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Enhanced Electrocatalytic Activity for the NADH Oxidation with Oxidatively Treated Carbon Nanotubes Incorporated on a Redox Polymer Modified Electrode

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The electrochemical oxidation of dihydronicotinamide adenine dinucleotide (NADH) has drawn many attentions for use of its oxidized form (NAD^+) as an enzymatic cofactor in many dehydrogenase based biosensors and biofuel cells.[1,2] At neutral pH, NADH/NAD^+ couple has a formal potential of −0.320 V (*vs* NHE),[3] but large oxidation overpotentials are generally required.[2,4] In addition to this problem, direct oxidation of NADH shows unwanted side reactions or adsorption of the product.[5,6]

To avoid these problems, many studies using various electrocatalysts were reported. In this point of view, mediator-modified electrodes and electrochemical sensing designs were reviewed.[1,2,7] The oxidation of NADH is known to follow an ECE mechanism with a rate-limiting step in the first electron transfer.[2,6] Various redox mediators with quinone groups making two-electron and hydride transfer possible were reported as good candidates.[2,7,8-10] Recently, carbon nanotubes were employed as a promising electrode material giving enhanced performances with less overpotentials and enhanced sensitivities.[11-14] The carbon nanotubes (CNTs) in the nanocomposite films show good charge transport properties resulting in less overpotentials and good stabilities.[12,15]

In the present study, a redox polymer, the copolymer of polyacrylamide and poly(*N*-vinylimidazole) complexed with $[Os(4,4'\text{-dimethyl-}2,2'\text{-bipyridine})_2Cl]^{+/2+}$ (PAA-PVI-$[Os(dme\text{-}bpy)_2Cl]^{+/2+}$), was employed for the electron transfer mediator for NADH oxidation. The redox polymers have been utilized for mediating electrons between the redox centers of enzymes and electrode surfaces.[16,17] Some oxidase enzymes were "wired" to carbon electrodes and efficient electrocatalytic reactions such as glucose oxidation and O_2 reductions were reported.[16-19] At an electrode with a hydrogel film of the redox polymer, which is not a quinone-like mediator, catalytic electro-oxidation of NADH, but only a partial fraction, was measured. A composite film of the redox polymer and oxidatively treated single-walled carbon nanotubes (SWNTs) was applied on an edge plane graphite electrode surface. At this newly prepared electrode, an enhanced activity showing most NADH catalytically oxidized with less overpotential was achieved. As far as we know, the present redox polymer-CNT composite system, which is not a quinone type mediator, is first for the electrocatalytic NADH oxidation.

Cyclic voltammograms obtained in a 2.0 mM NADH solution at bare EPG and RP/EPG electrodes for Figures 1(a) and 1(b), respectively, were shown. (*For abbreviations of electrode types, consult the experimental section.*) Voltammograms obtained in a pure supporting electrolyte were also shown as dashed curves. In Figure 1(a), only one oxidation wave for NADH oxidation was observed with a peak potential of 0.34 V. In Figure 1(b), in the absence of

