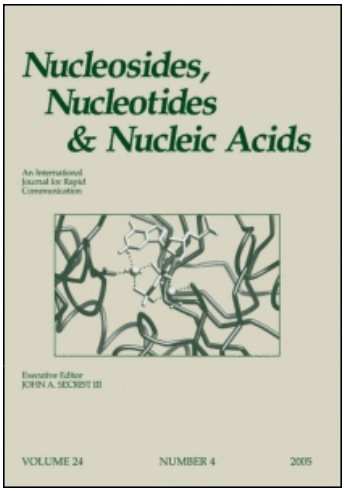

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Synthesis of Modified DNAs Bearing Two Different Fluorescent Probes and Its Application to Fluorescence Resonance Energy Transfer

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Synthesis of Modified DNAs Bearing Two Different Fluorescent Probes and Its Application to Fluorescence Resonance Energy Transfer

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Fluorescence resonance energy transfer (FRET) has become useful for structural studies of nucleic acids,[1] especially the analysis of the bending of DNA and RNA. In general, fluorophores were attached at 5′- and 3′-end of DNA or RNA owing to the easy preparation of labeled molecules. However, these end-labeled molecules are inapplicable to the large (long) molecules, to the estimation of the direction of a DNA bending, and to the estimation of the helix twist caused from bending. We have been studying on the labeling at the nucleobases and the internucleotidic linkages for FRET experiments. In this study, a bulged DNA as a model of bending was used. A bulge structure presents frequently in RNA and the bend angle of five-nucleotide bulges is from approximately 45 to 75°.[2]

The fluorescent probes bearing coumarin as a donor and fluorescein as an acceptor were introduced into a single strand DNA at C5-position of 2′-deoxyuridine derivatives. The substituent at C5 of 2′-deoxyuridine does not inhibit the base pairing with adenine on the complementary strand and is placed in a major groove. The modified DNAs were synthesized by the combination of pre- and post-synthetic

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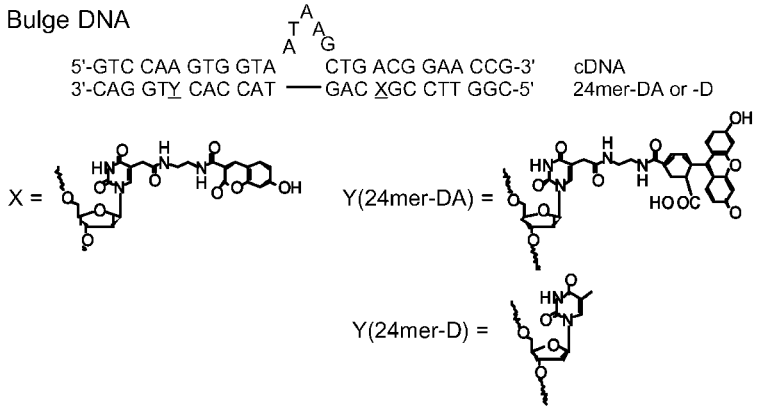

Figure 1. Sequences and structures of modified DNAs in this study.

modification using two C5-substituted 2′-deoxyuridine derivatives; a fluorescent-labeled 2′-deoxyuridine phosphoramidite and an active ester-bearing 2′-deoxyuridine phosphoramidite.[3] Fig. 1 shows the sequences and structures of the modified DNAs (24mer-DA and 24mer-D) in this study. Briefly, the synthesized DNA on CPG support was treated with 50% 1,2-diaminoethane in ethanol and then conc. aq. NH_3. The obtained modified DNA was labeled with coumarin succinimide to give a donor- and acceptor-labeled DNA (24mer-DA). Also, a donor-labeled DNA (24mer-D) was synthesized by a similar procedure. A donor- and acceptor-labeled duplex and a donor-labeled duplex were formed by the combination of the 24mer-DA or 24mer-D, and cDNA (Fig. 1). Fluorescence emission spectra of these duplexes are shown in Fig. 2. A decrease in the fluorescence intensity of donor emission around 450 nm was observed by the addition of the acceptor on DNA to indicate FRET. From the difference in this intensity, FRET efficiency was calculated on 0.47. The distance between the donor and the acceptor measured 36 Å from this FRET experiment for the bulge-containing DNA. In model study, the distance between two C5-positions of 2′-deoxyuridine derivatives in 24mer-DA was estimated

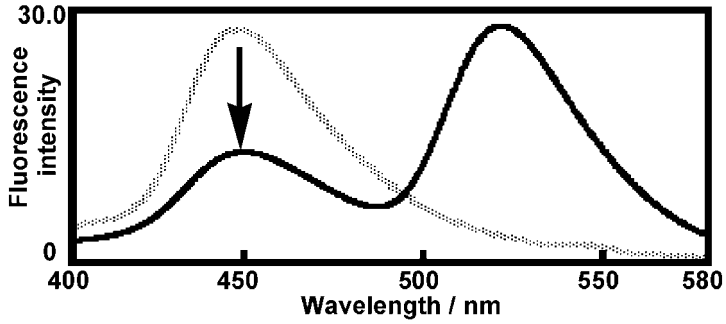

Figure 2. Fluorescence emission spectra of coumarin-fluorescein doubly-labeled DNA (black line) and coumarin-labeled DNA (gray line).

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at 40 Å. As the experimental value was smaller than the value established in the model study, the distance between two dyes is closer than that between two C5-positions of 2′-deoxyuridine derivatives. This may suggest that the reporter groups are not vertical against DNA helix axis and one or both of the dyes interact with DNA. This method will be used to analyze the bulge structure after the orientation of the probes against DNA is determined from more detailed studies.

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