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Establishment of dsDNA/GNs/chit/GCE biosensor and electrochemical study on interaction between 6-mercaptopurine and DNA

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Abstract. In this paper, the anti-cancer drug 6-mercaptopurine (6-MP) was taken as the detection object. The biosensor of dsDNA/GNs/chit/GCE was established using the grapheme (GNs) and chitosan (chit) as the compound modified material. The electrochemical behavior of 6-MP on the sensor was discussed, and the damage and its mechanism of 6-MP on DNA were studied. The experimental result showed that, after the modification of GNs-chit, the electrode activation area of GNs/chit/GCE increased remarkably, which was improved from 1.76cm^2 to 8.64 cm^2, and the responsive oxidation peak current of GNs/chit/GCE to $K_3[Fe(CN)_6]$ also increased remarkably. At the meantime, it was demonstrated that DNA was effectively fixed on the GNs/chit/GCE electrode;6-MP caused obvious damage to dsDNA, and the damage degree on the adenine was bigger than that on the guanine; the interaction between 6-MP and dsDNA was preliminarily deduced as the intercalation, and its electrochemical oxidation process was an irreversible process controlled by the adsorption.

Keywords: Biosensor,grapheme,6-MP, intercalation, DNA damage

1. Introduction

6-mercaptopurine (6-MP) is an important antitumor drug, which is commonly used for the antitumor and transplant rejection, and clinically used for treating the acute leukemia and chorionic epitheliomaand etc. Its pharmacologic action is considered to be related with the sulfydrylsin it and their influence on the synthesis of RNA and DNA, which is also considered as the reason of their carcinogenesis, thus the 6-MP has the double function.

The oxidative damage of DNA would result in gene mutation, cell dysfunction, necrocytosis and even cancer [1]. Comparing with traditional detection technique [2][3], biosensors, which were constructed based on prominent conductive capacity of electroactive polymers and surface effect of nanophase materials, provide a sensitive and rapid method to detect DNA damage [4],[5].

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Graphene(GNs) has a surprisingly high electronic mobility, and its large specific surface greatly increases the adhesive rate of other molecules on its surface. At the meantime, grapheme has a great biocompatibility. As an electrode modified material, grapheme has been applied to the sensor area to detect micromolecules and biomolecules in recent years [6],[7]. Chitosan (chit) is a kind of natural high-polymer material with great physicochemical property and film-forming property. It has the chelate and adsorption function for many ions, organics and biomolecules, and has been used to prepare the composite material by electrostatic self-assembly method with the chitosan [8].

Research on the interaction between the anti-cancer drug and the DNA would be of a great significance on the carcinogenesis mechanism of drugs, the pharmacology and toxicity of anti-cancer drugs, also on the design and synthesis of new drugs. The study of the interaction of 6-MP and DNA by utilizing the biosensor has been paid much attention [9],[10].

In this paper, the 6-MP is taken as the detection object, and the dsDNA/GNs/chit/GCE electrode is established using chitosan and grapheme as the modification composite material. The cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS) and differential pulse voltammetry (DPV) etc. methods are used to discuss the electrochemistry behavior of 6-MP on DNA biosensor, and the damage mechanism of 6-MP on DNA are also researched.

2. Experimental method

2.1. Reagent and material

The glassy carbon electrode (GCE, GC130, Φ3mm) is from Tianjin Aidahengsheng Technology Co. Ltd. The Herring sperm double-stranded DNA (dsDNA) is of biochemistry pure from Sigma.

2.2. Pretreatment for GCE

The electrode is treated with “8” form polish on the alumina powder with the diameter of 0.3, 0.1 and 0.05 μm, respectively. The electrode is washed by the distilled water and the ethanol and then ultrasonically washed by the distilled water.

2.3. Preparation of modified electrode

Chit/GCE electrode: a certain amount of chitosan is added into 1% (w/w)acetic acid solution, then the solution is treated with ultrasound until it is homogeneous and transparent, and the chit-acetic acid solution of 0.7%(w/v)is obtained. 10μL chit-acetic acid solution is drawn and homogeneously coated on the GCE, then dried in the air.

