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Electrogeneration of Superoxide Ion on Thiophenol-Modified Gold Electrode in Aqueous Solution

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1 INTRODUCTION

Superoxide ion (O_2^-) is the primary component of the so-called reactive oxygen species (ROS) that are produced in biological respiration and metabolism, and it is believed that this free radical ion may be closely related to a number of biological phenomena such as aging, disease, cancer and inflammation. In an attempt to gain better understanding of the biomolecular reactivity of O_2^- , we have been investigating its electrochemical and homogeneous redox behavior using molecularly modified electrodes¹⁻⁵⁾. This communication reports on the preliminary results concerning the electrogeneration of O_2^- via one-electron reduction of dioxygen O_2 at thiophenol-modified gold electrode in aqueous media.

2 EXPERIMENTAL

Electrochemical measurements were performed at laboratory temperature ($20 \pm 2^\circ C$) using a BAS 100B/W electrochemical system and a rotating electrode system (Nikko Keisoku Co.). An Ag/AgCl (saturated KCl) electrode was used as the reference electrode and a Pt wire as the counter electrode. A gold disk electrode (diameter: 1 mm) served as the working electrode. It was, before use, polished with 0.3 μm alumina powder (Marumoto Kogyo Co.) on a

microcloth wetted with Milli-Q water, carefully sonicated in water, rinsed with water, and finally air-dried. Surface modification was carried out by dipping the freshly polished electrode into a thiophenol/methanol (1/10 (volume ratio)) solution for 3 h. Irreversible modification of the surface was checked by measuring double-layer capacitance, which is lower than that of a bare clean Au electrode.

A Hitachi spectrophotometer U-3300 was used along with a quartz cell with light-path length of 1 cm to measure UV-Vis spectra of the reduced form of cytochrome *c*, which was produced by the redox reaction between its oxidized form and the electrogenerated O_2^- .

Bovine erythrocyte copper-zinc superoxide dismutase (SOD, 3000 units mg^{-1}) and thiophenol were purchased from Wako Pure Chemical Industries. Cytochrome *c* from horse heart (purity > 99 %) was obtained from Sigma. Water was purified by passage through a Milli-Q purification train. Other chemicals were of reagent grade and were used as received.

3 RESULTS AND DISCUSSION

Figure 1 shows the typical steady-state voltammograms for O_2 reduction at the bare and thiophenol-modified rotating disk Au electrodes in O_2 -saturated 0.5 M KCl solutions containing 0.1 mM ($M = mol\ dm^{-3}$) NaOH (pH 10) in the absence and presence of SOD. The voltammograms with half-wave potential around $-0.25\ V$, which were obtained at the unmodified Au electrode, correspond to the two-electron reduction of O_2 to H_2O_2 . At the thio-

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phenol-modified Au electrode the voltammograms shifted by ca. 0.2 V to the negative direction of potential, and at a given electrode rotation rate the limiting current (i_{lim}) was smaller compared with that at the unmodified electrode. At the thiophenol-modified electrode, an addition of a small amount of SOD enzyme (0.5 μM) into the electrolyte solution resulted in a significant increase in i_{lim} , which is likely due to the O_2^- dismutation(disproportionation) by SOD. SOD is a metallo-enzyme which efficiently catalyzes the dismutation of O_2^- to O_2 and H_2O_2 via a cyclic oxidation-reduction mechanism,⁶⁾

The SOD-catalyzed dismutation of O_2^- is almost diffusion-limited and the reaction rate is independent of pH in its range from 7 to 9.5⁷⁾. Thus, the data in Fig.1 suggest the electrogeneration of O_2^- via one-electron reduction of O_2 at the thiophenol-modified

Au electrode. Here it should be noted that the i_{lim} value obtained at the modified electrode in the absence of SOD exceeds that expected when the O_2 reduction moves from two-electron process to one-electron process (i.e., one half of the i_{lim} value at the unmodified electrode) by about 30–40%. This excess value is by far too large to correspond to the regeneration of O_2 from the pH-dependent spontaneous dismutation of O_2^- (its rate constant is ca. $10^3 \text{ M}^{-1}\text{s}^{-1}$ at pH10⁸⁾).⁹⁾ The contribution of the spontaneous dismutation of O_2^- to an increase in i_{lim} is calculated to be only a few percents at pH 10⁹⁾. Such an excess i_{lim} value is probably due to the further reduction of O_2^- with the intervention of water molecule acting as a proton donor:

as in the case of the electrogeneration of O_2^- at the mercury electrodes with a hydrophobic adsorption layer of quinoline and its derivatives^{1–5,10)}. The reaction(2) is thermodynamically more favorable than the first electron-transfer step:

