Detection of base pairing of an oxidatively damaged guanine using colloidal stability change of DNA-linked polymer micelles

Tohru Takarada¹, Emi Imaizumi¹, Katsuhiro Kino², Keitaro Yoshimoto¹, Shinzi Ogasawara¹, Naoki Kanayama¹ and Mizuo Maeda¹

¹Bioengineering Laboratory, Discovery Research Institute, RIKEN, Saitama 351-0198, Japan and ²Department of Pharmaceutical Technology, Faculty of Pharmaceutical Sciences at Kagawa Campus, Tokushima Bunri University, Kagawa 769-2193, Japan

ABSTRACT

A DNA-linked polymer micelle was prepared through a phase transition of an amphiphilic copolymer consisting of single-stranded DNA as the hydrophilic part and thermo-responsive poly(N-isopropylacrylamide) as the hydrophobic part. Duplex formation on the surface of micelle decreased the colloidal stability, whereas a terminal single-base mismatch at the interface between the DNA shell and the disperse medium increased the colloidal stability. Based on this change, we successfully detected base pairing of 7,8-dihydro-8-oxo-2'-deoxyguanine with adenine. This method will be useful for discovering a Watson-Crick or non Watson-Crick base pair of an unknown oxidative-damaged base with each four canonical one.

INTRODUCTION

Oxidative damage of DNA is known to be a cause of aging and a variety of diseases such as carcinogenesis and Alzheimer disease.¹ Guanine (G) is highly susceptible to one-electron oxidation in the genome because it has the lowest redox potential of the four natural DNA bases.² Several products are formed directly from G, including 7,8-dihydro-8-oxo-2'-deoxyguanine (8-oxoG), which is a major oxidative DNA lesion. Since 8-oxoG can pair with not only cytosine (C) in the Watson-Crick mode but also adenine (A) in the Hoogsteen mode (Fig. 1), base-pairing of 8-oxoG:A is believed to cause G:C to T:A transversion mutations during DNA replication.³ Moreover, since 8-oxoG has a lower redox potential than G, it can easily be further oxidized to yield several additional oxidation products, whose base-pairing mode is not yet completely elucidated.⁴

We recently found that a polymer micelle with a dehydrated poly(N-isopropylacrylamide) core surrounded by a double-stranded DNA (dsDNA) corona had drastically increased colloidal stability when a terminal single-base mismatch existed at the interface between the DNA shell and disperse medium.⁵ Similar colloidal stabilization was observed with dsDNA-linked gold⁶ or polystyrene⁷ nanoparticles, suggesting that the stabilization was independent of the properties of the hydrophobic core. The stabilization is at least partially attributed to a decrease in entropic repulsion between the micelles. In this study, we demonstrated the detection of base pairing between 8-oxoG and A using the terminal mismatch-induced colloidal stability change.

RESULTS AND DISCUSSION

We prepared a single-stranded DNA (ssDNA)-linked polymer micelle according to the previous reports.⁸⁻⁹ Poly(N-isopropylacrylamide) grafted with ssDNA (PNIPAAm-g-DNA; Fig. 2) was synthesized by radical copolymerization between N-isopropylacrylamide and 10-base ssDNA modified with a methacryloyl group at the 3′-end (DNA macromonomer).

The cloud-point temperature of PNIPAAm-g-DNA (the weight average molecular weight: 8.2 x 10⁶; the number average molecular weight: 6.1 x 10⁵; the DNA macromonomer fraction: 0.34 mol%) in 10 mM Tris-HCl

![Figure 1. Hydrogen bonding patterns of (a) 8-oxoG:C and (b) 8-oxoG:A pairs.](https://academic.oup.com/nass/article/51/1/305/1023900)

![Figure 2. Chemical structure of PNIPAAm-g-DNA.](https://academic.oup.com/nass/article/51/1/305/1023900)
buffer (pH 7.4) containing 300 mM NaCl was determined to be 31 °C, based on a slight decrease of transmittance at 500 nm. The concentration of DNA in 0.1 mg/ml PNIPAAm-g-DNA was determined to be 2.5 μM. When the same buffer solution of PNIPAAm-g-DNA was heated at 35°C, the copolymers immediately self-assembled to form a polymer micelle with a PNIPAAm core surrounded by ssDNAs (Fig. 3, without sample DNA and probe DNA). The hydrodynamic radius of micelle was determined to be 55 nm by dynamic light scattering measurement.

The colloidal stability of the polymer micelles was evaluated by the critical coagulation concentration (CCC) of the supporting electrolyte (NaCl). The CCC was determined by measurement of the transmittance at 500 nm of the micellar dispersion with a UV-vis spectrometer. Figure 4 shows the colloidal stability of the polymer micelle when the sample DNA (12 mer; X = C) and the probe DNA (22 mer; Y = C, G, T or A), both of which were equimolar to the surface DNA (2.5 μM), were added to form a singly nicked duplex (Fig. 3). The resulting dsDNA-linked polymer micelle drastically decreased the colloidal stability when the complementary probe DNA (Y = G) was added to the dispersion: the CCC was determined to be 400 mM. In contrast, the transmittance was almost 100% when the terminal mismatched dsDNA was formed on the surface (Y = C, T or A).

![Figure 3](https://example.com/figure3.png)

**Figure 3.** Schematic illustration of the DNA-linked polymer micelle used in this study.

![Figure 4](https://example.com/figure4.png)

**Figure 4.** Transmittance at 500 nm of the dispersion of dsDNA-linked polymer micelles as a function of NaCl concentration. The concentration of the polymer micelle was 0.1 mg/ml. Each concentration of sample DNA (X = C) and probe DNA (Y = C, G, T or A) was 2.5 μM.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Critical coagulation concentrations of NaCl (mM) for the DNA-linked polymer micelles</th>
</tr>
</thead>
<tbody>
<tr>
<td>probe DNA (Y)</td>
<td>sample DNA (X)</td>
</tr>
<tr>
<td></td>
<td>G</td>
</tr>
<tr>
<td>C</td>
<td>300</td>
</tr>
<tr>
<td>G</td>
<td>400</td>
</tr>
<tr>
<td>T</td>
<td>&gt;1500</td>
</tr>
<tr>
<td>A</td>
<td>1200</td>
</tr>
</tbody>
</table>

Using the identical method, we determined all of the CCC values with the sample DNA (X = G, C, A, T or 8-oxoG) and the probe DNA (Y = C, G, T or A). The results are summarized in Table 1. Non Watson-Crick base pairs (G:G and G:A) as well as Watson-Crick base pairs (G:C and A:T) destabilized the dsDNA-linked polymer micelles although the terminal mismatches such as A:A and T:T induced the remarkable stabilization of the micelles. Interestingly, the smaller CCC for 8-oxoG:A (600 mM) than that for G:A (1200 mM) was obtained, corresponding to the base pairing of 8-oxoG with A. These results strongly indicates that the present methodology will be useful for discovering a Watson-Crick or non Watson-Crick base pairing between an unknown oxidative-damaged base and each four canonical one.

**ACKNOWLEDGEMENTS**

This work was supported by a Grant-in-Aid for Young Scientists (B) (KAKENHI 16700376) from the Ministry of Education, Culture, Sports, Science and Technology of Japan, and by a grant for Eomolecular Science Research provided by RIKEN.

**REFERENCES**


*Corresponding Author. E-mail: tkird@riken.jp