EDTA. It is important to note, however, that although the
mediating effect of the combination in the range 0–0.2 mM thionine is larger than the total of the effects which would be expected from each mediator acting independently, there appears to be a levelling-off of the combined mediator effect at higher concentrations.

Fig. 1. Cell voltage versus time curves for microbial fuel cells. The arrow indicates when the cell was loaded (500 Ω). (A) 0.2 mM thionine; (B) 10 mM Fe(III)EDTA; (C) 0.2 mM thionine + 10 mM Fe(III)EDTA.

Fig. 2. Thionine concentration dependence of coulombic outputs of fuel cells containing E. coli (0.05 g dry weight), glucose (10 μM) and thionine (0–1.0 mM) with (curve A) and without Fe(III)EDTA (1.0 mM) (curve B).
The coulombic outputs of bio-fuel cells containing several ferric chelate–thionine mediators for the first 24 h are listed in Table 1 together with those for cells containing only ferric chelate compounds as single mediators for comparison. The figures show that although all the chelates appear to be effective at 1 mM concentration as mediators in combination with thionine, no further advantage is observed for the higher concentration of ferric chelate compounds.

A correlation between the voltage of a bio-fuel cell and the concentration of Fe(III)EDTA in the anode solution containing Fe(III)EDTA and thionine as a coupled mediator is shown in Fig. 3. At first, during the period in which the cell was left on open circuit, the cell voltage rose up to ca. 0.6 V, while the Fe(III)EDTA concentration decreased rapidly. The plot of $\log(C_{Fe(II)EDTA}/C_{Fe(III)EDTA})$ against the cell voltage during this period gave a straight line with a slope of 60 mV, which is

![Image](image_url)
approximately equal to the value from the Nernst equation assuming a one-electron transfer reaction. When the cell was placed under a 200 Ω load, the change in cell voltage directly reflected the change in concentration of Fe(III)EDTA. That is, the Fe(III)EDTA concentration at first gradually increased while the cell voltage gradually decreased, and then a sudden increase was observed, accompanied with a rapid decrease in the cell voltage after 2–3 h under load. These facts suggest that the cell voltage is mainly established by the concentration ratio of Fe(III)EDTA and Fe(II)EDTA. During the period when *E. coli* was metabolizing the glucose in the anode compartment, the reduction of Fe(III)EDTA apparently outpaced the electrode re-oxidation reaction, as indicated by the low concentration of Fe(III)EDTA accompanying the higher cell voltage under load. When most of the glucose had been consumed after 2–3 h under load, the reduction of Fe(III)EDTA was too slow to keep up with the electrode re-oxidation, which resulted in the increase in Fe(III)EDTA concentration.

*Reaction kinetics of thionine and Fe(III)EDTA as a coupled mediator*

Although both mediators can be reduced by *E. coli*, thionine is reduced over a hundred times faster than Fe(III)EDTA [3]. It is likely, therefore, that electrons obtained from the oxidation of glucose in the presence of *E. coli* are transferred mainly to thionine under the operational conditions of the cell. The reduced thionine is rapidly re-oxidized by Fe(III)EDTA, the rate of which has been shown to be very rapid (rate constant $k_2 = 4.8 \times 10^4$ mol$^{-1}$ dm$^3$ s$^{-1}$) by a stopped flow kinetic study [4]. Finally, the reduced chelate compound gives electrons to the anode by the electrode reaction of a Fe(III)EDTA/Fe(II)EDTA couple with a sufficiently large rate constant (standard rate constant $k_3 = 1.5 \times 10^{-2}$ cm s$^{-1}$) [3]. Considering the results of these rate studies, we proposed the following scheme for consecutive redox reactions in the anode compartment of the microbial fuel cell,

$$
\text{Fe(III)EDTA} \rightarrow \text{Fe(II)EDTA} \rightarrow \text{Thox} \rightarrow \text{Thred}
$$

where Thox and Thred stand for the oxidized and reduced forms of thionine, respectively. The rate of the overall electron transfer from *E. coli* to the anode is determined by the first step, i.e. the reduction of thionine by *E. coli*.

