Electrocatalytic oxidation of NADH at glassy carbon electrodes modified with an electropolymerized film of nile blue A

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The kinetic parameters for the electrocatalytic oxidation of di-hydronicotiamide adenine dinucleotide (NADH) at glassy carbon electrodes modified with an electropolymerized film of nile blue A (PNB) have been determined based on rotating disk electrode measurements. The rate constant for the chemical reaction between NADH and PNB is strongly influenced by NADH concentration and the pH value of solution, and it decreases with increasing NADH concentration, indicating that the electrocatalytic process proceeds *via* the formation of an intermediate of charge-transfer complex between NADH and PNB.

Keywords   Electrocatalysis, modified electrode, mediator, NADH, nile blue A

Introduction

The electrochemical oxidation of dihydronicotia-mide adenine dinucleotide (NADH) to the corresponding oxidized form (NAD^+) in aqueous solution has been widely studied, because the coenzyme NADH can be used in amperometric enzyme electrodes for many substrates. Direct oxidation of NADH at a bare electrode only proceeds at high overpotentials, which generally leads to fouling of the electrode surface. Many efforts have been made to improve the kinetics of NADH oxidation. One of efficient ways to reduce the high overpotential for the oxidation of NADH is to use mediators, either in solution or immobilized on the electrode surface, which can shuttle electrons between the electrode and NADH.[1-3] Mediators include quinones,[4] redox dyes,[5,6] ferrocene derivatives,[7] inorganic metal complexes[8] and polymers,[9-14] including both electronically conducting polymers and redox polymers. Bartlett and coworkers[15] recently studied the NADH oxidation at the poly(aniline)-poly(vinylsulfonate) composite-coated glassy carbon electrode. Their experimental results indicate that the modified electrode shows stable and reproducible electrocatalytic responses toward NADH oxidation.

We[13] recently reported that the electrooxidation of nile blue A (NB) on the glassy carbon electrodes gives rise to a stable electroactive thin film PNB, which exhibits high electrocatalytic activity for the oxidation of NADH. In the present work, the rate constants for the electrooxidation of NADH at glassy carbon electrodes modified with PNB have been determined and the effects of solution pH have been studied.

Experimental

Chemicals

NADH (Shanghai Lizhu Dongfeng Biotechnology Co., Ltd.) and nile blue A (chloride, cat. No. 12,147—9, dye content 90%, Aldrich) were used as received. All other chemicals were of analytical grade. The 0.1 $mol \cdot dm^{-3}$ of phosphate buffer solution (pH 6.8) was employed as supporting electrolyte. The B-R buffer was employed for the needs of various pH values. All the solutions were prepared with doubly deionized water and deaerated by passing through highly pure nitrogen for 20 min before the electrochemical experiments, and a continuous flow of nitrogen was maintained over the sample solution during experiments. The experimental temperature was controlled at 25 ± 0.1 ℃ with a Type 501 thermostat (Shanghai, China).

Apparatus

Electrochemical experiments were carried out with EG&G PARC Model 273 Potentiostat/Galvanostat and the software of PARC Model 270 Electrochemistry Analysis in a three-electrode cell, with a PTFE-shrouded glassy carbon electrode (3 mm diameter) as the working electrode, a saturated calomel electrode (SCE) as the reference electrode and a coiled platinum wire serving as the counter electrode. Rotating disk electrode (RDE) measurements were carried out with the EG&G PARC Model 616 disk electrode system, using a glassy carbon electrode (4 mm diameter). The pretreatment of the working electrode was previously reported.[13]

Procedures

The PNB was deposited by cyclic voltammetry. The procedures for the electropolymerization of NB were previously reported.[13] After the PNB film had formed on the electrode surface, the electrode was rinsed thoroughly with water, and transferred into the buffer solution to test its electrocatalytic activity toward NADH oxidation. The RDE measurements were carried out at 2 $mV \cdot s^{-1}$ from -0.35 to $+0.40$ V, and the catalytic current, which was taken as the plateau current after the background correction, was measured.

