Studies on Electrochemical Behavior of Bleomycin and Its Interaction with DNA at a Co/GC Ion Implantation Modified Electrode

HU, Jing-Bo(胡劲波) LI, Qi-Long*(李启隆) SHANG, Jun(尚军)
Department of Chemistry, Beijing Normal University, Beijing 100875, China

Electrochemical investigation of Bleomycin (BLM) and its interaction with DNA at a Co/GC ion implantation modified electrode was reported for the first time. The electrochemical behavior of BLM at Co/GC modified electrode has been studied by linear sweep and cyclic voltammetry, The reaction of BLM with DNA formed an electrochemically nonactive complex, which resulted in a decrease in the BLM equilibrium concentration and its reduction current. The decrease in peak current was proportional to DNA concentration and could be used to determination DNA concentration. The equilibrium constant of DNA-BLM complex β was determined to be 5.16×10^{16}. The experiments of AES and XPS showed that Co was surely implanted into the surface of GCE(glassy carbon electrode) and the depth distribution of Co was in good agreement with Gaussian normal distribution; the implanted Co at GCE improved the electrocatalytic activity.

Keywords Co/GC ion implantation modified electrode, linear sweep voltammetry, bleomycin, DNA

Introduction

Ion implantation is a kind of new material surface modification technique. Recently, Li *et al.*[1] have led this technique into analytical chemistry and made modified electrode with particular function. It has been applied to study the electrochemical behaviors and the determinations of organic drugs, which offers the following advantages: (1) It can implant different ions into different material surfaces according to different desires and requirements to make modified electrodes with catalytic activity. (2) Because the implanted surface causes defect and partial dislocation to form many active centres, its catalytic activity is much higher than that of the raw material. (3) The modified electrode has good stability and reproductivity.[1]

For several reasons the interaction of anticancer drugs with DNA has attracted considerable interest. The bleomycin (BLM) exhibits antitumor and antibacterial activity,[2] and has been employed for treatment of human squamous cell carcinoma. The properties and determinations of BLM by gas chromatography and high performance liquid chromatography,[3-5] immunological method,[6,7] microbial method,[8,9] polarography[10] and adsorptive stripping voltammetry[11,12] have been reported. The interaction of free porphyrin TMAP with DNA[13] and the interaction of BLM with DNA have been reported.[14] In this work, electrochemical behavior of BLM and its interaction with DNA at Co/GC ion implantation modified electrode were studied for the first time and a method for the determination of DNA was developed. In HOAc-NaOAc (0.1 mol/L, pH 4.62), a reductive peak of BLM was obtained by voltammetry. The peak potential was −0.73 V(vs. SCE). The peak current was proportional to the concentration of BLM over the range of 5.0×10^{-8}—1.0×10^{-6} mol/L and 1.0×10^{-6}—1.0×10^{-5} mol/L. The reaction of DNA with BLM formed an electrochemically nonactive complex, which resulted in a decrease in the BLM equilibrium concentration and its reduction current. The decrease in

* E-mail: liqilong@bnu.edu.cn
Received May 23, 2001; revised October, 29, 2001; accepted November 23, 2001.
Project supported by the National Natural Science Foundation of China (No. 29875003) and the Research Fund for the Doctoral Program of Higher Education of China (No. 98002709).

peak current was proportional to DNA concentration and could be used to determine DNA concentration. The experiments of AES and XPS showed that Co was surely implanted into the surface of GCE. The implanted Co at GCE improved the electrocatalytic activity.

Experimental

Apparatus

The electrode material was glassy carbon (GC), which was cut, polished and cleaned by acetone, NaOH (1 mol/L), HNO_3(1 mol/L) and water, successively. JY2 8010 Metal Vacum Arc Ion Source was used for ion implantation at an accelerating voltage of 40 keV in the dosage of 5×10^{17} Co^{2+}/cm^2. The electrode surface area was 1.0 cm^2. CHI660M Electrochemical Workstation CH Instruments Inc. (USA) was used for linear sweep and cyclic voltammetry, with a three electrode system consisting of a Co/GC electrode as working electrode, a SCE as reference electrode and a platinum as counter electrode. A Model 501 thermostat (Experimental Instrument Factory, Shanghai, China) was used to maintain a temperature of 25 ± 0.20 ℃. A Model DF 807 digital pH/ion meter was used for pH determination.