To test the proposed mechanism, the reduction of thionine by *E. coli* in the presence of Fe(III)EDTA, and that of Fe(III)EDTA in the presence of thionine were studied in phosphate buffer solutions of pH 7.0 at 30 °C. The change in concentration of thionine was followed spectrophotometrically, and that of Fe(III)EDTA was followed polarographically from the cathodic limiting current of Fe(III)EDTA at $-0.4$ V versus s.c.e.
Figure 4 shows an example of \((A_t - A_\infty)\) versus time plots for the reduction of thionine by \(E. coli\), where \(A_t\) and \(A_\infty\) are absorbances at time \(t\) and after completion of the reaction, respectively. In the absence of Fe(III)EDTA, thionine was reduced completely within a few minutes. On the other hand, in the presence of Fe(III)EDTA, induction periods were observed for the reduction of thionine by \(E. coli\) which were very marked at higher concentrations of Fe(III)EDTA.

The concentration change of Fe(III)EDTA is shown in Fig. 5, where the cathodic limiting current is plotted against time. A comparison of curves B in Figs. 4 and 5 which were obtained under the same experimental conditions shows that Fe(III)EDTA is being reduced at the same time as the reduction of thionine is.

Fig. 4. Reduction of thionine (20 \(\mu M\)) by \(E. coli\) (ca. 1 mg/cm\(^3\) dry weight) in phosphate buffer solutions containing different concentrations of Fe(III)EDTA: (A) 0.2 mM; (B) 1 mM; (C) 2 mM.

Fig. 5. Change in concentration of Fe(III)EDTA in phosphate buffer solutions containing Fe(III)EDTA (1 mM), \(E. coli\) (1 mg/cm\(^3\) dry weight), and different concentrations of thionine: (A) 5 \(\mu M\); (B) 20 \(\mu M\); (C) 60 \(\mu M\).
having an induction period. During the induction period, thionine reduced by \textit{E. coli} is apparently re-oxidized immediately by Fe(III)EDTA until most of the Fe(III)EDTA in the solution becomes reduced. After the induction period (e.g., after ca. 15 min for the example cited above), the oxidized form of thionine begins to decrease with a slope depending on the concentration of Fe(III)EDTA, as shown in Fig. 4.

In the absence of an anode, the reaction scheme (1) gives the following expressions for the concentration changes of Th$\text{ox}$ and Fe(III)EDTA, neglecting the contribution of backward reactions:

$$\frac{d[Th_{ox}]}{dt} = -k_1[Th_{ox}] + k_2[Fe(III)EDTA][Th_{red}] \quad (2)$$

$$\frac{d[Fe(III)EDTA]}{dt} = -k_2[Fe(III)EDTA][Th_{red}] \quad (3)$$

Considering the relative magnitude of rate constants $k_1$ and $k_2$, we can assume the condition $k_1 \ll k_2[Fe(III)EDTA]$ in the presence of an excess amount of Fe(III)EDTA.

During the induction period, the steady-state condition is satisfied with respect to Th$\text{ox}$:

$$\frac{d[Th_{ox}]}{dt} = 0 \quad (4)$$

Under these conditions, the concentration change of Fe(III)EDTA with time is given by the equation

$$\frac{d[Fe(III)EDTA]}{dt} = -k_1[Th_{ox}]_0 = -k_{Fe(III)EDTA} \quad (5)$$

where $[Th_{ox}]_0$ is the initial concentration of Th$\text{ox}$, and $k_{Fe(III)EDTA}$ is the apparent rate constant for the reduction of Fe(III)EDTA in solutions containing \textit{E. coli} and thionine.

![Fig. 6. Relationship between $k_{Fe(III)EDTA}$ and the concentration of thionine.](image)
The reduction of Fe(III)EDTA by \textit{E. coli} in the absence of thionine is extremely slow \cite{3}, but it was accelerated by the addition of thionine to the systems. This is shown in Fig. 5, where the current \textit{versus} time plots give steeper slopes in the presence of higher concentrations of thionine. Apparent rate constants, $k_{\text{Fe(III)EDTA}}$, were estimated from the initial slopes of these curves. A linear dependence of $k_{\text{Fe(III)EDTA}}$ thus obtained on the thionine concentration was observed as shown in Fig. 6, in agreement with equation (5). A linear relationship between $k_{\text{Fe(III)EDTA}}$ and the amount of \textit{E. coli} is expected from equation (5) because the rate constant $k_1$ is known to be proportional to the amount of \textit{E. coli} \cite{5}; this prediction was also confirmed experimentally.

The results of these rate studies, together with the evidence on the changes in concentration of ferric chelate in a working fuel cell, support the mechanism of coupled redox reaction proposed above.

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\textbf{REFERENCES}

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