given in much lower yield when isopropyl group was used instead of *t*-butyl group (Table 3).

In conclusion, three factors are important in the nitrolysis of *N*-alkly protected amine; NO_2^+ concentration (nitrating power), basicity of the amine, and the carbocation stability of the leaving alkyl group. Among them,the influence of basicity is not quite understood. In synthetic point of view, the nitrolysis can be applied to the cyclic compound having other nitro groups in the cyclic skeleton, as in the preparation of TNAZ. Otherwise the reaction proceeds in much lower yield.

Experimental

Caution. Some of the compounds reported herein should be handled with appropriate care. Most of the compounds were previous reported.

General procedure for the nitrolysis. To 20.2 mL (0.143 mol) of TFAA was added 6.4 mL (0.143 mol) of 100% HNO_3, cooling being required to maintain the temperature at -5 °C to 0 °C. This solution was cooled to -20 °C and amine (0.00204 mol) was added cautiously. The resulting solution was stirred for 1-2 hr, and poured into ice-water. The aqueous mixture was extracted with ether several times.The ether extracts were dried and concentrated in vacuo to give a nitramine.[9]

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9. N-Nitropiperidine[2a]; ^1H NMR ($CDCl_3$) 1.54-1.81 (m, 6H), 3.80 (m, 4H). N-Nitromorpholine[2a]; ^1H NMR ($CDCl_3$) 3.85 (s). ^{13}C NMR ($CDCl_3$) 48.6, 65.1. TNAZ[3]; mp 101 °C. ^1H NMR ($CDCl_3$) 5.2 (s).

Electrochemical Behavior of Safranine O in a Thick Lipid Film

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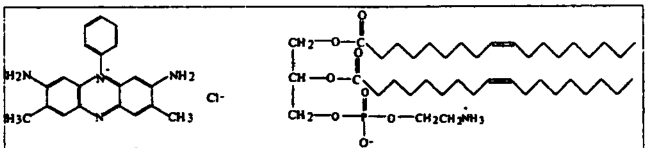
During last two decades, much attention has been paid to the microbial fuel cell[1-3] as a means of direct energy conversion. Electricity is produced by confining living microorganisms in an anodic compartment of the cell and feeding them with substrates. Electrons yielded from the catabolic action of microorganisms are transferred through the cell wall to the anode, and thus electrical energy can be drawn with a suitable cathode. Microbial fuel cells are promising as future energy sources because they are very environment friendly and the operation with a high efficiency is possible.[4,5] For example, electricity can be obtained during the waste treatment. Other than this, many advantages[6] over conventional methods have been pointed out. Among efforts that optimize the cell operational conditions, it has been found that a remarkable enhancement[7,8] in the efficiency can be achieved by adding redox mediators in an anodic part of the cell. The role of the mediator is believed to help electrons produced from the oxidation of substrates to be transferred to the anode, with itself undergoing a redox reaction. Although many have attempted to find suitable mediators and tested cell performance with them, the basic research on the interaction between mediators and lipids which consist of cell membranes is scarce. Only Bennetto *et al.*[9,10] measured rates of reduction of phenothiazine derivatives by *E. coli* and NADH as far as we know.

In this paper we report our preliminary results on the electrochemical behavior of safranine O(SFO) in a thick film of phosphatidylethanolamine (PE) as a model system for the study of mediator-lipid interaction. To our knowledge, there have been no reports on this subject although Petrova *et al.*[11] studied redox properties of phenazine-derivatives such as phenazine ethosulfate, phenosafranine and safranine T. SFO is a well known staining agent[12] in a Gram test and its redox potential is negative enough so that an appreciable cell potential could be achieved in combination with a suitable cathode. Moreover, the strong absorption of light in a visible region makes it possible to follow microbial activities spectrophotometrically. PE is known as a main constituent of cell membranes.



Experimentals

PE dissolved in chloroform (99%, 10 mg/mL) was purchased from Sigma and used as received. Whenever necessary, the solution was diluted with chloroform. SFO was purchased from Aldrich and used without further purification. 1 mM solution of SFO was prepared from 0.1 M phosphate buffer (pH 7) using 18 Mohm deionized water. All other reagents were of the best quality available. The cast layer of PE was formed by applying a measured volume of PE solution onto the glassy carbon (GC) electrode and allowed to dry. Thus prepared electrode was transferred into the deaerated SFO solution immediately. The conventional three-electrode system (BAS100BW) was used to record voltammograms. GC was subjected to polishing with 0.05 alumina until mirror finish and ultrasonication for 30 sec everytime new sets of data were acquired. All the experiments were done under a room temperature and atmospheric pressure.

