Electroreduction of O$_2$ on Cytochrome c Oxidase Modified Electrode for Biofuel Cell
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Bovine cytochrome c oxidase is immobilized in a gold substrate-supported lipid bilayer membrane to investigate reduction of oxygen using flow through detection technique. The results shown that system temperature, pH and component of solution affect the electroreductive rate of oxygen. In each case the reduction rate of oxygen is controlled by electron transfer. The oxidase modified electrode can be used for the direct electroreduction of oxygen to water in a biofuel cell.

Introduction

Oxygen reduction plays a crucial role in electrochemical energy conversion and biological transformations. The reductive kinetics of oxygen is slow, even using the best catalyst, platinum [1-2]. The outcome of slow electron transfer kinetics at the cathode is a fuel cell with inadequate power density. Thus, there is a remarkable need for developing alternative, less expensive, and more efficient electrocatalysts. Because biocatalysts are catalytic at moderate temperature allowing for the use of inexpensive fuel cell component, they are an attractive alternative to transition metal catalysts. Therefore, the uses of biocatalysts in the cathode compartment of biofuel cells were investigated [3-4].

![Figure 1](image-url) Figure 1, schematic of a cytochrome c oxidase modified electrode (not drawn to scale).

In order to control protein orientation and mimic the native protein environment, this group [5] have developed an approach that couples the self-assembly of thiol-based adlayers with deoxycholate dialysis for the creation of oxidase containing lipid bilayer membranes that are supported on gold electrodes as shown in figure 1. The preparation of a modified oxidase electrode was described previously in detail. Briefly this involves the use of a vapor-deposited gold on a quartz crystal as the substrate. The surface is
derivatized with an octadecanethiolate submonolayer, which serves to anchor the bilayer to gold substrate. The lipid bilayer containing cytochrome c oxidase is formed through the process of deoxycholate dialysis. The large hydrophilic end of molecule containing cytochrome c binding site protrudes from the lipid bilayer membrane mimicking its conformation in vivo. The oxidase modified electrodes exhibit direct electron communication between cytochrome c oxidase and electrode substrate. The interaction between cytochrome c in solution and cytochrome oxidase on the modified electrode has been investigated [5, 11-13].

Dutton et al. [6] created films of cytochrome c oxidase on the gold electrode coated with 3-mercaptop-1-propanol and failed to support any detectable redox contact between electrode support and cytochrome oxidase for electroreduction of oxygen, implying that the distances between the oxidase redox cofactors in the adsorbed oxidase are too far away from the electrode to promote significant electron transfer rates. Willner et al. [4] also assembled a cytochrome c oxidase monolayer on the gold electrode without the base cytochrome c layer, and did not get bioelectrocatalytic activity towards the reduction of oxygen. Therefore, cytochrome c oxidase is immobilized in a gold electrode-supported lipid bilayer membrane in the absence of chemical modification/mediators to investigate reduction of molecular oxygen by sampled-current voltammetry here.

**Experimental**

Bovine cytochrome c oxidase was isolated from fresh beef hearts using the procedure of Soulimane and Buse [7]. The procedure used for the preparation of the DOPC/DOPE lipid (Avanti Polar Lipid, 99%)/deoxycholate solution was identical that described in previous studies [8]. Bovine cytochrome c oxidase modified electrodes were prepared by mixing equal volumes of the lipid/deoxycholate solution and 0.4 mg/ml cytochrome c oxidase in 0.001 M tris/HCl pH =7.6 and 0.1% triton X-100 solution. Lipid modified electrode was prepared with the lipid/deoxycholate solution. The 1 ml solutions mentioned above were injected into the sample chamber in the electrochemical dialysis cell in order to make the corresponding modified electrodes. The 0.1 M phosphate buffer (pH=7.4) was flowed through the chamber of the dialysis for 18 hours at a flow rate of 25 μl/min. The phosphate buffer was then flushed through the sample chamber for 20 hours at a flow rate of 5 μl/min. The corresponding modified electrodes were used as investigation of electroreduction of oxygen. The water used in the experiments was deionized and further purified using Milli-Q academic system (Millipore Corporation) to exhibit a resistivity of 18.2 MΩ/cm. All chemicals were of analytical grade.

The electrochemical cells and instruments used in these investigations were previously described in this laboratory [8, 9]. The oxidase modified electrodes and a large area of Pt wire were used as working and counter electrodes, respectively. The area of the working electrode is 0.196 cm². An Ag/AgCl/1M KCl electrode was used as reference electrode. All potentials were shown with respect to standard hydrogen electrode (SHE).

In the temperature experiments, the cell housing the modified electrode was placed in a thermostated oven where it was heated to different temperatures. Sampled-current voltammograms were constructed from chronoamperometric current-time transients recorded on the modified electrode at the flow rate of 50 μL/min. The current transient was obtained by stepping the electrode potential from the open circuit potential to the potential of interest; the current was sampled at 300 s following the application of each potential pulse. After the desired data were collected, the modified electrode was held for
at least 10 min at the open circuit potential. The complete sequence was repeated at another potential.

