Functional Polymer-Modified Electrode Prepared by Electrolytic Polymerization of o-Nitroaniline

Yoshiharu NAKAMURA*, Jing-Yuan CHENG, Isao TABATA, Shin-ichiro SUYE and Mitsugi SENDA

Received June 15, 1994; Accepted August 29, 1994

1 INTRODUCTION
Regeneration of pyridine nucleotide coenzyme (NAD(P)H) is important for carrying out enzymatic syntheses of useful compounds by using NAD(P)-linked dehydrogenases. A number of ways to regenerate reduced cofactor have been proposed and tested: chemical, enzymatic, and electrochemical methods. Although the electrochemical method has an advantage of not requiring specific substrates, there is no effective regeneration system of NAD(P)H regeneration, because large overpotential is necessary to reduce NAD(P)− and inactive dimer of NAD(P)H is formed in the direct electrode reaction. Usually, for electrolytic regeneration of NAD(P)H a mediator and an oxidoreductase are used to decrease overpotential and avoid dimerization of NAD(P)H. Also, immobilization of the mediator as well as the enzyme and coenzyme on electrode surface is necessary to eliminate the contamination of the system by these substances.

This study is an attempt to prepare a new polymer-modified electrode, on which these substances can effectively be immobilized: the electrolytic polymerization by electro-oxidation and -reduction of o-nitroaniline in acidic solution was examined to prepare an amino group-carrying polymer-modified electrode.

2 EXPERIMENTAL
2.1 Preparation of the polymer-modified electrodes and immobilization of viologen derivative onto them
A basal pyrolytic graphite (BPG) electrode was fabricated from graphite rod (Megachem Co.), and glued into a teflon cap. The geometrical area of the electrode surface was 0.071 cm². A Pt plate and a saturated calomel electrode (SCE) were used as the counter and reference electrodes. The electrode potential was referred to SCE. Poly (o-nitroaniline)-modified electrode was obtained by electrolysis in a solution containing 0.01 mol·dm⁻³ o-nitroaniline and 1.0 mol·dm⁻³ H₂SO₄ as described in Results and Discussion below.

Viologen covalently bound to poly (aminoaniline) modified electrode was prepared by soaking polymer-modified electrode in 0.01 mol·dm⁻³ 1-methyl-1'-bromobutyl-4,4'-bipyridinium iodide bromide 0.1 mol·dm⁻³ Tris-HCl buffer (pH7.5) solution at 30°C for 12 h. 1-Methyl-1'-bromobutyl-4,4'-bipyridinium iodide bromide was synthesized from 4,4'-bipyridine (85 mmol, 13.1 g) and methyl iodide (via 1-methyl-4,4'-bipyridinium iodide); An equivalent mixture of the two compounds in acetonitrile was refluxed for 20 h at 70°C. The residue was obtained after rotary evaporation of acetonitrile and washed with acetonitrile. Then, this
intermediate compound (1-methyl-4,4'-bipyridinium iodide) and 1,4-dibromobutane were dissolved in methanol and refluxed at 63°C for 24 h. The residue was washed with methanol and after removal of solvent, the desired compound was obtained. The products was dried in vacuo. The overall yield was 32\%.

3 RESULTS AND DISCUSSION

3.1 Preparation of poly o-nitroaniline film coated electrode

At first it was tried to prepare poly (o-nitroaniline) film electrode by means of constant potential electrolysis of o-nitroaniline in aqueous sulfuric acid. When o-nitroaniline was subjected to the electro-oxidation at +1.3 V in 1.0 mol·dm⁻³ H₂SO₄, the anodic current rapidly decayed to decrease almost to zero after a few minutes and no appreciable polymerization took place on the electrode surface. This is probably due to the electron-attracting effect of nitro groups. Then, cyclic voltammograms of o-nitroaniline in 0.1 mol·dm⁻³ H₂SO₄ was investigated with a disc BPG electrode (Fig. 1). When the electrode potential was swept from +1.5 to -1.0 V, a cathodic peak current was produced at Ep = -0.5 V and, at the same time, the anodic peak remained not disappearing in the succeeding cycles, indicating that the electrolytic polymerization can take place by the repetition of electro-oxidation and reduction in o-nitroaniline solution. Therefore, o-nitroaniline was subjected alternatively to electro-oxidation at +1.5 V for 10 sec and electro-reduction at -0.5 V for 50 sec. As the electrolysis proceeded for 1 h, an cohesive black film was formed on the surface of BPG electrode. The polymer-modified electrode was further subjected to electro-reduction at -0.5 V to convert the residual nitro groups to amino groups on the polymer. The IR spectra of the polymer showed the absorption bands at 3300 and 1630 cm⁻¹ assignable to NH₂ group. The absorptions at 3130, 1570 and 815 cm⁻¹ were the well-documented spectra bands characteristic of aromatic compound. Although the absorptions based on nitro
group at 1510 and 1345 cm⁻¹ were observed for o-nitroaniline, these absorption bands were not detected for PAA. ¹H-NMR spectra also supported the presence of amino groups (data not shown). Therefore, reduced poly (o-nitroaniline) structure can be expressed poly (o-aminoaniline), (PAA). PAA obtained with given conditions was hardly soluble in typical organic solvent (eg. dimethyl sulfoxide, acetonitrile, tetrahydrofuran, alcohols, ethers).

3.2 Viologen immobilized electrode

Viologen group was introduced to the polymer on electrode surface by treating the amino group-carrying polymer-modified electrode with 1-methyl-1'-bromobutyl-4,4'-bipyridinium iodide bromide at 30°C for 24 h. Fig. 2A shows cyclic voltammograms of the viologen-immobilized polymer-modified electrode; two cathodic-anodic peaks at -0.72 and -1.15 V were observed, indicating the formation of electrochemically active viologen group on the polymer layer on the electrode surface. In fact, in the presence of NAD⁺ the height of the two cathodic peaks assignable to viologen group were enhanced: the height increased with increasing concentration of NAD⁺ (Fig. 2B), indicating that NAD⁺ can catalytically be reduced at about -0.9 V on the viologen-immobilized PAA film-modified electrode. On the other hand, viologen derivative could not be immobilized to the polymer from electro-oxidation of o-phenylenediamine. Its cyclic voltammogram did not show peaks observed on viologen. This is due to the fact that PAA was not prepared by electro-oxidation of o-phenylenediamine owing to the formation of phenazine ring.

These results indicate that the electro-polymerized PAA film-modified electrode has unique properties that the PAA has free amino acid groups carrying chemical reactivity to various compounds such as mediator, coenzyme and enzyme. Application of the PAA electrode to the construction of electrochemical reactor systems with an NAD(P)H recycling device is under study in this Laboratory.

The authors wish to thank Ministry of Science Culture of Japan for Grant-in-Aid for Scientific Research on Priority Areas (No. 05235105).

REFERENCES