H-Aggregation of Methyl Reds by the Hybridization of DNA-Dye Conjugates

Hiroyuki Asanuma,* Kenji Shirasuka, and Makoto Komiyama*
Research Center for Advanced Science and Technology, The University of Tokyo, 4-6-1 Komaba, Meguro-ku, Tokyo 153-8904

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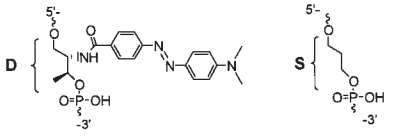
Two DNA-dye conjugates, which are complementary with each other, were prepared by incorporating 1,3-propanediol and Methyl Reds on D-threoninol alternately into natural DNA. By hybridizing the two strands, Methyl Reds H-aggregates were formed in the duplex.

Oligodeoxyribonucleotide (Oligo-DNA) is a natural supramolecule which is characterized by spontaneous hybridization with its complementary strand. Double helices are stably formed through complementary hydrogen bondings and stacking interactions. By mimicking DNA, various organized assemblies have been designed for the creation of new supramolecules.[1] While most of the supramolecules are completely artificial,[2,3] half-artificial systems involving both natural DNA and artificial scaffolds carrying functional molecules are also promising for this purpose.[4] Merits of natural DNA, such as hybridization with its complementary strand, can be efficiently utilized to create and regulate the supramolecular structure of functional molecules. Here, we choose a dye as a functional molecule and dye aggregates are prepared by use of DNA. Since dye aggregates exhibit distinct optical properties which their monomer does not have, they are practically and scientifically important.[5,6] One of the important themes on this field is how to prepare stable dye aggregate of pre-determined size and regulate its aggregated structure.[7] Dye aggregates are conventionally prepared by the self-association of individual dye monomers in solution so that size of aggregates is difficult to control. In the present paper, we create stable and size-controlled H-aggregate of Methyl Reds from two complementary DNA-dye conjugates. Predominance of this conjugate as a supramolecule is clearly demonstrated.

The phosphoramidite monomer carrying Methyl Red was synthesized from D-threoninol instead of natural deoxyribose framework in a similar manner reported previously (**D** residue in Scheme 1).[8] 1,3-Propanediol (**S** residue in Scheme 1) was used as a counterpart of Methyl Red moieties. The modified DNAs carrying both **D** and **S** residues were synthesized on an automated DNA synthesizer by using the above monomer and conventional ones. All the modified DNAs listed in Scheme 1 were purified by reversed-phase HPLC and characterized by MALDI-TOFMS.[9]



DS3A: 5'-GGT-ATC-**DSDSDS**-GCA-ATC-3'
DS3B: 3'-CCA-TAG-**SDSDSD**-CGT-TAG-5'
DS2A: 5'-GGT-ATC-**DSDS**-GCA-ATC-3'
DS2B: 3'-CCA-TAG-**SDSD**-CGT-TAG-5'
DS0A: 5'-GGT-ATC-GCA-ATC-3'
DS0B: 3'-CCA-TAG-CGT-TAG-5'

Scheme 1. DNA-Methyl Red conjugates and natural DNAs synthesized in this study.

In the single stranded state, all the modified DNAs involving **D** and **S** residues alternately showed almost the same electronic spectra. In Figure 1, UV-Vis and CD spectra of **DS3A** having three **D** and **S** residues are shown as typical ones.[10] The absorption maximum of Methyl Red appeared at 465 nm at 0 °C corresponding to the monomeric π–π^* transition (Figure 1A), which was almost independent of the temperature. Although **DS3A** is chiral and optically pure, CD was almost nil (Figure 1B). All these results indicate that Methyl Reds in the single-stranded DNA are in the monomeric state and are essentially isolated, since each dye in the strand is separated with **S**.[11]

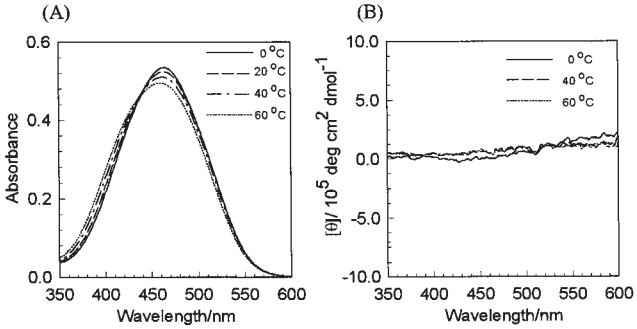


Figure 1. UV-Vis and CD spectra of **DS3A** at various temperatures in the presence of 100 mM NaCl (M = mol dm^{-3}) at pH 7.0 (10 mM phosphate buffer). [**DS3A**] = 5 μM.

Significantly, both hypsochromic shift and narrowing of the band were observed when the two complementary **DS3A** and **DS3B** were hybridized.[12] Absorption maximum of Methyl Red shifted from 465 nm (λ_{max} in the monomeric state) to 445 nm by mixing **DS3A** and **DS3B** at 0 °C (solid line in Figure 2A), and half-line-width became 93 nm (4670 cm^{-1}) which was 15 nm (457 cm^{-1}) smaller than that of single-stranded **DS3A** or **DS3B**.