The film thickness was dependent on the number of cycles, and the surface coverage, Γ, was calculated from $\Gamma = Q/nFA$, where F is Faraday's constant, Q is the charge obtained from the peak area (peak II) in cyclic voltammograms after the background correction, n is the number of electrons involved in the electrode process ($n = 2$ in the present case), and A is the surface area of glassy carbon electrode (the geometric area of the electrode was employed in all calculations because the surface of the electrode is microscopically rough and it is difficult to estimate the roughness factor). The value of Γ was obtained at a scan rate of 100 $mV \cdot s^{-1}$.

Results and discussion

Electrochemical polymerization of NB and the electrocatalytic activity of PNB toward NADH oxidation

As reported previously,[13] the potential range, especially the upper limit, is the most important factor for forming the PNB films. If the potential sweep is confined within the region of -0.6 to $+0.4$ V, a simple cyclic voltammogram will be obtained with a formal potential of -0.3 V, which corresponds to the redox reaction of monomer NB/leuco NB (structures I and II in Fig. 1). When the anodic potential is extended to $+1.2$ V, a shoulder around $+0.9$ V appears. This anodic peak is attributed to the one-electron oxidation of NH_2 group of monomer NB, forming a cation radical (structure III in Fig. 1). Bauldreay and Archer[16] studied polymerization mechanisms of thionine, nile blue *etc*. According to their results, the oxidation of NH_2 group is the initial step for the NB polymerization and the unpaired electron can be delocalized through the NB molecule with the aid of several canonical forms. The unpaired electron is sited either on amine group or at *ortho* positions with high probability. Radical dimerization might occur *via* carbon-nitrogen coupling routes as shown in Fig. 1, and form a dimeric species (structure V). The oxidation of the NH_2 group in the dimer might occur again, and so an insoluble, oligomeric, probably cross-linked film of PNB is readily produced. This is indicated by the fact that the peak currents increase with successive scanning during polymerization process. Curve (a) in Fig. 2 is the cyclic voltammogram of PNB film in 0.1 $mol \cdot dm^{-3}$ of phosphate buffer solution (pH 6.8) at a scan rate of 50 $mV \cdot s^{-1}$, two couples of well-defined redox peaks were observed. As shown previously,[13] couple I corresponds to the redox reaction of the monomer units containing in PNB film and couple II to the redox reaction of the nitrogen bridges. The formal potentials, $E^{0'}$, are -430 mV and -67 mV for couple I and couple II, respectively, at a scan rate of 20 $mV \cdot s^{-1}$. The electrochemical responses of the PNB resulted from surface-confined redox couples because the peak currents of two redox couples were directly proportional to the scan rate at least to 150 $mV \cdot s^{-1}$.

The heterogeneous charge transfer rate constant, k_s, of a surface-confined redox couple can be evaluated from cyclic voltammetry as reported by Laviron.[17] The dependence of k_s, which was obtained using Laviron's method in the case of $\Delta E_p/n < 200$ mV, on the solution pH is listed in Table 1.

The values of k_s for couple I and couple II increase with increase of the solution pH value. Gorton and coworkers[18] pointed out that the electron transfer from aromatics is too fast to be evaluated as long as no proton

transfer is involved. Proton transfer reduces the rate drastically. The local pH changes might be responsible for the increase of k_s.

As mentioned above, there have been numerous reports of the electrocatalytic activity toward NADH of adsorbed or electrodeposited mediators. Typical values of electron transfer rate constant between mediator and electrode are 6—17 s^{-1}, depending on solution pH, for adsorbed phenoxazines,[18,19] 3—6 s^{-1} for adsorbed quinones[20,21] and 1 s^{-1} for oxidized carbon black.[22] Un-