Results and Discussion

Figure 1 shows cyclic voltammograms of 1 mM SFO at GC immersed in a 0.1 M phosphate buffer. The first scan (curve a) gives well-defined cathodic (A, B) and corresponding anodic peaks (A', B'). The repeated cycling continuously increased A' and moved B' a little in a positive direction, however, cathodic peaks were not much affected. This is more evident from potential-holding experiments at -1.0 V where SFO is fully reduced (curves b-d). With an increasing accumulation time, A' continued to grow and move positively while the magnitude of B' became smaller. This observation may indicate that there exist two different states of the film on the electrode surface whose exact nature is not certain at the moment, and the state B' is transformed to the state A' as charge is injected into the film. Peak A could arise from the adsorption or capacitance change due to the molecular reorientation on the surface. The film growth with charge injection shows that reduced SFO molecules are easily associated with each other to form a conductive film. The charge in the reoxidation process was found approximately proportional to the accumulation time. The fact that the only reduced form accumulates indicates that the charge on a molecule plays an important role in deciding molecular association. Once reduced, a SFO becomes uncharged, capable of being associated with other SFO molecules while positively charged SFOs are not easily associated due to the electrostatic repulsion. This view is supported by experiments in an acidic solution (0.5 M sulfuric acid) where no accumulation of SFO has been observed although SFO was fully reduced. Protonation in a strong acidic solution causes SFO more positively charged and prevents SFO moleules from being associated even when reduced. This property is typical of phenothiazine- or phenazine-derivatives. For example, methylene blue ((dimethyl)aminophenothiazinium chloride) is also accumulated on platinum or gold electrodes to form a thicker film.[13,14]

Figure 2 shows the behavior of SFO inside a PE film. 3 μL of PE/chloroform (5×10^{-7} mol/cm^2) was applied to the GC surface and allowed the solvent to be evaporated. Despite some loss of PE due to the deposition on the shroud of the electrode, a thick PE layer could be discerned even with naked eyes. The gradual incorporation of SFO molecules into the film is clearly seen from an increase of voltammetric peaks as the scan continues, reaching at a steady-state value eventually (*ca.* 2×10^{-8} mol/cm^2). Comparing with the case of no PE film, what is peculiar is that peaks remained well separated as the cycling was repeated and the charge involved in the cathodic process was com-

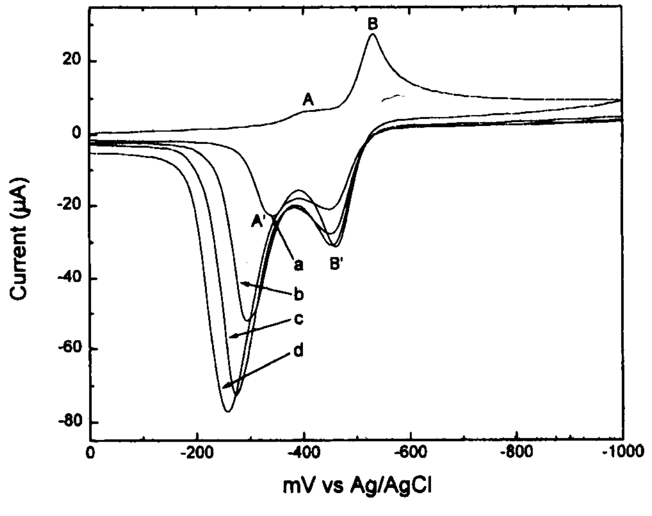


Figure 1. Cyclic voltammograms of 1 mM SFO on GC in a phosphate buffer (pH 7) depending on a generation time at -1.0 V, 0 sec (a), 30 (b), 60 (c), 120 (d). Scan rate: 50 mV/sec; Electrode area: 0.080cm^2.

