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Macrocyclic metal complexes for selective recognition of nucleic acid bases and manipulation of gene expression

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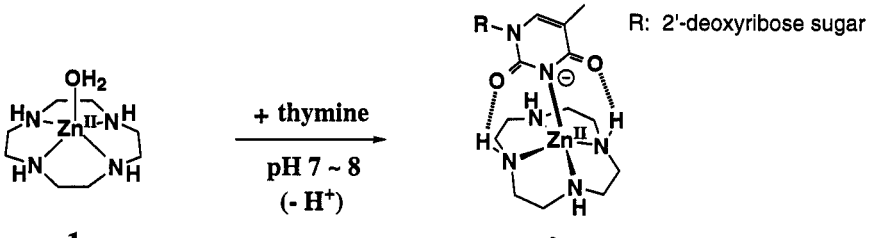
Abstract: Interaction of Zn^{II}-cyclen complexes **1**, **3**, **6**, and **7** with uracil and thymine bases in double-stranded poly(A)·poly(U) and poly(dA)·poly(dT) has been investigated. These zinc(II) complexes lowered the melting temperatures (T_m) of poly(A)·poly(U) and poly(dA)·poly(dT) in 5 mM Tris-HCl buffer (pH 7.6) containing 10 mM NaCl as their concentrations increased, indicating that they destabilized the duplex structure of polynucleotides. The comparison of circular dichroism (CD) spectra of poly(A)·poly(U), poly(A), and poly(U) in the presence of zinc(II) complex **3** led us to conclude that the spectral changes of poly(A)·poly(U) were due to a structural change from double to single-strand, caused by zinc(II) complex **3** binding exclusively to uracils in poly(U). The destabilization effect of zinc(II) complexes was not observed with poly(dG)·poly(dC) in thermal denaturation experiments (50 % formamide aqueous solution, 2.5 mM Tris-HCl buffer (pH 7.6) and 5 mM NaCl). However, the acridine-pendant cyclen complex **3**, which associates with guanine at N_7 and O_6, and through π-π stacking, interacted with poly(dG) in the double helix and greatly *stabilized* the poly(dG)·poly(dC) double-strand, as was indicated by the higher T_m than those with reference intercalating agents. Poly(A)·poly(U) double-strand was most effectively disrupted with a bis(Zn^{II}-cyclen) bridged by para-xylyl group **6** that was designed as a host molecule to bind to two neighboring uracils in a 1:2 complex. Zn^{II}-cyclen complexes thus may become a prototype of small molecules that can affect the biological properties of nucleic acids at the molecular level.

INTRODUCTION

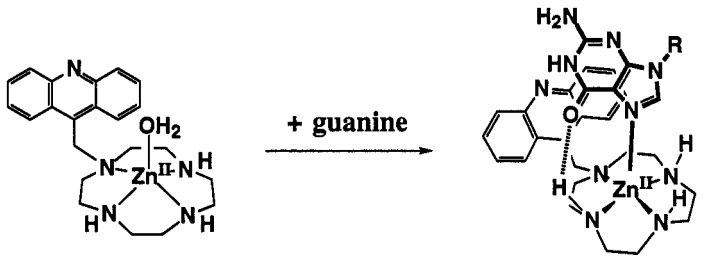
Small molecules that bind to specific sites on DNA or RNA *in aqueous media* are of much interest for synthetic gene-targeted drugs. Compounds capable of converting duplex DNA structures at the regulatory domain of specific genes into unrecognizable forms (e.g. *triple-helix formation, relaxation or distortion, dissociation of double-stranded conformation*) may be applied in novel therapies for the treatment of gene disorders.