der these conditions, the rate constants for chemical reaction between NADH and mediators are typically one order of magnitude smaller than the corresponding values for the electron transfer process between the mediators and electrodes. Based on the given value of the electron transfer rate constant of PNB in the present case, we could assume that the electron transfer process is faster than that of the chemical reaction between NADH and PNB.
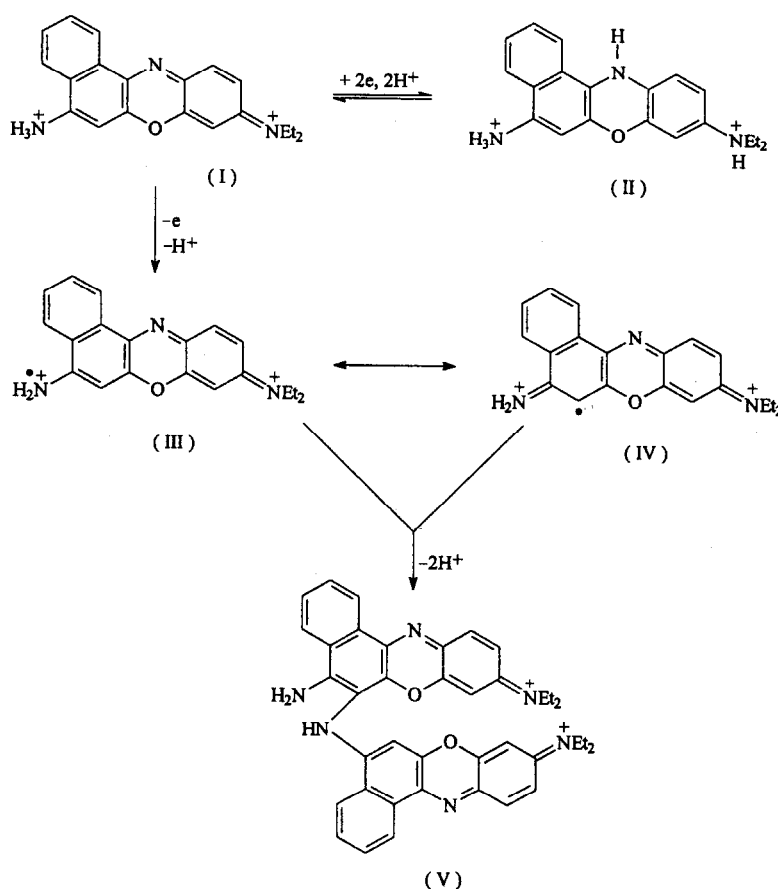


Fig. 1   Proposed mechanism for nile blue A coupling.

Table 1   Dependence of k_s on the solution pH for a PNB film electrode

pH	4.0	6.8	8.3
$k_s/(\mathrm{s}^{-1})$ for couple I	1.70	2.80	4.25
$k_s/(\mathrm{s}^{-1})$ for couple II	1.23	1.91	2.64

It has been indicated[13] that only the redox couple II has high electrocatalytic activity toward NADH oxidation, and the redox couple I has little electrocatalytic ac-

tivity for NADH oxidation, because the anodic currents increase dramatically and the cathodic currents decrease dramatically for couple II, and anodic currents increase slightly for couple I upon addition of NADH in solution. Fig. 2 shows cyclic voltammograms of the electrode in 0.1 mol·dm^{-3} phosphate buffer solution (pH 6.8) at 50 mV·s^{-1} in the absence (a) and presence (b) of NADH (5.0 × 10^{-4} mol·dm^{-3}). The difference in electrocatalytic efficiency between couple I and couple II is due to the different formal potentials. Based on the above re-

