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Poly-intercalators Carrying Threading Intercalator Moieties as Novel DNA Targeting Ligands

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Abstract. Threading intercalators are a novel class of intercalators that carry two substituents along the diagonal positions of an aromatic ring. These substituents are projecting out in DNA grooves when bound to DNA. Poly-intercalators carrying threading intercalating parts are quite novel and were recently found to show a unique DNA binding behavior. We review herein two types of poly-intercalators. First, tris-intercalators carrying a threading intercalator part in the middle of the molecule are described. These intercalators appear to intercalate into double stranded DNA in a special binding manner, which we call the penetrating mode, in which all the three intercalating units are arranged linearly with one of them penetrating into the DNA ladder. We synthesized two tris-intercalators (**3** and **4**) of this type and studied their binding behavior for double stranded DNA. All the experimental results were consistent with the proposed penetrating mode. Another type of threading poly-intercalators is a macrocyclic bis-threading intercalator (**5**). We found that this compound can bis-intercalate to double stranded DNA when the base pairing is disrupted temporarily to form a complex with a unique structure like a catenane. On the basis of a study of the interaction of such intercalators we envisage that DNA is a flexible and dynamic entity. These novel families of poly-intercalators will expand the scope of DNA poly-intercalation chemistry with possible medicinal applications.

Key words: DNA double strand, threading intercalator, tris-intercalator, macrocyclic bis-threading intercalator, catenane.

1. Introduction

Polyaromatic compounds often with a cationic center can insert or intercalate between base pairs of double stranded DNA [1, 2]. Hence, such a family of compounds are called intercalators and this DNA binding process is called intercalation. The driving force of intercalation is mainly based on a hydrophobic interaction between the plane of base pairs of double stranded DNA and the aromatic plane of the intercalator of similar area [1, 2]. Several biological activities

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were associated with the interaction of the intercalator with DNA and intercalators have been the target of intensive study [1, 2]. Threading intercalators are also becoming known. Nogaramycine is a representative of such threading intercalators [3]. Nogaramycine has two bulky substituents on a diagonal line of the intercalating plane. Therefore, one of the substituents needs to go through base pairs to form an intercalated complex with double stranded DNA. The double strand of DNA is moving dynamically with several motions because base pairs are not made from static bonds. Stacked base pairs are moving up and down and are disrupted temporarily. This base disruption occurs over several lengths and is called breathing of DNA [4]. One of the substituents of nogaramycine can go through between base pairs despite the fact that it is larger than the space available between the base pairs. In other words, double stranded DNA can accommodate several types of intercalators even when they are so bulky that stacking of the base pairs of DNA is seemingly impaired. 1,5-Disubstituted anthraquinone [5] and naphthalene diimide carrying two substituents [6] are known as synthetic threading intercalators. When a poly-intercalator contains threading intercalating parts, they will have a unique character in their DNA binding mode. In this paper, we discuss two types of poly-intercalators carrying threading intercalator part(s): One is a tris-intercalator carrying a threading intercalating part in the middle of the molecule and another is a macrocyclic threading bis-intercalator.

2. Tris-intercalator Carrying a Threading Part in the Middle

We synthesized a tris-intercalator carrying intercalator moieties at both terminal positions of the central threading intercalator. Figure 1 shows its possible binding process with double stranded DNA. In order for the middle threading intercalating part to intercalate fully into DNA, one of the terminal intercalating parts needs to go through between base pairs (Figure 1, the process from I to II). During this process, the two terminal intercalating parts protrude in the major and minor grooves of DNA. Then they intercalate from both sides (Figure 1, the formation process of III) to finally form a unique tris-intercalated complex, in which the three intercalating parts are arranged linearly to penetrate the DNA ladder. We call this binding mechanism the penetrating mode for tris-intercalation.

In the other binding mode, only the two terminal intercalating parts intercalate into the same groove, whereas the middle intercalating part remains un-intercalated (Figure 1, IV). In other words, the middle intercalating part of the tris-intercalator cannot be intercalated in this binding mode. We call this binding mechanism the covering mode for bis-intercalation. The first penetrating mode is expected to give more stable complex than the covering mode, despite the fact that one of the terminal intercalating parts needs to go through between base pairs of double stranded DNA during this complex formation. Since classical intercalating molecules do not distinguish major and minor grooves in the associating or dissociating processes [1], very high accuracy is needed for the intercalator to go through between base