1
Zn^{II}-cyclen

2
a ternary complex with thimine

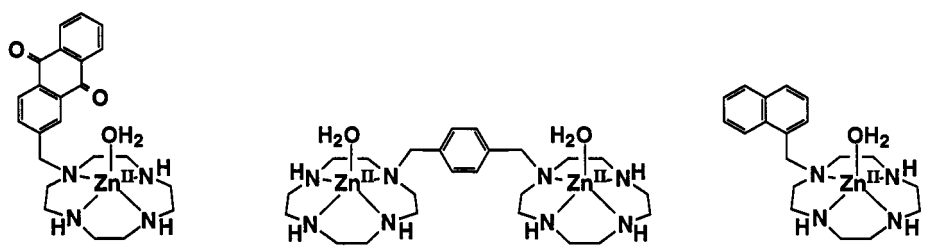
In the course of our continuing research into the intrinsic properties of Zn^{II} in zinc enzymes using macrocyclic polyamine complexes (ref.1), we have discovered that Zn^{II}-cyclen complex **1** selectively binds to thymidine (dT) and uridine (U) and its homologues containing an "imide" functionality in aqueous solution at physiological pH. Other nucleosides (dG, dA, dC) did not exhibit substantial interaction with Zn^{II}-cyclen complex **1** (ref.2). In the resulting ternary complexes such as **2**, **1** binds to dT derivatives through one coordination bond between the deprotonated N(3) and Zn^{II} ion and two hydrogen bonds between the two carbonyl oxygens of imide group and the two NH groups of **1**. We subsequently designed an acridine-pendant Zn^{II}-cyclen **3** to bring about higher efficiency and base selectivity with an enhanced binding through a π-π stacking interaction between the acridine moiety of **3** and thymine base (ref.3). Moreover, **3** showed an appreciably strong association with guanine base through a Zn^{II}–N_7 (guanine) coordination bond and the π–π stacking force (see, **4**). We further synthesized an anthraquinone-pendant cyclen zinc(II) complex **5** which forms stable 1:1 complexes with thymines in a fashion similar to **3**, and has enabled us to detect thymine bases by electrochemical methods (ref.4).



3
Acridine-pendant
Zn^{II}-cyclen

4
a ternary complex
with guanine

It is now of great interest to see whether or not such selective interactions between Zn^{II}-cyclen complexes and monomeric nucleobases can be extended to polynucleotides to affect the double strand structure. Only a few metal complexes (e.g., *cis*-$Pt(NH_3)_2Cl_2$) have shown selective interaction with nucleobases in DNA/RNA (ref.5). Recently, we synthesized a bis(Zn^{II}-cyclen) complex **6** as a novel barbiturate receptor in aqueous solution (ref.6), which was also designed so as to bind to two thymine bases and subsequently cause even stronger perturbation of poly(dA)·poly(dT) double-stranded structure. A naphthalene-pendant cyclen zinc(II) complex **7** was newly synthesized as a reference compound.



5
Anthraquinone-pendant
Zn^{II}-cyclen

6
Bis(Zn^{II}-cyclen)

7
Naphthalene-pendant
Zn^{II}-cyclen

RESULTS AND DISCUSSION

Binding of Zn^{II}-cyclen complexes 1 and 3 with single-stranded polynucleotides

To confirm the interaction between **1** and the uracil bases in poly(U), a UV titration of poly(U) with **1** in buffer solution at 25 °C and pH 7.6 was carried out. Figure 1a shows the effect of increasing concentration of **1** on the UV spectrum of poly(U) (100 μM in terms of phosphate). The λ_{max} and isosbestic point (240 nm) match the results for monomeric uridine (100 μM) (Fig.1b), in both position of absorption maximum and to the degree of absorption lowering. It is evident that the interaction of Zn^{II}-cyclen with uracil in poly(U) occurs through the deprotonation of uracil N(3) without interference by the anionic phosphate bridges of poly(U). As anticipated, from our earlier knowledge of poor affinity of Zn^{II}-cyclen **1** to dA, dC, and dG (ref.2), almost no UV absorption change of poly(A), poly(C), and poly(G) upon addition of Zn^{II}-cyclen was observed. Thus, the equilibrium as shown in Scheme 1 was assumed to be correct. The mechanism for the denaturation of poly(A)·poly(U) and poly(dA)·poly(dT) by the zinc(II) complexes is probably by disruption of A-U and A-T base pair hydrogen bonds through the formation of the ternary complex **8**.