sults, it can be seen that the electrocatalytic activity of PNB toward NADH oxidation is attributed predominantly to the redox couple II.
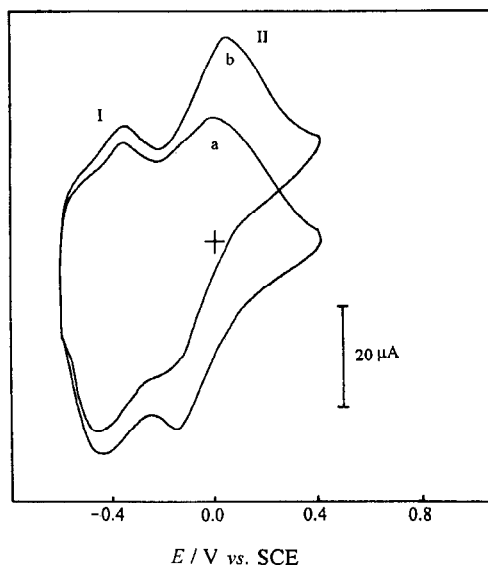
Fig. 2   Cyclic voltammograms for a PNB modified glassy carbon electrode in 0.1 mol·dm^{-3} of phosphate buffer solution (pH 6.8) at 50 mV·s^{-1} in the absence (a) and presence (b) of NADH (5.0 × 10^{-4} mol·dm^{-3}).

The dependence of the electrocatalytic peak current (for peak II) of NADH oxidation on Γ was studied. The catalytic current increases with increase of Γ up to about 5.0 × 10^{-10} mol·cm^{-2}, whereas the catalytic current levels off for the Γ above this value. In order to ensure that the electrocatalytic current was independent of the Γ, a coverage of 5.4 × 10^{-10} mol·cm^{-2}, which corresponds to cycling the glassy carbon electrode in NB solution in the potential range of -0.6 to +1.2 V (100 mV·s^{-1}) for ca. 50 min, was employed in the following experiments.

Dependencies of the rate constant (k_1) for electrocatalytic oxidation of NADH *on* C* *and solution* pH

We[13] have shown that the overall electrocatalytic oxidation of NADH at PNB modified glassy carbon electrodes might be controlled by the diffusion of NADH in solution and the cross-exchange process between NADH and the redox site of the PNB, and the rate-controlled process is complicated. The rotating disk electrode

method was used for evaluating the kinetic parameters in the present study.

The plots of electrocatalytic currents, i_{cat}, for NADH oxidation against $\omega^{1/2}$ at different concentrations of NADH were found to be nonlinear, suggesting that the reaction is limited by kinetics and not by mass transport. In such a case, the overall rate constant, k_1, for the chemical reaction between NADH and the mediator PNB can be obtained from the plot of $1/i_{cat}$ versus $1/\omega^{1/2}$ using the following equation:[18]

$$\frac{1}{i_{cat}} = \frac{1}{nFA\Gamma k_1 C^*} + \frac{1}{0.62nFA\nu^{-1/6}D^{2/3}C^*}\frac{1}{\omega^{1/2}} \tag{1}$$

where C^* is the bulk concentration of NADH, ω is the rotational speed in rad s^{-1}, ν is the kinematic viscosity (0.00896 cm^2·s^{-1} for aqueous solution[23]), D is the diffusion coefficient of NADH in solution (2.4 × 10^{-6} cm^2·s^{-1} [24]), and the other parameters have their usual meaning. Fig. 3 presents such plots for different concentrations of NADH. As anticipated, linear relationship between the $1/i_{cat}$ and $1/\omega^{1/2}$ was obtained. The k_1 decreases significantly with increase of the bulk concentration of NADH. The results are shown in Fig. 4A. A similar observation has been previously reported for the electrocatalytic oxidation of NADH at electrodes modified with different mediators.[12,18,19] In order to rationalize such a concentration dependence of the rate constant, Gorton et al.[19] has postulated that the electrocatalytic process involves an intermediate of charge-transfer complex between NADH and the mediator by a mechanism similar to Michaelis-Menten kinetics.

