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Abasic site-switched structure conversion of neutral red for selective DNA recognition

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ABSTRACT

DNA-binding-switched conversion of small molecule's structure and property provides a promising tool for achieving a high selectivity in identification of a DNA specific site. Herein, the optical properties of neutral red in response to its abasic site (AP site) binding were investigated for this purpose. At pH 8.4, neutral red exists in solution mainly at the neutral form (NR). However, the presence of an AP site in DNA converts NR to a protonated form (NRH^+), following a 100-nm red shift in absorption spectra. This conversion is inefficient for the fully matched DNAs without the AP site. Relative to the absorption spectra, the fluorescence in response to the binding-switched conversion is more dependent on the sequences near the AP site. The AP site's hydrophobic microenvironment and electron transfer between the AP site-bound NRH^+ in excited state and the nearby context bases govern the emission behavior. It was found that the AP site having a large void space favors occurrence of dipole interaction between the bound NRH^+ and the context bases. This AP site-dependent conversion of neutral red in structure and then its optical properties would find wide applications in the field of DNA assay with a high selectivity and sensitivity.

1. Introduction

External stimuli-directed formation of deoxyribonucleic acid (DNA) second structures, such as K^+-induced G-quadruplex [1], target-induced aptamer folding [2], B-to-Z conformation transformation [3], and cytosine semi-protonated i-motif [4], have long been used as the critical elements in developing biosensors having a high selectivity and screening genetic drugs possessing high therapy efficacy and low side effect. On the other hand, DNA specific binding-switched conversion of small molecule' structures and properties are more promising in DNA biosensing because the already existed DNA structures are mostly undisturbed. It is widely recognized that many of the DNA-binding enzymes and proteins adopt conformations different from the unbinding states at their binding sites [5,6]. However, this is seldom achieved for small molecules on the basis of less structure diversity of small molecules relative to biomacromolecules. Sometimes external triggering events have been needed to produce the DNA-binding state of small molecules, for example, photoswitchable spiropyran [7] and photoisomerizable azobenzene dyes [8].

However, DNA-binding-switched conversion of small molecule' structures and properties without external triggering is more advantageous in response to a DNA specific site binding due to more simplification in application. Thus, the converted state adaptive for DNA specific binding could have a characteristic property different from the state free in solution. Pethö et al. reported that porphyrin core was potonated upon binding to DNA grooves even at pH 7.0 [9]. Maiti et al. found that DNA intercalation can convert the sanguinarine alkanolamine form to iminium form [10]. We investigated the interaction of sanguinarine with abasic site (AP site)-containing DNAs and the results showed that the conversion always occurs, being independent on the presence of the AP site or not [11].

In living cells, damaged DNA bases are removed through in vivo base excision repair (BER) path [12], and thus an AP site is produced after this depurination or depyrimidination event. If not repaired, this site would have the potential to cause the commonly observed DNA mutagenic and carcinogenic lesions [13]. Therefore, recognition of the AP site holds great promise for diagnostic and therapeutic applications [14]. On the other hand, due to the confined cavity size and hydrophobic microenvironment, the AP site was designedly introduced into DNA to oppose the unpaired target base for the purpose of DNA mutation recognition. Some of fluorescent small molecules, such as derivatives of naphthyridine [15], naphthalene [16], flavin [17], and 3-hydroxyflavone [18], can selectively recognize the AP site with a target-base-dependent 'turn-off' or 'turn-on' emission response. Nevertheless, no any conversion in molecular structure was observed, although these fluorophores experienced a change in emission mechanism, mostly resulting

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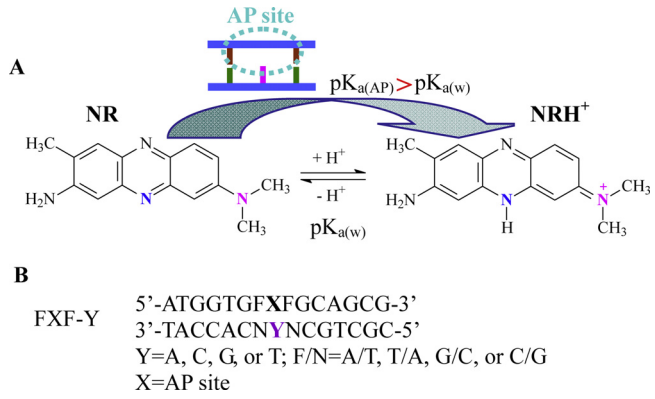

Scheme 1. (A) Structures of NR and NRH^+ in equilibrium and schematic representation of the AP site-targeted conversion of NR to NRH^+. (B) For the AP site-containing DNAs, X = AP site (dspacer, tetrahydrofuran residue) that is opposed by base Y and flanked by base Fs (Y, F = A, C, G, and T). Thus, the used DNAs were named as FXF-Y. Fully matched DNAs (FM-DNA) with X/Y = A/T, C/G, G/C, and T/A were used as control.

from the altered existing microenvironment upon entering into the AP site.