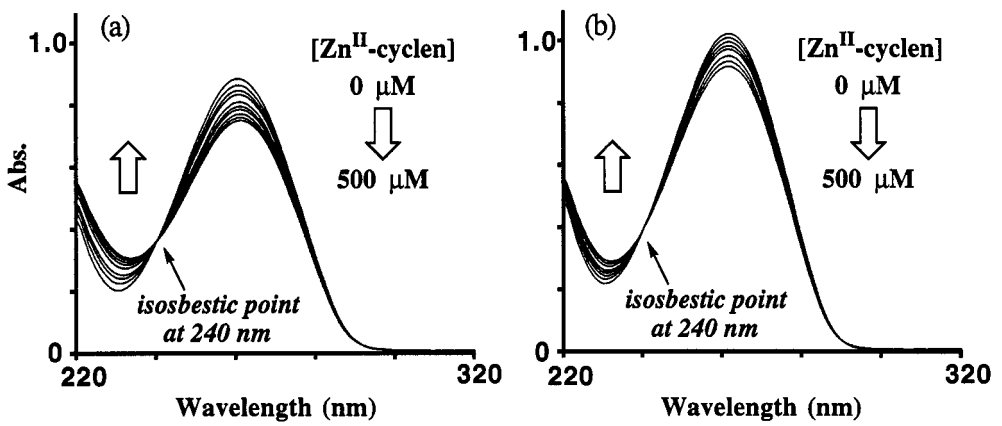
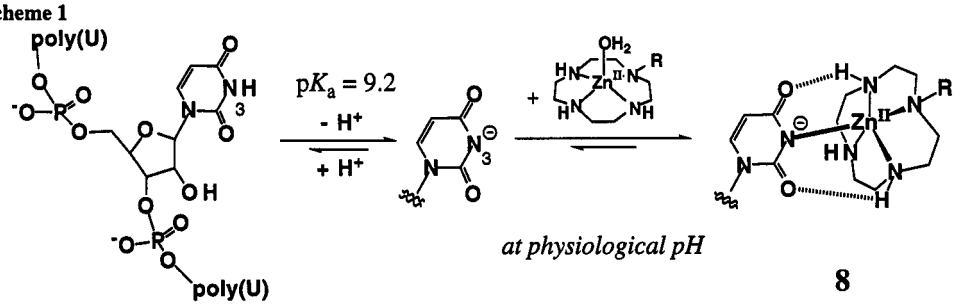


Fig.1 UV absorption spectra of (a) 100 μM(P) poly(U), and (b) 100 μM uridine titrated with Zn^{II}-cyclen **1** (0 - 500 μM) at 25 °C and pH 7.6 (5 mM Tris-HCl buffer, 10 mM NaCl).

Scheme 1



8

An attempt was made to confirm the interaction between **3** and **7**, and poly(U) independently by measuring UV spectral changes of the nucleosides around 270 nm as done with **1**. However, the UV absorption band of each guest was concealed behind those for the acridine and the naphthalene in **3** and **5**, respectively. It is most likely from the pH titration studies that all these zinc(II) complexes bind to N(3)-deprotonated uracils in poly(U) in the same manner with the Zn^{II}–N^- coordination interaction.

Interactions of Zn^{II}-cyclen complexes 1, 3, 6, and 7 with double-stranded poly(A)·poly(U) and poly(dA)·poly(dT)

It is interesting to see whether the selective binding of Zn^{II}-cyclen to poly(thymine) or poly(uracil) leads to a perturbation of double-stranded poly(A)·poly(U) and poly(dA)·poly(dT) structure.

When the double-stranded DNA/RNA polymers are slowly heated, the double helix "melts". The helix to random coil transition occurs over a range of a few degrees, resulting in an increase in absorbance. The midpoint temperature of this process corresponds to the melting temperature (T_m) of double helical DNA/RNA polymers. It is well established that cations (*e.g.* Na^+, Mg^{2+}, Ca^{2+}), outside-binding compounds (*e.g.* spermine, distamycin, netropsin) and intercalating agents (*e.g.* ethidium bromide or acridine derivatives) in general, stabilize double-stranded DNA/RNA to raise the melting temperature (T_m). A lowering of the melting temperature should result if the Zn^{II}-cyclen derivatives bind strongly to thymine or uracil bases in double-stranded DNA/RNA in an analogous way to binding found for the corresponding mononucleosides: the A-T and A-U base pair hydrogen bonding will be disrupted, resulting in weaker bonding between the strands and, consequently, a lower temperature for denaturation.