$$\text{NADH} + M_{ox} \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} [\text{NADH}\cdots M] \xrightarrow{k_{+2}} \text{NAD}^+ + M_{red} \tag{2}$$
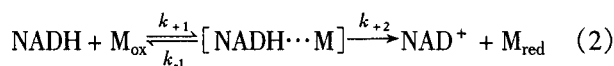
where M represents the mediator. Combination of the rate constants k_{+1}, k_{-1} and k_{+2}, as in the Michaelis-Menten kinetics model, yields:

$$K_M = (k_{-1} + k_{+2})/k_{+1} \tag{3}$$

The overall rate constant, k_1, for the chemical reaction between NADH and PNB, can be expressed as:[19]

$$k_1 = k_{+2}/(K_M + C^*) \tag{4}$$

Inversion of Eq. (4) gives:

$$1/k_1 = K_M/k_{+2} + C^*/k_{+2} \qquad (5)$$

Eq. (5) indicates that a plot of $1/k_1$ versus C^* will be a straight line if a complex is involved in the reaction between NADH and the mediator. Thus, such a plot can be used for a diagnostic test of the kinetic model proposed in Eq. (2).
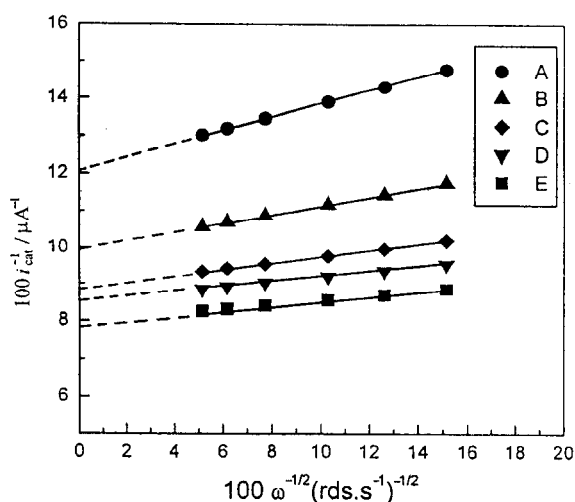
Fig. 3   Plot of i_{cat}^{-1} versus $\omega^{-1/2}$ for the electrocatalytic oxidation of NADH [(A) 1.0, (B) 1.5, (C) 2.0, (D) 2.5 and (E) 3.0 × 10³ mol·dm⁻³ at a PNB modified glassy carbon electrode in 0.1 mol·dm⁻³ of phosphate buffer solution (pH 6.8)].

Substitution of k_1 in Eq. (1) with Eq. (4) gives:

$$\frac{1}{i_{cat}} = \frac{1}{nFAk_{+2}\Gamma} + \left(\frac{K_M}{nFAk_{+2}\Gamma} + \frac{1.61\nu^{1/6}}{nFAD^{2/3}\omega^{1/2}} \right) \frac{1}{C^*} \qquad (6)$$

It is clear that the linearity of plot of $1/i_{cat}$ vs. $1/C^*$ can also be used to analyze the reaction mechanism involving a charge-transfer complex. That is, if a charge-transfer complex is involved in the catalytic reaction, the plot of $1/i_{cat}$ vs. $1/C^*$ should be linear.

Fig. 4A shows a relationship between k_1 and C^* of NADH. Obviously, it is not a straight line. Fig. 4B is a plot of $1/k_1$ against C^*, whose linearity is as ex-

pected from Eq. (5). A line regression equation $k_1^{-1}/$ $(10^{-3} \text{ mol}^{-1} \cdot \text{dm}^3 \cdot \text{s}^{-1})^{-1} = (0.78 \pm 0.01) + (0.80 \pm 0.03) C^* /(\text{mmol} \cdot \text{dm}^{-3})$ can be obtained, with a correlation coefficient of 0.9994. A plot of i_{cat} versus NADH concentration, shown in Fig. 5A, has the characteristic Michaelis-Menten shape. A reciprocal plot from these data is a straight line (Fig. 5B), as predicated by Eq. (6). The regression equation is $i_{cat}^{-1}/10^{-2} \cdot \mu A^{-1}$ $= (5.72 \pm 0.05) + (6.37 \pm 0.07)(C^*)^{-1}/(\text{mmol} \cdot \text{dm}^{-3})^{-1}$, with a correlation coefficient of 0.9999. Thus, we can conclude that the electrocatalytic oxidation of NADH at PNB modified glassy carbon electrodes proceeds via an intermediate complex. The rate constants k_{+2} and K_M could be evaluated according to Eq. (5). At pH 7.0, the k_{+2} and K_M values are 1.26 s⁻¹ and 9.83×10^{-4} mol·dm⁻³, respectively.

