An Enzyme Switch Sensitive to NADH

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A microarray electrode coated with pyrrole–N-methylpyrrole copolymer containing diaphorase shows an on–off response upon addition of NADH, since diaphorase catalyses the reduction of the polymer by NADH from a conductive to an insulating state.

Electropolymerisation of pyrrole and its derivatives has been recognized as a useful way to immobilise enzymes on electrode surfaces.[1-6] The enzyme immobilised electrodes have been applied to biosensors transducing the quantity of enzyme substrates to analogue electric signals. We report here a digital enzyme device based on the microarray electrode coated with pyrrole–N-methylpyrrole copolymer containing diaphorase (Dp), a flavin enzyme catalysing the reduction of oxidized dyes by NADH.[7] The basic concept of the enzyme-based device is shown in Fig. 1. When the polymer is conductive (oxidized form), the array device is in 'on' state. The addition of NADH turns the array device 'off', since Dp immobilised in the polymer catalyses the reduction of the polymer by NADH into an insulating state.

The array electrode used in the present study was an interdigitated type[8] with two sets of Pt arrays on a glass substrate; basically, each array exposed to the electrolyte solution has 6 electrode elements 50 nm thick, 10 μm wide, 3 mm long, and separated by 10 μm from the adjacent elements (total electrode area; 5.0×10^{-3} cm^2, see Fig. 2). The array electrode was dipped in a 20 mmol dm^{-3} octadecyltriethoxy-

silane–benzene solution for 12 h to make the glass surface hydrophobic. This process is essential to obtain a thin polymer formation at the gap area[9] and rapid responses. The electro-
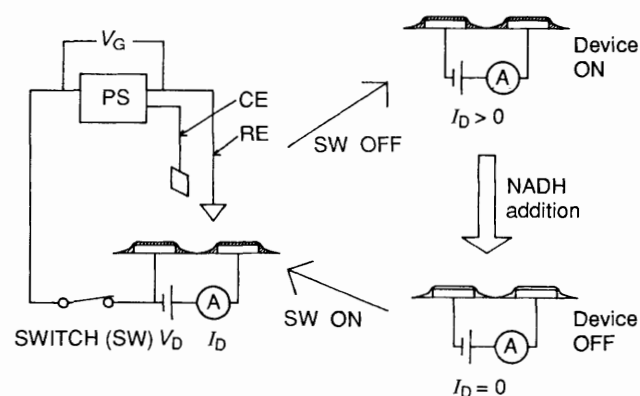

Fig. 1 Basic concept of the enzyme-based switch and the measurement system. CE = counter electrode, RE = reference electrode.
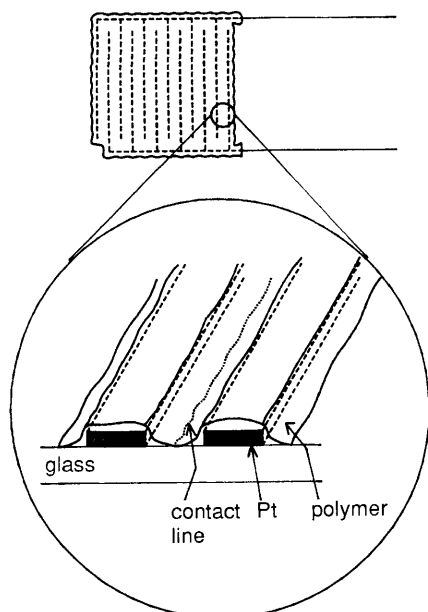
Fig. 2 Schematic illustration of the microarray device coated with a conductive polymer

