Electrochemical Investigation of DNA Adsorbed on Conducting Polymer Modified Electrode

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Detection of DNA is a very important task for molecular biology and biomedical field. We have investigated electrochemical behavior of double-stranded DNA and single-stranded DNA adsorbed on conducting polymer modified electrode in presence of cobalt complex. The possibility of using such electrode as gene detector is discussed.

Key words DNA, sensor, polypyrrole, immobilization.

In the past years, gene detection based on DNA hybridization¹ for use in clinical analysis^{2,4}, environmental monitoring⁵, and so on, has become a research area of great interest. However, the traditional methods, radioisotope technologies, were not applicable to a routine environment owing to the radioisotopic contaminants. Various novel techniques, such as non-radioactive labeling^{6,7}, the polymerase chain reaction (PCR)⁸ have been developed to replace traditional methods. However, the cost of the techniques are too high to be applied widely.

Recently, DNA sensors⁹⁻¹², novel techniques for detecting DNA specific sequence, are developed quickly due to its great promise for rapid and low-cost DNA detection. Generally, the first step of the techniques is the immobilization of a single-stranded DNA (ssDNA) probe onto the electrochemical, optical¹³ or acoustic transducers¹⁴. Then, the ssDNA immobilized ssDNA recognizes the

complementary in a sample solution and hybridization happens at the same time. Finally, the signal of hybridization is converted into an electrical signal by special transducer.

In all DNA sensors, the voltammetric DNA sensor (VDS) is attractive¹⁵ because the sensitivity of VDS is high owing to the straight conversion of electrical signal, and the DNA sensor can be performed by simple cyclic voltammetry or linear sweep voltammetry. The important step of studying the VDS is the immobilization of DNA probe. Hashimoto¹⁶ and Mikkelsen¹⁷ immobilized the DNA probe by adding a mercaptohexyl group to 5'-phosphate end or adding polydeoxyguanosine to the 3'-end and obtained high detecting sensitivity. However, the methods require the procedures of synthesis and purification which raise the cost and complexity of DNA detection. Wang's method of immobilizing DNA¹⁸ is simple and rapid by

adsorbing DNA onto carbon paste electrode surface at positive potential, but the DNA sensor is difficult to be reused under the denatured conditions of heating and 8 mol/L urea.

In this paper, we report a new, rapid, low-cost method of immobilizing DNA using polypyrrole (PPy) film as a medium to adsorb DNA molecules. The PPy film adsorbs DNA stably and is synthesized by simple electropolymerization.

Experimental

Materials

Calf thymus DNA (CT DNA) was purchased from Sino-American Biotechnology Company and was used without further purification. Denatured CT DNA was produced by heating native CT DNA in a water bath at 100 °C for about 15 min following by rapid cooling in an ice bath. Pyrrole (98%, Aldrich) was purified prior to use by distilling pyrrole under N₂. Tris(1,10-phenanthroline) cobalt(III) perchlorate (Co(phen)₃(ClO₄)₃) was prepared from cobalt(II) chloride, 1,10-phenanthroline, perchloric and bromine according to published procedures¹⁹.

Briefly, the mixture of CoCl₂·6H₂O (2.0 g) in water (40 ml) and 1,10-phenanthroline hydrate (5.0 g) in methanol (5 ml) was set to reflux for 30 min with Br₂ water (20 ml). Then HClO₄ (70%, 20 ml) was added to the hot solution. The suspension was cooled (ice) and the product was recrystallised twice from hot water. Analytical reagent grade cobalt(II) chloride, bromide, perchloric and hydrochloric acids, citrate, sodium chloride, NaH₂PO₄, Na₂HPO₄ and urea were used as received. The water used for all solutions was purified using a four-filter Milli-Q System and had a final resistivity of 18.2 MΩ/cm.

Apparatus

Cyclic voltammetry was performed with a CHI 660 Electrochemical Workstation (USA), in three electrodes cell using glassy carbon (4 mm inner diameter) as working electrode, saturated calomel electrode (SCE) as reference electrode and a platinum wire coil (0.8 mm diameter) as auxiliary electrode. The glassy carbon (GC) working electrode

was mechanically polished successively with α-Al₂O₃ powder of 1.0, 0.3 and 0.05 μm and washed with double-distilled water in an ultrasonic water bath prior to use.

Preparation of PPy/GC electrode

Polypyrrole film modified electrode was prepared by cyclic voltammetry (CV) (-0.2 ~ 0.7 V) electrooxidizing an unstirred aqueous solution of 0.1 mol/L pyrrole containing 0.1 mol/L NaCl as electrolyte in a 0.02 mol/L phosphate buffer (pH 7.0). Thickness of PPy film was controlled by the charge consumed during electrolysis.