It has been shown that the stability of the intermediate complex depends on the polarity of the solvent and the reaction is strongly influenced by the solution pH.[1] The values of K_M/k_{+2}, k_{+2} and K_M at various solution pH values are listed in Table 2. As shown in Table 2, the kinetics of the reaction between the PNB and NADH is influenced by the solution pH value. The k_{+2} increases with the decrease of the solution pH value, however, the K_M/k_{+2} and K_M increase with increase of the solution pH value.

Conclusion

The kinetic parameters for electrocatalytic oxidation of NADH at PNB modified glassy carbon electrodes have been determined from rotating disk electrode measurements. The rate constant for the chemical reaction between NADH and the PNB mediator is dependent on the NADH concentration and the solution pH value, indicating that the electrocatalytic process involves the formation of an intermediate of charge-transfer complex between NADH and the PNB.

Table 2   Rate constants at various solution pH values for the electrocatalytic oxidation of NADH at PNB modified electrodes

pH	$10^4 \, K_M/k_{+2}(\text{mol} \cdot \text{dm}^{-3} \cdot \text{s})$	$k_{+2}(\text{s}^{-1})$	$10^4 \, K_M(\text{mol} \cdot \text{dm}^{-3})$
4.0	3.7	1.87	6.92
6.8	7.8	1.26	9.83
8.3	9.8	1.13	11.07
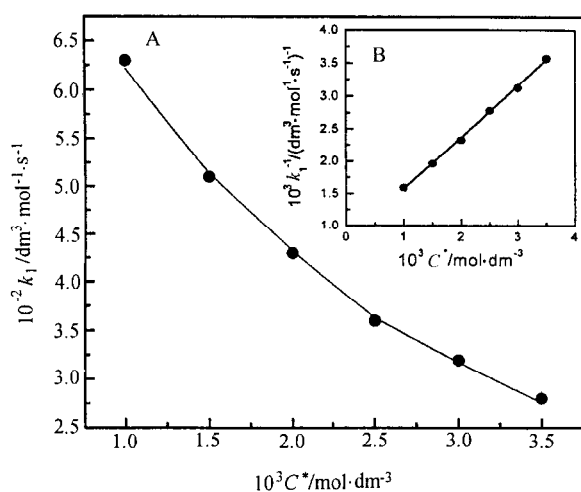
Fig. 4 Plots of k_1 vs. C^* (A) and k_1^{-1} vs. C^* (B) for the electrocatalytic oxidation of NADH at a PNB modified glassy carbon electrode in 0.1 mol·dm^{-3} phosphate buffer solution (pH 6.8).
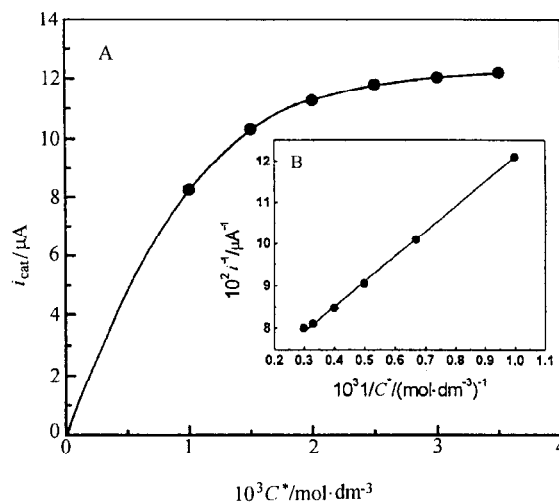
Fig. 5 Plots of i_{cat} vs. C^* (A) and $1/i_{cat}$ vs. $1/C^*$ (B) for the electrocatalytic oxidation of NADH at a PNB modified glassy carbon electrode in 0.1 mol·dm^{-3} of phosphate buffer solution (pH 6.8).

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