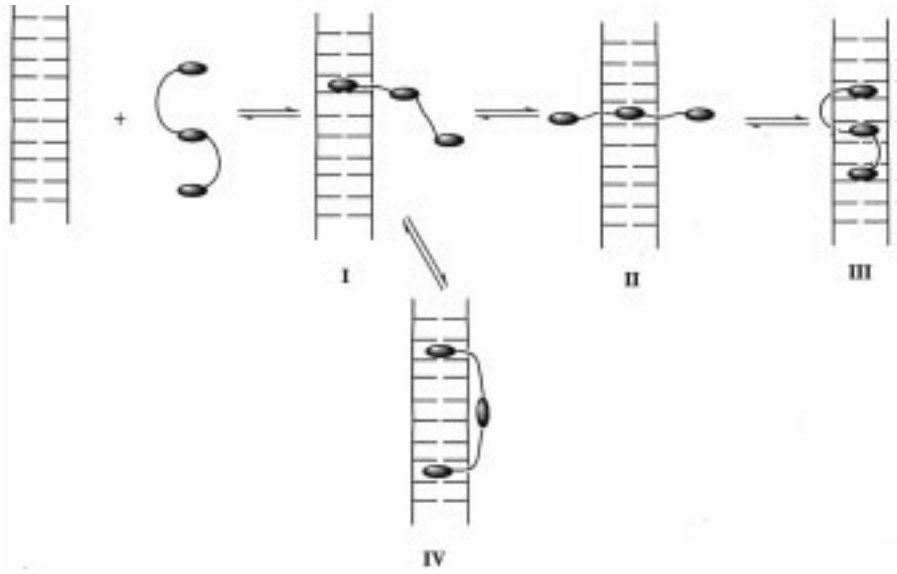

Figure 1. A possible intercalating process of a linear tris-intercalator.

pairs. This desired tris-intercalated complex formation is expected by this novel tris-intercalator.

To test the possibility of such a complex formation with tris-intercalators, we synthesized an anthraquinone derivative (**1**) carrying two acridine moieties at its 1- and 5-positions as shown in Figure 2 [7, 8]. It should be noted that 1, 5-disubstituted anthraquinone derivatives act as a threading intercalator [5].

Tris-intercalator **1** has absorption maxima at 360 and 539 nm for the acridine and anthraquinone chromophores, respectively (Table 1). The molar absorptivity of **1** at 539 nm is 59% smaller than that of the anthraquinone (**2**) carrying no terminal intercalating parts, suggesting the intramolecular stacking of the anthraquinone and acridine parts. When calf thymus DNA was added, the anthraquinone part of **1** underwent further hypochromicity of 17%. Thus, the total hypochromicity for the anthraquinone part of **1** is 66% upon its binding to double stranded DNA. We do not have a molar absorptivity of the monomeric acridine part, but the acridine parts of **1** should show large hypochromicity when bound to DNA, although the acridine parts of **1** show 6% hypochromicity after DNA binding. Both absorption maxima of **1** underwent a slight red-shift upon DNA binding. Since these phenomena are in agreement with those of ordinary intercalators [1] and were seen with both the acridine and anthraquinone parts, all the three intercalating parts of **1** intercalate into the double stranded DNA simultaneously. Tris-intercalator **1** should bind to DNA with the penetrating mode because full tris-intercalation is possible only with this mode.

Circular dichroism (CD) spectra of calf thymus DNA showed an additional Cotton effect at both 360 and 540 nm in the presence of tris-intercalator **1**. The

