Dye Aggregates

Alternating Hetero H Aggregation of Different Dyes by Interstrand Stacking from Two DNA–Dye Conjugates**

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DNA is a naturally occurring supramolecule that spontaneously forms a stable duplex with its complementary strand. Recent developments in phosphoramidite chemistry have made it possible to introduce various functional molecules or metal complexes into DNA.[1] In these modified DNA structures, non-natural molecules or metal ions are aligned in an ordered manner and exhibit interesting properties that are rarely found in the monomeric state, such as the "fluorosides" demonstrated by Kool and co-workers.[2]

Dye aggregates are known to exhibit properties that the corresponding monomers do not show. Control of the aggregation is important,[3] especially in the fields of photoinduced electron transfer[4] and enhancement of nonlinear optical properties.[5] Previously, we introduced multiple methyl red moieties into DNA and successfully prepared stable H* and H aggregates that are characterized by a

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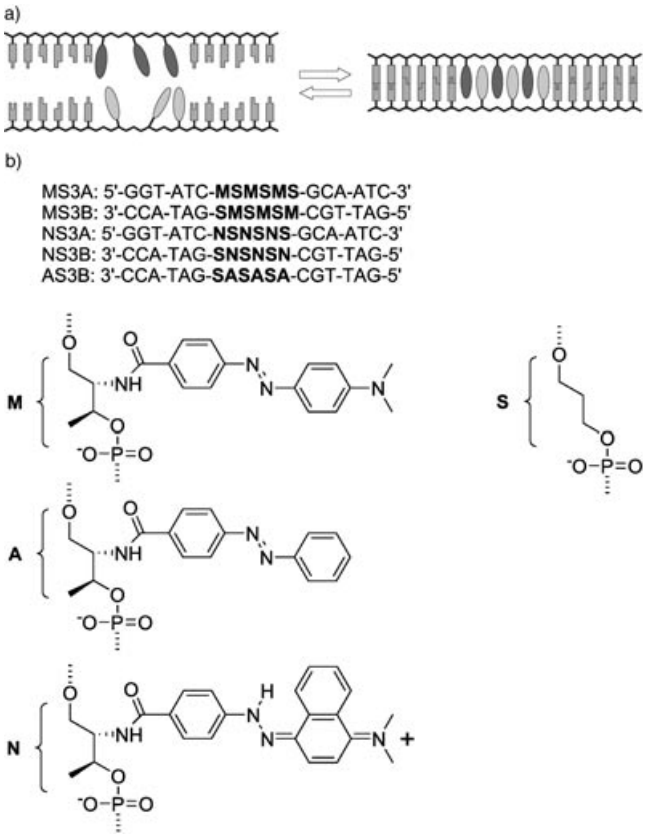
narrowing of the absorption band as well as hypsochromic shift.[6,7] The number of dye molecules and their orientation are easily controlled by programming the automated DNA synthesizer with the correct sequence. Various aggregates that are impossible to prepare by self-association of dyes are easily programmable through the covalent attachment of dye to DNA (conjugation of dye and DNA). One example of this is a heteroaggregate in which two different dyes are stacked alternately (Scheme 1a). Dye aggregates are conventionally



Scheme 1. Schematic illustration of a) preparation of heteroaggregates and b) modified DNA synthesized in this study.

prepared by the spontaneous self-association of dye monomers,[3] so alternating alignment of two different dyes is very difficult in solution. In our study, dye molecules are introduced into each strand and dye aggregates are prepared by hybridization of these strands. As far as we know, preparation of this type of heteroaggregate has not yet been reported. It is widely known that H aggregation of identical dyes (homo H aggregate) shows both a narrowing and hypsochromic shift of the band because of the strong exciton coupling, as predicted by McRae and Kasha.[8] However, there has been little investigation of whether similar narrowing of the band occurs in heteroaggregation, both from experimental and theoretical viewpoints.

Here, we report for the first time hetero H aggregates, which are difficult to prepare with a conventional method,[3] by the use of two DNA–dye conjugates (Scheme 1a). This heteroaggregate showed a new sharp absorption band that was different from those of the individual dyes, which indicates that exciton coupling occurs even between the different dyes.

