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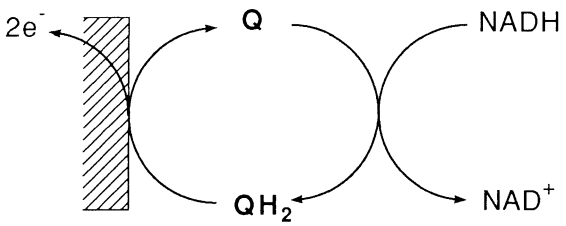
Heterocyclic *o*-Quinones. Mediator for Electrochemical Oxidation of NADH

Shinobu ITOH, Hajime FUKUSHIMA, Mitsuo KOMATSU, and Yoshiki OHSHIRO*
Department of Applied Chemistry, Faculty of Engineering, Osaka University,
Yamada-oka 2-1, Suita, Osaka 565

Heterocyclic *o*-quinones such as phenanthrolinequinone derivatives are demonstrated to act as a mediator in electrochemical oxidation of NADH, which can be used as an NAD^+-recycling system for HLADH-catalyzed oxidation of alcohols.

Enzymatic catalysis in organic synthesis has recently attracted a great deal of attention upon its inherent high stereo-, substrate-, and chemoselectivities. In particular, NAD(P)(H)-requiring oxidoreductases are the most useful and widely investigated enzymes.[1] In order to apply such enzymes to organic synthesis, much effort has been devoted to development of an efficient coenzyme-recycling system by means of chemical, electrochemical, and enzymatic strategies.[2] As a chemical method for alcohol oxidation, we have already reported an efficient NAD^+-regeneration system with heterocyclic *o*-quinones and molecular oxygen, which is much more effective than the well known system of FMN/O_2.[3] In this paper, several heterocyclic quinones were examined as a mediator in the electrochemical oxidation of NADH (Scheme 1). So far, quinonoid compounds have been well demonstrated to act as the electrochemical mediator,[4] but detailed study has not been carried out on the heterocyclic quinones.[5]



Scheme 1.

In cyclic voltammetric experiments, it was obvious that 1,7-phenanthrolinequinone (**2**) acts as a mediator in the electrochemical oxidation of NADH (Fig. 1). Addition of a catalytic amount of **2** to an NADH buffer solution caused a drastic shift of the anodic peak potential from 412 mV to -127 mV vs. SCE and an increase of the anodic peak current. Thus in the

following discussion, the catalytic efficiency was estimated by i_{Med}/i_Q, where i_{Med} is the anodic peak current of the mediated reaction and i_Q is the anodic peak current of the quinone itself.

