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Studies on the interaction mechanism between hexakis(imidazole) manganese(II) terephthalate and DNA and preparation of DNA electrochemical sensor

Shu-Sheng Zhang *, Shu-Yan Niu, Bin Qu, Gui-Fen Jie, Hua Xu, Cai-Feng Ding

College of Chemistry and Molecular Engineering, Qingdao University of Science and Technology, Zhengzhou Road 53, Qingdao, Shandong 266042, PR China

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Abstract

The interaction between hexakis(imidazole) manganese(II) terephthalate ($[Mn(Im)_6](teph) \cdot 4H_2O$) and salmon sperm DNA in 0.2 M pH 2.30 Britton–Robinson buffer solution was studied by fluorescence spectroscopy and cyclic voltammetry. Increasing fluorescence was observed for $[Mn(Im)_6]^{2+}$ with DNA addition, while quenching fluorescence phenomenon appeared for EB-DNA system when $[Mn(Im)_6]^{2+}$ was added. There were a couple quasi-reversible redox peaks of $[Mn(Im)_6]^{2+}$ from the cyclic voltammogram on the glassy carbon electrode. The peak current of $[Mn(Im)_6]^{2+}$ decreased with positive shift of the formal potential in the presence of DNA compared with that in the absence of DNA. All the experimental results indicate that $[Mn(Im)_6]^{2+}$ can bind to DNA mainly by intercalative binding mode. The binding ratio of the DNA-$[Mn(Im)_6]^{2+}$ association complex is calculated to be 1:1 and the binding constant is 4.44×10^3 M^{-1}. By using $[Mn(Im)_6](teph) \cdot 4H_2O$ as the electrochemical hybridization indicator, the DNA electrochemical sensor was prepared by covalent interaction and the selectivity of ssDNA modified electrode were described. The results demonstrate the use of electrochemical DNA biosensor in the determination of complementary ssDNA.

Keywords: Hexakis(imidazole) manganese(II) terephthalate; Cyclic voltammetry; Differential pulse voltammetry; Intercalative binding; Electrochemical DNA biosensor; Fluorescent sensor

1. Introduction

The knowledge of the structure of DNA and its interactions with other biological compounds can lead to advances in pharmacology and diagnosis basis of many diseases [1–5]. A variety of methods for DNA-based diagnostics have been studied in the past decades [1–7]. Simple optical detection schemes, such as UV absorbance and fluorescence detection, have been used extensively when coupled with separation methods [5]. Gel electrophoresis of DNA sequences performed by polymerase chain reaction (PCR) is a simple and effective tool to detect specific DNA sequence [2]. Electrochemical DNA biosensors are widely used to detect DNA hybridization events, because of their high sensitivity and compatibility [2,6,7]. A DNA electrochemical biosensor generally is an electrode with an oligonucleotide immobilized on the surface [8–10]. These devices can be used sequence-specific hybridization events directly [11,12] or by DNA intercalators (metal coordination complexes, antibiotics, etc.), which form complexes with the nitrogenous bases of DNA [4,8,10, 13,14].

Imidazoles are a common component of a large number of natural products and pharmacologically active molecules [15]. The imidazole ring functions as a ligand toward transition metal ions in a number of biologically important systems [15,16]. These facts make imidazole and its derivatives important target analytes. It was reported that the metal coordination compound of imidazole could inhibit tumor growth by interacting with DNA [17–20]. For exam-

* Corresponding author. Tel.: +86 532 84022750; fax: +86 532 4023927.
E-mail address: shushzhang@126.com (S.-S. Zhang).

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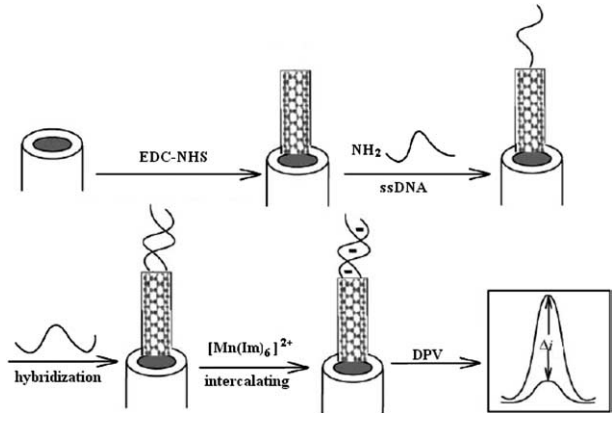
ple, the nichel(II) coordination compounds of imidazole can bind with DNA and has been regarded as efficient anti-tumor drugs [17]. Tamura reported that $Cu(OAc)_2(Im)_2$ also has the anti-tumor effect [20]. It was also reported that synthesized zinc(II) porphyrin conjugate with an appended pyrene subunit exhibit significant fluorescence sensing toward imidazole derivatives [15]. As one of the several transition metal elements, manganese has been found to play an important role in the metabolism process in the biological system and in the redox process in the organism [21–23]. However, the complexes of Mn(II) with imidazole ligand are rarely reported. To our knowledge, the study on the interaction of hexakis (imidazole) manganese(II) terephthalate with DNA has not been reported yet.

