Electrogeneration of Superoxide Ion at Polyaniline-modified Electrodes in Aqueous Media

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Received June 18, 2001 ; Accepted July 18, 2001

Electrogeneration of superoxide ion (O_2^-) with polyaniline (PAn)-modified indium-tin oxide (ITO) electrodes has been examined in alkaline aqueous media. The PAn-modified electrode showed electrocatalytic activity for O_2 reduction. Specific reactions of cytochrome *c* (cyt. *c* (Fe^{3+})), nitroblue tetrazolium salt analogue (WST-4) and superoxide dismutase (SOD) with O_2^- have been successfully applied to confirm the electrogeneration of O_2^- . The results demonstrate that the PAn-modified electrode is useful for the electrogeneration of O_2^- via a redox-mediated reduction of O_2 in alkaline aqueous media.

Key Words : O_2 Reduction, Superoxide Ion, Polyaniline, Superoxide Dismutase

1 Introduction

Recently, it has been recognized that superoxide ion (O_2^-) may convert to most of other reactive oxygen species as a primary precursor, and that many of biological phenomena such as aging, inflammation and various diseases may be attributed to direct or indirect action of O_2^- on biological tissues.¹⁾ The electrogeneration of O_2^- at electrodes is a useful tool in the elucidation of the reaction of O_2^- in a living body and, especially, kinetic analysis of fast biochemical reactions between O_2^- and physiologically important substances, because an arbitrary amount of O_2^- can be supplied quickly to the system examined. Our group has demonstrated that O_2^- is generated by one-electron reduction of molecular oxygen (O_2) on the electrode covered with hydrophobic layer using quinolines²⁾ and thiophenol³⁾ in aqueous alkaline solutions. Recently, Morita⁴⁾ reported that adding chemically synthesized polyaniline (PAn) powder into O_2 -saturated water produced H_2O_2 via a disproportionation of O_2^- generated by one-electron transfer reaction from the reduced PAn to O_2 . This report has encouraged us to study the O_2^- generation at PAn-modified electrodes in aqueous solutions. In this study, we examined the O_2^- generation via a redox-mediated reduction of O_2 at PAn-modified indium-tin oxide (ITO) electrodes in aqueous media.

2 Experimental

Aniline (Kanto Chemical Co.) was of reagent grade, and was used after purification. Cytochrome *c* (cyt. *c* (Fe^{3+})) from horse heart (cyt.*c*; type VI, purity >99%) and superoxide dismutase (SOD) from bovine erythrocyte (SOD; EC. 1. 15. 1. 1, copper-zinc type, 3800 units

mg⁻¹) were purchased from Sigma Chemical Company and Wako Pure Chemical Industries, respectively. WST-4,⁵⁾ a novel analogue of nitroblue tetrazolium salt, was available from Dojin Chemical Company. All other chemicals were of reagent grade. PAn was prepared from 10 mM aniline solution containing 0.1 M sodium dodecylbenzenesulfonate (SDBS) by scanning repeatedly the electrode potential between -0.2 and 1.0 V vs. Ag | AgCl | KCl (sat) at 50 mVs⁻¹ for 50 times. The reduced form of PAn was obtained by the reduction treatment of the electrochemically synthesized PAn with hydrazine monohydrate for a week.⁶⁾

3 Results and Discussion

3. 1 O_2 reduction at PAn-modified ITO electrodes

Figure 1 shows the typical cyclic voltammograms obtained at bare and PAn (film thickness: 10 nm)-modified ITO (0.5 cm², 10 Ω cm⁻¹) electrodes in O_2 or N_2 -saturated 0.1 M $Na_2B_4O_7/NaOH$ buffer solutions (pH 9.4). The redox response obtained at the PAn-modified electrode under N_2 atmosphere is very broad, but it is apparently distinguishable from the blank response obtained at the bare electrode. Under O_2 atmosphere much larger cathodic current was observed at the PAn-modified electrode than at the bare electrode and at the same time the anodic current corresponding to the reoxidation of PAn decreased remarkably, as can be seen from the comparison of Figs. 1-(b) and (c). Therefore, the O_2 reduction at the PAn-modified ITO electrode was considered to proceed electrocatalytically. Such an electrocatalytic O_2 reduction at the PAn-modified ITO electrode was not observed in acidic and neutral solutions.

Fig. 1 Typical cyclic voltammograms recorded at (a) bare and (b, c) PAN-modified ITO electrodes in 0.1 M $\text{Na}_2\text{B}_4\text{O}_7/\text{NaOH}$ buffer solution (pH 9.4) under (a, c) O_2 and (b) N_2 atmosphere. The potential sweep rate is 50 mVs^{-1} .

Scheme 1 Schematic depiction of electrogeneration of O_2^- at PAN-modified electrode and its detection reactions. PAN (Ox) and PAN (Red) represent the oxidized and reduced forms of PAN, respectively. Reactions (1), (2) and (3) represent the reduction of O_2 by the PAN (Red), the reduction of cyt. c (Fe^{3+}) (or WST-4) by O_2^- and the spontaneous and SOD-catalyzed disproportionation of O_2^- , respectively.