Methyl red and naphthyl red (designated as **M** and **N**, respectively, in Scheme 1b) were introduced into the DNA on D-threoninol linkers from the corresponding phosphoramidite monomers, as reported previously.[6,9] The pK_a values of methyl red and naphthyl red in the single-stranded DNA were 4.1 and 5.8,[9] respectively, as determined from the change of their absorption maxima. At pH 5.0, where all the spectroscopic experiments were conducted, methyl red was neutral whereas naphthyl red was protonated (Scheme 1b). 1,3-Propanediol (**S** residue in Scheme 1b) and dye residues were alternated in the middle of the sequence.[10,11] Aqueous solutions of **MS3A** and **NS3B** exhibited absorption maxima at 462 and 520 nm, respectively (Figure 1).[12] Interestingly, a

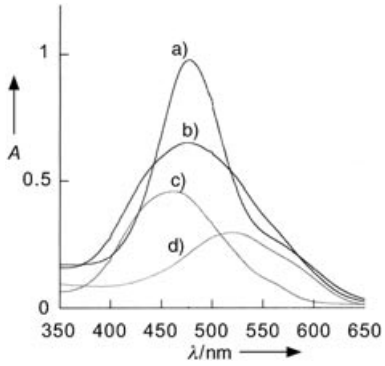


Figure 1. UV/Vis spectra of **MS3A/NS3B** duplex (a), single-stranded **MS3A** (c), **NS3B** (d), and simple sum of the spectra of **MS3A** and **NS3B** (b) at 0°C, pH 5.0 (10 mM MES buffer) in the presence of 100 mM NaCl.

new single sharp band appeared at 478 nm when these two strands **MS3A** and **NS3B** were hybridized (Figure 1). A simple sum of the spectra of the single-stranded **MS3A** and **NS3B** solution was very broad and different from the spectrum of the **MS3A/NS3B** duplex (compare the lines in Figure 1). In addition to the new absorption band, a single strong circular dichroism (CD) band with a sharp and symmetrical positive Cotton effect was induced at around 480 nm (Figure 2b). These spectroscopic behaviors demonstrate that methyl red and naphthyl red in the heteroaggregates optically interacted with each other (H band) and had different spectroscopic properties from those in the monomeric states.

This sharp new band became broad on elevating the temperature, and at 60°C, which is higher than the melting temperature T_m of the **MS3A/NS3B** duplex,[13] the spectrum almost coincided with the simple sum of the two individual spectra of **MS3A** and **NS3B** (Figure 2a). This result also supports the hypothesis that the new band is attributed to heteroaggregation. Concurrently, the strong induced CD signal disappeared at 60°C (Figure 2b).

The peak maximum of the heteroaggregates appeared in the middle of those for homoaggregates (H aggregates) of methyl red (**MS3A/MS3B**) and naphthyl red (**NS3A/NS3B**) in

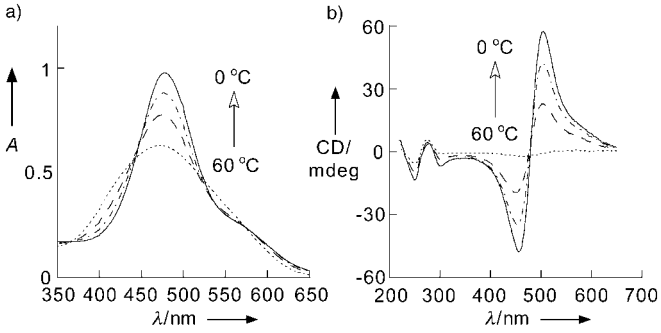


Figure 2. Effect of the temperature on a) UV/Vis and b) CD spectra of **MS3A/NS3B** duplex (20 °C interval) at pH 5.0 (10 mM MES buffer) in the presence of 100 mM NaCl.

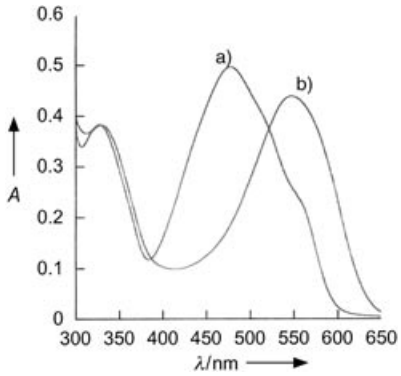


Figure 4. UV/Vis spectra of **MS3A/AS3B** (a) and **NS3A/AS3B** (b) at 0 °C, pH 5.0 (10 mM MES buffer) in the presence of 100 mM NaCl.

both UV/Vis and CD spectra (Figure 3). This fact indicates that the new narrow band for the heteroaggregates is an H band derived from exciton coupling among the chromophores.[14–16] This assignment is further supported by the fact that an increase in the aggregation number resulted in a larger hypsochromic shift (see the Supporting Information).[8] To our knowledge, the appearance of an H band from two different kinds of dyes has rarely been reported and is not even predicted in the exciton theory.[17]

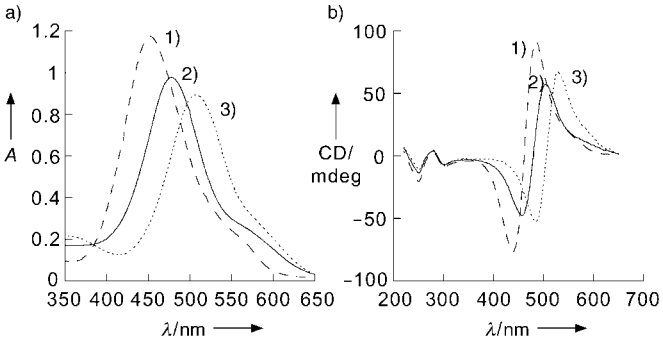


Figure 3. a) UV/Vis and b) CD spectra of heteroaggregates (**MS3A/NS3B**; 2), naphthyl red homoaggregates (**NS3A/NS3B**; 3), and methyl red homoaggregates (**MS3A/MS3B**; 1) at 0 °C, pH 5.0 (10 mM MES buffer) in the presence of 100 mM NaCl.