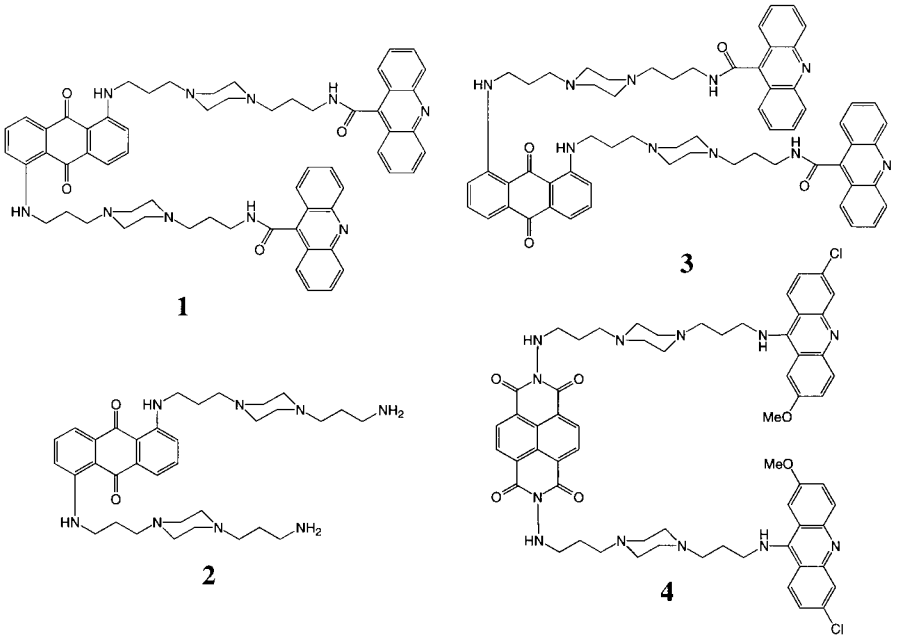

Figure 2. Chemical structures of tris-intercalators (**1**, **3**, and **4**) and threading intercalator (**2**).

Table I. Spectroscopic properties of compounds **1**, **2**, **4**, and **5** in free and DNA-bound form

	UV-visible absorption				
	Free ligand		Bound ligand		
Compound	λ_f (nm)	$10^{-3}\epsilon_f$ (cm^{-1} M^{-1})	λ_b (nm)	$10^{-3}\epsilon_b$ (cm^{-1} M^{-1})	H[d]
1[a]	360	8.7	362	8.2	6
	539	4.7	540	3.9	17
2[a]	536	11.5	541	7.9	31
4[b]	383	23.0	388	10.0	57
	424	12.0	430	10.0	17
4[e]	388	9.0			
	427	9.0			
Quinacrine[b]	388	9.0	427	9.0	
5[c]	385	36.0	388	22.0	39
6[c]	381	30.0			

Experiments were conducted in: [a]0.3 mM sodium acetate buffer at pH 4.5, [b]pure water, and [c]10 mM MES buffer and 1 mM EDTA at pH 6.24.
[d]H stands for percentage of hypochromicity [%H = $(1 - \epsilon_b/\epsilon_f) \times 100$].
[e] 0.5 M NaCl.

induced CD of this region derives from the tris-intercalator chromophores and demonstrated that all the chromophores are located in a dissymmetric and very restricted environment such as the one created by base pairs in double stranded DNA.

We measured the unwinding angle of base pairs in double stranded DNA caused by a bound tris-intercalator to prove the intercalation of their middle intercalating units. Using the viscometric titration with supercoiled plasmid DNA, an unwinding angle of 49° was obtained for the tris-intercalator. The unwinding angle of classical mono-intercalators is known to be nearly 17° [9] and the value obtained above was almost 3 times that for mono-intercalators. This is more evidence of the penetrating mode for tris-intercalation. Penetrating tris-intercalation is expected to stabilize the DNA double strand. We compared the effect of tris-intercalator on the DNA melting temperature. We chose tris-intercalator 1,8-isomer (**3**) as the control, in which the two substituents are located in the same groove when intercalated into DNA base pairs [5]. Tris-intercalator **3** intercalates into DNA base pairs by the covering mode for bis-intercalation. Tris-intercalator **1** stabilized the DNA double strand with ΔTm = 28 °C and this effect was larger than that for **3** (ΔTm = 24 °C).

We studied the preference of tris-intercalator **1** for DNA bases by C_{50} measurements. C_{50} values represent the tris-intercalator concentration necessary to displace 50% of DNA-bound ethidium bromide. The smaller the C_{50} values, the stronger the binding. Comparison of natural DNAs carrying different GC (guanine and cytosine) contents, tris-intercalator **1** exhibited a very high AT (adenine and cytosine)-preference, although monomeric acridine and anthraquinone intercalators exhibit a GC-preference. We studied DNase I footprinting to examine the DNA sequence preference of tris-intercalator **1** in more detail. The result revealed that **1** bound to AT-contiguous sequences with very high selectivity.

We synthesized tris-intercalator **4** (Figure 2) to expand the scope of this class of tris-intercalators [10]. Tris-intercalator **4** has a naphthalene diimide as a threading intercalator and two 9-aminoacridine moieties connected through alkylamino chains. The molar absorptivity of the acridine and naphthalene diimide parts of **4** increases with an increase in the buffer or salt concentration of the medium. Table I shows the example in the case of NaCl. Likewise, the fluorescence intensity of the acridine parts of **4** decreased drastically with an increase in the buffer or salt concentration. These results suggested that tris-intercalator **4** also underwent intermolecular stacking as the salt concentration in the medium is increased. This behavior is similar to those observed previously, though for monomeric systems [11]. Since **4** assumes an extended conformation in pure water, we were able to titrate **4** with sonicated calf thymus DNA by monitoring the absorption or fluorescence changes. The binding constant obtained according to the Scatchard analysis was 10^7 M^{-1} [12].