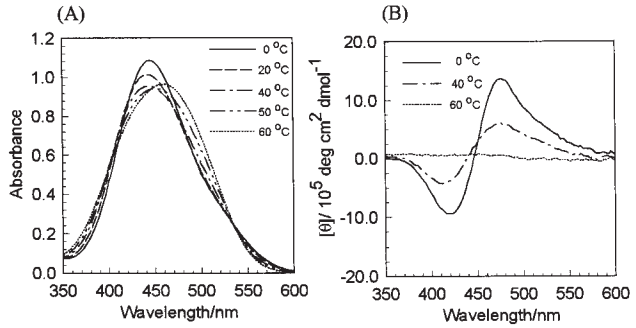
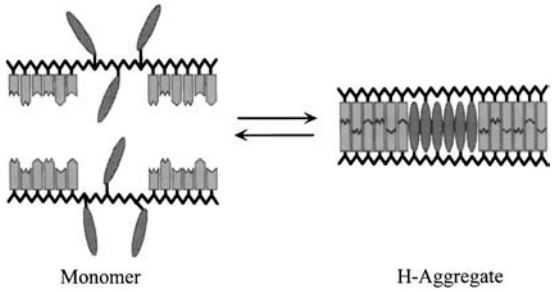


Figure 2. Effect of temperature on UV-Vis and CD spectra of **DS3A/DS3B** duplex in the presence of 100 mM NaCl at pH 7.0 (10 mM phosphate buffer). [**DS3A**] = [**DS3B**] = 5 μM.

Furthermore, strong CD was also induced as shown by the solid line in Figure 2B. This hypsochromism clearly demonstrates that H-aggregate of Methyl Reds was formed in the duplex: totally six Methyl Reds from each strand are alternately stacked along the helix axis by hybridization as schematically illustrated in Scheme 2. Similar hypsochromic shift and induced CD were also observed for **DS2A/DS2B** duplex which involves four Methyl Reds in total. Peak maximum of **DS2A/DS2B** duplex appeared at 451 nm at 0 °C whereas that of **DS2A** without **DS2B** appeared at 465 nm.[10] Induced hypsochromicity of **DS2A/DS2B** duplex with respect to its monomeric transition was 14 nm, which was smaller than that of **DS3A/DS3B** (20 nm). This result is consistent with the molecular exciton theory predicting that larger hypsochromic shift is induced with increase in the number of aggregation. Thus, H-aggregates of pre-determined size are prepared on the hybridization of two complementary DNAs involving Methyl Reds and spacers (**S** residues) alternately.[7] It should be noted that the T_ms of **DS2A/DS2B** and **DS3A/DS3B** were 51.5 and 50.9 °C, respectively, which were even higher than the corresponding natural **DS0A/DS0B** duplex without **D** and **S** residues (47.7 °C).[12] Stacking interaction between the Methyl Reds of each strand sufficiently compensated the destabilization of the duplex which should be caused by non-natural 1,3-propanediol and threoninol linkers.



Scheme 2. Schematic illustration of the reversible formation of H-aggregated Methyl Reds by hybridizing two complementary DNA-Methyl Red conjugates.

When the temperature of the solution was raised above T_m of **DS3A/DS3B** duplex, λ_{max} shifted toward longer wavelength because two hybridized DNAs were dissociated into two single strands and thus Methyl Reds were in the monomeric state (see dotted line in Figure 2A). Accordingly, induced CD almost disappeared above T_m (dotted line in Figure 2B). This change was completely reversible and H-aggregate was formed again below the T_m. Thus, structural change of Methyl Reds between monomer—H-aggregate was reversibly induced as illustrated in Scheme 2.

In conclusion, novel H-aggregate was prepared from two modified DNAs involving Methyl Reds and spacers. Various aggregates of functional molecules are programmable.

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References and Notes

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6 Aggregation of dye molecules is known to affect spectroscopic behaviors due to the coherent coupling of dye excitation. Depending on the aggregated structure and the number of aggregation, bathochromic (J-aggregate) or hypsochromic (H-aggregate) shift is induced.
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9 MALDI-TOFMS for **DS3A**: obsd 5311 (calcd for [**DS3A**-H^+]: 5311). **DS3B**: obsd 5311 (calcd for [**DS3B**-H^+]: 5311). **DS2A**: obsd 4756 (calcd for [**DS2A**-H^+]: 4755). **DS2B**: obsd 4757 (calcd for [**DS2B**-H^+]: 4755).
10 All the spectroscopic measurements were carried out under the following conditions: [DNA] = 5 μM (M = mol dm^{-3}), [NaCl] = 100 mM, pH 7.0 in the presence of 10 mM phosphate buffer.
11 Consistently, both hypsochromic shift and distinct CD were observed when **D** residues were sequentially introduced.
12 Melting temperatures (T_ms) of **DS3A/DS3B**, **DS2A/DS2B**, and **DS0A/DS0B** were 50.9, 51.5, and 47.7 °C, respectively, as estimated from the melting curves monitored at 260 nm.