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The Effect of Environmental Stresses on the Performance of Microbial Fuel Cells

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A study was performed to examine the effect of temperature and ethanolic stresses on the coulombic efficiency of a microbial fuel cell. It was found that the coulombic yields were altered by environmental stresses such as temperature shock or ethanol treatment to the bacteria. While high-temperature or ethanolic shock led to a remarkable decrement in coulombic output, the low-temperature shock induced a slight increase in microbial fuel cell efficiency, indicating that the membrane fluidity is considerably affected by the environmental stress, which in turn affects the electron transfer process through the bacterial cell membrane to and from the electrode. Cyclic voltammetric study with an electrode modified with the total lipids showed markedly different electrochemical behaviors depending on the environmental stress. A reciprocal relationship between coulomb output and the ratio of saturation/unsaturation of fatty acids has been observed.

Introduction

A microbial fuel cell is the biological device, which converts chemical energy into electrical energy, in which anodic electrode potential develops when electrons produced from the oxidation of substrates by microorganisms are transferred to the anode. In the development of a microbial fuel cell, especially notable is the work by Bennetto and coworkers since early 1980s. They not only tested a number of microorganisms and mediators in an effort to construct better fuel cells but demonstrated that an appreciable amount of energy could be available. Among tested dyes such as phenoxazine, phenothiazine, phenazine, indophenol, and bipyridium derivatives, thionin was found to be very effective in maintaining relatively high cell voltage. The high performance was achieved in combination of *P. vulgaris* as a microorganism, thionin as a mediator, and glucose as a substrate, where coulombic yield of 30-60% was obtained in a phosphate buffer at pH 7.

In this study, we had concentrated our standpoint on the mediator-membrane interactions. It has been well known that microorganisms vary their fatty acyl chain compositions for the maintenance of optimal membrane fluidity against the various growth conditions, which is so called the homeoviscous adoptation. On the basis of this mechanism, we demonstrated that the mediator-bacterial membrane interaction is a decisive factor controlling coulombic efficiency in a microbial fuel cell.

Experimental

Preparation of Microorganism

Proteus vulgaris (ATCC 6059) was obtained from the culture collection of KCCM (Korean Culture Center of Microorganisms) and kept on a nutrient agar plate at 4 . Experimental cultures were grown aerobically at 37 in a nutrient broth containing 3 g of beef extract and 5 g of peptone per liter. Various temperature (10, 25, 46) shocks or different ethanol treatments (0.5, 1.0, 3.0%) were applied for 3 h at the mid log phase of the microorganisms. After the shock treatments, each bacterial cell was harvested by centrifuging at 3,000 × g for 5 min and washed twice with 0.05 M phosphate buffer solution (pH 7.0). The washed microorganisms were resuspended in the same phosphate buffer solution to give 20 mg (dry wt.) per ml for the experiments.

Structural Analysis of Fatty Acyl Components of Membrane Lipids

Experiments were performed on harvested whole cells by treatment with methanolic HCl to prepare fatty acid methyl esters. Cells suspended with 0.5 mL of chloroform and 1.5 mL of 5% methanolic HCl solution were sealed in a teflon-lined screw-capped vial and heated in a dry oven at 72 for 24 h. Chloroform (3 ml) was added every 8 h, followed by mild sonication for 5 min. After concentration to dryness under nitrogen gas, samples were partitioned between water and chloroform, and the aqueous layer was washed several times with chloroform or hexane. The combined solutions were filtered through the glass wool. Prepared fatty acid methyl esters were subjected to the gas chromatography analysis on a 25 M J&W scientific DB1 column using nitrogen as the carrier gas. The fatty acid identification and molecular weight were determined by GC/MS.

Cyclic Voltammetric Analysis of Thionin in a Lipid Film

Total lipid extracts were prepared by using a modified Bright-Dyer method from the *P. vulgaris* cells, which were treated by different environmental shocks. The purified lipids were diluted with chloroform to give 10 mg/mL for the experiment. A half (0.5) mM solution of thionin was prepared with 0.05 M phosphate buffer solution (pH 7.0) using 18 MΩ deionized water. The cast layer of the lipid was formed by applying the measured volume of lipid solution onto the glassy carbon electrode and allowed to dry. Thus, prepared electrode was transferred into the deaerated 30 mL of thionin solution immediately. Lipids used for the control experiments were extracted from *P. vulgaris* cultured at 37 without shock treatment. A glassy carbon electrode with an area of 0.8 cm^2 was used as a working electrode.

Results and Discussion

Effects of the Environmental Stresses on the Bacterial Membrane Composition and Coulombic Output in the Microbial Fuel Cell

Fig. 1 shows coulombic responses of the microbial fuel cell containing *P. vulgaris* against the temperature shocks. Upon thermal shock treatment, coulombic yield was significantly altered. When the bacteria were subjected to a cold shock at 10 and 25, coulombic output were changed just slightly (+0.04 and +0.07 C, respectively). However, with a high-temperature shock at 46℃, coulombic output was declined extremely (-0.44 C). This result could be explained by the change of the bacterial membrane fluidity, not with a thermal damage to it. Because *P. vulgaris* is able to grow up even at this high temperature and some cellular thermal damages could be protected by the various heat shock proteins. Temperature-induced alterations in fatty acid compositions are known to play a significant role in the thermal compensation of membrane fluidity. Temperature effects on the composition of the membrane fatty acids have been thoroughly studied for *E. coli*. The configuration of unsaturated carbons (*cis*- or *trans*-) or the hydrocarbon chain length of fatty acids are

known to be responsible for temperature adaptive changes, because configurations of unsaturated carbons and increased chain length influence the packing density of membrane lipids and hence the membrane fluidity. Effects of ethanolic shock on the microbial fuel cell efficiency were also investigated in the same manner as temperature shock. The potency of ethanol as an inhibitor of a cellular function is directly related to its hydrophobicity as measured by the partitioning of ethanol between aqueous and hydrophobic environments, and the detrimental effects of ethanol on bacterial cells appear to result from colligative effects of ethanol rather than from damage via a specific receptor. Enteric bacteria such as *E. coli* are relatively sensitive and grow very little in ethanol concentrations above 6 % by volume. However, under 4% concentration, their growth was not significantly inhibited by the ethanol treatment. Concerning the lethal effect of high concentration ethanol treatment upon bacteria, we carried out the experiments below this upper limit value.