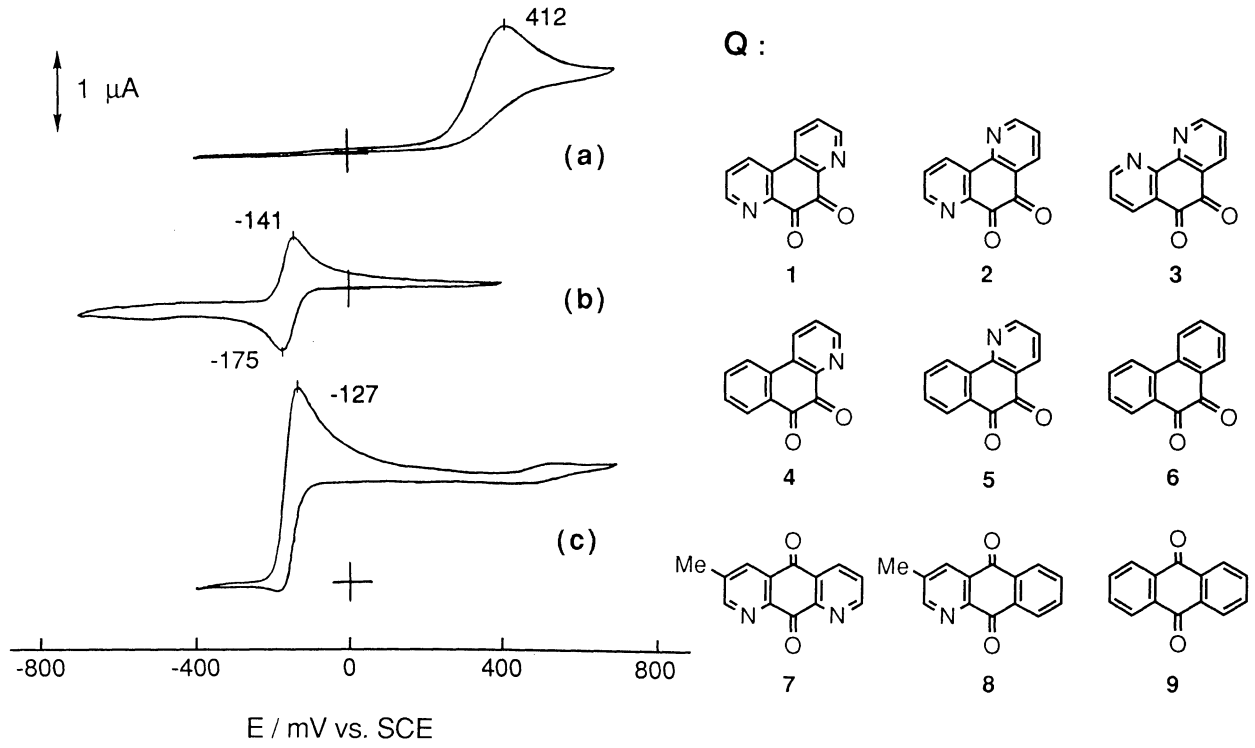


Fig. 1. Cyclic voltammograms; v = 1 mV/s, 0.1 M phosphate buffer (pH 8.3 - 8.4). (a) 1.0 mM NADH, (b) 0.2 mM **2**, (c) 1.0 mM NADH and 0.2 mM **2**.

The effect of the pyridine nitrogens and the difference between *ortho*- and *para*-quinone functions were examined by using several quinones (**1** - **9**).[6] In Table 1 are shown the catalytic efficiency of the quinones together with their redox potentials (E_m) determined by cyclic voltammetry at pH 8.3. Catalytic effect of diaphorase, a flavoprotein which catalyzes electron-transfer of NAD(H), was also investigated.[7] In the absence of diaphorase, only **1**, **2**, and **4** showed catalytic activity and the order of the efficiency was **1** > **2** > **4**. It is apparent that the redox potential is an important factor and -200 mV vs. SCE seems to be a lower limit under these conditions. It could be also said that the existence of the peri pyridine nitrogen in *ortho*-quinones is favorable.

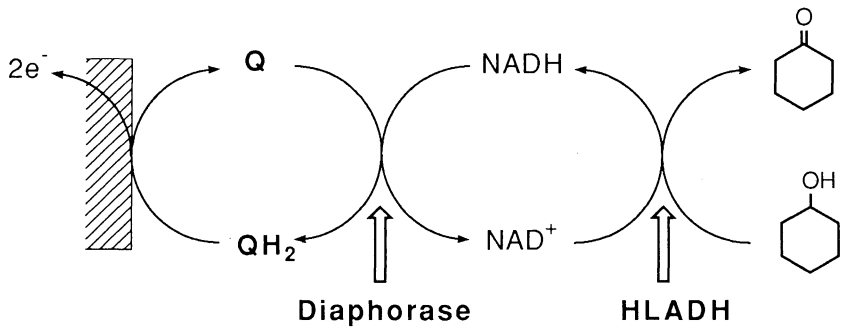
Diaphorase drastically improved the efficiency of the systems and decreased the lower limit to ca. -300 mV vs. SCE. Again in this case, not only the redox potential but also the quinone structures are important. This may be attributed to the substrate specificity of

diaphorase. In the case of **6**, more than 700 mV decrease of the anodic peak potential was achieved.

Table 1. Catalytic Efficiency for the Electrochemical Oxidation of NADH

Q	E_m [a]	i_{Med}/i_Q [b]	
		without Diaphorase	with Diaphorase [c]
1	- 150	3.7	3.9
2	- 160	2.1	2.9
3	- 230	1.0	2.5
4	- 240	1.5	3.7
5	- 280	1.0	3.2
6	- 300	1.1	2.1
7	- 450	1.0	1.1
8	- 550	1.1	1.1
9	- 670	1.0	1.0

a) $E_m = (E_p^a + E_p^c) / 2$ vs. SCE at pH 8.3 in 0.1 M phosphate buffer, b) i_Q: anodic peak current of Q (0.2 mM), i_{Med}: anodic peak current of the mediated oxidation of NADH (1.0 mM), ν = 1 mV/sec, 0.1 M phosphate buffer (pH 8.3 - 8.4), c) [diaphorase] = 20 U/ml



Scheme 2.