Herein, neutral red was employed as a new fluorophore to achieve a molecular structure conversion accompanying its selective binding to the AP site. Neutral red belongs to the quinone-imine dye, and exists in aqueous solution in the forms of protonated (NRH^+) and neutral (NR) species (Scheme 1) by an equilibrium having a pK_a about 6.81 [19]. NRH^+ has a structure feature that is significantly different from NR (Scheme 1). Thus, the dye has long been used as a stain for cells or tissues, an intracellular pH indicator, and an environment toxicology biomarker [20–24]. Although interaction of the dye with natural DNAs [25–27] was previously reported, a weak binding was observed and sequence selectivity binding was not found. Additionally, the natural DNAs are suspected to contain a number of the AP sites. In this work, the AP site recognition of the dye was carried out in order to throw light on the DNA binding specificity of neutral red. At weak alkaline solution, the AP site-containing DNA converts NR to NRH^+ by a sequence-dependent efficiency, while the fully matched DNA without the AP site is inefficient for this conversion. Thus, NR can serve as a binding-induced converter for DNA damage detection.

2. Experimental

2.1. Materials and reagents

DNA species (Scheme 1) were synthesized by TaKaRa Biotechnology Co., Ltd. (Dalian, China) and purified by HPLC. A tetrahydrofuran residue was used as the chemically stable abasic site (AP site) for replacement of the naturally-occurred unstable deoxyribose structure. The DNA concentrations were measured by UV absorbance at 260 nm using extinction coefficients calculated by the nearest neighbor analysis. Neutral red was obtained from Sigma Chemical Co. (St. Louis, USA) and used as received. Milli-Q water (18.2 mΩ; Millipore Co., Billerica, USA) was used in all experiments. All the other chemicals were analytical-reagent grade (Aladdin Reagent Co., Shanghai, China) and were used without further purification.

2.2. Fluorescence measurements

Fluorescence spectra were acquired with a FLSP920 spectrofluorometer (Edinburgh Instruments Ltd., Livingston, UK) at $18 \pm 1\,^{\circ}C$, equipped with a temperature-controlled circulator (Julabo Labortechnik GmbH, Seelbach, Germany). To prepare DNA duplex solutions, the target strand and the AP site-containing probe strand were mixed in equimolar amounts and annealed in a thermocycler (first at 92 °C, then cooled down to room temperature slowly) in 0.1 M phosphate buffer (at the desired pH) containing 1 mM ethylenediaminetetraacetic acid disodium salt (EDTA). Neutral red was added to the duplex DNA solution to an appropriate molar ratio in 0.1 M phosphate buffer at the desired pH containing 1 mM EDTA. After mixing, the solution was incubated for 15 min with gentle stirring. The resulting solution was examined at room temperature within 2 h.

2.3. UV/vis absorption spectra and melting temperature (T_m) measurements

UV/vis absorption spectra and melting temperatures (T_m) of DNA in the presence and absence of neutral red were determined with a UV2550 spectrophotometer (Shimadzu Corp., Kyoto, Japan), equipped with the accessory of a TMSPC-8 T_m analysis system which can simultaneously control the chamber temperature and detect up to eight samples with a micro multi-cell.

3. Results and discussion

3.1. AP site-dependent selective conversion of neutral red from neutral to protonated form

The previously reported interaction of neutral red with natural DNAs [25–27] was carried out at high dye concentrations. However, neutral red tends to aggregate in aqueous solution especially at high concentration and basic pH [28]. In this work, not more than 5 μM neutral red was used to avoid the aggregation. Neutral red exists in aqueous solution in the neutral (NR) and protonated (NRH^+) forms (Scheme 1) with the corresponding absorption bands located at 450 nm and 536 nm, and their population is dependent on solution pH (Fig. S1) with a pK_a value of about 6.72 in our condition, which is in agreement with the reported value [19]. In order to investigate the possible conversion of the dye between the two forms upon binding to various DNAs, the experiments were carried out at pH 8.4 where the neutral form (NR) of the dye was dominant in solution. As shown in Fig. 1, the optical property of the dye exhibits a strong sequence-dependent change upon binding to the AP site-containing DNAs (AP-DNA). Addition of AP-DNAs having context thymines flanking the AP site (TXT-Ys) decreases the 450 nm NR absorption band that is accompanied by an increase in the NRH^+ absorption band (Fig. 1A). These changes in the absorption spectra are more pronounced for the AP-DNAs with the unpaired cytosine and thymine opposite the AP site (TXT-C and TXT-T) than the adenine and guanine opposite the AP site (TXT-A and TXT-G). The absorption wavelengths seem to also experience a sequence-dependent change. For example, upon resolving the overlapped peaks by fitting (Fig. S2), the NRH^+ absorption band appears at 550 nm in the presence of TXT-C, having a red shift in comparison to the NRH^+ free in solution (536 nm, Fig. S1), indicating that a portion of NR is converted into NRH^+ in the AP-DNA-bound state. Furthermore, this red shift in absorption band means that the converted NRH^+ has a strong electron interaction with the DNA bases. Based on the calculated absorption coefficient of $1.42 \times 10^4\ M^{-1}\ cm^{-1}$ (in agreement with the previously reported values [29]) for the TXT-C-bound NRH^+ that was obtained in excess of TXT-C, we estimated that about 40% of NR was converted into NRH^+.

The conversion of NR to NRH^+ upon binding to the AP-DNAs was further investigated by changing the bases flanking the AP site to adenines, guanines, and cytosines. Interestingly, we found that as observed for TXT-Ys, the NRH^+ conversion preference to the unpaired pyrimidines opposite the AP site over the unpaired