Reagents

BLM was obtained from the Institute of Materia Medica, Chinese Academy of Medical Science (Beijing, China), with a purity of 98%. Stock standard solution of BLM (1.7×10^{-4} mol/L) was stored in the dark. Calf thymus DNA (CTSNA) and Fish sperm DNA (FSDNA) were purchased from Beijing Baitai Technology Company. The stock solution of CTDNA and FSDNA was prepared by directly dissolving them in triple distilled water. Their concentrations were determined by the absorbance at 260 nm in 0.05 mol/L NaCl/0.005 mol/L Trihydroxymethyl aminomethane (pH 7.1) solution. Unless otherwise stated, the DNA in this paper represents CTDNA. The mixture of DNA and BLM was incubated in 1.0 mol/L HOAc-NaOAc solution (pH 4.62) for 2 h at 37 ℃. All the chemicals were of analytical grade. All solutions were prepared from triple distilled water.

Procedure

A 10 mL of HOAc-NaOAc (0.1 mol/L, pH 4.62) containing a specific amount of sample solution was added to cell and purged with purified nitrogen for 4 min to remove oxygen. The voltammogram was recorded by using linear sweep voltammetry at a scan rate of 100 mV/s. The scan was terminated at −1.4 V.

Results and discussion

Electrochemical behavior of BLM

In HOAc-NaOAc (0.1 mol/L) buffer solution, a sensitive reduction peak of BLM was obtained by linear sweep voltammetry at a Co/GC ion implanted modified electrode (Fig. 1). The peak potential was −0.73 V (vs. SCE) (curve c), illustrating that Co/GC electrode had higher catalytic activity for the reduction of BLM. The peak current was proportional to the concentration of BLM over the range of 5.0×10^{-8}—1.0×10^{-6} mol/L and 1.0×10^{-6}—1.0×10^{-5} mol/L with a detection limit of 1.0×10^{-8} mol/L. It can be determined the trace of BLM.

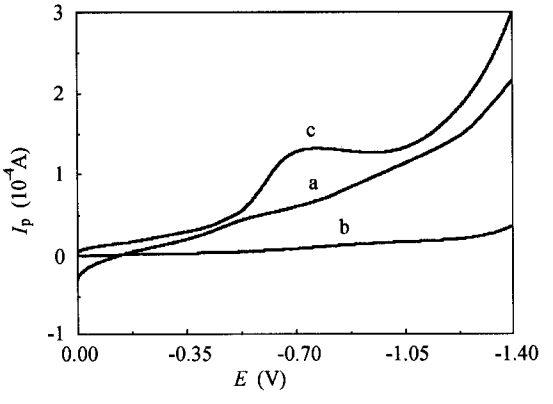

Fig. 1 Linear-sweep voltammograms. (a) 0.1 mol/L HOAc-NaOAc at Co/GCE, (b) 0.1 mol/L HOAc-NaOAc + 1.0×10^{-6} mol/L BLM at GCE; (c) 0.1 mol/L HOAc-NaOAc + 1.0×10^{-6} mol/L BLM at Co/GCE.

Repetitive cyclic voltammograms

The repetitive cyclic voltammograms for 1.0×10^{-6} mol/L BLM were shown in Fig. 2. The cathodic and anodic current hardly changed, indicating that BLM had little adsorption characteristic at the Co/GC electrode. A

well-defined peak was observed on the anodic branch, and $I_{pa}/I_{pc} \neq 1$, $\triangle E_p = 0.20$ V, indicating that the reduction process of BLM at the Co/GC electrode was quasi-reversible.

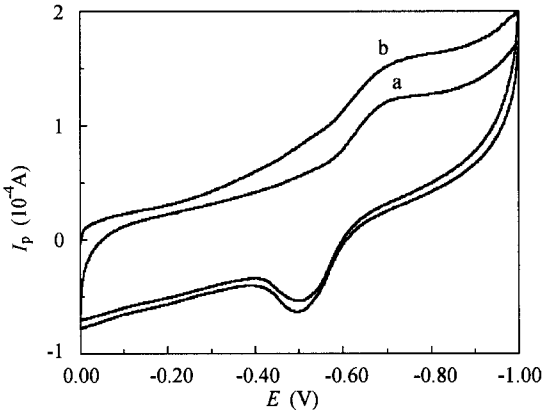

Fig. 2 Repetitive cyclic voltammograms. (a) First scan, (b) second scan. 0.1 mol/L HOAc-NaOAc + 1.0×10^{-6} mol/L BLM, $v = 100$ mV/s.

