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Preferential binding specificity of silver cation to a single nucleobase over base pairs evaluated by abasic site-containing DNA

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ABSTRACT

The binding specificity of silver cations to abasic (AP) site-containing DNA was electrochemically investigated by comparison with the fully matched DNA without the AP site. AP site-containing DNA is designed in a way that only the nucleotide opposite the AP site is variable to allow for coexistence of an unpaired nucleotide and a number of DNA base pairs. The surface of a gold electrode was modified by AP site-containing DNA duplex on which Ag^+ binding specificity was evaluated. Electrochemical investigations on the AP-DNA-modified electrodes reveal that Ag^+ preferentially associates to the unpaired nucleotides instead of the coexisted base pairs and shows sequence-dependant binding, especially stronger for purines than for pyrimidines. Additionally, the hydrogen bond pattern moieties of the unpaired nucleotides should be involved in Ag^+ binding evidenced by a decrease of the redox signal when introducing a ligand with its hydrogen bond moiety complementary to the nucleotide deoxycytidine. This is the first attempt to make a comparison in one DNA molecule for metal ion binding to coexisted unpaired nucleotide and DNA base pairs. The present method demonstrates an easy way for investigating binding specificity of heavy metal ions to AP site in the presence of coexisted DNA base pairs.

1. Introduction

Metal ion-DNA interaction is responsible for a wide range of biochemical processes such as DNA stability [1], initiation of antitumor agent therapeutics [2]. Metalation of DNA is an alteration for construction of nanomaterials, for example, silver (Ag) nanowires [3]. The interaction of Ag^+ with DNA was convinced at very early stage by potentiometric titrations and spectroscopy and at least three types of binding model (types I, II, and III) were suggested [4,5] (binding around base pairs for the type I, conversion of the N–H···N hydrogen bond of a complementary base pair to an N–Ag–N bond for the type II and binding at higher silver/DNA concentration ratio for the type III). DiRico et al. [6] reported that guanine instead of cytosine or thymine in DNA was available for Ag^+ binding. However, binding of Ag^+ to backbone phosphate groups was excluded. Arakawa et al. [7] suggested that Ag^+ associated to guanine N7 and adenine N7. Nevertheless, the binding sites of nucleobases for Ag^+ are not very clear [8]. Up to now, Ag^+ binding preference to single nucleotide or base pairs is still not evaluated. Additionally, compared with that occurred to DNA base pairs, difficulties arise when investigating the interaction of metal ion with free single nucleobase because multiple sites [8] in nucleobase are available for metal ion binding and in some cases insoluble products were formed [9].

Here, a series of abasic site (AP site)-containing DNAs are designed to allow for coexistence of an unpaired nucleotide and base pairs in one DNA molecule. The unpaired nucleotide is embedded within the DNA helix and freely approaching each other in the presence of Ag^+ for formation of the insoluble polymeric products [9] is avoided. It is, therefore, convenient to compare the Ag^+ binding specificity within these two types of involved sites in this design. AP site-containing DNA (AP-DNA) is also in vivo produced in cell by removal of a damaged nucleotide [10]. So the binding specificity of metal ions to the AP site should be clearly useful for evaluating unfound toxicity of metal ions in gene level. We have recently discovered that AP site binding of a ligand (2-amino-7-methyl-1,8-naphthyridine, AMND) with its hydrogen bond pattern complementary to the base opposite the AP site can facilitate electron transfer through DNA [11]. In the present study, we describe the voltammetric studies of self-assembled monolayers of the AP-DNA duplexes upon Ag^+ association (Scheme 1), by variety of the nucleotide opposite the AP site and comparison with fully matched DNA without the AP site (FM-DNA).

2. Experimental

DNAs were synthesized by Shanghai Sangon Biotechnology Co. Ltd. (Shanghai, China) and purified by HPLC. AMND was

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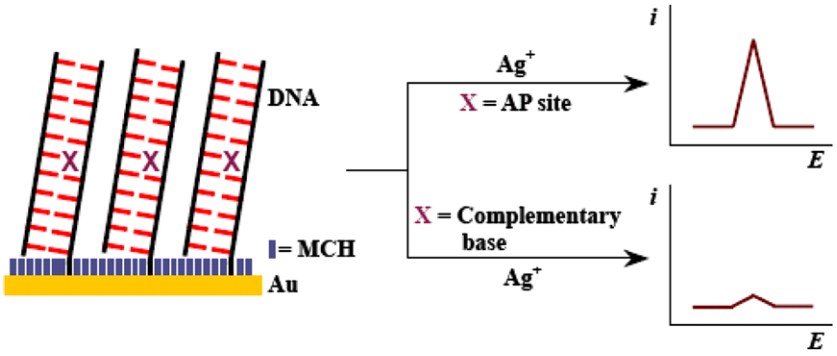

Scheme 1. Demonstration for construction of DNA duplex-modified gold electrode for evaluation over the binding specificity of silver cation to AP-DNA and FM-DNA.