Immobilization of DNA

Adding 1 μl hydrochloric acid (1 mol/L) into 1 ml DNA solution containing CT DNA (0.5 ~ 1.0 mg/mL) and NaCl (0.1 mol/L) to keep the solution acidic; then, the PPy/GC electrode was immersed into the DNA solution for several minutes, and gently washing the DNA/PPy/GC electrode for 30 s with a 0.02 mol/L NaCl, 0.02 mol/L phosphate buffer (pH 7.0) to wash off non-adsorbed DNA.

The surface was rinsed carefully with a 0.02 mol/L NaCl and 0.02 mol/L phosphate buffer (pH 7.0) for a short time (5 s) prior to each medium exchange. Successive measurements were carried out by renewing the surface and repeating the above procedure (using a new solution). All data were obtained at room temperature (17 ± 2 °C).

Results and Discussion

The fact that the bare smoothing GC electrode surface does not adsorb DNA in a solution had been demonstrated by Mikkelsen¹⁷ and our experiments. To detect DNA at GC electrode, we modified a layer of PPy film on GC as a medium to adsorb DNA molecules. PPy is a kind of conducting polymer material and its characteristics has been well studied²⁰⁻²⁴. PPy on electrode surface forms three-dimensional porous structure with high surface-to-volume ratio²⁵⁻²⁶; its average pore size has been estimated as 5.0-10.0 nm²⁶. The pores benefit DNA hybridization owing to reducing the contact

Fig. 1. Cyclic voltammograms of 0.3 mmol/L Co(phen)_3^{3+} (a) with and (b) without DNA adsorbed on PPy/GC electrode; conditions: 0.02 mol/L phosphate buffer (pH 7.0) with 0.02 mol/L NaCl, scan rate 100 mV/s. The PPy modified electrode was prepared by CV electrooxidizing 0.1 mol/L pyrrole solution containing 0.1 mol/L NaCl and 0.02 mol/L phosphate buffer (pH 7.0) (potential scan range: -0.2~0.7, scan rate: 100 mV/s, potential cycle number: 20); then putting the electrode in a 1 ml 0.75 mg/ml (A) dsDNA or (B) ssDNA solution containing 1 μL HCl (1 mol/L) and 0.1 mol/L NaCl for 5 min.

points between PPy and DNA adsorbed on PPy film. Further, there are positive charges on the surface of PPy film at the potential of over -0.3 V (vs. SCE)²⁷, thus DNA with a great number of negative charges on molecule surface can be stably adsorbed on the PPy^+ film.

To detect the electrical signal of DNA molecule which is electroinactive at the range of -0.9~1.0 V (vs. SCE)¹⁵, we selected Co(phen)_3^{3+} as an

Fig. 2. Cyclic voltammograms of 0.3 mmol/L Co(phen)_3^{3+} (a) with and (b) without DNA adsorbed on PPy/GC electrode; film thickness: 75 cycles; other conditions as in Fig 1.

electroactive indicator. Co(phen)_3^{3+} is reversibly electroactive (1e) with the peak potentials of $E_{pc} = 0.095$ V and $E_{pa} = 0.160$ V (vs. SCE) at glassy carbon-working electrode [28]. The complex as an indicator intercalates between the stacked base pairs of native dsDNA.

Figure 1A shows the difference between adsorbed and non-adsorbed dsDNA on the GC/PPy electrode surface. The anodic peak currents are 5.99 and 5.01 μA respectively, the difference of the peak currents (Δi_p) is 0.98 μA ; further, after more than 20 cycles, the peak currents do not change (not shown). These data demonstrate that DNA molecule had been adsorbed on PPy film surface stably by electrostatic interaction and Co(phen)_3^{3+} had been concentrated around the DNA molecule by intercalation and static electricity. Figure 1B shows the difference between adsorbed and non-adsorbed ssDNA on PPy film. The difference of the peak currents (Δi_p) is 0.10 μA , which is much lower than dsDNA. The result suggests that the adsorption of Co(phen)_3^{3+} onto ssDNA molecule by static electricity was weak compared to intercalation. Overall, the difference between results of ssDNA and dsDNA indicates that the GC electrode modified by PPy film can be used as a substrate electrode to distinguish between ssDNA and dsDNA.