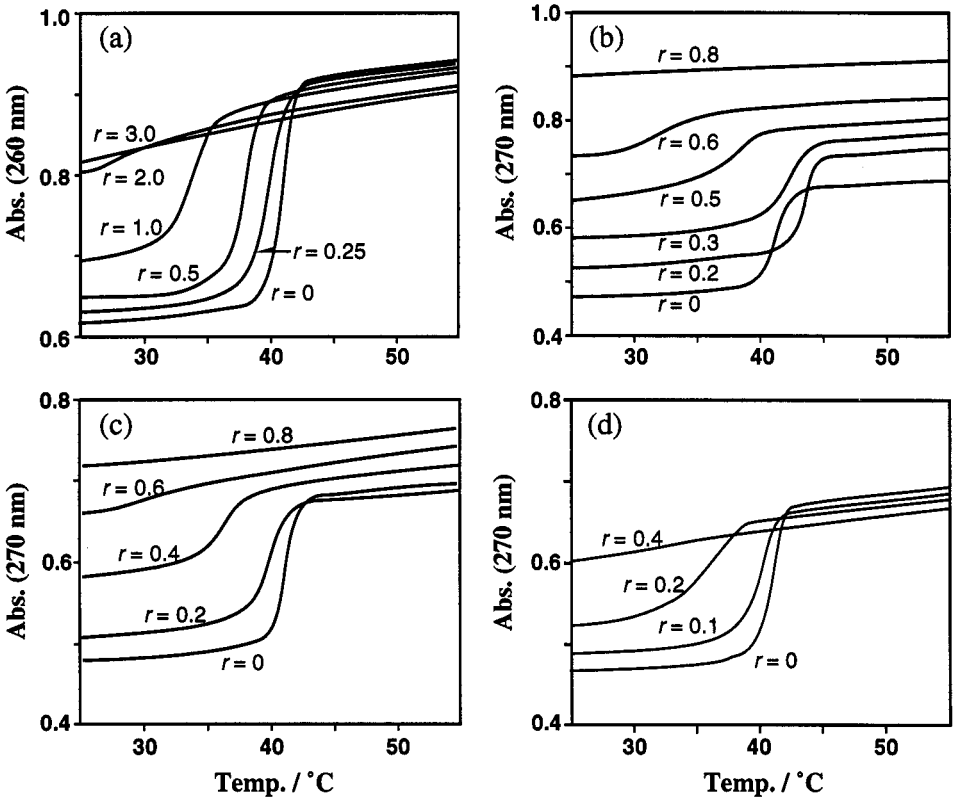


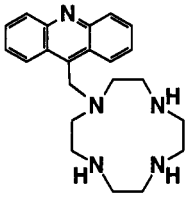
Fig.2 Melting curves for poly(A)·poly(U) in the presence of various concentrations of Zn^{II}-cyclen complexes, **1** (Fig.2a), **3** (Fig.2b), **7** (Fig.2c), and **6** (Fig.2d).

Thermal melting curves for poly(A)·poly(U) (100 μM(P)) in the absence and presence of Zn^{II}-cyclen complexes (**1**, **3**, **6**, and **7**) were obtained by following absorption changes at 260 or 270 nm as a function of temperature in buffer solution (pH 7.6, 5 mM Tris-HCl and 10 mM NaCl) (Fig.2a-d). Interaction of zinc(II) complexes with the duplexes reached equilibration within 15 min. In the absence of Zn^{II}-cyclen complexes (r = 0, where r = [Zn^{II}]/[uracil bases in poly(U) (= 50 μM)]), a single transition was observed at T_m in A_{260} or A_{270}. Upon increasing the concentration of Zn^{II}-cyclen complexes, the break in the absorbance took place at lower temperature with smaller hyperchromicity. This indicates that as more A-U base-pairs are disrupted by Zn^{II}-cyclen complexes, there is less hybridization. As shown in Fig.2,