synthesized according to the literature [12]. The DNA concentration was measured by UV absorbance at 260 nm using extinction coefficient calculated by the nearest neighbor analysis [13]. The DNA strand containing the AP site with (for electrode assembly) or without (for homogeneous solution investigation) a thiol linker terminus at the 5′ end (HS-$(CH_2)_6$-5′-TCTGCGTCCAG**X**GCAACGCACAC-3′, **X** = tetrahydrofuranyl residue as AP site model) was mixed in an equimolar amount with its complementary strand containing four different bases opposite the AP site (5′-GTGTGCGTTGC*N*CTGGACGCAGA-3′, *N* = A, C, G, or T) and annealed (for FM-DNA, **X** = G, *N* = C). Then 20 μl of 10 μM DNA duplex with the thiol linker terminus in 0.1 M phosphate buffer solution (PBS, pH 7.0) containing 0.1 M $MgCl_2$ with or without 50 μM AMND was dropped onto a gold disk electrode (1.6 mm in diameter, BAS, USA) to form a monolayer and the electrode was kept under the saturated vapor pressure condition overnight. The electrode was again immersed into a 1 mM 6-mercapto-1-hexanol (MCH) buffer solution for 1 h. After triply washing with 0.1 M PBS containing 1 mM EDTA (for removal of Mg^{2+}) and 0.1 M PBS, the electrode modified by DNA duplexes was immersed into a 0.1 M PBS containing desired Ag^+ concentration ($AgNO_3$, 99%, Sigma, St. Louis, USA) for 15 min. After washing and immersing the electrodes in 0.1 M PBS 10 min, electrochemical experiments were performed using CHI 1030A (CHI, Austin, USA) at 25 °C in 0.1 M PBS free of Ag^+. The gold electrode modified by DNA duplexes with Ag^+ loading, Ag/AgCl (sat. KCl, BAS), and platinum wire (Nilaco, Japan) were used as working, reference, and counter electrodes, respectively. About 0.1 M PBS was deoxygenated by purging with purified nitrogen gas. For simplicity, the formed AP-DNA duplexes were referred to AP-[N] (N = A, C, G, or T) according to the bases opposite the AP site.

3. Results and discussion

The binding of Ag^+ to DNAs without the thiol linker terminus was first analyzed by potentiometric titration [4] with freshly prepared silver wire and Ag/AgCl (sat. KCl, BAS) as indicator and reference electrodes, respectively. No significant difference in potential response was observed for AP-DNAs and FM-DNA in identical Ag^+ concentrations (data not shown). This could be caused by the fact that the used DNA sequences are identical (for the total 22 base pairs) except the AP site and any weakly binding event will make the specific binding at the AP site undetectable. So it is unsuitable at homogeneous aqueous solution to distinguish the Ag^+ binding preference to the unpaired nucleotide from the coexisted base pairs. Therefore, the DNA self-assembled monolayer at electrode surface was constructed by reaction of DNA thiol terminus with gold [14]. In order to minimize non-specific adsorption and passivate occasional void gold surface between duplexes, the electrode was again immersed into 1 mM 6-mercapto-1-hexanol (MCH) buffer solution 1 h. As shown in Fig. 1a, after immersion of the DNA-loaded electrodes into 5 μM Ag^+ buffer solution 15 min and washing with PBS, almost symmetrical redox peaks clearly appear at AP-[N]-modified electrodes (scan rate 0.1 V s^{-1}), while negligible small peaks are observed for FM-DNA-modified electrode (the inset of Fig. 1a). The fact that no significant difference is observed for the redox peak potentials at FM-DNA- and AP-DNA-modified electrodes suggests that the possible difference in Ag^+ binding sites

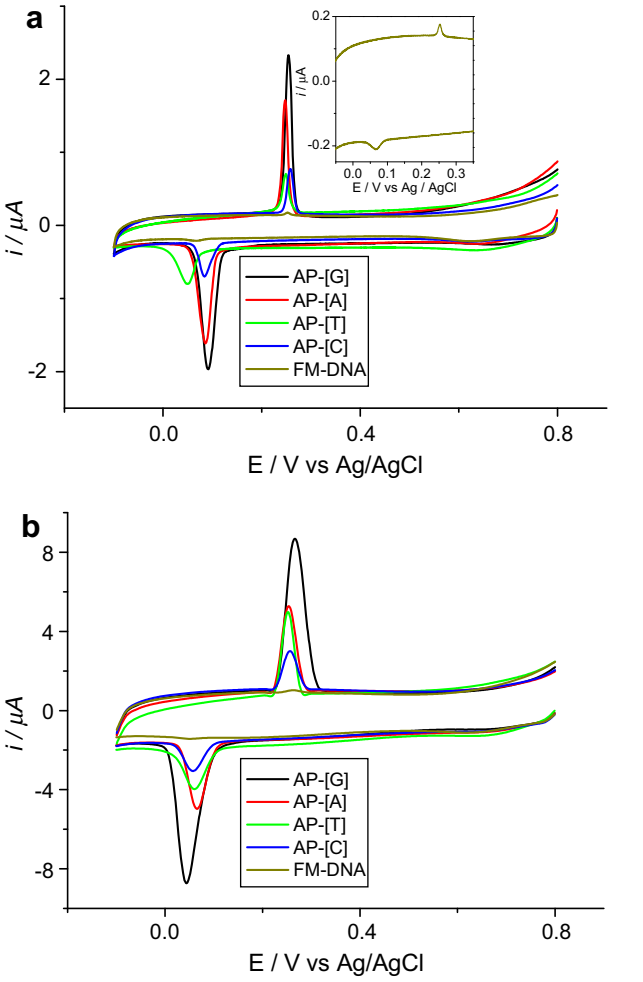

Fig. 1. CVs of DNA-modified gold electrodes in a 0.1 M phosphate buffer solution (pH 7.0) after 5 μM (a) or 10 μM (b) $AgNO_3$ pretreatment. Inset in (a) is the CV at FM-DNA-modified electrode partially presented for displaying the small peaks. Scan rate: (a) 0.1 V s^{-1}; and (b) 1 V s^{-1}.