The electrogeneration of O_2^- at the thiophenol-modified Au electrode could be confirmed from the observation of the absorption spectra of the ferrous cytochrome $c(\text{cyt}.c(\text{Fe}^{2+}))$ which is produced by the reduction of its ferric form($\text{cyt}.c(\text{Fe}^{3+})$) with O_2^- ;

This reaction has been widely used for in vitro O_2^- assay in which the amount of the produced $\text{cyt}.c(\text{Fe}^{2+})$ can be sensitively monitored at ca. 550 nm.¹¹⁾ The related experimental results are shown in Fig.2, where the constant potential electrolysis at -0.5 V was carried out at the thiophenol-modified rotating disk Au electrode in O_2 -saturated 0.5 M KCl + 0.1 mM NaOH solution(pH10) containing 1 μM $\text{cyt}.c(\text{Fe}^{3+})$ for 30 min, and then absorption spectra of the electrolysis solution were measured. The new specific α, β absorption bands around 520 and 550 nm are significantly larger compared with that in the

Fig.1 Typical steady-state voltammograms for the O_2 reduction at the bare(1,2,3) and thiophenol-modified(1',1'',2',2'',3',3'') rotating Au disk electrodes in O_2 -saturated 0.5 M KCl + 0.1 mM NaOH solution (pH10) in the absence(1,1',2,2',3,3') and presence(1'',2'',3'') of 0.5 μM SOD. Electrode rotation rate: (1,1',1'')400, (2,2',2'') 900 and (3,3',3'') 1600 rpm. Potential scan rate: 5 mVs^{-1} .

Fig.2 Visible absorption spectra of (A,C) O_2 or (B) N_2 -saturated 0.5 M KCl + 0.1 mM NaOH solution containing cyt.c(Fe^{3+}) ((A,B) $1\mu M$, (C) $2.5\mu M$) before(---) and after(—) the electrolysis with (A,B) the thiophenol-modified and (C) unmodified Au electrodes at -0.5 V vs. Ag/AgCl for 30 min. The electrode was continuously rotated at 400 rpm during the electrolysis.

blank experiment under N_2 atmosphere(Fig.2(B)). In this case, the red shift of Soret band around 415–425 nm was, as expected¹²⁾, observed (not shown here). The absorption spectrum of Fig.2(A) was essentially the same as that observed for the cyt.c(Fe^{2+}) solution which was prepared by reducing the cyt.c(Fe^{3+}) solution with sodium dithionite. Thus, these facts demonstrate that cyt.c(Fe^{2+}) is produced via the mediated reduction of Eq.4 and that the direct reduction of cyt.c(Fe^{3+}) does not actually occur at the thiophenol-modified Au electrode. In addition, it is also obvious from Fig.2(C) that the reduction of cyt.c(Fe^{3+}) by H_2O_2 , which is produced by two-electron reduction of O_2 , as well as its direct electrode reduction are almost negligible under the present experimental conditions. Finally, it should be mentioned that the rate constant (ca. $3 \times 10^{-2} M^{-1}s^{-1}$ ¹³⁾) of the backward reaction of Eq.4 is smaller about seven orders of magnitude than that ($1.5 \times 10^5 M^{-1}s^{-1}$ ¹⁴⁾) of the forward reduction. The slowness of the oxidation of cyt.c(Fe^{2+}) by O_2 is very important from the point of view of its role in vivo, because it should react with cytochrome *c* oxidase but not with O_2 , which is

present in its surroundings.

In conclusion, we could for the first time observe the electrogeneration of O_2^- via one-electron reduction of O_2 at the surface-modified Au electrode in aqueous media. The thiophenol layer on the Au electrode surface is considered to function as a hydrophobic layer which makes difficult the access of water molecules to the electrogenerated O_2^- ion. The similar studies have been previously carried out exclusively using mercury(or mercury-coated Pt and Au) electrodes with hydrophobic surfactants^{1-5,7,15)} and in the only one case using carbon paste electrodes¹⁶⁾. A related study using other electrode surface modifiers is in progress.

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