Incorporation of cationic dyes into DNA for distinct stabilization of duplex.

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ABSTRACT

In this study, cationic dyes (methylstilbazole) were introduced into ODN. When two complementary ODNs, both of which tethered this dye, were hybridized, the melting temperature drastically increased. The duplex was further stabilized by introducing multiple dyes.

INTRODUCTION

Modification of nucleotides is one of the most active research fields in chemical biology. Recently, oligodeoxyribonucleotides (ODNs) have been utilized not only for biological uses but also as a scaffold for preparing supramolecular arrays.¹ ² In these reports, metal ligands or fluorescent dyes were introduced at the C1’ position of D-ribose as non-natural bases. One of the reasons for utilizing D-ribose is that functional molecules can be incorporated into natural ODNs without distorting their natural B-type structure. Furthermore, functional molecules on natural nucleotides can be substrates of DNA or RNA polymerase, and in some cases are enzymatically incorporated into ODNs. This latter purpose inevitably requires the natural D-ribose backbone. However, there is no limitation on the structure of a scaffold when the ODNs are designed for supramolecular function and are not enzymatically but chemically synthesized.

Threoninol is an amino acid derivative that can be synthesized by a one-step reduction from threonine methyl ester.¹ Previously, we have reported on novel “base-pairs” of functional molecules on D-threoninol (threoninol-nucleosides).³ In our design, these nucleosides are introduced at the counterpart of each strand to form a pseudo “base-pair”. Larger molecules than natural bases can be introduced and pseudo “base-pairs” stabilize the duplex by intermolecular stacking interactions. By this design, highly organized molecular cluster can be easily constructed without disturbing duplex. Such molecular clusters are applicable to nonlinear optical materials and molecular wire as well as detection of single nucleotide polymorphisms and so on.

Here, we introduce cationic dyes into DNA on D-threoninol linker for further stabilization of the duplex. The cationic residues are expected to stabilize the duplex by electrostatic attraction between dye cation and phosphodiester anion. It is relatively difficult to tether cationic molecules on D-ribose because glycosidic bond are labile to acidic/basic conditions.⁵ Thus, D-threoninol is much more suitable for this purpose. We use methylstilbazole (Z in Scheme 1) because it has a positive charge on pyridine ring and a stable “base pair” is expected to form in the duplex. In addition, stilbazole dyes are known to have strong nonlinear optical properties.⁶ Therefore, highly organized stilbazole cluster in the duplex would be promising for SHG material. In this study, modified ODNs tethering methylstilbazole are synthesized and their stability is investigated by measuring melting temperatures.

RESULTS AND DISCUSSION

Incorporation of methylstilbazole (Z) into DNA was conducted by a phosphoramidite method. Pyridine ring was quaternized after phosphoramidation because methylated dye was hardly soluble in organic solvents. As shown in

<table>
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<th>Table 1. Effects of dye number on melting temperatures.</th>
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<td>Dye number</td>
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<td>0</td>
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³Conditions: 5 μM duplex, 100 mM NaCl, pH 7.0 (10 mM phosphate buffer). ⁴Melting temperature of native duplex (N.C.)
Scheme 1, 1 to 3 Zs were incorporated into DNA. In addition, methylazobenzene (X) and stilbazole (B) were introduced into DNA as a control.

When Z1a involving single Z residue was hybridized with its complementary Z1b (also involves single Z residue), its melting temperature ($T_m$) was determined as 57.7 °C (see Table 1), which was 10 °C higher than that of native duplex (N/C: 47.7 °C). Thus, duplex was greatly stabilized by introducing a base pair of cationic methylstilbazole on $D$-threoninol. In order to evaluate the effect of positive charge, $T_m$s of Bla/B1b and X1a/Xb duplexes were measured. As listed in Table 1, $T_m$ of Bla/B1b was 52.7 °C, which was 5 °C lower than that of Z1a/Z1b duplex. Similarly, $T_m$ of X1a/X1b, each of which tethers single methylazobenzene moiety, was determined as only 51.4 °C. It should be noted that the structure of methylazobenzene was almost the same as that of methylstilbazole except for the positive charge on the dye. Thus, we could conclude that fairly large $T_m$ of Z1a/Z1b duplex is attributed to electrostatic interaction between the positive charges on dyes and negative charges on phosphate.

To investigate the structure of a base pair of methylstilbazole, UV-Vis spectra of Z1a/Z1b were measured (Fig. 1). At 80 °C where duplex was completely dissociated, a broad peak was observed at 347 nm. However, the peak became much narrower upon lowering the temperature below 40 °C. In addition, the peak shifted to shorter wavelength. These spectral shifts are characteristic to dye aggregates where dyes stacked vertically to the helical axis: dyes are firmly stacked in face-to-face manner in spite of facing positive charges. The melting profiles of Z1a/Z1b monitored at 260 and 360 nm are shown in Fig. 2. Melting temperature of Z1a/Z1b determined from the absorbance at 360 nm was 58.8 °C, which is almost the same as that at 260 nm (57.7 °C). These results clearly show that base pairing of natural bases and stacking of dyes are fairly synchronized.

Duplex was extremely stabilized by multiplying base pairs of methylstilbazole. As listed in Table 1, $T_m$ of Z3a/Z3b was as high as 68.5 °C, which was 20 °C higher than that of native duplex. On the other hand, multiplication of methylazobenzene did not show such stabilization of the duplex: $T_m$ of B3a/B3b was 50.6 °C and even lower than that of Bla/B1b (52.7 °C). These results demonstrate that accumulated positive charges on the dyes strongly stabilized duplex.

**CONCLUSION**

Highly stable “base pairs” of methylstilbazole on $D$-threoninol were successfully prepared. By introducing these base pairs, optical materials and supramolecular clusters are expected to be prepared without the assistance of natural bases.

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