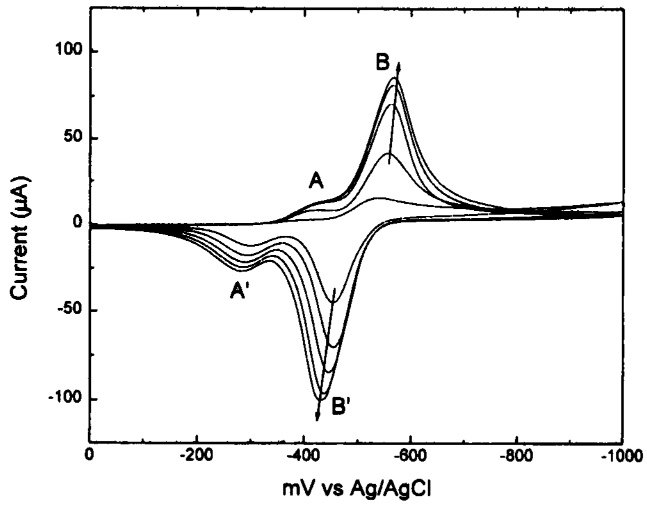


Figure 2. Cyclic voltammograms of SFO inside PE film (5.0×10^{-7} mol/cm^2) in a phosphate buffer containing 1 mM SFO. Scan rate: 50 mV/sec.

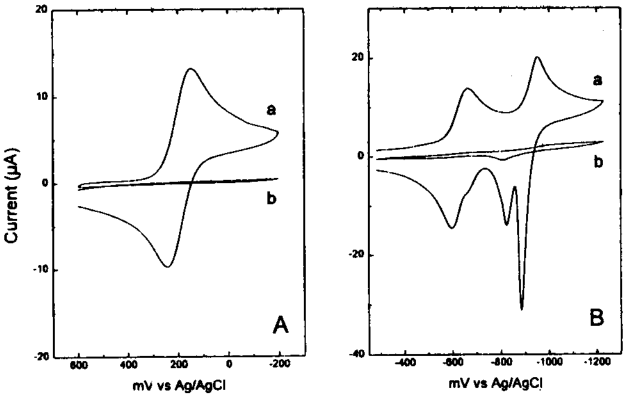


Figure 3. Voltammetric response of 1 mM $Fe(CN)_6^{3-}$ (panel A) and ethyl viologen (panel B) in a phosphate buffer (pH 7) with (curve b) and without (curve a) PE film (5.0×10^{-7} mol/cm^2) on the electrode surface. Scan rate: 50 mV/sec.

parable with that in the anodic one, indicating reoxidized SFO did not leave the film but confined within the PE matrix. This also implies that the interaction between SFO molecules is minimal in the film and thus each molecule behaves independently still maintaining electrochemical activity. The easy incorporation of SFO could be explained by the hydrophobic interaction between PE and SFO. Despite amphiphilic nature of PE, two long alkyl chains of a PE form a hydrophobic environment for SFO to go into the film. SFO in the PE film is still electrochemically active, transferring electrons through the film. To confirm our reasoning, a series of control experments have been done. Experiments with a highly charged inorganic species, $Fe(CN)_6^{3-}$ in the presence of the film gave very low currents, compared to the bare GC electrode (panel A in Figure 3). A highly ionic species is hydrophilic and therefore excluded by the lipid layer and electrons cannot be tunneled to that species outside the film. The same results were also obtained with a relatively hydrophilic organic molecule, ethyl viologen (panel B in Figure 3). Short alkyl chains do not provide much hydrophobicity to the molecule, thus preventing it from being partitioned into the film. The pH effect on hydrophobicity was also investigated. In 0.5 M sulfuric acid, almost no faradaic current was observed, which suggests the hydrophobic interaction was not being operated any more because of protonation of PE as well as SFO, while in 0.1 M NaOH the similar incorporation was observed. The similar observation has been reported by Kaifer and coworkers[15] for the study of phosphatidylcholine and organic molecules. They varied the chain length of alkyl groups of viologen and ferrocene to give different extent of hydrophobicity to the molecule. As the chain length becomes longer, more incorporation was observed. In our experiments, hydrophobicity could be controlled by adjusting a solution pH, not by changing the chain length. It was found, however, that SFO was not physically strongly held in the PE matrix from the fact that the transfer of the electrode covered with PE and SFO into a fresh supporting electrolyte resulted in an appreciable loss of faradaic current, indicating SFO gradually diffues out into the solution if no SFO is present.

Our results may imply some important aspects for the application to the microbial fuel cell. When selecting mediators, their interaction with lipids should be considered and that can be easily done by considering hydrophobicity. In this sense, SFO could be a good candidate as a redox mediator for the fuel cell application. It is easily reduced at ordinary electrodes such as GC and readily incorporated into the PE layer without losing any electroactivity. Good voltammetric characteristics at pH 7 also offers beneficial effects, for most microorganisms are active at this pH. The elucidation of the exact nature of each voltammetric peak and spectroscopic properties of SFO in the PE film is in progress and will be reported in due course.

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