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Electrocatalytic Oxidation of NADH on Thin Poly(acrylic acid) Film Coated Graphite Felt Electrode Coimmobilizing Ferrocene and Diaphorase

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A thin poly(acrylic acid) film coated graphite felt electrode was chemically bonded with aminoferrocene and 2-aminoethylferrocene, and the properties of the latter bonded electrode (El 1) showed better electron transfer than the former bonded one. Then, El 1 was further bonded with diaphorase (Dp) to construct ferrocene (Fc)- and Dp-comodified electrode (El 2). El 2, which is stable and transfers electrons smoothly, was successfully used for the preparative and selective electrocatalytic oxidation of NADH to NAD^+, being capable of repeated use without any appreciable inactivation.

An electrode of large surface area and modified with electroactive functional groups is effective to preparative and advanced organic electrosynthesis. For this purpose, we have been interested in the use of a graphite felt (GF, 0.7 m^2/g) coated with a thin poly(acrylic acid) (PAA) layer.[1] The carboxylic acid groups of the PAA layer can be bonded with various kinds of functional compounds by amido and ester linkages, and the attached electroactive functional groups are easy to migrate and to transfer electrons in the network of the PAA layer.[2] One of the present authors (Osa) studied electrocatalytic oxidation of NADH using ferrocene (Fc) derivatives as mediator in solution.[3,4] Further electrocatalytic acceleration of NADH oxidation was carried out by Matsue and coworkers[5-7] on diaphorase (Dp) modified electrode in the presence of ferrocene derivatives in solution. In this communication, we report the first study on the characteristics of PAA coated electrode coimmobilizing Fc and Dp and application of the electrode to macro-electrocatalytic oxidation of NADH.

GF electrode (National Electric Carbon Corp., WDF, 5.0 x 2.0 x 0.5 cm^3) was coated with PAA (MW : 1400000) by immersion in a 0.25% methanol solution. This coated electrode was then treated with 40 mM (M=mol·dm^{-3}) of aminoferrocene[8] or 2-aminoethylferrocene[9] in dimethylformamide (DMF) in the presence of dicyclohexylcarbodiimide (DCC) (1.2 equiv. to aminoferrocene or 2-aminoethylferrocene) for 12 hours at 4 °C and 60 hours at room temperature. The electrodes were then crosslinked with 7 mM of hexamethylenediamine in DMF in the presence of DCC (2 equiv. to hexamethylenediamine) for 12 hours at room temperature. Thus, the carboxyl groups of polymer layers on the GF electrodes were attached to aminoferrocene (42%) or 2-aminoethylferrocene (46%) and crosslinked with hexamethylenediamine (36 and 33%, respectively). The free carboxylic acids of both electrodes were 21-22%.[10] The density of Fc-amido and Fc-ethylamido groups was 10.5 and 11.5 $\mu mol/cm^3$, respectively. The oxidation potentials of the Fc-ethylamido-modified GF electrode (El 1) and the Fc-amido-modified electrode were almost the same of +0.24 and + 0.23 V vs. SCE (Fig. 1), but the former electrode (El 1) gave larger current in cyclic voltammetry (CV)

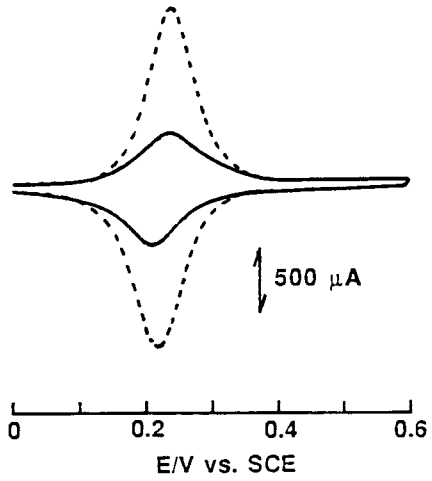


Fig. 1. Cyclic voltammograms of Fc-modified GF electrodes (1.0 x 1.0 x 0.5 cm^3) in phosphate buffer (pH 7.0) at the scan rate of 50 mV/s. ——: ferrocenyl-amido modified, ·····: ferrocenylethyl-amido modified.