Kinetic analysis on **4** and DNA was carried out in buffered solution by monitoring the absorption of the naphthalene chromophore and the absorption and fluorescence of the acridine chromophore. A dissociation rate constant of 0.02 s^{-1}

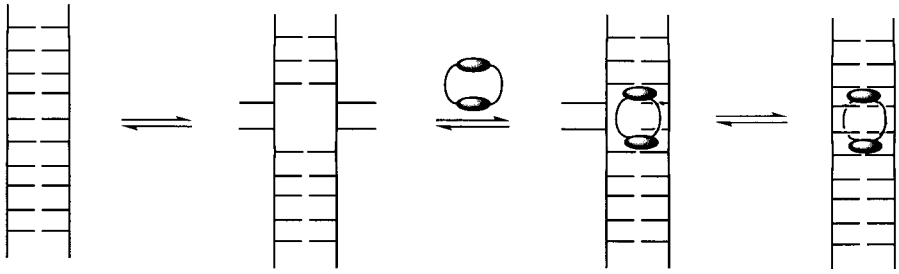

Figure 3. The base pair disruption of double stranded DNA and the intercalation of a macrocyclic bis-threading intercalator, resulting in a catenated complex.

was obtained from the data by Wilson's treatment [14] and the results obtained by the two methods were in good agreement with each other. Since naphthalene diimide threading intercalators have a dissociation rate constant of 0.1–10 s^{-1}, this value for tris-intercalator **4** is at least 5 times smaller than that for ordinary threading mono-intercalators [12]. This also supported the penetrating mode for tris-intercalation of **4** with double stranded DNA.

The penetrating mode for the tris-intercalation is novel and expected to occur in the weak double stranded DNA region where DNA breathing is more frequent. Such a DNA sequence is known to be important for gene expression; the DNA double strand must open for this process to take place [14]. Therefore this series of tris-intercalators may be used as a labeling reagent for such DNA sequences. From the stabilization ability the DNA double strand, they could also be used as a reagent which can stop the proper gene expression.

3. Macrocyclic Threading Bis-intercalator

A macrocyclic threading bis-intercalator can be formed by connecting two chromophores with linkers. The base pair breaking is necessary to form a bis-intercalated complex with DNA. Figure 3 depicts the bis-intercalating process of a macrocyclic bis-intercalator with double stranded DNA. A macrocyclic bis-intercalator will intercalate when the base pairs are open to form a complex with a catenated structure. This is a less favorable process thermodynamically than that for the tris-intercalator discussed above.

Zimmerman and co-workers were the first to synthesize this series of compounds by connecting the 4- and 9-positions of acridine parts with linkers [15]. Since 4,9-disubstituted acridine also is known to serve as a threading intercalator, the macrocyclic bis-acridine served indeed as such. In collaboration with Wilson's group they studied the binding behavior in great detail [16] and found the expected bis-intercalation mode as described above. This result suggested that double stranded DNA dynamically moves with several motions and can accept many types of poly-intercalators. It is noted that triostin, an antibiotic bis-intercalator, bis-

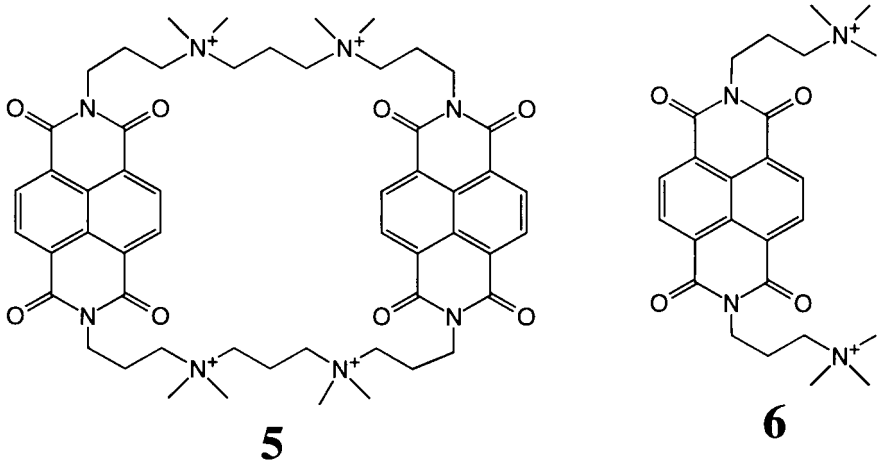

Figure 4. Chemical structures of macrocyclic threading intercalator (**5**) and threading mono-intercalator (**6**).

intercalates into double stranded DNA and the base pairs of its binding sites change from the Watson–Crick base pairing to the Hoogsteen base pairing [17].

