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Modulation of base selectivity for a base-discriminating fluorescent nucleobase by addition of mercury ion

Akimitsu Okamoto,[a,*] Yuji Ochi[a] and Isao Saito[b,*]

[a]*Department of Synthetic Chemistry and Biological Chemistry, Faculty of Engineering, Kyoto University, Kyoto 615-8510, Japan*
[b]*NEWCAT Institute, School of Engineering, Nihon University and SORST, Japan Science and Technology Corporation, Tamura, Koriyama 963-8642, Japan*

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Abstract—We altered the fluorescence emission selectivity of a base-discriminating fluorescent base, ^{Py}U, from A-selective to T-selective by the addition of mercury ion. The strong fluorescence from a duplex containing the ^{Py}U/T base pair was specific to the mercury ion among divalent metal ions, providing a unique method for sensing mercury ions in aqueous solutions.

1. Introduction

Fluorescence-labeled DNA probes have wide applications, ranging from genomic sequencing to the rapid detection of infections and genetic diseases.[1] We have recently demonstrated the new concept of base-discriminating fluorescent (BDF) nucleobases, which can distinguish the type of base opposite the BDF base by a fluorescence change.[2,3] For example, ^{Py}U base, one of such BDF bases, in a duplex DNA selectively emits strong fluorescence only when the base opposite ^{Py}U is an adenine base (Fig. 1a).[3] Thus, these BDF probes provide a powerful alternative to conventional SNP typing methods. If the base selectivity of fluorescence intensities of BDF probes can be switched by external additives, then the utility of BDF probes will be greatly expanded. Mercury ion may be a candidate for such an external additive. Mercury(II) ions are known to bind to N3 of thymines and uridines.[4] In particular, it has recently been reported that mercury ion selectively binds to T/T mismatched base pairs in 3-(*N*-morpholino)propanesulfonic acid (Mops) buffer (pH 7.0) (Fig. 1b).[5,6] Herein, we report the modulation of the fluorescence intensity of BDF oligodeoxynucleotides (ODN) by the unique binding of mercury ion to the ^{Py}U/T base pair. By the addition of mercury ion to ^{Py}U-labeled duplexes, the selectivity of the ^{Py}U fluorescence emission to the complementary base was greatly altered. The strong fluorescence from a duplex containing the ^{Py}U/T base pair was specific to the mercury ion among divalent metal ions, providing a unique method for sensing mercury ions in aqueous solutions.

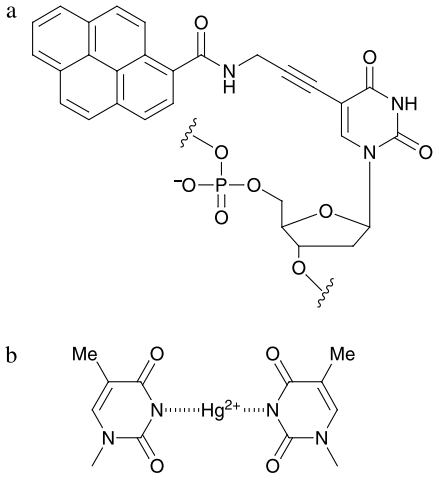

Figure 1. (a) Structure of a base-discriminating fluorescent (BDF) nucleotide, ^{Py}U. (b) Proposed structure of a T/T base pair formed through a mercury ion.

Keywords: Base-discriminating fluorescent nucleobases; Mercury ion; Fluorescence; Base pair.
* Corresponding authors. Tel.: +81 75 383 2757; fax: +81 75 383 2759 (A.O.); tel.: +81 24 956 8911; fax: +81 24 956 8924 (I.S.); e-mail addresses: okamoto@sbchem.kyoto-u.ac.jp; saito@mech.ce.nihon-u.ac.jp

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2. Modulation of base selectivity

A ^{Py}U-containing ODN probe 5′-d(CGCAAT^{Py}U-TAACGC)-3′ (**ODN1**) was prepared according to the protocol reported earlier.[3] Initially, we measured the fluorescence spectra of 2.5 μM **ODN1**, hybridized with 5′-d(GCGTTANATTGCG)-3′ (**ODN1′(N)**, **N** = A, G, T, or C), in a buffer solution of 10 mM Mops (pH 7.0) and 0.1 M sodium nitrate at 25 °C (Fig. 2a). The fluorescence spectrum of a matched duplex, **ODN1/ODN1′(A)**, showed a strong fluorescence at 400 nm by 350 nm excitation (Fig. 2b). In contrast, the fluorescence intensities for single-stranded **ODN1** and mismatched **ODN1/ODN1′(N)** duplexes (i.e., **N** = G, T, or C) were much weaker. This A-selective fluorescence emission of ^{Py}U was in good agreement with the fluorescence behavior of ^{Py}U reported earlier.[3]