polymerisation was conducted in a KNO_3 solution (0.1 mol dm^{-3}) containing 70 mmol dm^{-3} pyrrole, 30 mmol dm^{-3} N-methylpyrrole and 10 μm Dp (EC 1.6.99.–, purified from *Bacillus stearothermophilus*)[7] under potentiostatic conditions. The polymerisation potential for one of the two arrays was set at 650 mV and the other at 670 mV vs. standard calomel electrode (SCE) by means of a bipotentiostat. Since the potential of the two arrays is slightly different, ohmic contact of the polymers formed from the two arrays induces sudden change in the current due to superimposition of the ohmic current on the faradaic current for polymerisation. Therefore, *in situ* monitoring of the contact time as well as the conductivity of the polymer is possible (see Fig. 1). We terminated the polymerisation when the apparent conductivity between the two arrays became 1.5×10^{-4} S (total charge; 1.0 mC). From the total charge passed for the polymerisation, the average thickness of the polymer is calculated to be ca. 0.7 μm.[2] However, the polymer film is not uniform and the conductivity is governed by the contacting parts (or lines) between the polymers growing from adjacent electrodes (see Fig. 2). The conductivity of a thick polymer (average thickness, 5 μm) was determined by the *in situ* method and was found to be 1.3×10^{-3} S cm^{-1}, in satisfactory agreement with the value reported in the literature.[10] The average concentration of Dp in the polymer would be of the order of 10^{-5} mol dm^{-3}, since the previous study[6] demonstrated that the concentration of Dp trapped in polypyrrole is almost the same as that in the coating solution.

The array device produced by the above procedure was rinsed thoroughly with water and transferred into a 0.10 mol dm^{-3} KNO_3–20 mmol dm^{-3} phosphate buffer solution (pH 6.8, 20 °C) to investigate the electrochemical characteristics.[10,11] Fig. 3 depicts the V_G vs. I_D plot for the microarray electrode; the polymer is practically an insulator at potentials below −0.4 V vs. SCE. The conductance of the polymer increases with increasing V_G and reaches almost constant value above 0.0 V vs. SCE. The V_G vs. I_D characteristics indicate that chemical reducing agents with formal potentials less than −0.4 V can potentially reduce the polymer from a conductive to an insulating state and thus turn this array device 'off'. NADH is one of such chemicals; the formal potential of NADH/NAD^+ is −0.56 V vs. SCE. However, the direct electron-transfer from NADH to the polymer is
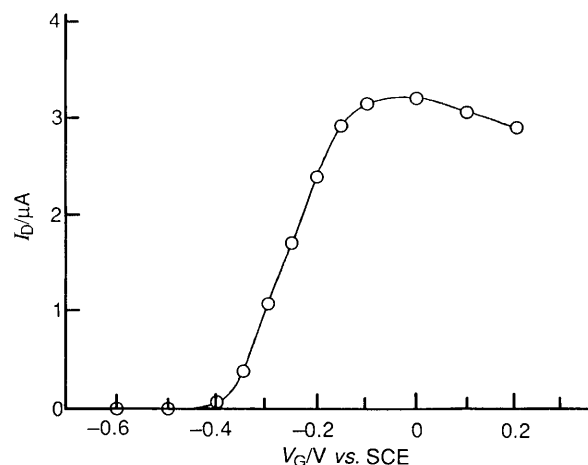

Fig. 3 Plot of I_D vs. V_G for the microarray device. V_D: 20 mV. Solution: 0.10 mol dm^{-3} KNO_3–20 mmol dm^{-3} phosphate buffer (pH 6.8).
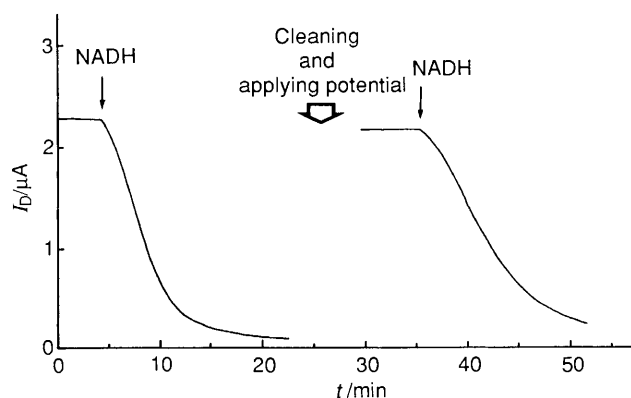