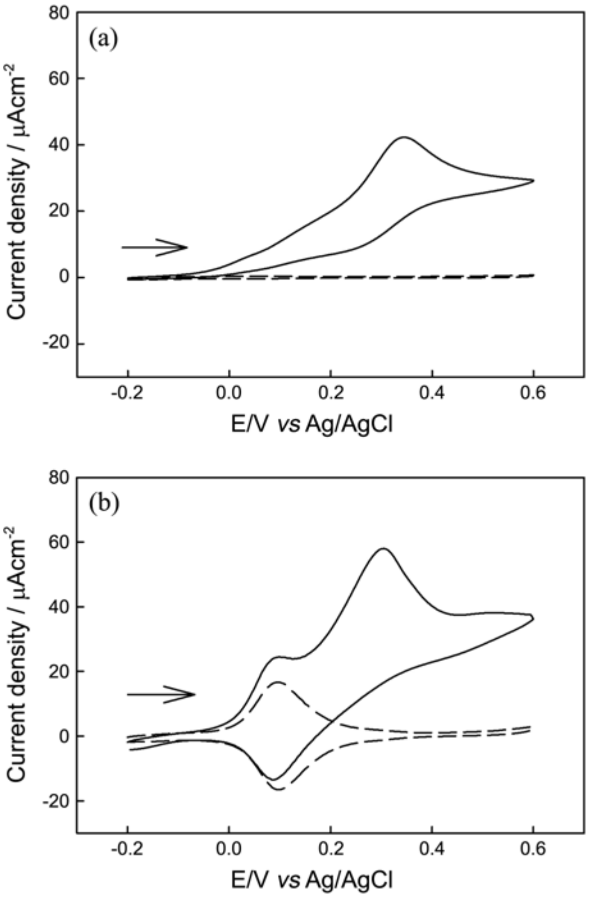

Figure 1. Cyclic voltammograms in a 2.0 mM NADH solution at (a) EPG and (b) RP/EPG Electrodes. For the redox polymer film on RP/EPG electrode, an aliquot of 1.28 μL from the mixture of 32.12 μL 4.5 mg/mL redox polymer and 5.02 μL 3.2 mg/mL PEGDGE was coated. The dashed curves were obtained in a pure supporting electrode. Supporting electrolyte: pH 7.4 20 mM phosphate buffer. Scan rate: 1 mV·s^{-1}.

NADH, a couple of redox waves due to the redox reactions of the osmium complexes (-[Os(dme-bpy)$_2$Cl]$^+$) tethered to the polymer chain was shown and a formal potential of 0.10 V was measured. In a scan rate as low as 1 mV/s, the peak separation was negligible indicating the reversible reactions of the osmium complexes.[20] In the presence of NADH, two oxidation waves were measured at 0.10 V and 0.31 V. The first oxidation wave at 0.10 V showed a little increase of the current in comparison to that in the absence of NADH. The increased current at such an early potential was due to the NADH oxidation catalyzed by the redox polymer. Possible reaction mechanism with the osmium complexes, which is a non-quinine mediator, is suggested as with other transition metal complex.[2]

$$2(\text{-[Os(dme-bpy)}_2\text{Cl]}^+) \rightarrow 2(\text{-[Os(dme-bpy)}_2\text{Cl]}^{2+}) + 2e \quad (1)$$

$$\text{-[Os(dme-bpy)}_2\text{Cl]}^{2+} + \text{NADH} \rightarrow \text{-[Os(dme-bpy)}_2\text{Cl]}^+ + \text{NADH}^{\cdot+} \quad (2)$$

$$\text{NADH}^{\cdot+} \rightarrow \text{NAD}^{\cdot} + \text{H}^+ \quad (3)$$

$$\text{-[Os(dme-bpy)}_2\text{Cl]}^{2+} + \text{NAD}^{\cdot} \rightarrow \text{-[Os(dme-bpy)}_2\text{Cl]}^+ + \text{NAD}^+ \quad (4)$$

The second oxidation wave at 0.30 V, whose potential was similar to the wave in Figure 1(a), was mainly assigned as the direct oxidation of NADH at the EPG surface, which was not catalyzed by the redox polymer. However, higher current of the second wave than that in Figure 1(a) was measured and this might be due to the presence of the current catalyzed by the oxidized osmium complexes in addition to the current from the direct NADH oxidation. The increase of the first wave was relatively small and the size of the second wave was still much larger. Only a small fraction of NADH was catalytically oxidized by the osmium complexes even at a slow scan rate as low as 1 mV/s. As the scan rate was increased, the increased part of the current appearing in the first oxidations region, decreased. At a scan rate as high as 10 mV/s, no increase of the current in the first oxidation wave was measured and most of NADH was oxidized at a more positive potential directly on the EPG surface. The scan rate dependence of the current increase in the first oxidation wave region confirms the oxidation of NADH catalyzed by the redox polymer.