GNs/chit/GCE electrode: a certain amount of GNs is added into the chit-acetic acid solution above and treated with ultrasound until they are homogeneously mixed, and the GNs/chit dispersion liquid of 0.5mg/mL is prepared. 10μL dispersion liquid is drawn and homogeneously coated on the GCE, then dried in the air.

DNA/GNs/chit/GCE electrode: a certain amount of dsDNA is dissolved in Tris-HCl, and the dsDNA solution of 9 mg/mL is prepared. The dsDNA is homogeneously coated on the GNs/chit/GCE surface, stored in the refrigerator at 4℃.

2.4. Electrochemical test

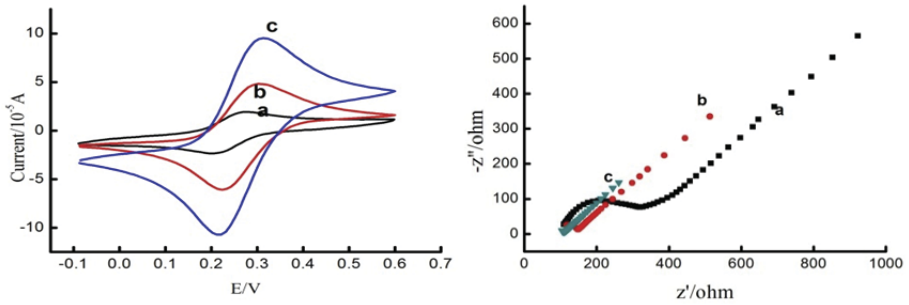
The electrochemical performance is tested by the Autolab model PGSTAT30. The platinum electrode is the counter electrode, and the saturation calomel electrode is the reference electrode.

The sweeping speed of CV test is 50mV/s, the electric potential amplitude is 5mV and the scanned area is from -0.1 to 0.6V; The frequency range of EIS testis 10000-0.1Hz with the electric potential amplitude of50mV; The modulation amplitude tested by DPV is 0.05V, the awaiting electric potential is 0V, the interval time is 0.2s, and the pulse time is 0.05s; The equilibrium time tested by chronocoulometry method is 2s, the interval time is 0.1s, and the potential duration is 0.25V/s.

3. Results and Discussion

3.1. CV and impedance of different modified electrodes

It could be observed from Fig.1(A) that a couple of redox peaks are presented by the three electrodes (a), (b) and (c). Compared with (a), the redox current of (b) modified with chitosan increases a little, while the redox current of (c) increases a lot. One reason is that, the GNs material has strong nanoscale and surface effect, increasing the adsorption amount of $[Fe(CN)_6]^{3-}$ on the electrode surface; the other is that GNs has great electric conduction, and the electric signal is enhanced with the combined action of the two above, thus, the redox peak current of (c) increases a lot.



(A)(B)

Fig.1.CV and Impedance curves of different modified electrodes: (A) The CV of different modified electrodes in the 0.2 M KCl solution containing 5×10^{-3} M $K_3[Fe(CN)_6]$: (a) GCE, (b) chit/GCE, (c) chit/GNs/GCE; (B) The EIS of different modified electrodes in the 0.1M KCl solution containing 5×10^{-3} M $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (1:1): (a) GCE, (b) chit/GCE, (c) chit/GNs/GCE.

As shown in Fig.1(B), the resistance value of (a) is the biggest for there are no modifications. The resistance value of (b) is smaller than (a) for there is amino azyl in the chitosan molecule, thus, it could be better combined with the negative ion in the solution, thereby improving the transfer of the charge. The resistance value of (c) is smaller than (b) due to the good conductivity, superior size effect and surface effect of the GNs, which significantly enhances the transfer of the charge.

