Chinese Chemical Letters Vol. 15, No. 8, pp 997-1000, 2004 997
http://www.imm.ac.cn/journal/ccl.html

DNA Nano-netting Intertexture on Carbon Electrodes

Xiang Qin LIN*, Xiao Hua JIANG, Li Ping LU

Department of Chemistry, University of Science and Technology of China, Hefei 230026

Abstract: Native calf thymus double stranded DNA (ct-dsDNA) is successfully immobilized from solution onto carbon substrates by covalent linkages under an optimized deposition potential of 1.8±0.3 V *vs*. 50 mmol/L NaCl-Ag/AgCl. The long chain DNA fabricates a layer of well conductive nano-netting intertexture, which is stable in pH 14 alkaline solution and in boiling water. The ct-dsDNA modified carbon fiber disk electrode shows two to three orders of magnitude enlarged electrode effective surface area and similarly enlarged voltammetric responses to $Co(phen)_3^{3+}$ and dopamine. Thermal dissociated single stranded ct-DNA can also lead to similar result. This modified electrode will find wide applications in the fields of DNA-based electrochemical biosensors.

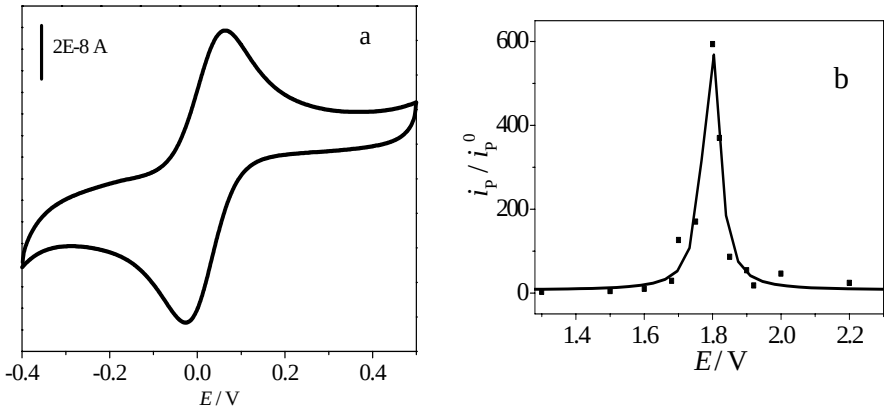
Keywords: Calf thymus DNA, immobilization, carbon fiber electrode, electrochemical biosensor.

Since Jaqueline Barton observed the electronic conductivity of deoxyribonucleic acid (DNA) in the early nineties, various techniques for assembling DNA on solid substrate have been developed for studying the charge transfer mechanism inside the double helix, which is important not only in the field of nanoelectronics, but also for better understanding biological functions of DNA and related molecules in living cells[1-2]. Besides, the DNA modified electrode provides a new type of biosensors based on the interaction of DNA and small bioactive molecules[3-4].

Native DNA molecules have been successfully attached onto carbon surface from solution by covalent bond under exactly controlled DC potential in our work. Under optimized conditions, the planted calf thymus DNA (ct-DNA) forms a nano-netting intertexture on the substrates such as carbon fiber and HOPG electrodes. This intertexture is featured as an electric conductive layer resulting great enhancement of effective surface area up to 1000 times the original. This process was controlled by electrochemical deposition method under a steady state potential in the range of 1.6 to 2.1 V *vs*. 50 mmol/L NaCl-Ag/AgCl. **Figure 1a** shows a typical electrochemical response of $Co(phen)_3^{3+}$ on the DNA modified carbon fiber disk electrode (CFDE) in THB (5 mmol/L pH 7.1 Tris-HCl + 50 mmol/L NaCl). It can be measured from the cyclic voltammetry (CV) curves. The results showed that the modification of ct-dsDNA led to an enlargement of about 500 times for the charging current and 200 times for the Faradic current. By using differencial pulse voltammetry (DPV) 600 times enhancement was obtained (not shown). The Faradic current enhancement is attributed to the enlargement of the surface

* E-mail: xqlin@ustc.edu.cn

Figure 1 Cyclic voltammetry of $Co(phen)_3^{3+}$(a) and DPV peak current enlargement *vs.* DC depositing potential (b)



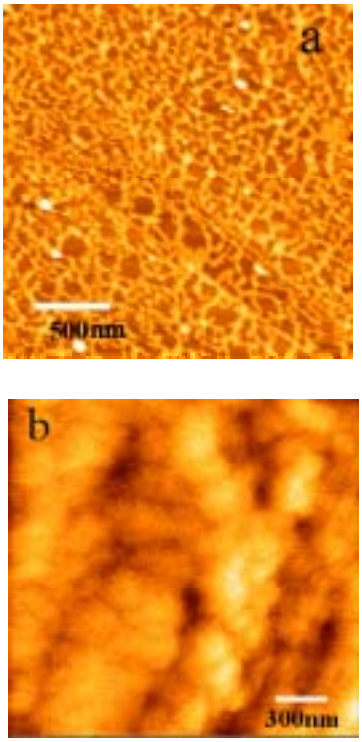
(a) at ct-dsDNA/CFDE prepared at 1.8 V for 30 min and at bare CFDE (insert); (b) at ct-dsDNA/CFDEs prepared under different deposition potentials in 0.1 mg/mL ct-dsDNA for 30 min. CV: 50 mV/s; DPV: 25 mV amp. Solution: 0.3 mmol/L $Co(phen)_3^{3+}$ + THB.

effective area of the disk electrode, which was as a result of a special shape and structure of DNA deposition.