Table 1. Effect of PPy film thickness on the peak current.

cycle number	2	5	10	15	20	25	30	45
dsDNA	Ad $\odot$	7.35	7.33	6.74	6.87	5.99	5.63	5.16
	B $\odot$	6.93	6.80	5.83	5.34	5.01	4.85	4.49
	$\Delta d\odot$	0.42	0.53	0.91	1.53	0.98	0.78	0.67
ssDNA	As $\odot$	7.92	7.49	6.93	6.07	5.28	5.14	5.56
	B	7.62	7.35	7.03	6.23	5.18	5.07	5.50
	$\Delta s\odot$	0.30	0.14	-0.10	-0.16	0.10	0.07	0.06

① Ad; As: the peak current of dsDNA and ssDNA adsorbed on PPy electrode, respectively.

② B: the peak current of PPy electrode without DNA adsorbed.

③ Δd ; Δs : the peak current difference between Ad and B, or As and B.

Conditions as in figure 1.

Fig. 3. The difference of anodic peak current for 0.3 mmol/L Co(phen)_3^{3+} between DNA and no DNA adsorbed on PPy/GC electrode surface as a function of DNA solution pH; other conditions as in Fig 1.

Influence of the thickness of PPy film

The number of potential cycles during electropolymerization of pyrrole can be used to estimate the thickness of the polypyrrole on glassy

carbon electrode owing to the more charge consumed, the thicker PPy forms on the GC electrode. Figure 2 shows the CV result of 0.3 mmol/L Co(phen)_3^{3+} on a thicker PPy modified electrode (PPy/GC electrode was prepared by electropolymerization of pyrrole with 75 cycles). In the curve, no apparent peak is found and ΔE ($E_{pa} - E_{pc}$) is wider than that in figure 1A. The result suggests that thicker PPy film is not suitable to detect DNA. Table 1 shows the influence of thickness of PPy film on Δi_p (the difference between the peak currents of DNA and no DNA adsorbed on the PPy film). For dsDNA, firstly, the Δi_p increases with increasing the thickness of PPy film and reaches the maximum at the film thickness of 15 cycles; Then the difference value decreases with continuing to increase the number of cycles. This fact indicates that the increasing of the thickness of PPy film in some content strengthens the adsorption of DNA molecule due to the increasing of positive charge on PPy film surface; however, as the thickness of PPy film continues to increase, the transfer of charge becomes slower, as a result, the Δi_p becomes smaller and the sensitivity of detecting DNA decreases.

The influence of pH

The pH of the solution containing DNA drastically influences the adsorption of DNA molecule onto PPy film surface. Figure 3 shows that the Δi_p increases quickly as pH decreases. The result suggests that the quantity of the surface charge of PPy film has a functional relation with pH. The relation may be explained by the following reaction²⁹:

The positive charge on PPy film surface will increase as plenty of doping hydrogen ion in an acidic solution, as a result, the PPy film adsorbs DNA molecule much more and more compacted. However, for the detection of DNA molecule, the pH value cannot be lower than 4.0 owing to the depurination. On the basis of the above discussion, the pH 3.0~4.0 may be the better pH range of DNA adsorption in a solution for a short time.

The influence of DNA adsorbing time

Figure 4 shows the time curve of DNA adsorption. In the first one minute, the Δi_p increases rapidly; after one minute, the Δi_p keeps stable. The result suggests that the adsorption of DNA molecules onto PPy film surface is controlled by dynamics and the DNA adsorption reaches saturation in one minute. Once the surface is saturated by DNA, the DNA molecules would repulse the other non-adsorbed DNA molecules by electrostatic interaction, as a result, the other molecules can not be adsorbed onto PPy film surface any more.

Fig. 4 . The difference of anodic peak current for 0.3 mmol/L Co(phen)_3^{3+} between dsDNA and no DNA adsorbed on PPy/GC electrode surface as a function of adsorption time. Conditions: potential cycle number: 20; 0.02 mol/L phosphate buffer (pH 7.0), containing 0.02 mol/L NaCl; scan rate: 100mV/s. The PPy/GC electrode was immersed in a 1 ml DNA solution containing 0.75 mg/ml dsDNA, 0.1 mol/L NaCl and 1 μl HCl (1mol/L) for 5 min.