In this paper, the $[Mn(Im)_6](teph) \cdot 4H_2O$ was synthesized according to our previous report [24]. The interaction between $[Mn(Im)_6](teph) \cdot 4H_2O$ and DNA has studied by cyclic voltammetry and fluorescence spectroscopy. The experimental results have proved that $[Mn(Im)_6](teph) \cdot 4H_2O$ could interact with DNA mainly by intercalative binding. Using $[Mn(Im)_6](teph) \cdot 4H_2O$ as the electrochemical hybridization indicator, the electrochemical DNA sensor was prepared and tested by covalent interaction. The results demonstrate that $[Mn(Im)_6](teph) \cdot 4H_2O$ is a promising electrochemical DNA biosensor to determinate the complementary ssDNA. This will bring further insight about the interaction mechanism between $[Mn(Im)_6](teph) \cdot 4H_2O$ and DNA, and is helpful for further research to design novel anti-tumor drugs and/or diagnosis disease. Schematic representation of the electrochemical DNA biosensor based on covalently DNA immobilization and target-specific DNA detection is shown in Scheme 1.

2. Experimental

CHI 832 electrochemical analyzer was obtained from Shanghai Chenhua Instrument Company of China; the three electrode system consisted of the glassy carbon electrode (GCE) as the working electrode, a Ag/AgCl as the reference electrode and a platinum wire as the auxiliary electrode. Hitachi F-4500 flurospectrophotometer was obtained from Hitachi company of the Japan. pHS-25 pH meter was obtained from Shanghai Leici Instrument Factory of China. KQ-50B ultrasonic cleaner was obtained from Kunshan ultrasonic Imstrument Company. DF-101B magnetic stirrer with constant temperature was obtained from Gongyi Yingyuyuhua Instrument Company. 78-1 magnetic heating-up stirrer was obtained from Jiangsu Jintan Zhengji Instrument Factory.



Scheme 1.

Salmon sperm DNA (10 mg mL^{-1}) was purchased from Shanghai Huashun Biological Engineering Company ($A_{260}/A_{280} > 1.8$); the concentration was determined by the ultraviolet absorption at 260 nm ($\varepsilon = 6600\ M^{-1}\ cm^{-1}$). Ethidium bromide (EB, 10 mg mL^{-1}) was also purchased from Shanghai Huashun Biological Engineering Company and was diluted to the needed concentration with double distilled deionized water. The $[Mn(Im)_6](teph) \cdot 4H_2O$ was homemade. 2.50×10^{-2} M $[Mn(Im)_6](teph) \cdot 4H_2O$ solution: 0.1749 g $[Mn(Im)_6](teph) \cdot 4H_2O$ was dissolved in the distilled water and then diluted to 10 mL. 0.2 M pH 2.3 Britton–Robinson buffer solution (BR); 5.00×10^{-2} M, pH 7.4 phosphate buffer solution (PBS); 2.00×10^{-2} M, pH 7.0 tris(hydroxymethy) ammomethane-HCl buffer solution (Tris–HCl); 1.00×10^{-3} M ethylenediamine tertraacetic acid solution (EDTA). Tris–HCl–EDTA buffer solution (TE): 1.00×10^{-2} M Tris–HCl + 1.00×10^{-3} M EDTA, adjusted to pH 8.0; 5.00×10^{-2} M 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide solution (EDC); 8.00×10^{-3} M *N*-hydroxysuccinimide solution (NHS); 2.00×10^{-2} M NaCl solution; 1.00×10^{-2} M KCl solution. Other chemicals employed were of analytical grade and doubly deionized water were used in all solutions. The 20-base oligonucleotides neomycin-3′-phosphotransferase gene (NPTII) were purchased from Shanghai Huashun Biological Engineering Company; their base sequences are as follows: S_1: 5′-GCC GAG AAA GTA TCC ATC AT-3′; S_2: 5′-ATG ATG GAT ACT TTC TCG GC-3′. The 20-base sequence S_1 is complementary to 20-base sequence S_2. All oligonucleotide stock solutions of the 20-base oligomers (100 $\mu g \cdot mL^{-1}$) were prepared with TE solution.

2.1. Fluorescence spectroscopic studies of the interaction between $[Mn(Im)_6]^{2+}$ and DNA

0.25 mL of 2.50×10^{-2} M $[Mn(Im)_6]^{2+}$solution and 2 mL of BR buffer solution were transferred into each of four 10 mL colorimetric tubes, and then different amounts of salmon sperm DNA solution were added respectively. The mixture was diluted to the mark and reacted for 9 min at room temperature. The measurements of fluorescence were made by using Hitachi F-4500 flurospectrophotometer in 1.0 cm quartz cell, and the excited wavelength was 300 nm.

One milliliter of 1.27×10^{-3} M EB solution and 2 mL BR buffer solution were transferred into each of three 10 mL colorimetric tubes, and then different amounts of DNA and $[Mn(Im)_6]^{2+}$ solution were in turn added, respectively. The mixture was diluted to the mark and reacted for 9 min at room temperature. The measurements of fluorescence were