3. 2 Detection of electrogenerated O_2^- by cyt. c and WST-4

The O_2^- generation based on the O_2 reduction at the PAN-modified ITO electrode was examined using ferric cyt. c (Fe^{3+}) and WST-4 (scheme 1). The reductions of cyt. c (Fe^{3+}) and WST-4 with O_2^- are well known to result in the specific absorption spectra of cyt. c (Fe^{2+}) and the reduced form (formazan) of WST-4 at *ca.* 550 nm ⁷⁾ and *ca.* 537 nm ,⁵⁾ respectively. The measurement of these absorption spectra has been frequently made to confirm the generation of O_2^- *in vivo* and *in vitro* systems.

Figure 2 shows the visible absorption spectra of cyt. c (Fe^{3+}) solution which was electrolyzed at -0.5 V for 1 h using the bare and PAN-modified ITO electrodes under O_2 or N_2 atmosphere. The strong absorption peak characteristic of cyt. c (Fe^{2+}) was clearly observed at 550 nm when the cyt. c (Fe^{3+}) solution was electrolyzed at the PAN-modified electrode under O_2 atmosphere. In addition, in this case, the intensity of the absorption peak at 550 nm was found to largely depend on the presence and the absence of SOD enzyme, which efficiently catalyzes the disproportionation of O_2^- to O_2 and H_2O_2 ,⁸⁾ in the electrolytic solution. That is, the intensity was, as expected, higher in the absence of SOD than in its presence (see

Fig. 2 Typical visible absorption spectra obtained before (a) and after (b, c) the electrolysis of 0.1 M $\text{Na}_2\text{B}_4\text{O}_7/\text{NaOH}$ buffer solution (pH 9.4) containing $8 \mu\text{M}$ cyt. c (Fe^{3+}) and 5 mM EDTA with (A) PAN-modified and (B) bare ITO electrodes under (a, c) O_2 and (b) N_2 atmosphere. The electrode potential in each electrolysis was kept at -0.5 V vs. Ag | AgCl | KCl (sat) for 1 hr.

Fig. 3 Typical visible absorption spectra recorded after the electrolysis of O_2 -saturated 0.1 M $\text{Na}_2\text{B}_4\text{O}_7/\text{NaOH}$ buffer solution (pH 9.4) containing $8 \mu\text{M}$ cyt. c (Fe^{3+}) and 5 mM EDTA with PAN-modified ITO electrode in the absence (a) and presence (b) of $0.5 \mu\text{M}$ SOD. The electrode potential in each electrolysis was kept at -0.5 V vs. Ag | AgCl | KCl (sat) for 1 hr.

Fig. 3). The absorption at 550 nm was also observed, even though weak, when the electrolysis was carried out at the PAN-modified electrode under N_2 atmosphere (Fig. 2A-b) and at the bare electrode under N_2 and O_2 atmosphere (Figs. 2B-b and c). This may be due to the direct reduction of cyt. c (Fe^{3+}) on the electrode surface. These results conclude that O_2^- can be generated via an electroreduction of O_2 at the PAN-modified ITO electrode in weak alkaline aqueous solution. In other words, the reduced form of PAN can function as an effective one-electron reducing agent for O_2 . The generated O_2^- ions spontaneously disproportionate into O_2 and H_2O_2 . Thus, the above-mentioned detection reaction of O_2^- using cyt. c (Fe^{3+}) competes with the spontaneous disproportionation of O_2^- .

To further confirm the electrogeneration of O_2^- at the PAN-modified electrode, the following experiment was carried out. That is, we examined the reactions of O_2

Fig. 4 Visible absorption spectra of (A) cyt. *c* (Fe^{3+}) and (B) WST-4 solutions before (a) and after (b) 65 mg of PAn powder (reduced form) was added into O_2 -saturated 0.1 M $\text{Na}_2\text{B}_4\text{O}_7/\text{NaOH}$ buffer solution (pH 9.4) containing 20 μM cyt. *c* (Fe^{3+}) (or WST-4) and 5 mM EDTA.

with the reduced form of the electrochemically synthesized PAn powder using cyt. *c* (Fe^{3+}) and WST-4 in the same aqueous solution as that used above. The results are shown in Fig. 4. When the reduced form of the PAn powder was added into O_2 -saturated solutions containing cyt. *c* (Fe^{3+}) and WST-4, the absorption peaks characteristic of cyt. *c* (Fe^{2+}) and the formazan of WST-4 could be clearly observed at *ca.* 550 and 537 nm, respectively. Of course, such characteristic absorption peaks could not be obtained when the reduced form of the PAn powder was added into N_2 -saturated solutions of cyt. *c* (Fe^{3+}) and WST-4. These results along with the ESR data reported by Otsuka *et al.*⁴⁾ support the above-mentioned conclusion.

4 Conclusions

The O_2^- generation by the electroreduction of O_2 with

the PAn-modified ITO electrode in alkaline aqueous media was investigated. The PAn-modified electrode showed the electrocatalytic activity for O_2 reduction. The efficient generation of O_2^- via a redox-mediated reduction of O_2 at the PAn-modified electrode could be confirmed by use of indicator methods with cyt. *c* (Fe^{3+}), SOD and WST-4. The PAn-modified electrode can be used for the electro-generation of O_2^- in alkaline aqueous media.

Acknowledgements

The present work was financially supported by Grant-in-Aids for Scientific Research (No. 12875164) and Scientific Research (A) (No. 10305064), for T. Ohsaka, from the Ministry of Education, Science, Sports, Culture and Technology, Japan.

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