Oligonucleotide-bound Eu(III) as a Probe for Microenvironments at Specific Site in DNA

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By attaching Eu(III)/β-diketone complex covalently to oligonucleotide, microenvironments at a specific site in DNA have been probed. The conjugate efficiently emits luminescence, since coordination of water to the Eu(III) is competitively inhibited by the internal phosphodiester groups. In the presence of complementary DNA, however, this inhibition is diminished because of rigidity of double-stranded DNA and the emission becomes much weaker. The emission intensity is also affected by other environmental changes.

Precise evaluation of the environments where biomolecules are placed is very important so that the biological functions are understood sufficiently. Until now, many methods for macroscopic analysis of the surrounding environments of biomolecules have been developed. However, there are few methods for measuring microenvironments at a specific site in biomolecules.

Luminescence of lanthanide compounds shows various features such as long lifetime, large Stokes shift, and sharply-spiked emission bands. Moreover, the intensity and the lifetime of the emission can give information on both the coordination environments and degree of hydration of these ions.[1–4] Lanthanide ions can form complexes with many biological substances bearing negatively charged donor groups (e.g., carboxylates or phosphates). Binding also occurs as a consequence of metal coordination to the oxygen of carbonyl or hydroxy groups (e.g., in sugars or nucleotides). For these reasons, lanthanide ions have been useful luminescent probes for the structure of biological macromolecules. In almost all previous researches, metal ions in biomolecules were replaced by lanthanide ions, and environments around these ions (in particular, the presence or absence of the coordinating functional groups) were analyzed. However, the use of spontaneous substitution of metal ion limits the scope of application. Therefore, chemical modifications of biomolecules to bind metal ligands should be useful for analyzing microenvironments at desired site.[5,6] Here, we have synthesized an oligonucleotide-bound Eu(III) complex and developed a new system for analyzing microenvironments at desired site in DNA. The effect of duplex formation of the conjugate was investigated to show the validity of this strategy. In the presence of fully complementary DNA, the emission intensity of Eu(III) complex greatly decreased compared to that in its absence. Furthermore, the structural change of the complex upon the duplex formation was confirmed by the analysis of emission lifetimes. The emission intensity from the conjugate was also notably affected by one mismatch between the probe DNA and sample DNA.

The DNAs used in this study are summarized in Figure 1. CDPP–DNA_1 was prepared by conjugating amino-modified DNA with 5-(4-chlorosulfonyl-1,1′-biphenyl-4′-yl)-1,1,1,2,2-pentafluoro-3,5-pentanedione,[6] and purified by both polyacrylamide gel electrophoresis and reversed-phase HPLC (MALDI-TOF; see Supporting Figure 1).

CDPP–DNA_1 5'- CCAAGTGTCG***Z***AGTGCC -3'
DNA_2 3'- GGTTCACAGCATCACGG -5'
DNA_3 3'- TTTGGCATTGGTGGCAGACG -5'
DNA_4 3'- GGTTCACAG***G***ATCACGG -5'
DNA_5 3'- GGTTCACA***C***CATCACGG -5'
DNA_6 3'- GGTTCACAGC***G***TCACGG -5'
DNA_7 3'- GGTTCACAGCA***A***CACGG -5'
DNA_8 3'- GGTTCACAGCAT***G***ACGG -5'

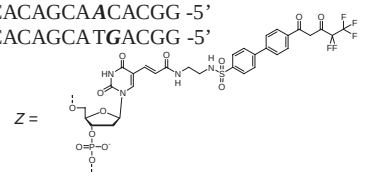


Figure 1. DNA sequences used in this study and the structure of CDPP-modified thymidine *Z*. The mismatching sites are designated in bold italics.

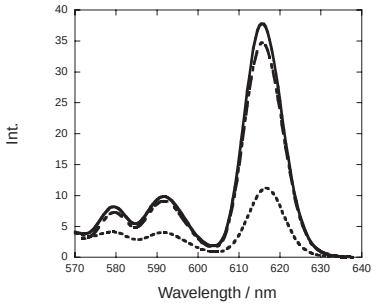


Figure 2. Emission spectra of CDPP–DNA_1/Eu(III) complex (excitation at 340 nm) in the presence of complementary DNA_2 (dotted line) and non-complementary DNA_3 (broken line). The solid line shows the spectrum in the absence of DNA additive. [CDPP–DNA_1] = [DNA additive] = [Eu(III)] = 1 μM.