Notably, not all combinations of chromophores exhibit a hetero H band. In the case of the methyl red/naphthyl red combination, a new sharp H band appeared. But when **MS3A** or **NS3A** was hybridized with **AS3B** involving azobenzenes in which the absorption maximum was located at 330 nm, no significant optical interaction was observed (Figure 4): the UV/Vis spectrum of either **MS3A/AS3B** or **NS3A/AS3B** almost coincided with the simple sum of that of each strand (see the Supporting Information).[18] A certain degree of spectral overlap between the two chromophores is required to produce a hetero H band.[19]

In conclusion, heteroaggregates in which two dyes are stacked alternately can be prepared by interstrand stacking from two DNA–dye conjugates. Even different dyes can exhibit an H band as a result of aggregation. The present method is applicable to other functional molecules, and various novel materials can be produced by the attachment of dyes to DNA.[17]

Experimental Section

Synthesis of the modified DNA involving dyes: The modified DNA molecules carrying **A**, **M**, **N**, and **S** residues were synthesized with an automated DNA synthesizer by using the corresponding phosphoramidite monomer prepared according to previous papers,[6,9,20] and conventional ones. All the modified DNA molecules listed in Scheme 1 b were purified by reversed-phase HPLC and characterized by MALDI-TOF mass spectrometry.

MALDI-TOF mass spectrometry: **NS3A**: found *m*/*z* 5463 (calcd for [**NS3A**−H]$^+$: *m*/*z* 5462); **NS3B**: found *m*/*z* 5464 (calcd for [**NS3B**−H]$^+$: *m*/*z* 5462); **MS3A**: found *m*/*z* 5311 (calcd for [**MS3A**−H]$^+$: *m*/*z* 5311); **MS3B**: found *m*/*z* 5311 (calcd for [**MS3B**−H]$^+$: *m*/*z* 5311); **AS3B**: found *m*/*z* 5183 (calcd for [**AS3B**−H]$^+$: *m*/*z* 5182).

Spectroscopic measurements: The UV/Vis spectra and CD spectra were measured on JASCO model V-530 and JASCO model J-725 instruments with 10-mm quartz cells, respectively. Both of them were equipped with programmed temperature controllers. The conditions for the sample solutions were: [NaCl] = 100 mM, pH 5.0 (10 mM β-morpholinoethanesulfonic acid (MES) buffer), [DNA] = 5 μM. The T_m value was determined from the maximum in the first derivative of the melting curve, which was obtained by measuring the absorbance at 260 nm as a function of temperature. The temperature ramp was 1.0 °C min^{-1}.

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[7] An H aggregate is a dye cluster that causes a hypsochromic shift in the absorption spectrum with respect to the monomer spectrum. Dye molecules in H aggregates are known to be stacked in a face-to-face manner. See the Supporting Information.
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[10] Recently, Brotschi and Leumann reported interstrand stacking of bipyridyl C-nucleotides that were consecutively introduced into the DNA. In our design, dye moieties are separated by **S** residues to avoid firm H* aggregation of methyl red moieties in the single-stranded state. See Refs. [6] and [11].
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[12] Absorption maxima of monomers of methyl red and naphthyl red in the DNA appeared at around 484 and 546 nm, respectively. See the Supporting Information.
[13] The melting temperature of **MS3A/NS3B** was 50.7 °C, as estimated from the change in absorbance at 260 nm as a function of temperature. See the Supporting Information for the actual melting curve.
[14] The possibility of a charge-transfer band is ruled out because the absorption band of the charge-transfer complex usually appears at a longer wavelength than those of individual dyes. See Ref. [15].
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[16] The relative alignment of the chromophores will be discussed in detail elsewhere.
[17] Since exciton coupling occurs over very short ranges (within 1.0 nm), our present finding would be applicable to the detection of short-distance interaction.
[18] A contribution of the reduction of the conformers or local polarity change to the band narrowing of **MS3A/NS3B** is unlikely, because significant narrowing was not observed for the combination with **AS3B** involving **A** residues that are structurally similar to **M**.
[19] We attributed the optical inactivity of the azobenzene/naphthyl red or azobenzene/methyl red combinations to the large difference in the absorption maximum, but more detailed analyses are needed.
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