Overall results are summarized in Table 1. Obvious decrements in coulombic output were observed when the high-temperature or ethanolic shock was applied. This information gives an explanation for the importance of the mediator-membrane lipid interaction in a microbial fuel cell operation. The alteration in fatty acyl chain composition is the most frequently observed response to various growth conditions in microorganism. One of the most common responses of Gram-negative bacteria to high temperature shock appears to be an increase in the degree of fatty acid saturation. Saturated fatty acids are packed more compactly due to their conformational property, and show much higher melting points than their unsaturated homologs. The adaptive response to ethanol treatment in the enteric bacteria is to increase the proportion of nonpolar lipids and average fatty acid chain length. Therefore, the ratio of saturated fatty acids to unsaturated fatty acids can be used as a parameter for the bacterial membrane fluidity. Our experimental results illustrate cold shock treatment induces increasing of mediator-permeability in *P. vulgaris*, while the ethanolic or high-temperature shock treatments make the membrane more rigid so that it lowers the mediator-permeability through the membrane. Obvious reciprocal relationship between the S/U ratio and the coulombic output was found.

Table 1. Effects of thermal and ethanolic stress on the degree of saturation of the fatty acids and coulombic output variations in *P. vulgaris*.

	Temperature shock				Ethanolic shock			
	10 ℃	25 ℃	37 ℃	46 ℃	0%	0.5%	1.0%	3.0%
[a]S/U ratio	1.84	1.75	2.27	2.60	2.28	1.94	2.59	2.88
Coulombic output	0.73	0.76	0.69	0.25	0.71	0.64	0.48	0.40

Electrochemical Behavior of Mediator in the Lipid Film

Fig. 2 shows the successive cyclic voltammograms of thionin depending on the stresses inside the lipid film, which is composed of total lipid extracts isolated from *P. vulgaris*. In the control experiment (Fig. 2A), thionin shows well-defined redox peaks corresponding to two-electron process. The gradual accumulation of thionin molecules into the lipid film is clearly observed from an increase of voltammetric peaks as the scan is repeated. This phenomenon was explained by the hydrophobic interaction between the lipid film and thionin. The long alkyl chains of lipid molecules provide a hydrophobic environment for thionin to go into the film. Thionin inside the film is still electrochemically active, transferring electrons through the lipid layer. Highly charged species such as $Fe(CN)_6^{3-}$, in the mean time, are excluded by the lipid layer, giving very low current because of their hydrophilic nature. Almost invariant voltammograms were obtained after heat treatment at 25 , showing the lipid structures were not affected. This explains why the discharging pattern of a fuel cell

was almost identical (Fig. 1). A dramatic change, however, has occurred after the temperature shock at 46 (Fig. 2B). Almost no thionin redox peaks were present, indicating that thionin molecules are excluded by the lipid layer. These results finely coincide with the very low efficiency of the fuel cell at this temperature (Fig. 1). The similar pattern of cv was observed after ethanolic shock.

The effect of these environmental shocks on the fuel cell operation and electrochemical behavior of thionin could be explained by the compositional change of the fatty acids in response to the adaptive change of membrane fluidity. Heat or ethanolic shocks induced the high ratio of saturated per unsaturated fatty acids of bacterial membrane lipids, which caused thionin less permeable to the inside of the cell. Rigid lipid molecules also form a tightly packed layer on the electrode surface, through which thionin hardly penetrates.

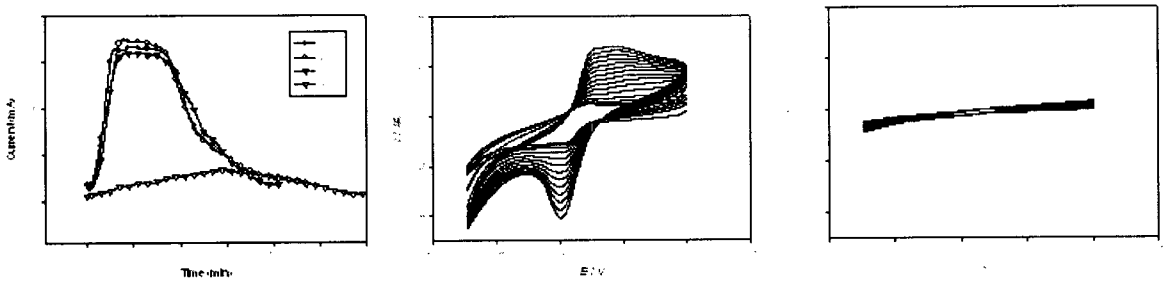

Fig. 1 (left). Variation of current output with time through the 560-Ω external load for fuel cells containing *P. vulgaris* with different temperature shock treatment. Cyclic voltammograms of 0.5 mM thionin at a glassy carbon electrode coated with a total lipid film (4.3×10^{-7} mol/cm^2) of *P. vulgaris* with control (middle) and 46 °C temperature shock treated (right).

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