Results and Discussion

The typical sampled-current voltammograms at the flow rate of 50 µL/min on the oxidase modified electrodes in different pHs of 0.1 M phosphate buffer solution were shown in figure 2. When the 172 mV potential is applied, the small cathodic current was observed on the cytochrome oxidase modified electrode in each solution. The current on the cytochrome oxidase modified electrode increased obviously in the step potential range from 172 to −128 mV as the step potential decreased. The result suggests that the distance between cytochrome c oxidase and the electrode substrate is close enough to promote direct electron transfer for reduction of oxygen. To ascertain that the current observed in potential step experiments was caused by reduction of dioxygen by cytochrome c oxidase. The cell was thoroughly deoxygenated and then oxygen was reintroduced. After purged with high pure nitrogen for 30 min, the phosphate buffer solution was injected to make the cell anaerobic at the flow rate of 0.5 ml/min. After 20 min of this treatment, the magnitude of the current was severely diminished. But when oxygen was introduced to the phosphate buffer solution again, the current recovered to the same magnitude as the solution without nitrogen purging. The results confirmed that the electroreduction of oxygen was the process-taking place on the oxidase modified electrode. By the way, when the 22 mV potential is applied, the small cathodic current without the cytochrome oxidase is much smaller than that on the cytochrome oxidase modified electrode at the same step potential. The results further confirmed that the larger current results from the catalytic reduction of dioxygen by cytochrome c oxidase.
At the same step potential the current increases with the decrease of pH. At lower pH values, more protons would be available in solution for proton uptake, which may result in a bigger current. The results indicate that the cytochrome c oxidase immobilized on the lipid bilayer membrane retains proton-pumping capability. Malmstrom et al [10] studied the pH dependence of the steady state kinetic parameters of cytochrome c oxidase in detergent solution in the pH range from 5.4 to 8.4 with the stopped flow technique. The results shown that the catalytic constant increases continuously with decreasing pH of solution, which is in agreement with the change tendency of the sampled current with pH of solution in our potential step experiments.

The solution environment such as the concentration of the buffer strongly affects the direct electron transfer of cytochrome c oxidase. A typical dependence of the sampled current of oxygen reduction on concentration of phosphate buffer solution (pH=7.4) at the flow rate of 50 µL/min on the oxidase modified electrode was shown in Figure 3. In each solution (pH= 7.4) the current on the cytochrome c/cytochrome oxidase modified electrode increases monotonously as the cathodic bias increases. The reductive current of oxygen on the electrode declines upon increasing the concentration of phosphate buffer solution.

The solution component can also affect reduction rate of oxygen owing to the binding of anions. A typical dependence of the sampled current for electroreduction of oxygen on the solution component (0.1 M) at the flow rate of 50 µL/min on the oxidase modified electrode was shown in Figure 4. In each component the current on the oxidase modified electrode increases monotonously as the cathodic bias increases. The current decreases in the following order: Perchlorate > nitrate > phosphate.

The system temperature may affect the direct electron communication between cytochrome c oxidase and electrode substrate. A typical dependence of the sampled current for electroreduction of oxygen on the system temperature in 0.1 M phosphate buffer solution (pH=7.4) at the flow rate of 50 µL/min on the oxidase modified electrode...
was shown in Figure 5. At different temperatures the current on the oxidase modified electrode increases monotonously as the cathodic bias increases. The amplitude of the current at 37 °C is larger than that at 22°C as expected. Upon lowering the temperature to room temperature, the currents identical to those shown at 22 °C in figure 5 were obtained.

At different component, pH and ionic strength solutions and system temperatures the current of oxygen reduction on the oxidase modified electrode increases monotonously as the cathodic bias increases, and no current plateaus was observed in the potential window of interest. The results imply that the electrocatalyzed reduction of oxygen is limited by electron transfer. When the volume flow rate was changed in the range of 20 to 500 µl/min the sampled current at the same potential on the oxidase modified electrode is almost constant, which further confirms that the electroreduction of oxygen is limited by electron transfer. Although the direct electron transfer between protein and electrode substrate takes place, the protein architecture on the surface is probably in a non-optimized configuration.

Conclusion

The direct electron transfer for electroreduction of oxygen was observed on the modified electrode. The pH, component, ionic strength of solution and temperature affect reductive rate of oxygen. Although the direct electron transfer takes place on the oxidase modified electrode, the electroreduction of oxygen is controlled by electron transfer. Slow electron transfer is due to non-controlled immobilization, which traps the metalloprotein in non-optimal orientations. If the assembled protein to the electrode is optimized, the cytochrome c oxidase modified electrode has the potential use for the direct electroreduction of oxygen to water in the cathode compartment of a biofuel cell.

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References