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Biomolecular sensor based on a polypyrrole-coated microelectrode array*

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Conducting polymers have been studied extensively because of the fundamental interest and the potentiality in practical applications. An outstanding property of the polymers is that the conductivity of some of them, such as polypyrrole (PPy), polyaniline and so on, is affected by the applied voltage. For the polypyrrole, the oxidized state is of an electronic conductor and the reduced state is essentially insulating. Using this property, the polymer-based electronic devices can be fabricated. In the previous report, we showed that the conductivity of polypyrrole coated on a microelectrode array was changed with the external applied voltage and found that diodes can be prepared by using the array. The corresponding responses were measured[1]. On the other hand, the enzyme sensors for detecting the products of enzyme reactions have great potential as analytical tools for biologically important compounds. Numerous sensors have been fabricated so far by immobilizing enzyme onto various transducers, such as ion- or gas-sensitive electrodes and optical fibers. As far as we know, however, no report on immobilization of the enzyme on the surface of microelectrode array and fabrication of the corresponding electrochemical sensor has been found up to now. The molecule-based device can be used to make a microsensor, which can be used in clinical chemistry, environment monitoring, process control and biological systems, etc. In the present work, we report a novel enzyme sensor which was fabricated by immobilizing the diaphorase (DP), a nicotinamide adenine dinucleotide (NADH) dehydrogenase, in polypyrrole films on interdigitated microelectrode array (IDA) surface. The diode characteristics of PPy/Dp modified IDA (defined as PPy/DP/IDA) was studied and the $I_D \sim V_G$ response was measured. The experimental results indicate that the reduced NADH can turn the PPy/DP/IDA device from "on" state to "off" state; moreover, the "on-off" time is the function of the concentration of NADH and thus the device can be used to determine the NADH quantitatively. This work is significant for fabrication of

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molecule-based microelectronic device.

1 Experimental

Diaphorase (DP, EC 1.8.1.4, from procine heart, 3 000 u/g, Sigma), NADH (Shanghai Institute of Biochemistry) and anthraquinone-2-sulfonic acid, sodium salt (AQ, Aldrich) were used as received. Pyrrole was obtained from Fluka Chemical Company and used after distilling. Other reagents used were of analytical grade. The phosphate buffer solution was made up with 0.1 mol/L Na_2HPO_4 and 0.1 mol/L NaH_2PO_4. All solutions were prepared with doubly distilled, deionized water. Most of the electrochemical experiments were carried out with an M270 electrochemical system (EG&G, PAR, USA). In some cases, an M366 bipotentiostat with an MRE0150 recorder (EG&G, PAR, USA) was used for controlling two sets of arrays potential, respectively. The IDA electrode surface was examined by using an X-650 scanning electron microscopy (Hitachi, Japan). All the experiments were performed at room temperature.

The microelectrode array used in the present study was an interdigitated type (IDA) with two sets of Au array on a glass substrate, the scanning electron microscopy (SEM) picture of the IDA is shown in fig. 1. Basically, each array consists of 50 microelectrode elements, each of which was 8 μm wide, 0.5 mm long and separated by 4.0 μm from the adjacent elements. The complete details of fabrication procedure is the same as previously reported[2]. To carry out electrochemical experiments, a Pt counter and Ag/AgCl reference microelectrodes were sealed on either sides of IDA to form a microelectrochemical cell. Then a droplet of ca. 30 μL of the sample solution was syringed onto the microcell so as to cover the working, reference and counter electrodes.

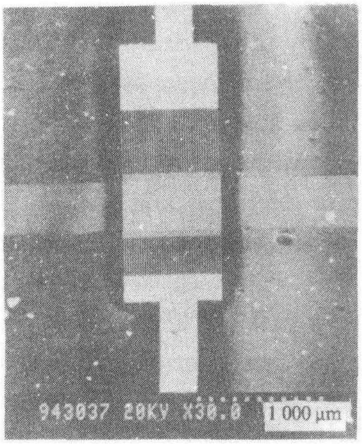


Fig. 1. Scanning electron microscope picture of IDA electrode.

The immobilization of DP and electropolymerization of pyrrole were conducted in a phosphate buffer solution (pH 7.0) containing 5×10^{-2} mol/L pyrrole and 1×10^{-3} mol/L DP under potentiostatic conditions. When DP and AQ were coimmobilized on the IDA, the phosphate buffer solution also contained 1×10^{-3} mol/L AQ. The polymerization potential for one of the two sets of arrays was at +900 mV, and for the other at +880 mV by means of a bipotentiostat. Since the potentials of the two sets of arrays were slightly different (20 mV), ohmic contact of the polymers formed from the two sets of arrays induced a sudden change in the current due to superimposition of the ohmic current on the faradaic current for polymerization. Once this sudden change in the current occurred, the polymerization process was terminated.