The oxidations of NADH by quinone mediators were reported to follow a low energy route through an "inner-sphere" electron transfer and provide rapid reaction rates.[2,10] However, the reaction rate of Eq. (2), which is a "outer-sphere' one-electron transfer by a transition metal complex, is slow to be a limiting step.[2] The overall reaction rate between NADH and the osmium complex may be slow producing low catalytic current. In the steps of electron transfer from NADH to the redox polymer, and to the electrode surface, the redox reactions of the osmium complexes of the polymer chain was quite reversible as shown in Figure 1(b) and it was expected that the electron transfer in the polymer chain might not be a rate-limiting step.

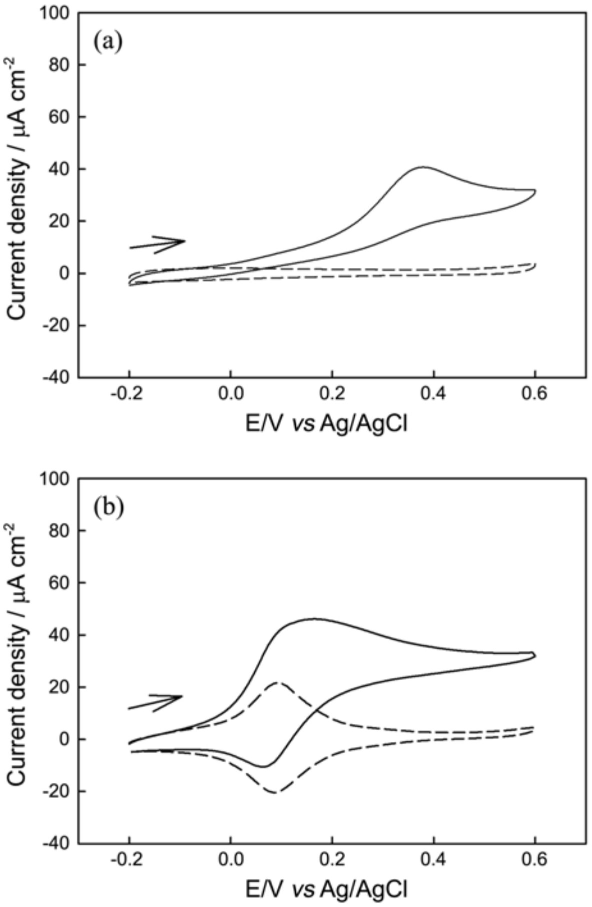

Figure 2. Cyclic voltammograms in a 2.0 mM NADH solution. Electrodes employed: (a) a CNT/EPG electrode. (An aliquot of 5.13 μL from the mixture of 4.00 μL 4.5 mg/mL CNT-COOH and 1.13 μL 3.2 mg/mL PEGDGE was coated). (b) a RP-CNT/EPG electrode. (An aliquot of 6.46 μL of the mixture of 4.00 μL 4.5 mg/mL CNT-COOH, 1.33 μL 4.5 mg/mL redox polymer, and 1.13 μL 3.2 mg/mL PEGDGE was coated). The dashed curves were obtained in a pure supporting electrolyte. Other conditions were the same as Figure 1.

CNT/EPG and RP-CNT/EPG electrodes were employed for the NADH oxidations. In Figure 2(a), with the CNT/EPG electrode, cyclic voltammograms were obtained in a 2.0 mM NADH solution (the solid curve) and in a pure supporting electrolyte (the dashed curve). In the presence of NADH, one oxidation wave was shown with a peak potential at 0.38 V, which was close to the value with the bare EPG electrode in Figure 1(a) and no catalytic effect for the NADH oxidation on the CNT coated electrode was observed. In Figure 2(b), with the RP-CNT/EPG electrode, the dashed curve obtained in the absence of NADH showed the redox waves of the redox polymer at 0.1 V. In a 2.0 mM NADH solution, only one oxidation wave with increased current at a potential of 0.16 V was measured. It is noted that the second oxidation wave at about 0.31 V, which was observed in Figure 1(b), disappeared and most NADH seemed to be oxidized at a more positive potential of 0.16 V. The difference between Figure 2(b) and Figure 1(b) is the inclusion of the CNT-COOHs inside the redox polymer film and much increase of the fraction of the first wave in the NADH oxidation. As