Because mercury ions selectively bind to mismatched T/T base pairs in duplexes,[5,6] we expected that the fluorescence from a duplex containing a ^{Py}U/T base pair would be changed by the addition of mercury salt. We added mercury (II) perchlorate to the duplex samples and measured their fluorescence spectra (Fig. 2c). The fluorescence intensity of a matched duplex, **ODN1/ODN1′(A)**, decreased to 63% on the addition of 1 equiv of mercury ion and to 16% on the addition of 5 equiv, as compared with that observed in the absence of mercury ions. Mercury ion is known to quench the fluorescence of aromatic hydrocarbons, such as pyrenes and naphthalenes.[6,7] The effect of the concentration of mercury ions on the fluorescence of ^{Py}U nucleoside was also investigated. The fluorescence quantum yield of ^{Py}U nucleoside was 0.21. However, the fluorescence quantum yield rapidly decreased with the addition of mercury perchlorate, and the fluorescence was quenched to 10% of the original fluorescence by the addition of 5 equiv mercury perchlorate. The remarkable decrease in the strong fluorescence from **ODN1/ODN1′(A)** can be explained by the fluorescence quenching of the pyrene fluorophore by mercury ions. The addition of mercury ions also weakened the fluorescence emission of mismatched duplexes, **ODN1/ODN1′(G)** and **ODN1/ODN1′(C)**.

In contrast, only the fluorescence intensity of a duplex containing a mismatched ^{Py}U/T base pair, **ODN1/ODN1′(T)**, increased considerably on the addition of 5 equiv of mercury ions, and the increment was approximately two times that without mercury ions. The fluorescence enhancement would be elucidated by investigating the effect of the addition of mercury ions on the duplex thermal stability. We examined the change of the melting temperatures (T_m) of **ODN1/ODN1′(T)** with the addition of mercury ions. The addition of 5 equiv of mercury ions to an **ODN1/ODN1′(T)** sample increased the T_m value of the duplex (41.8 → 42.8 °C), suggesting that the duplex was stabilized by the binding of mercury ions. On the other hand, the T_m value of **ODN1/ODN1′(A)** decreased (47.7 → 43.1 °C). The stabilization of a ^{Py}U/T base pair mediated by mercury ions would contribute to the emission enhancement of **ODN1/ODN1′(T)**, because the formation of ^{Py}U/T base pair with the assistance of mercury ions would force the

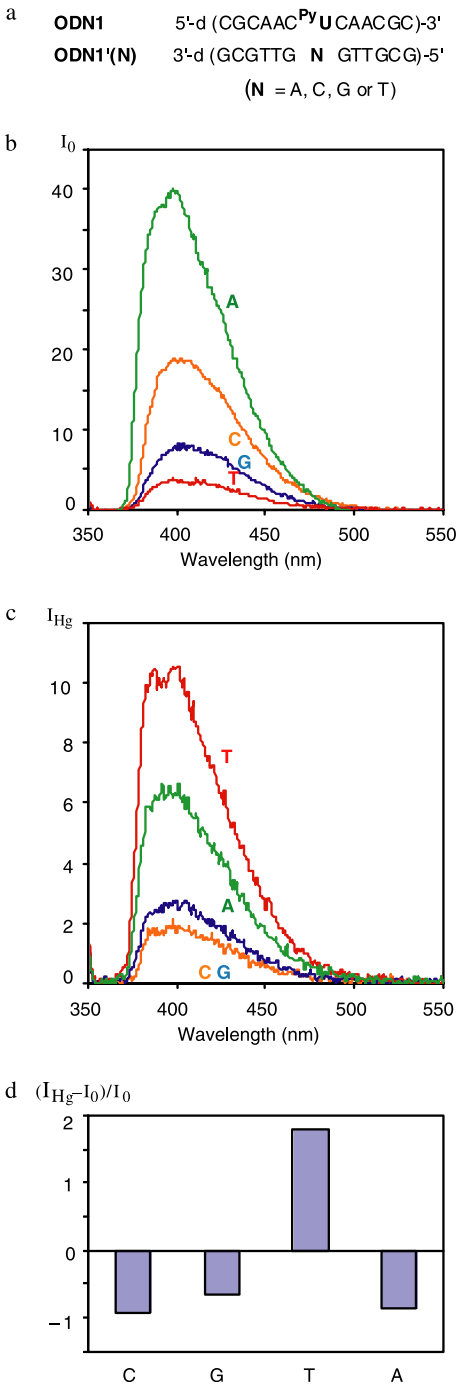

Figure 2. (a) A ^{Py}U-containing duplex used in this study (**ODN1/ODN1′(N)**). **N** = A, G, C, or T. (b) Fluorescence spectra of duplexes in the absence of mercury ion in a sample solution. Sample solutions consisted of 2.5 μM duplex, 10 mM Mops buffer (pH 7.0), and 0.1 M sodium nitrate, and were excited at 350 nm. (c) Fluorescence spectra of duplexes in the presence of mercury ion in a sample solution. Sample solutions consisted of 2.5 μM duplex, 10 mM Mops buffer (pH 7.0), 0.1 M sodium nitrate, and 12.5 μM mercury (II) perchlorate, and were excited at 350 nm. (d) Changes in the fluorescence intensities of ^{Py}U/N duplexes. The height of bars shows the increment/decrement of the fluorescence intensity of mercury ion-containing samples at 400 nm relative to that of mercury ion-free samples.

pyrenecarboxamide fluorophore to the highly polar region outside the duplex.[3]

The increment/decrement of fluorescence intensity of **ODN1/ODN1′(N)** accompanying the addition of mercury ions is summarized in Figure 2d. The fluorescence intensity was increased T-selectively by adding a mercury salt, and accordingly the base selectivity of the fluorescence emission of BDF base ^{Py}U was altered from adenine to thymine by addition of mercury ion.