The electrochemical oxidation of NADH catalyzed by the quinone was then applied to the horse liver alcohol dehydrogenase (HLADH)-catalyzed oxidation of alcohols (Scheme 2). Controlled potential electrolysis of cyclohexanol (2.0×10^{-3} M) was carried out by using 10 x 30

mm glassy carbon working electrode at -100 mV vs. SCE in the presence of **2** (1.0×10^{-4} M), NAD^+ (1.0×10^{-4} M), HLADH (5.0×10^{-7} M), and diaphorase (10 units/ml) in 0.1 M phosphate buffer (pH 8.2) under anaerobic conditions (35 °C). Cyclohexanol was quantitatively oxidized to cyclohexanone within 10 h. The rate of cyclohexanone formation in this electrochemical system was, however, relatively lower than that in the Q/diaphorase/O_2 system,[3b] indicating that the oxidation of the quinone on the electrode surface is the rate-limiting step. Mechanistic details and application to other enzymatic reactions are now under investigation.

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References

1) "Application of Biochemical Systems in Organic Chemistry," ed by J. B. Jones, C. J. Sih, and D. Perlman, John Wiley & Sons, New York (1976).
2) "Electroenzymology, Coenzyme Regeneration," Biotechnology Monographs, ed by K. Nakamura, M. Aizawa, and O. Miyawaki, Springer-Verlag, Berlin, Heidelberg (1988).
3) a) S. Itoh, M. Kinugawa, N. Mita, and Y. Ohshiro, *J. Chem. Soc., Chem. Commun.*, **1989**. 694; b) S. Itoh, N. Mita, and Y. Ohshiro, *Chem. Lett.*, **1990**, 1949.
4) D. C. Tse and T. Kuwana, *Anal. Chem.*, **50**, 1315 (1978); C. Degrand and L. L. Miller, *J. Am. Chem. Soc.*, **102**, 5728 (1980); H. Jaegfeldt, A. B. C. Torstensson, L. G. O. Gorton, and G. Johansson, *Anal. Chem.* **53**, 1979 (1981); A. Kitani and L. L. Miller, *J. Am. Chem. Soc.*, **103**, 3595 (1981); C. Ueda, D. C. Tse, and T. Kuwana, *Anal. Chem.*, **54**, 850 (1982); H. Jaegfeldt, T. Kuwana, and G. Johansson, *J. Am. Chem. Soc.*, **105**, 1805 (1983); B. W. Carlson and L. L. Miller, *ibid.*, **107**, 479 (1985); L. Gorton, *J. Chem. Soc., Faraday Trans. 1*, **82**, 1245 (1986).
5) C. A. Goss and H. D. Abruna, *Inorg. Chem.*, **24**, 4263 (1985).
6) Phenanthrolinequinone derivatives **1**, **2**, and **3** were prepared by HNO_3 oxidation from the corresponding phenanthrolines according to the reported method; T. S. Eckert, T. C. Bruice, J. A. Gainor, and S. T. Weinreb, *Proc. Natl. Acad. Sci. U.S.A.*, **79**, 2533 (1982). Benzoquinolinequinone derivatives **4** and **5** were prepared by I_2O_5 oxidation from the corresponding azaphenanthrenes according to the reported method; K. Kloc, J. Mlochowski, and Z. Szulc, *J. Prakt. Chem.*, **319**, 959 (1977). Azaanthraquinone derivatives **7** and **8** were prepared by cycloaddition of 1-(dimethylamino)-3-methyl-1-azabuta-1,3-diene and quinoline-5,8-dione and 1,4-naphthoquinone, respectively; K. T. Potts, E. B. Walsh, and D. Bhattacharjee, *J. Org. Chem.*, **52**, 2285 (1987).
7) In the previous study, we found that diaphorase catalyzes the electron-transfer between the heterocyclic quinones and NADH; see, Ref. 3b.

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