The influence of urea and heating

The drastically denatured conditions, urea and heating, destroy the PPy/DNA/GC electrode in different extents. The PPy film was destroyed partly

by putting PPy/GC electrode into boiling water bath for 5 min. The result demonstrates that heating denaturation of dsDNA molecules to reuse the modified electrode would not be successful. We also studied the influence of 8 mol/L urea and found that the results varied with temperature. When putting DNA/PPy/GC into 8 mol/L urea solution (50 °C) for 10 min, the Δi_p did not change. The result suggests that the DNA/PPy/GC was not destructed; however, when heating the modified electrode at 70 °C under the same other conditions, the Δi_p changed apparently, which indicates the DNA/PPy/GC had been destructed. All the results demonstrate that DNA/PPy film can resist the treatment of 8 mol/L urea at low temperature. Thus we may utilize the conditions to denature the short-chain dsDNA on PPy modified electrode in order to reuse the DNA sensor.

Conclusion

A new substrate, PPy modified electrode, for immobilizing DNA has been studied. The PPy modified electrode can be made conveniently and low-cost. The positive charges on PPy film surface in an acidic solution adsorb DNA stably and the PPy film resist mild denatured condition to a certain extent which provides the possibility of reusing the DNA sensor based on PPy film. These results imply that the newly developed method of immobilizing DNA is quick and convenient for the investigation of DNA sensors.

References

1. U. Landegren, R. Kaiser, C. Caskey, L. Hood, *Science*, **242**, 229 (1988).
2. K. Millan, A. Saraullo, S. Mikkelsen, *Anal. Chem.*, **66**, 2943 (1994).
3. A. M. O. Brett, S. H. P. Serrano, T. A. Macedo, *Electroanalysis*, **8**, 992 (1996).
4. J. Wang, X. Cai, G. Rivas, H. Shiraishi, *Anal. Chem.*, **68**, 2629 (1996).
5. J. Wang, G. Rivas, D. Luo, X. Cai, *Anal. Chem.*, **68**, 4365 (1996).

6. G. Gentilomi, E. Ferri, S. Girotti, *Anal. Chim. Acta.*, **255**, 387 (1991).
7. Y. Kan, F. Chehab, *Proc. Natl. Acad. Sci. U. S. A.*, **86**, 9178 (1989).
8. J. Sevall, H. Prince, G. Garratty, *Clin. Chem.*, **39**, 433 (1993).
9. K. Hashimoto, K. Ito, Y. Ishimori, *Anal. Chim. Acta.*, **286**, 219 (1994).
10. K. Ito, K. Hashimoto, Y. Ishimori, *Anal. Chim. Acta.*, **327**, 29 (1996).
11. N. Kawazoe, Y. Ito, Y. Imanishi, *Anal. Chem.*, **68**, 4309 (1996).
12. J. Wang, E. Palecek, P. Nielsen, X. Cai, *J. Am. Chem. Soc.*, **118**, 7667 (1996).
13. P. Piunno, U. Krull, R. Hudson, *Anal. Chim. Acta.*, **288**, 205 (1994).
14. H. Su, K. Kallury, M. Thompson, A. Roach, *Anal. Chem.*, **66**, 769 (1994).
15. S. Mikkelsen, *Electroanalysis*, **8**, 15 (1996).
16. K. Hashimoto, K. Ito, Y. Ishimori, *Anal. Chem.*, **66**, 3830 (1994).
17. K. Millan, A. Spurmanis, S. Mikkelsen, *Electroanalysis*, **4**, 929 (1992).
18. J. Wang, X. Cai, G. Rivas, H. Shiraishi, *Anal. Chim. Acta.*, **326**, 141 (1996).
19. L. Dollimore, R. Gillard, *J. Chem. Soc., Dalton Trans.*, 933 (1973).
20. A. Diaz, K. Kanazawa, G. Gardini, *J. Chem. Soc., Chem. Commun.*, 635 (1979).
21. S. J. Dong, J. Ding, *Synth. Met.*, **20**, 119 (1987).
22. S. J. Dong, J. Ding, *J. Chem. Soc., Faraday Trans. I.*, **85**, 1599 (1989).
23. E. K. Wang, Y. Liu, Z. Samec, *Electroanalysis*, **2**, 623 (1990).
24. E. K. Wang, Y. Liu, S. J. Dong, J. Ding, *J. Chem. Soc., Faraday Trans.*, **86**, 2243 (1990).
25. S.W.Feldberg, *J. Am. Chem. Soc.*, **106**, 4671 (1984).
26. M. Umana, J. Waller, *Anal. Chem.*, **58**, 2979 (1986).
27. H. Eisazadeh, G. Spinks, G. Wallace, *Materials. Forum.* **16**, 341 (1992).
28. M. T. Carter, A. J. Bard, *J. Am. Chem. Soc.*, **109**, 7528 (1987).
29. Q. B. Pei, R. Y. Qian, *Synth. Met.*, **45**, 35 (1991).