3. Mercury ion-selective sensing

The T-selective increase of fluorescence intensity of **ODN1/ODN1′(N)** by addition of mercury ions is applicable to a new mercury ion sensor. Contamination with mercury ions may have severe effects on our living environment and health. Thus, it must be desirable to selectively and easily detect a mercury ion in an aqueous media.[6] We designed **ODN2** as a unimolecular device containing a ^{Py}U/T base pair for sensing mercury ions (Fig. 3a), and compared the fluorescence intensity in the presence of mercury ions with that in the presence of other divalent metal ions. We added **ODN2** (2.5 μM) to a solution of 10 mM Mops buffer (pH 7.0) and 0.1 M sodium nitrate containing a metal ion (10 μM), and measured the change in the fluorescence intensity of **ODN2** at 400 nm. The addition of mercury ion remarkably enhanced the fluorescence intensity, whereas the fluorescence remained weak during the addition of other divalent metal ions such as Ca^{2+}, Co^{2+}, Cu^{2+}, Mg^{2+}, Mn^{2+}, Pd^{2+}, and Zn^{2+} (Fig. 3b). The binding of mercury to a ^{Py}U/T base pair is strong and highly selective, whereas other heavy metal ions do not show any notable effects on duplex stability. Thus, **ODN2** acted as a highly selective fluorescent sensor, which relies upon the selective binding of mercury ions with a ^{Py}U/T base pair. The photograph in Figure 3c clearly shows that the fluorescence emission from **ODN2** was highly selective to mercury ion. The fluorescent hairpin ODN containing a ^{Py}U/T base pair facilitated a mercury ion-selective sensing.

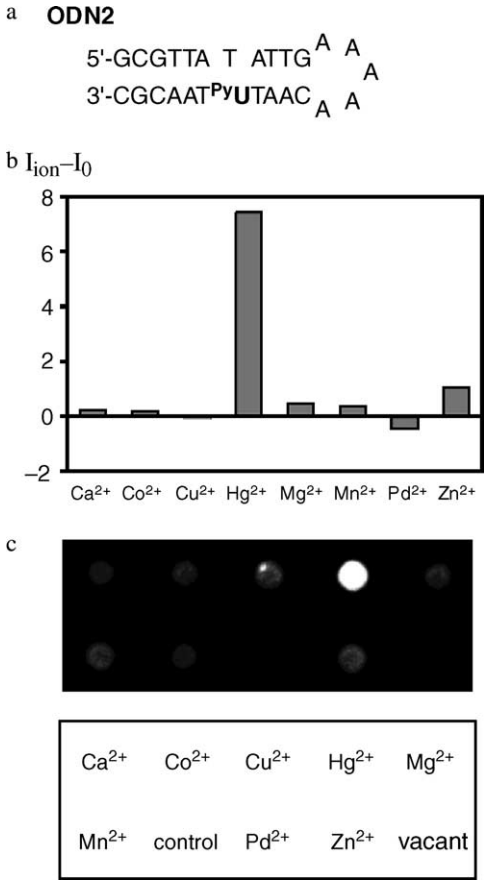

Figure 3. (a) A hairpin-shaped ^{Py}U-containing ODN (**ODN2**) as a mercury ion sensor. (b) Changes in the fluorescence intensities of **ODN2**. Sample solutions for fluorescence measurement consisted of 2.5 μM **ODN2**, 10 mM Mops buffer (pH 7.0), 0.1 M sodium nitrate, and 10 μM divalent metal ions, and were excited at 350 nm. The height of the bars shows the increment/decrement of the fluorescence intensity of divalent metal ion-containing samples relative to that of a divalent metal ion-free sample. (c) Fluorescence imaging of **ODN2** in the presence of divalent metal ions. The sample solutions were illuminated with a UV transilluminator (365 nm). The image was taken through a 380-nm long pass emission filter by means of a CCD camera.

4. Conclusion

In conclusion, we altered the fluorescence emission selectivity of a BDF base. The fluorescence of a BDF base, ^{Py}U, was modulated from A-selective to T-selective by the addition of mercury ion. This T-selective fluorescence enhancement was not observed with other divalent ions. Thus, the unique fluorescence behavior of a duplex containing a ^{Py}U/T base pair is applicable to a new mercury ion sensor.

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