Effect of scan rate

The effect of scan rate v on the peak current I_p was studied. The I_p was linear function of $v^{1/2}$; the relationship between the I_p and v showed a downward-inclined curve, indicating that the peak current of BLM was diffusion controlled.

The experiment of effect of the accumulation time on the peak current was also studied and indicated that the peak current hardly changed with increasing the accumulation time. This showed that this system had no adsorption property.

Effect of scan rate on catalytic efficiency

The ratio of the peak current at the Co/GC electrode I_c to the peak current at the GCE I_d in the solution containing BLM is an important parameter. It shows the catalytic efficiency of the Co/GC electrode. The ratio I_c/I_d in different scan rate showed in Fig. 3. The I_c/I_d decreased with increasing the scan rate, indicating that the slower the scan rate is, the more beneficial to the reduction of BLM at the Co/GC electrode. This is evidently a characteristic of electrocatalytic reaction.[15]

Surface analysis of Co/GC *electrode surface*

The depth distribution of Co element in the surface of the Co/GC electrode was in good agreement with Gaussian normal distribution.[16]

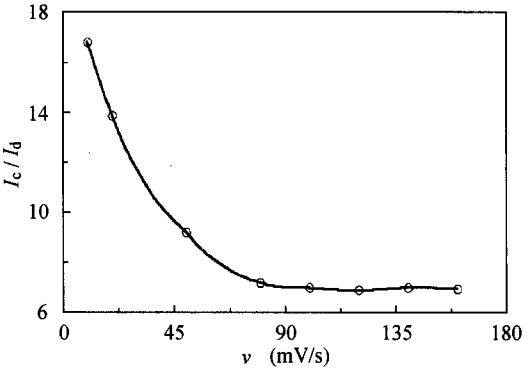

Fig. 3 Effect of scan rate on catalytic efficiency. 0.1 mol/L HOAc-NaOAc, 1.0×10^{-6} mol/L BLM

XPS of a GCE and Co/GC electrode showed that there were only two elements C and O in the surface of the GCE, while there were three elements C, O and Co in the surface of Co/GC electrode, indicating that Co was implanted into the GCE surface.[16]

Studies of the mixture of BLM *and* DNA

The experiments showed that in HOAc-NaOAc (0.1 mol/L) buffer solution (pH 4.62), BLM was reducible at the Co/GC electrode, while DNA was not reducible. Under the conditions of 0.1 mol/L HOAc-NaOAc buffer solution and the warming in water bath for 2 h at 37 ℃, the peak current of BLM decreased with increasing the concentration of DNA, indicating that the interaction of BLM with DNA had taken place to form an electrochemically nonactive complex.

Effect of temperature on the complex formation of BLM *with* DNA

The effect of temperature on the peak current was studied in the temperature over the range of 4—40 ℃ for 2 h and the results were shown in Fig. 4. The peak current decreased with increasing the temperature and tended to a stable value at the temperature of 30—40 ℃.

Effect of warming time on the complex formation of BLM *with* DNA

The effect of warming time on the peak current was also studied. Fig. 5 showed that the peak current decreased with increasing the warming time and tended to a stable value when the time is 1.5—3.0 h at 37 ℃, indicating that the interaction of BLM with DNA was completed.

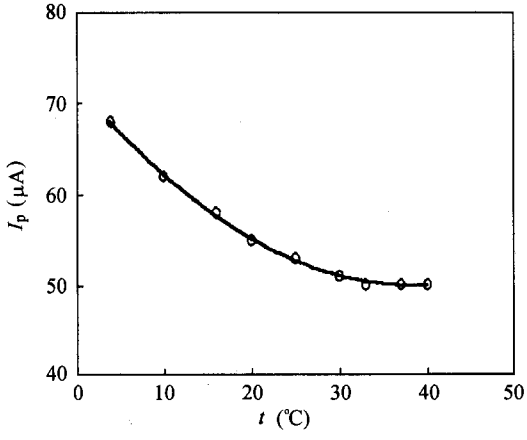

Fig. 4 Effect of temperature. 0.1 mol/L HOAc-NaOAc (pH 4.62), 1.7×10^{-6} mol/L BLM, 5.3×10^{-6} mol/L DNA, $v = 100$ mV/s, $t_{acc} = 2$ s.