The array device prepared by the above procedure was rinsed thoroughly with water. The electrochemical characteristics of the PPy/DP/IDA were investigated in phosphate bufffer solution. At the start of each measurement the device was held at +0.1 V for about 30 s to oxidize the polypyrrole, the device was disconnected from the potentiostat and a bias of 20 mV was applied between the two sets of microelectrodes, then NADH was added. The I_D, flowing through the polypyrrole between the two sets of microelectrodes, was recorded as a function of time and the effect of NADH concentration on the device "on-off" time was investigated.

2 Results and discussion

2.1 Characteristics of PPy/DP/IDA device

The basic principle of the PPy/DP/IDA device and its response to NADH are shown in fig. 2. Fig. 3 depicts the I_D vs. V_G plot for the PPy/DP/IDA device, which was obtained by changing the value of voltage V_G, at the same time I_D was measured (V_D = 20 mV was maintained). The polypyrrole is practically an insulator at the value of V_G below −0.2 V, and there is no current flowing between the two sets of arrays of IDA electrode, i.e. $I_D = 0$ and the PPy/DP/IDA device is in "off" state. When the V_G is higher than −0.1 V, there is a current flowing between the IDA electrodes. The conductance of the polypyrrole increases with the increasing value of V_G and keeps almost constant when its value is above +0.1 V. At this time, the polypyrrole is in oxidized form and conductive, and the PPy/DP/IDA device is in "on" state.

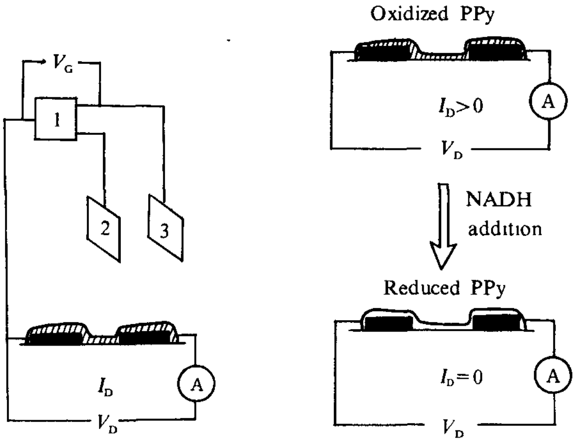


Fig. 2. Basic principle of the PPy/DP/IDA device and the configuration of electrochemical apparatus for the electrochemical measurements. 1, Potentiostat; 2, counter electrode; 3, reference electrode.

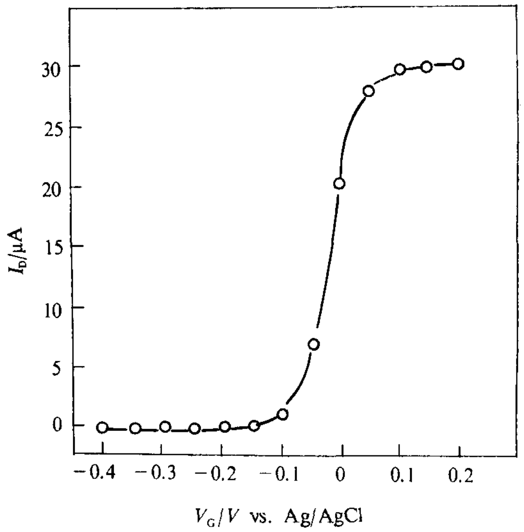


Fig. 3. Plot of I_D vs. V_G for the PPy/DP/IDA device (I_D = 20 mV).

2.2 Response of PPy/DP/IDA device to NADH

The I_D vs. V_D characteristic of PPy/DP/IDA device (fig. 3) indicates that the chemical reducing agents with formal potential less than -0.2 V can potentially reduce the polypyrrole from conductive state to insulating state and thus turn the PPy/DP/IDA device from "on" state to "off" state, consequently the PPy/DP/IDA can be used as a sensor to respond to the agent. NADH, whose formal potential is -0.56 V vs. SCE at pH 7.0, is one of such chemicals, which can be used as the "on-off" for PPy/DP/IDA device. However, the direct electron transfer rate from NADH to the polypyrrole is extremely slow, i.e. the "on-off" time is too long. It is not very useful for NADH analysis. Matsue's experimental results showed that the electron transfer rate between NADH and polypyrrole can be accelerated by DP in the presence of electron mediator, such as AQ. Since the AQ exists in anion form in aqueous solution and is negatively charged while the polypyrrole is positively charged in oxidized state, AQ can be incorporated as a dopant. AQ and DP can be coimmobilized into the polypyrrole films during the electropolymerization process. Fig. 4 shows a typical response of I_D of the PPy/DP/IDA device upon NADH. When the polypyrrole film contains DP and AQ, the addition of NADH caused a sharp decrease in the I_D value (fig. 4-2). After about 10 min, the I_D reached zero. This result indicates that NADH can change the polypyrrole from the oxidized state to the reduced state, and turn the PPy/DP/IDA device from "on" state to "off" state. Such an on-to-off response was not observed for the device when AQ was not contained in polypyrrole film (fig. 4-1).