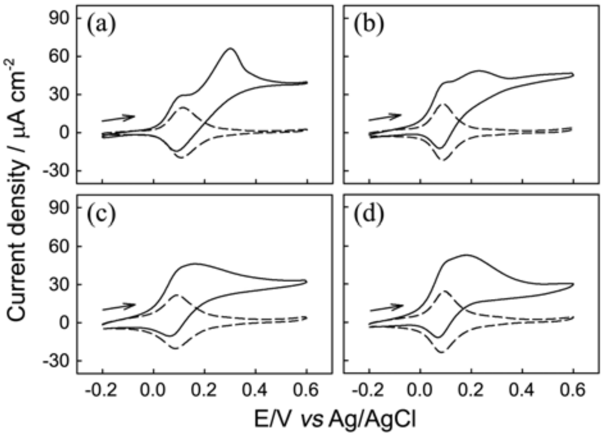

Figure 3. Effects of CNT-COOH contents on the cyclic voltammograms in a 2.0 mM NADH solution at a RP-CNT/EPG electrode. The amount of CNT-COOH in the coating mixture was varied as (a) 0 μg, (b) 6 μg, (c) 12 μg, and (d) 18 μg. To give each indicated amount of the CNT-COOHs, the compositions of the coating mixtures as described in the text were employed. The dashed curves were obtained in a pure supporting electrode. Other conditions were the same as Figure 1.

seen in Figure 2(a), with only CNTs on the electrode surface, no catalytic effect for the NADH oxidation was obtained.

Figure 3 shows changes of the voltammogram shape as the content of CNTs in the catalyst film was varied. In the coating mixture containing 1.33 μL 4.5 mg/mL PAA-PVI-$[Os(dme\text{-}bpy)_2Cl]^+$ and 1.13 μL 3.2 mg/mL PEGDGE as fixed amounts, the volume of 4.5 mg/mL CNT-COOH was varied as 0 μL, 1.33 μL, 2.67 μL, and 4.00 μL to give 0 μg, 6 μg, 12 μg, and 18 μg of CNT-COOHs, respectively, for each RP-CNT/EPG electrode. For each set of voltammograms, a comparison between the pure supporting electrolyte and the 2.0 mM NADH solution as the dashed and the solid, respectively, was made. In Figure 3(a), obtained with no CNT-COOH contained in the catalyst film, a small increase of the current in the first oxidation wave was observed and a large second oxidation wave was obtained. As the content of CNTs in the catalyst film layer was increased, the size of the second wave decreased with gradual enhancements of the first oxidation wave at about 0.16 V as shown in Figure 3(b) through 3(d). Effect of the inclusion of the oxidatively treated CNTs on the electrocatalysis for the NADH oxidation was evident. In Figure 3(d), with 18 mg CNT-COOH included in the coating mixture, the second oxidation wave almost disappeared showing a broad oxidation wave at a potential of 0.16 V and the composition of the three components was then 65.2 wt % CNT-COOH, 21.7 wt % redox polymer, and 13.1 wt % PEGDGE.

As an explanation for the enhanced catalytic effect on the RP-CNT/EPG electrode in comparison to the RP/EPG electrode, at this stage, it is suggested that the inclusion of CNT material in the catalyst film would increase the microscopic surface area and an increase of the density of the osmium complexes of the redox polymer on the outer surface was expected. The reaction rate of NADH with osmium complexes would increase resulting in the enhanced NADH oxidation current at an earlier potential of 0.16 V. The presence of CNTs in the catalyst film layer might not be a drawback to decrease down the overall electron transfer rate from NADH to the electrode surface. In the previous study, a bilirubin oxidase cathode wired by the redox polymer was constructed over the CNT layer and the electron transfer in the CNT layer in a slow scan rate was rapid enough not to be the rate-limiting step in the overall electrocatalytic reduction of oxygen.[21]

