Selective stabilization of a bulged duplex of d(GCGAAACGC) oligonucleotide by thymine base-substituted naphthalene diimide

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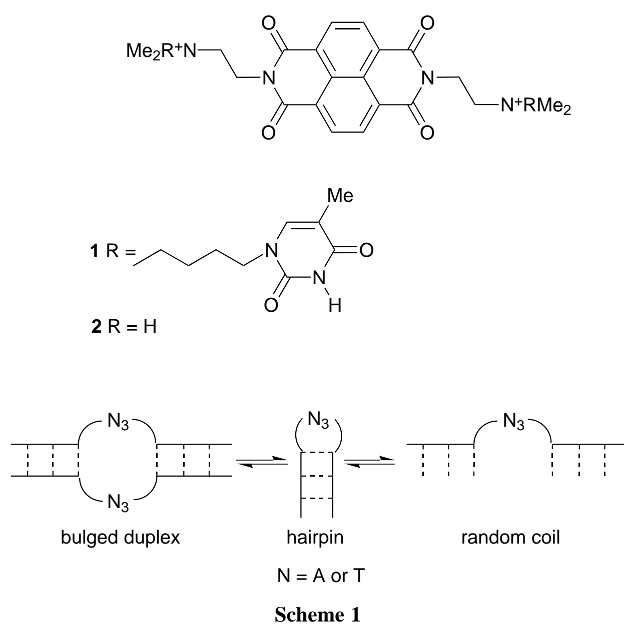
A naphthalene diimide carrying thymine moieties at the termini of its substituentsstabilized specifically the bulged duplex of d(GCGAAACGC), which is rarely found under ordinary conditions.

Nucleic acids have a variety of higher-order structures, such as base bulge and hairpin, which are associated with many biological functions.[1] Compounds recognizing these structures are important for studying nucleic acid structure and can serve as new DNA-targeting agents. Many molecules have been synthesized to exhibit this.[2–10] For example, a macrocyclic bisacridine developed by Lehn and co-workers showed a selective binding to the DNA hairpin of d(GCGAAACGC), named sA_3.[10] We have shown that thymine base-substituted naphthalene diimide **1** has a high affinity for polyA.[11] It occurred to us that compound **1** might bind to the hairpin region of sA_3 specifically as well, since this oligonucleotide consists of single stranded adenines. Contrary to this expectation, however, **1** produced a bulged duplex rather than a hairpin structure on sA_3, as shown in Scheme 1.

In sA_3, the bulged structure is in equilibrium with a hairpin. The latter is the predominant species in solution under ordinary conditions, as exemplified by a melting profile in which two transition temperatures were observed.[10] The higher melting temperature (T_{mH}) is attributed to intramolecular transition from a hairpin to random coil. The lower melting temperature (T_{mL}), which is rarely found and sA_3 concentration-dependent, is attributed to intermolecular transition from a bulged duplex to a hairpin.

Fig. 1(*a*) shows the melting curve of sA_3 with a major transition temperature at 54 °C due to the collapse of the hairpin structure as described previously.[10] Two transition temperatures appeared at about 18 and 60 °C in the presence of **1**, and the lower temperature transition was raised with an increase in the amount of **1**. Since the absorbance of **1** alone was independent of temperature over the 15–90 °C range, the lower transition temperature was attributed to T_{mL}. These data indicated that **1** can stabilize the bulged structure of sA_3 specifically, although it stabilized the hairpin structure as well, albeit to a smaller extent [*ca.* 6 °C, compare curves (i) and (ii) in Fig. 1(*a*)]. On the other hand, naphthalene diimide **2**, without thymine moieties, showed a transition curve featuring mainly a rise in the T_{mH} to 80 °C. This behaviour of **2** was largely unaffected by the presence of thymine. Ordinary intercalators insert themselves between adjacent base pairs in double stranded nucleic acids, thus effecting stabilization.[12] This is the case with **2**, where the T_{mH} was raised by 26 °C when compared with the T_{mH} of sA_3 alone. In summary, **1** stabilizes the bulged duplex of sA_3 much more than its hairpin structure, whereas **2** stabilizes mainly the hairpin structure, as do other ordinary intercalators. This difference arises presumably because **1** can bridge the two DNA strands through hydrogen bonding of its thymines moieties with the adenine moieties of sA_3. We estimate that 50 and 10% of sA_3 assumes the bulged structure in



Scheme 1

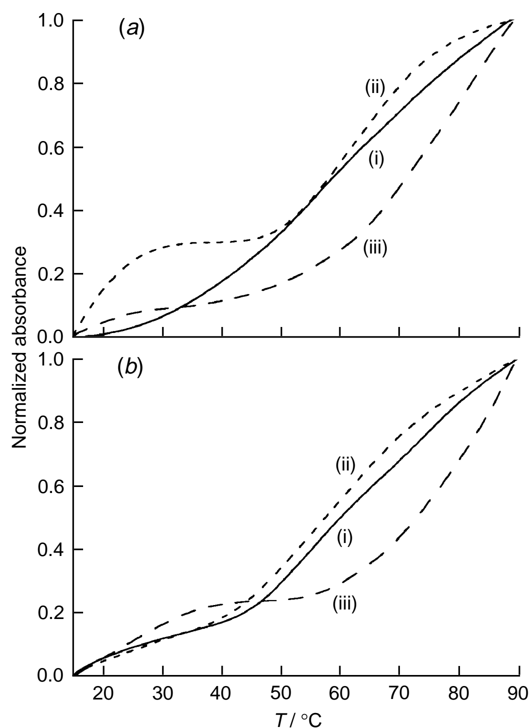


Fig. 1 Melting profiles for (*a*) sA_3 and (*b*) sT_3: (i) no additive; (ii) in the presence of **1**; (iii) in the presence of **2**. Carried out in 10 mm MES (2-morpholinoethanesulfonic acid) and 1 mm EDTA (ethylenediaminetetraacetic acid) buffer (pH 6.25); oligonucleotide (in the double strand form) and additive concentration = 5.8 μm.

the presence of an equimolar amount of **1** and **2**, respectively, at 20 °C.