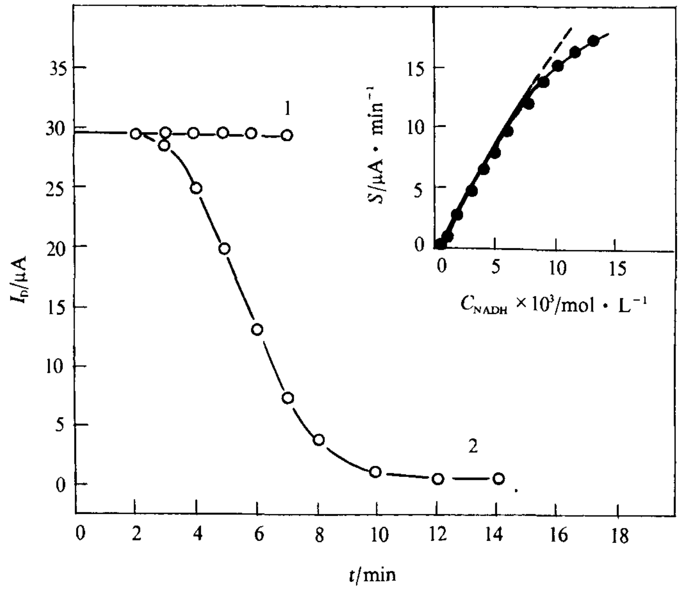
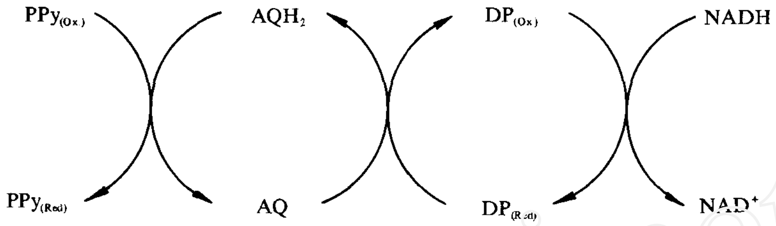


Fig. 4. Plot of the I_D as a function of time following addition of NADH to phosphate buffer solution (pH 7.0) with the final NADH concentration of 4×10^{-3} mol/L for PPy/DP (1) and PPy/DP/IDA (2) electrode respectively ($I_D=20$ mV). The inset shows a calibration plot of S as a function of the NADH concentration.

These results show that the DP immobilized in the polypyrrole accelerates the reduction of the polymer by NADH into an insulting state only in the presence of AQ. The overall reaction process can be shown by the following scheme:



From this scheme, one can conclude that the DP accelerates the reduction of AQ by NADH to the reduced form, AQH_2, which is the actual reductant for conductive polypyrrole. The "off" state device was rinsed with buffer and the polypyrrole was reoxidized by applying +0.1 V, then the NADH was added. The I_D vs. time response very similar to fig. 4-2 was obtained again. Such responses were observed at least more than ten times in the repeated measurements with the maximum average response current of 29.3 nA and the relative standard deviation of 1.8%.

The experimental results show that the response speed, i.e. the "on" to "off" time of PPy/DP/IDA device, is dependent on the concentration of NADH. High NADH concentration results in a rapid decrease in I_D value. We defined the response speed of the PPy/DP/IDA device to NADH as the slope (S) of the steepest part in the $I_D \sim t$ curve. The S depends on the NADH concentration. The inset of fig. 4 shows a plot of S as a function of the NADH concentration, C_{NADH}. The S increases with increasing C_{NADH}. The linear response between S and C_{NADH} is observed in the range of 5.0×10^{-4}—6.0×10^{-3} mol/L. The detection limit of NADH is found to be 1.0×10^{-5} mol/L. The results indicate that the PPy/DP/IDA device can be used as a detector for NADH, because a wide linear range and low detection limit can be achieved.

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