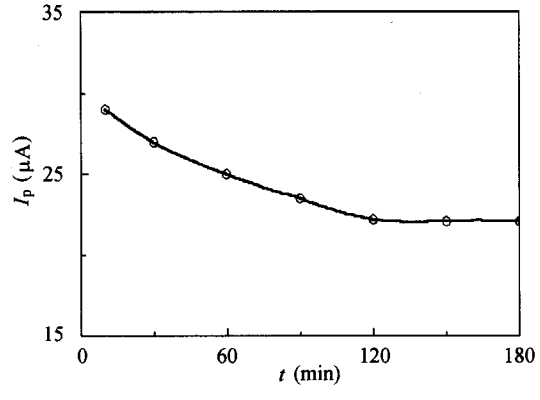

Fig. 5 Effect of warming time. The conditions are the same as in Fig. 4.

Determination of the stoichiometry of DNA-BLM *complex*

In Fig. 6 curve (b) typically represented the current I_p change at $c_{DNA} = 5.3 \times 10^{-6}$ mol/L on varying the concentration of BLM. Curve (a) showed the relationship between I_p and c_{BLM} in the absence of DNA. Curve (c) is the relationship between $\triangle I_p$, which is $I_{pa} - I_{pb}$, and the concentration of BLM.

According to the method,[13] it is assumed that the interaction of DNA with BLM forms only a single complex DNA-mBLM:

$$\text{DNA} + m\text{BLM} \Leftrightarrow \text{DNA-}m\text{BLM}$$

The equilibrium constant is

$$\beta = [\text{DNA-}m\text{BLM}]/([\text{BLM}]^m \cdot [\text{DNA}]) \quad (1)$$

And the following equations can be deduced:

$$\triangle I_{MAX} = k' c_{DNA} \quad (2)$$

$$\triangle I = k'[\text{DNA-}m\text{BLM}] \quad (3)$$

$$[\text{DNA}] + [\text{DNA-}m\text{BLM}] = c_{DNA} \quad (4)$$

$$\triangle I_{MAX} - \triangle I = k'\{c_{DNA} - [\text{DNA-}m\text{BLM}]\} \quad (5)$$

$$= k'[\text{DNA}] \quad (6)$$

Incorporating (3) and (6) into (1) leads to

$$\log[\triangle I/(\triangle I_{MAX} - \triangle I)] = \log\beta + m\log[\text{BLM}] \quad (7)$$

If DNA and BLM form a single complex, the plot of $\log[\triangle I/(\triangle I_{MAX} - \triangle I)]$ versus $\log[\text{BLM}]$ becomes linear with the slope of $m = 2.87 \approx 3$ (Fig. 7). From the intercept of the line the equilibrium constant β can be calculated to be 5.16×10^{17}.

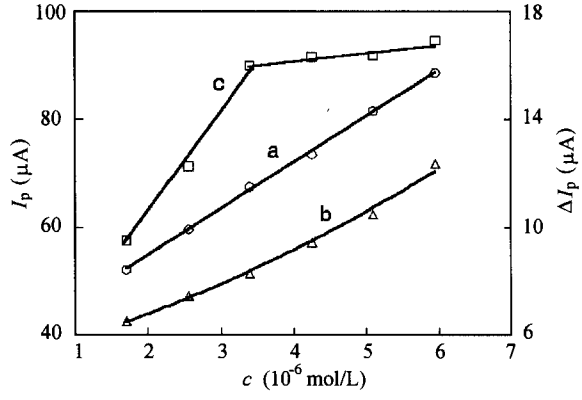

Fig. 6 Relationships between I_p and c_{BLM} (a, b) and between $\triangle I_p$ and c_{BLM} (c). (a) $c_{DNA} = 0$, (b) $c_{DNA} = 5.3 \times 10^{-6}$ mol/L, (c) $\triangle I_p = I_{pa} - I_{pb}$. Other conditions are the same as in Fig. 4.