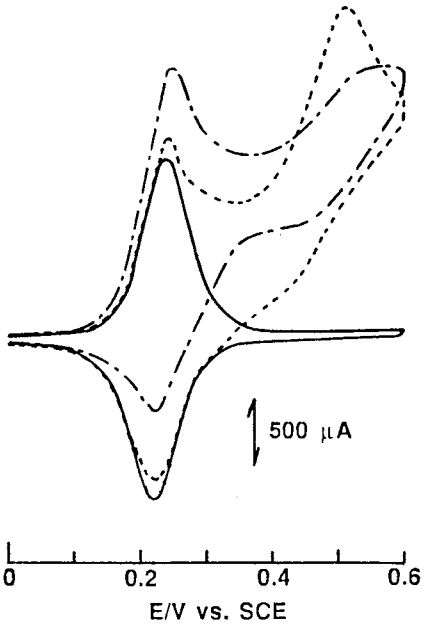
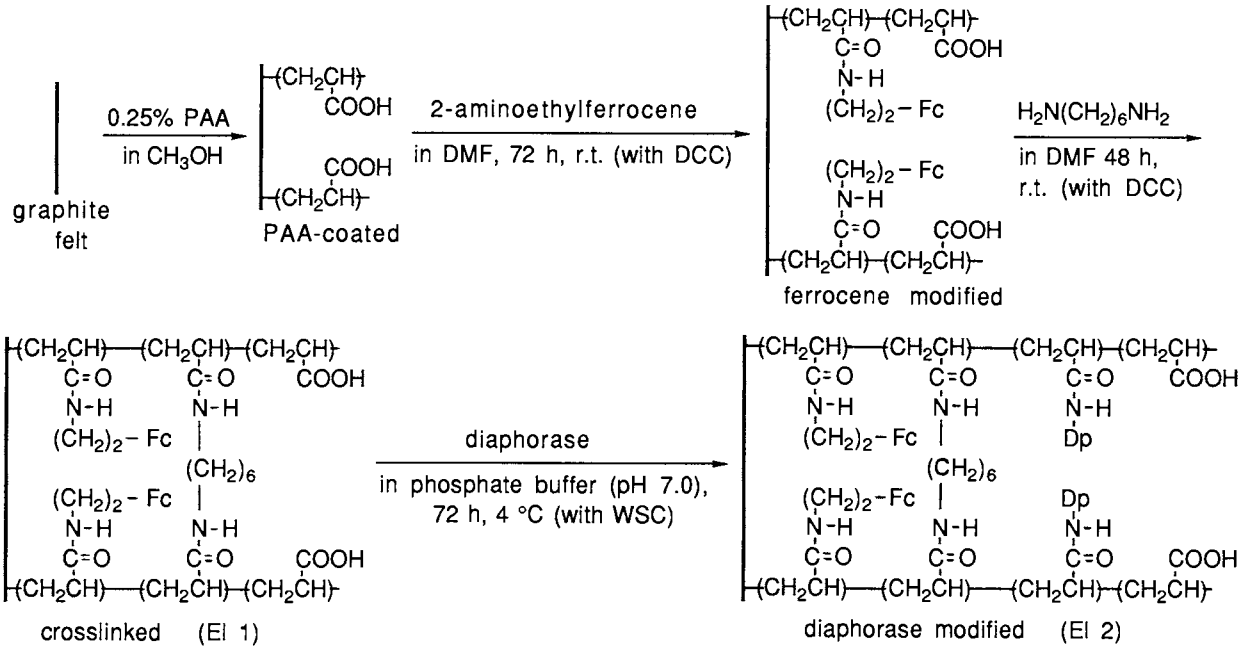


Fig. 2. Cyclic voltammograms on Fc- and Dp-comodified GF electrode (El 2) in the presence (—·—) and absence (——) of 10 mM NADH, and Fc-modified GF electrode (El 1) in the presence of 10 mM NADH (·····). Phosphate buffer (pH 7.0). Electrode area: 1.0 x 1.0 x 0.5 cm^3. The scan rate of 50 mV/s.



Scheme 1. Preparation method of thin PAA film coated graphite felt electrode coimmobilizing ferrocene- and diaphorase.

than the latter one. This means that the Fc moieties in the PAA layer of the former electrode is much contributed to electron transfer than those of the latter electrode, because of the presence of a flexible alkylene chain attaching Fc in El 1. Therefore, El 1 was used in the following experiments. The design of the chain length showed to improve the contribution of the Fc species to electron transfer.

El 1 (5.0 x 2.0 x 0.5 cm^3) was then treated with 5 ml of 20 μM Dp (EC 1.8.1.4) / phosphate buffer (pH 7.0) in the presence of 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (10 equiv. to Dp) for 72 hours at 4 °C (Scheme 1). The Fc- and Dp-comodified GF electrode (El 2) thus prepared afforded an electrocatalytic current for the oxidation of NADH in CV though El 1 scarcely showed it (Fig. 2). This result clearly demonstrates that Dp catalyzes effectively the oxidation of NADH by the oxidized Fc species.[5-7]