An optimal amount of the catalyst was determined. For the RP-CNT/EPG electrodes, different volumes from the mixture of 16.00 μL 4.5 mg/mL CNT-COOH, 5.32 μL 4.5 mg/mL redox polymer, and 4.52 μL 3.2 mg/mL PEGDGE, which was the composition used in Figure 3(d), were applied onto the EPG surfaces. Maximum catalytic current for NADH oxidation was obtained with the volume of about 10 μL. The microscopic surface area contacting to the solution phase reached its maximum point at this coated volume and, even with large amount, no additional effect was observed. The stability of the RP-CNT/EPG electrode was also tested. In the time dependence measurement of the NADH oxidation current by applying a potential of 0.25 V at a rotating electrode of 200 rpm speed, the current decayed about 2.5% until 1600 s. The result indicates that the RP-CNT/EPG electrode was quite stable during electrochemical measurements and is good for NADH-dependent biosensors.

This study demonstrates that the present redox polymer-CNT composite can be utilized for the electrocatalytic oxidation of NADH and this new effect of the oxidatively treated CNTs on the redox polymer mediated-electrocatalysis is now first reported. The redox polyer has been utilized for bioelectrocatalysis because of its unique properties such as an efficient electron transfer and forming a stable redox hydrogel films due to its enzyme wiring and this makes the present system promising for use in NADH-dependent dehydrogenase enzyme reactions. It is hoped that further studies are needed for better understanding of the mechanism in detail.

Experimental Section

NADH (β-Nicotinamide adenine dinucleotide) was purchased from Sigma (St Louis, MO). Poly(ethylene glycol) (400) diglycidyl ether (PEGDGE) was purchased from Polysciences Inc. (Warrington, PA) and single-walled carbon nanotubes (SWNTs, 1.1 nm diameter and 0.5-100 μm lengths) were from Aldrich. All other reagents were used as received. Deionized water purified by passing it through a purification train (Human Power II+, Human Co) was used for the preparations of the solutions. The pH 7.4 20 mM phosphate buffer (PB) was prepared for the supporting electrolyte.

The redox polymer, PAA-PVI-$[Os(dme\text{-}bpy)_2Cl]^+$, was synthesized as described in the literature.[22,23] SWNTs were cut and carboxylated by the reported procedure.[24-26] The carboxyl group was confirmed with IR spectrometry by the band at 1720 cm^{-1}, which was shown only with the treated

CNTs and assigned as the C=O stretch mode.[24,26] These carboxylated CNTs, CNT-COOH, were used as electrode materials.

The surface of an edge plane pyrolytic graphite (EPG) disk electrode (geometric area, 0.302 cm^2) was ground using 2000 SiC paper, sonicated, washed with deionized water, and dried in air. For working electrodes, in addition to the EPG electrode, three modified EPG electrodes coated with redox polymer (RP/EPG), CNT-COOH (CNT/EPG), and a composite of redox polymer plus CNT-COOH (RP-CNT/EPG) were prepared. For the RP/EPG electrode, an aliquot of the mixture of PAA-PVI-$[Os(dme\text{-}bpy)_2Cl]^{+/2+}$ redox polymer and PEGDGE cross-linker was dropped over the surface and dried for 18 h under the temperature of 42 °C. Similarly, an aliquot of the mixture of CNT-COOH and PEGDGE for the CNT/EPG electrode, and another aliquot of the mixture of PAA-PVI-$[Os(dme\text{-}bpy)_2Cl]^{+/2+}$, CNT-COOH, and PEGDGE for the RP-CNT/EPG electrode were used. The quantity of each component was fully described in detail for each electrode in Figure captions.

Electrochemical measurements were performed using an electrochemical analyzer (Model CHI 630 or CHI 600, CH-Instruments, Austin, TX) controlled through a personal computer. A two-compartment cell closed with a Teflon cap through which the electrodes and gas bubbling system were fitted was employed. A working electrode and a platinum auxiliary electrode were placed in one compartment separated from the other compartment, where the Ag/AgCl reference (3 M NaCl, 0.22 V *vs* SHE) electrode was held, by using a porous fritted-glass disk. Argon gas was bubbled to remove oxygen from the solutions. All experiments were carried out at ambient temperature (22 ± 2 °C).