3.2. Active area of modified electrode and electrochemical behavior of dsDNA on modified electrode

The active area of the electrode modified with the compound materials is calculated with the chronocoulometry and with 1×10^{-4}M $K_3[Fe(CN)_6]$ as the model compound.

According to the chronocoulometry formula:

$$Q_d = \frac{2nFAD_O^{1/2}C_O^* t^{1/2}}{\pi^{1/2}} + Q_{dl} + nFA\Gamma_O \quad (1)$$

Where Q_d is the charge or electricity (C); n is the electron transfer number for the electrode reaction; F is the Faraday constant; A is the electrode active area (cm^2); D_0 is the diffusion coefficient of 1×10^{-4} M $K_3[Fe(CN)_6]$ in 1 M KCl supporting electrolyte, which is 7.6×10^{-6} cm^2 s^{-1}[11]; t is the electrolysis time (s); $C_0$is the initial molar concentration (M) of the active substance in solution.

According to the above formula, a linear relation is presented between $Q_d$and$t^{1/2}$. $Q_{dl}+nFA\Gamma$ is the intercept of the obtained linear equation Q~/$t^{1/2}$.

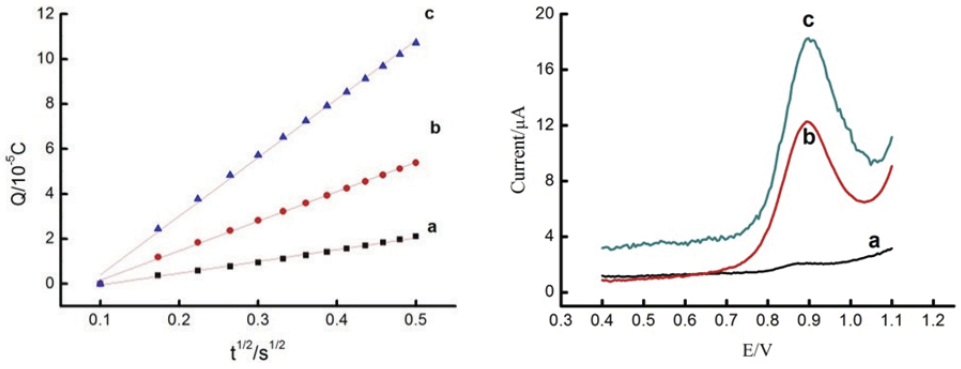
The relation of Q and $t^{1/2}$ for different modified electrodes in KCl solution is shown in Fig.2(A). The linear equations for the curves a, b and c in Fig.2(A)are obtained by the linear fitting:

$$y= 5.3\times10^{-5}x-6.3\times10^{-6}, (R^2=0.9959) \quad (2)$$

$$y=1.3\times10^{-4}x-1.0\times10^{-5}, (R^2=0.9986) \quad (3)$$

$$y=2.6\times10^{-4}x -1.9\times10^{-5}, (R^2=0.9976) \quad (4)$$

According to the above linear equations, combined with Eq. (1), the active areas of the electrodes are calculated, and the results are as follows: the GCE electrode 1.76cm^2, chit/GCE electrode 4.32cm^2, GNs/chit/GCE electrode 8.64cm^2, indicating that the specific surface area of the GCE is significantly increased with the modification of GNs-chit, which facilitates the electronic transfer.



(A)(B)

Fig.2.Active area of modified electrode and electrochemical behavior of dsDNA on modified electrode: (A) The Q~$t^{1/2}$ of different modified electrodes in the 1M KCl solution containing 1×10^{-4} M $K_3[Fe(CN)_6]$: (a) GCE, (b) chit/GCE, (c) GNs/chit/GCE; (B) Electrochemical behavior of dsDNA in different modified electrodes in 0.1 M phosphate buffer: (a) dsDNA/GCE, (b) dsDNA/chit/GCE, (c) dsDNA/chit/GNs/GCE.