We also synthesized macrocyclic bis-intercalator **5** consisting of naphthalene diimide units (Figure 4). All the ^{1}H-NMR peaks of **5** were broadened even at 130 °C, suggesting that the two aromatic rings of **5** are constrained so that their free rotation is hindered. Macrocyclic bis-intercalator **5** has absorption maxima at 363 and 385 nm with a blue shift of 4 nm from those with mono-intercalator **6** having the same aromatic skeleton as **5** (Table I). The absorption of **5** at 363 nm is larger than that at 385 nm, whereas the opposite was true for **6**. The molar absorptivity of **5** at 385 is 1.8×10^4 cm^{-1} M^{-1} per naphthalene diimide unit and that of **6** is 3.0×10^4 cm^{-1} M^{-1}, indicating that the naphthalene diimide part of dimer **5** is 39% hypochromic compared with that of monomer **6**. This would derive from intramolecular stacking of the aromatic rings of **5**, a phenomenon in agreement with the ^{1}H-NMR data.

We studied the interaction of **5** with a cytidylyl(3′ → 5′)-guanosine of C_pG to prove further its catenated structure. When C_pG was added, **5** underwent further hypochromicity as large as 63% in total. No absorption change was observed when C_pG was added to monomer **6**. Since this large hypochromicity is similar to that seen for intercalation into double strand DNA and since C_pG can form a mini double strand, a catenated structure was suggested for this complex as shown in Figure 5.

The complex of macrocyclic bis-intercalator **5** with calf thymus DNA undergoes very slow dissociation with a rate constant of 0.0007 s^{-1} [18]. This value is 10^3-times smaller than that of **6** (0.7 s^{-1}). This result supports the catenated structure for the complex of **5** with double strand DNA.

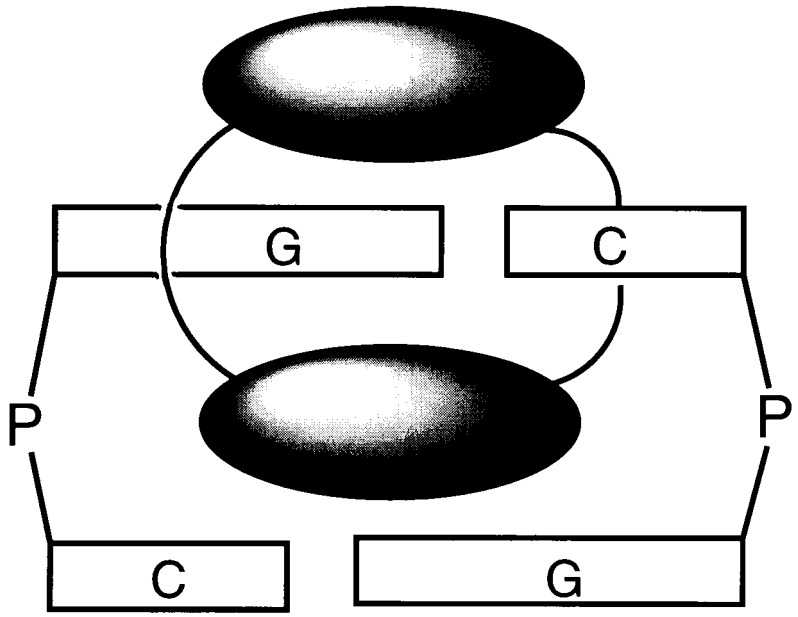

Figure 5. The proposed model for the complex of **5** with a C_pG mini helix.

4. Summary

The double stranded DNA is moving dynamically with several motions such as one base pair or longer base pair disruption (breathing). The tris-intercalator and macrocyclic bis-threading intercalator described here can recall the dynamics of the DNA double strand. These poly-intercalators are quite novel and have huge possibility of poly-intercalator chemistry.

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