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References

1. Radoi, A.; Compagnone, D. *Bioelectrochem.* **2009**, *76*, 126.
2. Lobo, M. J.; Miranda, A. J.; Tunon, P. *Electroanalysis* **1997**, *9*, 191.
3. Harris, D. C. *Quantitative Chemical Analysis*, 8th ed.; Freeman: New York, 2010; p 298.
4. Banks, C. E.; Compton, R. G. *Analyst* **2005**, *130*, 1232.
5. Moiroux, J.; Elving, P. J. *Anal. Chem.* **1978**, *50*, 1056.
6. Moiroux, J.; Elving, P. J. *J. Am. Chem. Soc.* **1980**, *102*, 6533.
7. Gorton, L.; Dominguez, E. *Rev. Mol. Biotechnol.* **2002**, *82*, 371.
8. Scherbak, N.; Strid, A.; Eriksson, L. A. *Chem. Phys. Lett.* **2005**, *414*, 243.
9. Pariente, F.; Lorenzo, E.; Abruna, H. D. *Anal. Chem.* **1994**, *66*, 4337.
10. Carlson, B. W.; Miller, L. L. *J. Am. Chem. Soc.* **1985**, *107*, 479.
11. Luz, R. C. S.; Damos, F. S.; Tanaka, A. A.; Kubota, L. T.; Gushikem, Y. *Electrochim. Acta* **2008**, *53*, 4706.
12. Raj, C. R.; Chakraborty, S. *Biosens. Bioelectron.* **2006**, *22*, 700.
13. Zhu, L.; Zhai, J.; Yang, R.; Tian, C.; Guo, L. *Biosens. Bioelectron* **2007**, *22*, 2768.
14. Wooten, M.; Gorki, W. *Anal. Chem.* **2010**, *82*, 1299.
15. Chakraborty, S.; Raj, C. R. *Electrochem. Commun.* **2007**, *9*, 1323.
16. Heller, A. *J. Phys. Chem.* **1992**, *96*, 3579.
17. Ohara, T. J.; Rajagopalan, R.; Heller, A. *Anal. Chem.* **1993**, *65*, 3512.
18. Barton, S. C.; Kim, H.-H.; Binyamin, G.; Zhang, Y.; Heller, A. *J. Am. Chem. Soc.* **2001**, *123*, 5802.
19. Mano, N.; Kim, H.-H.; Zhang, Y.; Heller, A. *J. Am. Chem. Soc.* **2002**, *124*, 6480.
20. Bard, A. J.; Faulkner, L. R. *Electrochemical Methods Fundamentals and Applications*, 2nd ed; Willy: New York, 2001; p 591.
21. Song, J.; Shin, H.; Kang, C. *Electroanalysis* **2011**, *23*, 2941.
22. Buckingham, D. A.; Dwyer, F. P.; Goodwin, H. A.; Sargeson, A. M. *Aust. J. Chem.* **1964**, *17*, 325.
23. Lumiey-Woodyear, T. D.; Rocca, P.; Lindsay, J.; Dror, Y.; Freeman, A.; Heller, A. *Anal. Chem.* **1995**, *67*, 1332.
24. Chen, J.; Hamon, M. A.; Hu, H.; Chen, Y.; Rao, A. M.; Eklund, P. C.; Haddon, R. C. *Science* **1998**, *282*, 95.
25. Liu, J.; Rinzler, A. G.; Dai, H.; Hafner, J. H.; Bradley, R. K.; Boul, P. J.; Lu, A.; Iverson, T.; Shelimov, K.; Huffman, C. B.; Rodriguez-Macias, F.; Shon, Y.-S.; Lee, T. R.; Colbert, D. T.; Smalley, R. E. *Science* **1998**, *280*, 1253.
26. Li, X.; Niu, J.; Zhang, J.; Li, H.; Liu, Z. *J. Phys. Chem. B* **2003**, *107*, 2453.