Fig. 4 Response of I_D upon addition of NADH. V_D: 20 mV. Solution: 0.10 mol dm^{-3} KNO_3–20 mmol dm^{-3} phosphate buffer (pH 6.8). Final NADH concentration: 1.0 mmol dm^{-3}.

extremely slow.[6] The electron-transfer can be speeded up by Dp in the presence of small electron-mediators.[6] We have investigated the V_G vs. I_D characteristics for array devices with various pyrrole–N-methylpyrrole copolymers. The initial potential and maximum I_D value in the V_G vs. I_D plots depend on the polymer composition. The copolymer synthesised from a solution of 7:3 monomer ratio shows the preferable characteristics for the switch device responding to NADH.

Fig. 4 shows the response of I_D of the microarray device upon addition of NADH to the electrolyte solution. The solution contains 1.0 mmol dm^{-3} anthraquinone-2-sulphonate (AQ) as an electron-mediator. The addition of NADH caused a sharp decrease in the I_D value to zero current level. Such an on–off response was not observed for the array device without Dp. These results indicate that trapped Dp catalyses the reduction of the polymer by NADH. Dp accelerates the reduction of AQ by NADH to the reduced form (AQH_2), which is the actual reductant for the conductive polymer. In the present case, the polymer on the microarray device was oxidised by applying −0.1 V vs. SCE, then the device with the oxidized polymer was disconnected from the potentiostatic control. From the V_G vs. I_D curve (Fig. 3), the polymer is partially conductive after the disconnection. We have chosen this oxidising potential since the direct reduction of the partially oxidised polymer by NADH does not proceed. The response time is dependent on the concentration of NADH added to the solution, *i.e.* high NADH concentration results in a rapid decrease in the I_D value. Similar responses were observed at least several repeated measurements.

Since AQ can be incorporated as a dopant, Dp and AQ were coimmobilized in the polymer on the array device. Although the device still responded to NADH, the response was not sharp. The optimisation of the polymerisation conditions is now underway. Such biochemical digital devices based on enzyme reactions will bring about new developments in the area of biosensors.

References

1 N. C. Foulds and C. R. Lows, *J. Chem. Soc., Faraday Trans. 1*, 1986, **82**, 1259; *Anal. Chem.*, 1988, **60**, 2473.

2 M. Umana and J. Waller, *Anal. Chem.*, 1986, **58**, 2979.

3 P. N. Bartlett and R. G. Whitaker, *J. Electroanal. Chem.*, 1987, **224**, 37.

4 C. Iwakura, Y. Kajiya and H. Yoneyama, *J. Chem. Soc., Chem. Commun.*, 1988, 1019.

5 S. Yabuki, H. Shinohara and M. Aizawa, *J. Chem. Soc., Chem. Commun.*, 1989, 945.

6 T. Matsue, N. Kasai, M. Narumi, M. Nishizawa, H. Yamada and I. Uchida, *J. Electroanal. Chem.*, 1991, **300**, 111.

7 T. Matsue, H. Yamada, H.-C. Chang, I. Uchida, K. Nagata, K. Tomita, *Biochim. Biophys. Acta*, 1990, **1038**, 29.

8 T. Matsue, A. Aoki, E. Ando and I. Uchida, *Anal. Chem.*, 1990, **62**, 407; A. Aoki, T. Matsue and I. Uchida, *Anal. Chem.*, 1990, **62**, 2206.

9 M. Nishizawa, T. Sawaguchi, T. Matsue and I. Uchida, *Synth. Met.*, submitted for publication.

10 G. P. Kittlesen, H. S. White and M. S. Wrighton, *J. Am. Chem. Soc.*, 1984, **106**, 7389.

11 E. W. Paul, A. J. Ricco and M. S. Wrighton, *J. Phys. Chem.*, 1985, **89**, 1141; C. Shu and M. S. Wrighton, *J. Phys. Chem.*, 1988, **92**, 5221.