The enlargement effect is a potential dependent factor. As seen in **Figure 1b**, only a narrow region of 1.6 ~ 2.1 V is characteristic for an effective deposition; an optimum potential of 1.8 V was demonstrated, and the enlargement of DPV current up to a maximal value of 600 was obtained. In the case of the electric potentials below +1.8 V the DNA base pairs could not get well-stacking form, while the electric potentials were higher than +1.8 V, this may cause a serious surface oxidation of the carbon electrode and disturb the immobilization of DNA. So the enlargement effect is less obvious in both cases.

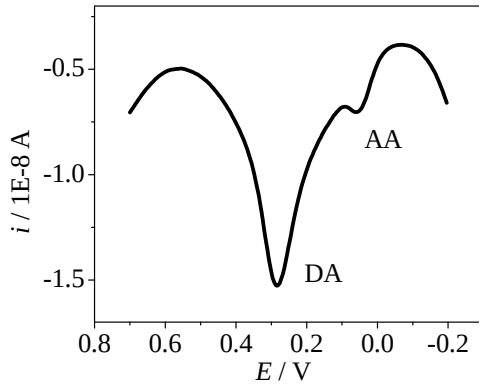
Figure 2 shows the AFM images of DNA deposition example on HOPG and carbon fiber column electrode (CFCE). It can be seen that the DNA long chain twisted and crossly linked forming a fine intertexture on HOPG. Similar but less resolved netting image on CFCE was obtained, indicating a little denser and thicker configuration was resulted by higher speed deposition. The AFM image on CFDE was not successfully observed due to technical difficulties. However, it can be suggested that the similar hollow netting intertexture was also fabricated on CFDE with even thicker structure due to the spherical diffusion condensation during the deposition process. Besides, AFM images on HOPG (not shown) indicated DNA was deposited either in uniform film or in separated nanoparticles at lower or higher potentials, which were evidenced less conductive by electrochemical methods. It is proposed that the DNA was attached to the electrode *via* its one end with its strand compressed and twisted, forming a three dimensional intertexture in the presence of electric field. This was expected for increasing the conductivity of the helix and making for electrochemical sensing.

Figure 2 Tapping mode AFM images of ct-dsDNA



Deposited on HOPG (a) and on a carbon fiber column electrode (b) at 1.8 V for 30 min.

Figure 3 DPV of DA-AA mixture at ct-dsDNA/CFDE



Solution: 5 μ mol/L DA + 5 mmol/L AA + 0.1 mol/L pH 7.4 PBS. DPV: 50 mV amp.

To testify the stability of ct-dsDNA modified CFDE (labeled as ct-dsDNA/CFDE), it was treated in strong basic solution and boiling water, respectively. The result showed that the response of cobalt complex on ct-dsDNA/CFDE treated in pH 14 NaOH solution

for 10 min had almost no change. This indicated that a strong covalent binding was formed between the DNA and the carbon surface. And it excluded the possibility of forming phosphate ester or carbonic ester which can be easily hydrolyzed in alkaline solution. After treated in boiling THB for 3 min, the electrode showed a doubled charging current and partial reduction of the Faradic current, which was attributed to denaturizing the dsDNA to ssDNA. This means thermal dissociated single stranded ct-DNA could also be used for this assembling. Thus, the dsDNA/CFDE could be applied in wide pH ranges and thermal conditions.

Preliminary application of this electrode was made for sensing some small molecules such as catecholamine neurotransmitters in mimic biological conditions. A typical DPV is shown in **Figure 3**. Similar enlargement effect was also observed for dopamine but much less enlargement for ascorbic acid and uric acid. This is probably due to electrostatic repulsion of the polyanionic sugar-phosphate backbone of DNA. This simple molecular recognition effect provided a tolerance ratio of ascorbic acid for the dopamine determination higher than 1000.

Conclusion

A novel nano-netting DNA intertexture is fabricated on carbon substrates at controlled deposition potential, which has good conductivity and is stable in alkaline solution and in boiling water. The modification can produce two to three orders of enlargement of both the effective electrode surface area and Faradic sensitivity for some species such as $Co(phen)_3^{3+}$ and dopamine. This modification technique is of great significance in the fields of DNA-based devices.

References

1. R. Holzel, N. Gajovic-Eichelmann, F. F. Bier, *Biosensors and Bioelectronics*, **2003**, *18*, 555.
2. C. R. Treadway, M. G. Hill, J. K. Barton, *Chem. Phys.*, **2002**, *281*, 409.
3. Y. Zhao, D.W. Pang, S. Hu, Z. D. Wang, J. K. Cheng, H. P. Dai, *Talanta*, **1999**, *49*, 751.
4. X. Q. Lin, L. P. Lu, X. H. Jiang, *Microchem. Acta*, **2003**, in press.

Received 14 July, 2003