To investigate whether **1** can stabilize the bulged adenines specifically, the melting curve of d(GCGTTTCGC) (sT_3) was measured in the absence and presence of **1** or **2** under the same conditions [Fig. 1(*b*)]. This oligonucleotide showed two transition temperatures at *ca*. 15 and 60 °C, which can be attributed to the transition from a bulged duplex to hairpin and from a hairpin to random coil, respectively [(i) in Fig. 1(*b*)]. Neither the bulged duplex nor the hairpin was stabilized by **1**, whereas **2** stabilized the hairpin structure exclusively [(ii) and (iii) in Fig. 1(*b*)]. This result proves that **1** stabilizes the bulged adenines specifically.

The competitive binding to sA_3 of **1** and **2** with ethidium bromide was performed by monitoring ethidium fluorescence.

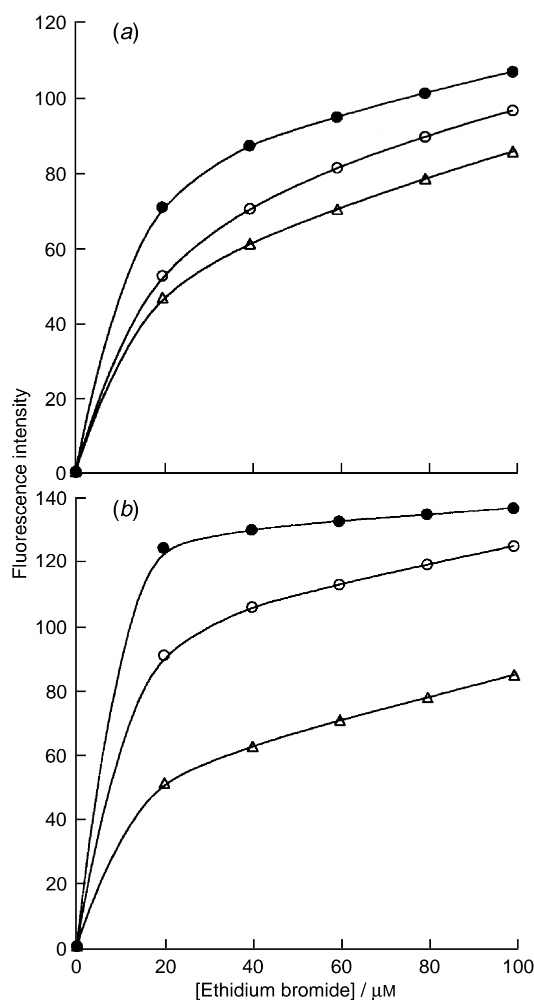


Fig. 2 Changes in the fluorescence intensity upon addition of ethidium bromide to (*a*) sA_3 and (*b*) sT_3: (●) no additive; (△) in the presence of **2**; (○) in the presence of **1**. Carried out in 10 mm MES buffer (pH 6.25) containing 0.3% Me_2SO at 20 °C; oligonucleotide (in the double strand form) and additive concentration = 5.8 μm.

Addition of ethidium bromide to sA_3 increased the fluorescence intensity at 595 nm (λ_{ex} = 546 nm) at 20 °C as a result of binding to DNA as shown in Fig. 2(*a*). The fluorescence of ethidium bromide was suppressed with a mixture of sA_3 and **1** or **2**, and the quenching was more pronounced with **2** than with **1** [Fig. 2(*a*)]. On the other hand, **1** was not as effective as **2** in the quenching of the fluorescence of sT_3-bound ethidium bromide [Fig. 2(*b*)], demonstrating that the binding of **1** with sT_3 is weaker than with sA_3, whereas **2** can bind to both sA_3 and sT_3 equally well.

Since ethidium bromide binds to DNA by intercalation,[13] those intercalators which bind to the double stranded region of DNA compete with ethidium bromide to suppress its fluorescence. It follows therefore that **2** binds mainly to the double stranded region of sA_3, whereas **1** is away from such a region and binds mainly to the single stranded loop region. Both the bulge and hairpin structures of sA_3 have a loop, but the curvature of the loop is smaller for the bulge. Hence, the naphthalene diimide ring of **1** can stack more effectively with the adenine rings of the bulge. Moreover, since **1** possesses two thymine bases, it can hold together the two strands of sA_3 through complementary hydrogen bonding of the thymines with the adenines. We believe that this is the reason why **1** stabilizes the bulged structure of sA_3 specifically. Whatever the mechanism, this is the first example of the alteration of the secondary structure of an oligonucleotide using a small molecule.

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