As shown in Fig.2(B), there is an oxidation peak of guanine in dsDNA at 0.90V, while the oxidation peak of dsDNA/GCE at 0.90V is quite weak. After the modification, an obvious dsDNA characteristic peak is observed at 0.90V (curves (b) and (c)), which indicates that dsDNA has been fixed on the modified electrode efficiently.

The oxidation peak current of dsDNA/chit/GCE is bigger than that of dsDNA/GCE, the reason is that the good biocompatibility the chitosan increases the fixed amount of dsDNA on

chit/GCEelectrode. Additionally, the large amount of amino azyl in the chitosan also improves the transmission capacity of the charge on the electrode.

The peak current of dsDNA characteristic peak increases obviously when GCE electrode is modified with GNs due to its good electrical conductivity, the big specific surface area of GNs and the large number of the active functional group on the GNs surface, which would improve the adsorption of dsDNA on the electrode.

3.3. Electrochemical behavior of 6-MP on modified electrode and its damage to dsDNA

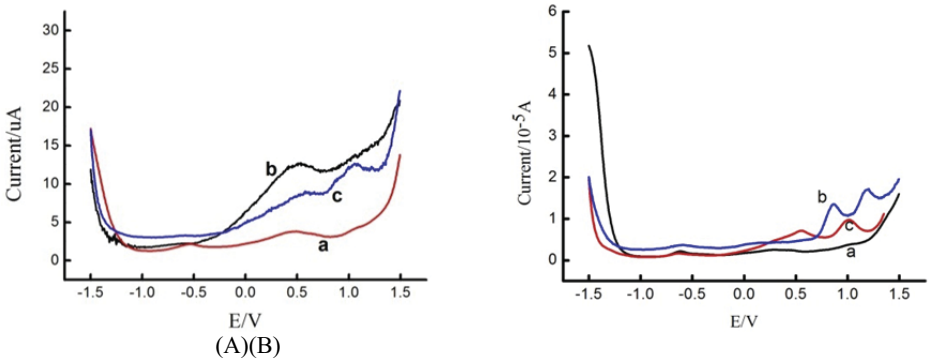
It can be seen from Fig.3(A) that, a weak buffer solution characteristic peak on the GNs/chit/GCE electrode is found for the buffer.

As the GNs/chit/GCE is scanned in the buffer with 6-MP, an oxidation peak is presented at around 0.5V. By the comparison of curves (a) and (b), for only 6-MP is added in the buffer solution, it could be deduced that the oxidation peak at around 0.5V is the characteristic oxidation peak of 6-MP.

By the comparison of curves (b) and (c), there are two oxidation peaks found at around 0.6V and 1.1V for curve (c). It is confirmed that the peak around 0.6V is the oxidation peak for 6-MP,and the peak around 1.1V is the guanine oxidation peak for dsDNA.

By the comparison of the peak position and value of 6-MP in curves (b) and(c), it is observed that the oxidation peak of 6-MP is shifted to the right obviously and the current peak value decreases. The reason for the decrease is that the dsDNA is a biomacromolecule, the electronic transmission capacity thereof is weaker than that of ionic compound, and the charge conduction capacity is decreased.

According to the Literature [12], as the micromolecule interacted with DNA, the oxidation peak potential is shifted to the right, which illustrated that the interaction between the material and DNA is the intercalation. Thus, it could be determined preliminarily that the combination mode between 6-MP and dsDNA is the intercalation.



(A)(B)