The emission spectra of the solutions containing CDPP–DNA_1 (1 μM) and Eu(III) ion (1 μM) at pH 7.8 (50 mM Tris-HCl), in the presence and absence of fully complementary additive DNA_2 (1 μM), are shown in Figure 2. The excitation wavelength is 340 nm which corresponds to the absorbance of the β-diketone moiety. These solutions give the same emission maximum at 615 nm (corresponding to the Eu(III) emission of $^5D_0 \rightarrow {}^7F_2$). These results indicate that the binary complex Eu(III)–CDPP is satisfactorily formed and the energy transfer from the β-diketone to the Eu(III) is efficient even in the absence of DNA additive. When fully complementary DNA_2 is added, the emission intensity becomes much weaker (compare the dotted line with the solid one). The ratio of the intensities in the presence and the absence of DNA_2 is approximately 1:3. On the other hand, DNA_3 (1 μM), which has almost no complementarity to DNA_1, hardly affects the intensity of emission from the Eu(III)–CDPP complex. Apparently, the Eu(III) complex of the CDPP-modified oligonucleotide can act as a useful probe for DNA

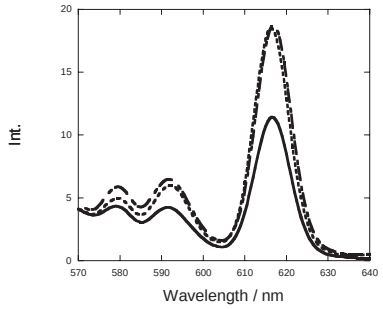
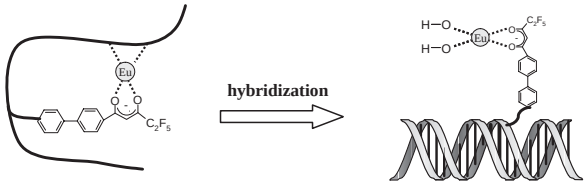


Figure 3. Emission spectra of CDPP–DNA_1/Eu(III) complex (excitation at 340 nm) in the presence of DNA_2 (solid line), DNA_4 (broken line), and DNA_5 (dotted line). [CDPP–DNA_1] = [DNA additive] = [Eu(III)] = 1 μM.



Scheme 1. Proposed mechanism for the change of luminescence intensity of CDPP–Eu(III) complex on DNA duplex formation.

duplex formation.

In order to investigate the effect of a mismatch on the luminescence, the additive DNAs having one base mismatch toward CDPP–DNA_1 were used. Typical emission spectra of the solutions are shown in Figure 3. Under the experimental conditions used, DNA_1 can form stable duplexes with both DNA_4 and DNA_5, and the resultant duplexes contain one base mismatch. Their emission intensities were far greater than that of fully matched DNA duplex. When the other DNA additives (DNA_6, DNA_7, and DNA_8) were used, the emission intensities of the resultant duplexes were somewhat weaker than those of both DNA_1/DNA_4 and DNA_1/DNA_5 (see Supporting Figure 2). The subtle structural differences between fully matching site and mismatching site in duplexes were distinguished.

From these results, the mechanism for the change of emission intensity upon the duplex formation is proposed in Scheme 1. The luminescence of Eu(III) is known to be remarkably quenched by its coordination water molecules.[2,3] When the CDPP–DNA_1/Eu(III) complex is single-stranded, the phosphodiesters of DNA_1 and the β-diketone moiety of CDPP are cooperatively coordinated to the Eu(III) due to the flexibility of single-stranded DNA. Therefore, the coordination of quenching water molecules to Eu(III) is repressed. Upon hybridization of the CDPP–DNA_1 with DNA_2, the backbone of DNA_1 becomes less flexible, and thus it is difficult for its phosphodiesters to bind the Eu (III) in the CDPP complex cooperatively. As a consequence, the number of coordinated water molecules increases and the emission intensity is greatly decreased due to the quenching by these water molecules. In the case of DNA duplex including a mismatching base pair, the DNA backbone is more flexible, and its phosphodiester linkages can weakly interact with the Eu(III). Therefore, the intensity of luminescence is between the value in the presence of fully matched DNA and that in its absence.

Table 1. Lifetime (τ) and the number of coordinated water molecules (q) of CDPP–DNA_1/Eu(III) complex in H_2O and D_2O[a]

	τ/μs		$\tau(H_2O)^{-1} - \tau(D_2O)^{-1}$	q
	H_2O	D_2O		
CDPP–DNA_1	93	190	5.5	5.8
CDPP–DNA_1/DNA_2	89	290	7.8	8.2

[a][CDPP–DNA_1] = [DNA_2] = [Eu(III)] = 1 μM.

In order to confirm this hypothesis still more, the numbers of coordinated water in the first coordination sphere of the Eu(III) (q) were directly determined by using eq 1.[2]

$$q = 1.05 \times [\tau(H_2O)^{-1} - \tau(D_2O)^{-1}], \quad (1)$$

where $\tau(H_2O)$ and $\tau(D_2O)$ are the lifetimes for luminescence decay of CDPP–DNA_1/Eu(III) complex in H_2O and D_2O, respectively. As summarized in Table 1, the number of coordination water (q) of the Eu(III) in the complex is increased by 2 when the DNA_1 forms the duplex with DNA_2. This result strongly indicates that the number of water molecules coordinated to the Eu(III)–CDPP complex is regulated by the fine conformational change at the specific site of DNA. This Eu(III) complex conjugate also has a potential as bio-probes to investigate ternary structures of RNA, single nucleotide polymorphisms (SNPs), and protein–DNA interactions.

In conclusion, we found that the luminescence intensity from oligonucleotide-bound CDPP–Eu(III) complex is very sensitive to the change of microenvironments. Therefore, these conjugates can be used for in situ analysis of interactions of DNA with various biomolecules.

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