Analytical application

Adding DNA to BLM solution resulted in a decrease in the I_p of BLM, which can be used to determine DNA concentration. When DNA was added to 1.7×10^{-6} mol/L BLM solution, the I_p of BLM decreased linearly with DNA concentration from 2.65×10^{-7} to 1.06×10^{-6} mol/L. The linear regression equation is $I_p = 72.62c - 5.654$ (I_p in μA, c in 10^{-6} mol/L), and the correlation coefficient $r = 0.9978$. DNA samples 1 and 2 were obtained from the Department of Biology, Beijing Normal University. The determination results were shown in Table 1. Table 2 showed consistency by two kinds of methods, *i. e.*, UV spectroscopy (UV-S) and electrochemical method (ECM), indicating that the ECM is suitable for the determination of DNA concentration.

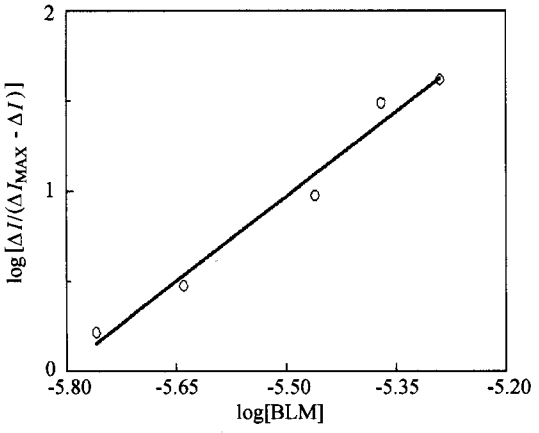

Fig. 7 Relationship between $\log[(\triangle I/\triangle I_{MAX} - \triangle I)]$ and log[BLM].

Table 1 Results of sample determinations

Sample	Added (10^{-6} mol/L)	Found (10^{-6} mol/L)	Recovery (%)	RSD (%)
CTDNA	1.54	1.50	97.4	1.2
	3.09	3.15	101.9	2.0
FSDNA	5.15	5.05	98.1	1.4
CTDNA + FSDNA	1.54 + 2.55	4.20	102.7	1.7
1		3.75		1.5
2		3.05		1.8

Table 2 Comparison of results determined by UV-S and ECM

Sample	UV-S (10^{-6} mol/L)	ECM (10^{-6} mol/L)
1	3.75	3.55
2	3.07	3.10

References

1 Li, Q.; Hu, J. *Huaxue Tongbao* **2000**, *3*, 32 (in Chinese).

2 Ishizuka, M.; Takayama, H.; Takeuchi, T.; Umezawa, H. *J. Antibiot., Ser. A* **1967**, *20*, 15.

3 Shiu, G. K.; Goehl, T. J. *J. Chromatogr.* **1980**, *181*, 217.

4 Aszalos, A.; Gawfor, J.; Vollmer, P. *J. Pharm. Sci.* **1981**, *70*, 878.

5 Chang, T.; Lee, T. M.; Borders, B. *J. Antibiot.* **1984**, *79*, 1098.

6 Gaver, R. C.; Dixon, C. W.; Smith, R. D. *Cancer Chemother. Pharmacol.* **1986**, *16*, 207.

7 Teale, J. D.; Clough, J. M.; Marks, V. B. *J. Cancer* **1977**, *35*, 822.

8 Elson, M. M.; Shafer, R. B. *Med. Pediatr. Oncol.* **1978**, *5*, 213.

9 Tom, W.; Lynch, W. E.; Sartiano, G. P. *Anal. Biochem.* **1980**, *108*, 306.

10 Tan, X.; Li, Q. *Fenxi Huaxue* **1997**, *25*, 789 (in Chinese).

11 Li, Q.; Tan, X.; Hu, J. *Chem. J. Chin. Univ.* **1997**, *18*, 37 (in Chinese).

12 Tan, X.; Hu, J.; Li, Q. *Analyst* **1997**, *122*, 991.

13 Qu, F.; Li, N.; Jiang, Y. *Anal. Chim. Acta* **1997**, *344*, 97

14 Hideo, S.; Kazuo, N.; Emiko, A.; Hiroshi, Y.; Bobuo, T.; Hamao, U. *J. Antibiot.* **1970**, *10*, 473.

15 Andricux, C. P.; Blocman, C.; Dumas-bouchiat, J. M.; Saveant, J. M. *J. Am. Chem. Soc.* **1979**, *101*, 3431.

16 Hu, J.; Huang, Q.; Li, Q. *Chin. Sci. Bull.* **2001**, *46*, 1355.

17 Laviron, E. *J. Electroanal. Chem.* **1974**, *52*, 355.

(E0105235 LI, L. T.; HUANG, W. Q.)