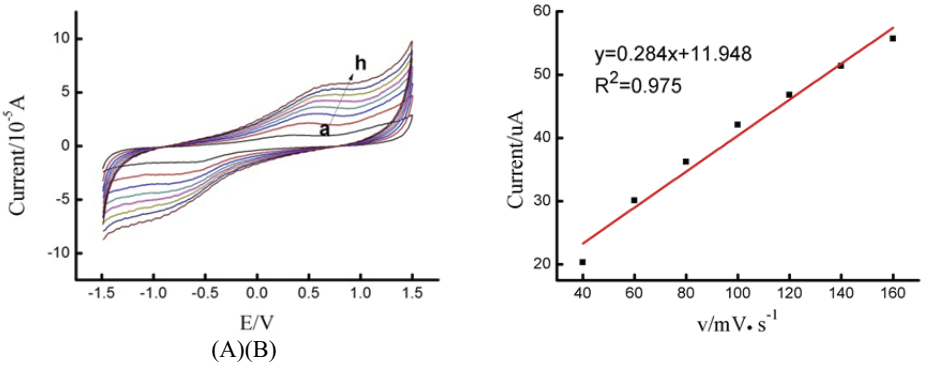
Fig.3.Electrochemical behavior of 6-MP on modified electrode and its damage to dsDNA: (A) The DPV of dsDNA/GNs/chit/GCE in 0.1M phosphate buffer with6-MP: (a) GNs/chit/GCE in buffer, (b) GNs/chit/GC in buffer containing 7.5×10^{-4} M 6-MP, (c) dsDNA/GNs/chit/GCE in buffer with7.5×10^{-4} M 6-MP; (B) The DPV of electrode in 0.1 M phosphate buffer containing different6-MP: (a)GNs/chit/GCE in buffer, (b) dsDNA/GNs/chit/GCE in buffer, (c) dsDNA/GNs/chit/GCE in buffer containing 7.5×10^{-4} M6-MP.

The damage of 6-MP on dsDNA has been showed in Fig. 3(B). It can be observed from curve (a) that the DPV curve of the GNs/chit/GCE electrode is smooth and no obvious oxidation peak is found, while two oxidation peaks are found at 0.9V and 1.2V for the dsDNA/GNs/chit/GCE (curve b). The two peaks are corresponding to the characteristic oxidation peaks of guanine and adenine of dsDNA.

As dsDNA/GNs/chit/GCE is scanned in the buffer with 7.5×10^{-4} M 6-MP (curve c), the oxidation peak of 6-MP is found at around 0.5V. By the comparison of curve (b) and (c), after 6-MP is added, the oxidation peak current of the guanine decreased obviously, and the characteristic oxidation peak of the adenine disappeared, which means that the damage of 6-MP on the dsDNA is obvious and the interaction of 6-MP and adenine is stronger.

3.4. Effect of scan speed on interaction between dsDNA and 6-MP

It could be observed from Fig.4(A) that, as the scan speed increases from 20 mV/s to 160mV/s, the oxidation peak current increases gradually.



(A)(B)

Fig.4.Effect of scan speed on interaction between dsDNA and 6-MP： (A) The CV of dsDNA/GNs/chit/GCE in phosphate buffer containing 6-MP under different sweep speed: (a), (b), (c), …, (h) were 20, 40, 60,…, 160mV/s, respectively; (B) The relationship between oxidation peak current of 6-MP on dsDNA/GNs/chit/GCE and sweep speed.

The curve for the scan speed and oxidation peak current is shown in Fig.4(B).The result indicated that the relation between 6-MP oxidation current and scan speed is linear, which means that the electrochemical oxidation of the 6-Mp on dsDNA/GNs/chit/GCE is an irreversible process controlled by adsorption. The relation of oxidation current and scan speed is fitted by the equation as follows:

$$i/(\mu A) = 0.28v/(mV/s) + 11.95, (R^2=0.975) \quad (5)$$

4. Conclusion

The electrode active area and current of GNs/chit/GCE increased obviously with the modification of GNs-chit. The dsDNA/GNs/chit/GCE electrode was successfully established by utilizing the good biocompatibility of the chitosan, the good nanometer effect and charge transmission capacity of the GNs. The damage of 6-MP on DNA was obvious. Compared with the guanine, the interaction of 6-MP and adenine was stronger. The combination mode between 6-MP and dsDNA might be the intercalation. The electrochemical oxidation of the 6-MP on dsDNA/GNs/chit/GCE was an irreversible process controlled by adsorption.

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