The electrocatalytic oxidation of NADH was carried out on El 2 and El 1 (5.0 x 2.0 x 0.5 cm^3) in 50 ml of 50 mM NADH/phosphate buffer (pH 7.0) at 0.30 V. The consumed amount of NADH in electrolysis solutions was analyzed by the absorbance decrease at 340 nm. The formation of NAD^+ was not identified directly, because NAD^+ does not show any intrinsic absorption bands in the UV and visible region differed from those of NADH. However, the addition of ethanol and alcohol dehydrogenase (EC 1.1.1.1) to the solutions after electrolysis, recovered the original absorbance of NADH at 340 nm. This fact means that NADH is selectively oxidized to NAD^+. For the electrolysis on El 1, 10 μmol of Dp was added. The results are shown in Fig. 3. The electrode of El 2 afforded complete conversion of NADH in 3 hours macro-electrolysis and quantitative oxidation to NAD^+. On the other hand, El 1 in the presence of Dp gave 20 % conversion of NADH to NAD^+ for 3 hours electrolysis. From those results, it is clear that the network of the thin PAA layer on GF allows electron transfers between Fc and Dp and diffusion of substrates such as NADH. The peak current of Fc of the used electrode was almost equal to that of new one (Fig. 4), and the

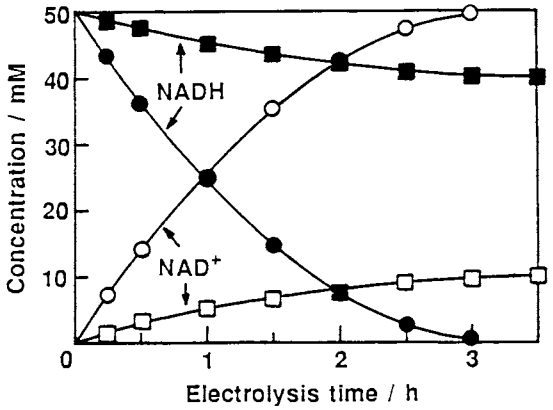


Fig. 3. Macroelectrolysis of NADH on Fc- and Dp-comodified GF electrode (El 2) (-○-), and Fc-modified GF electrode (El 1) in the presence of Dp (-□-). Electrode area : 5.0 x 2.0 x 0.5 cm^3. Oxidation potential : 0.30 V vs. SCE.

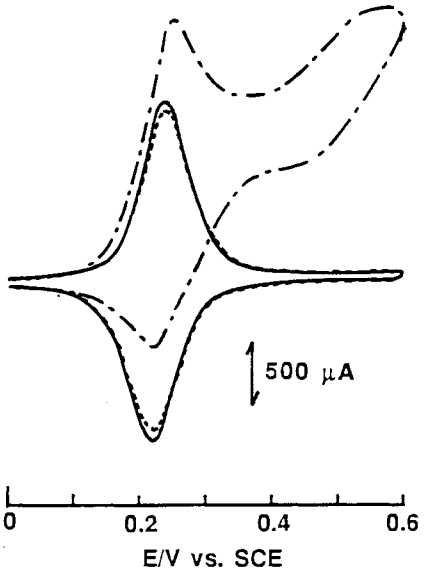
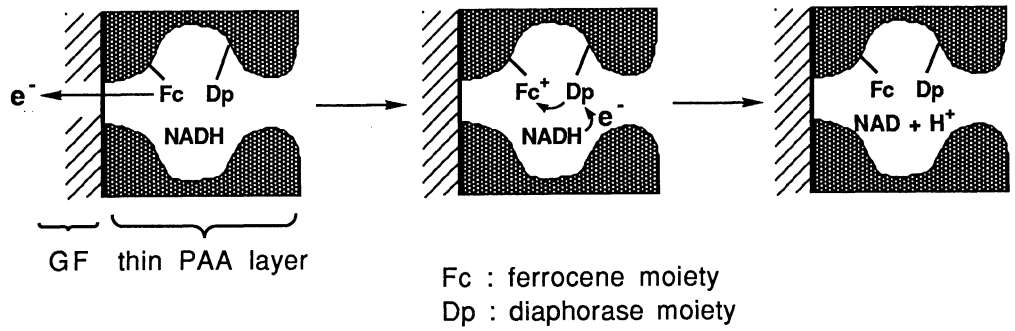


Fig. 4. Cyclic voltammograms of the new (——) and the used (· · · · ·) electrodes (1.0 x 1.0 x 0.5 cm^3), and the used electrode in the presence of 10 mM NADH (— · —) in phosphate buffer (pH 7.0). The scan rate of 50 mV/s.

used electrode also showed the nearly same electrocatalytic current for the oxidation of NADH as the new electrode. Therefore, it can be assumed that this modified electrode is not deactivated during macro-electrolysis and can be used repeatedly.

The mechanism of the electrocatalytic oxidation of NADH to NAD· on the thin PAA film coated GF electrode immobilizing Fc and Dp can be proposed as schematically shown in Scheme 2. The formed intermediate of NAD· is easily oxidized to NAD^+ by direct electron transfer from the GF electrode, via ferricinium, or via oxidized diaphorase.



Scheme 2. The proposed mechanism of oxidation of NADH to NAD on ferrocene- and diaphorase-comodified graphite felt electrode.

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