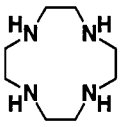
the addition of these zinc(II) complexes lowered the midpoint temperature of the transition. In the case of **1**, there was no break at all in the absorbance plot at $r = 3.0$, implying the complete dissociation of the double strand at 25 °C. The minimum concentrations, at which the double-strands are disrupted, are 150 μM ($r = 3.0$) for **1** (Fig.2a), 40 μM ($r = 0.8$) for **3** (Fig.2b), 30 μM ($r = 0.6$) for **7** (Fig.2c), and 10 μM ($r = 0.4$) for **6** (Fig.2d).

In the absence of **3** (*i.e.* $r = 0$, where r = [Zn^{II}]/[uracil bases in poly(U) (= 50 μM)]) a single transition was observed in A_{270} at $T_m = 41.5$ °C (Figure 2b). At a low concentration of **3** ($r = 0.2$), the double-stranded poly(A)·poly(U) was thermodynamically stabilized ($T_m = 43.5$ °C, $\Delta T_m = + 2.0$ °C). However, upon increasing concentration of **3** the break in absorbance occurred at lower temperatures with smaller hyperchromicity. In the presence of 40 μM of **3** ($r = 0.8$) there was no break at all in the absorbance change, implying that the transition of double to single-strand occurs below 25 °C.

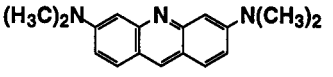
For the reference, the same experiment was carried out using acridine-pendant cyclen ligand **9** possessing both intercalator and polyamine moieties. However, the addition of **9** (0-40 μM) caused only a decrease in T_m ($\Delta T_m = + 8$ °C at $r = 0.8$) for poly(A)·poly(U). Furthermore, other references cyclen **10**, acridine type intercalator **11**, and divalent Cu^{II} complex **12** (little interaction with U and dT was observed in the potentiometric pH-titration) thermodynamically stabilized the double-strand structure of poly(A)·poly(U) ($\Delta T_m = + 5.5$ °C for **10**, + 18.5 °C for **11**, and + 6.5 °C for **12** at $r' = 1.0$ (r' = [compound]/[uracil in poly(U)])). The stabilization effects of these reference compounds may be ascribed to the fact that they interact with double-stranded polyanionic nucleic acid as cations neutralizing the repulsive negative charges on the phosphate backbone of duplex (for **8**, **9**, and **12**) and/or as intercalators (for **9**, and **11**) stabilizing the double-stranded structure.



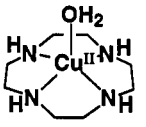
9
Acridine-pendant Cyclen



10
Cyclen



11
Acridine Orange



12
Cu^{II}-cyclen

Accordingly, **6** was the most potent disruptor of the poly(A)·poly(U) double strand. These differences are related not only to their binding constants for uridine but also to affinities for double-stranded polynucleotides (pendant functionality, electrostatic interaction, dimeric effect, etc.). Furthermore, binding experiments where the phosphate sugar backbone of the polynucleotide was changed from ribose to deoxyribose indicated that similar destabilization effects are observed with DNA.

From these results, we concluded that these zinc(II) cyclen complexes bind to thymine and uracil bases, breaking hydrogen-bonds of A-U and A-T base pairs in double-stranded poly(A)·poly(U) and poly(dA)·poly(dT), to destabilize the double-stranded structures.

Circular Dichroism (CD) Studies with Acridine-pendant Zn^{II}-Cyclen 3

Additional evidence for the denaturation of poly(A)·poly(U) duplex by **3** was obtained by the CD spectral changes. The CD spectra of polynucleotides exhibited significant changes upon addition of **3** in the region below 320 nm (Fig.3).

A comparison of (a) to (b) show that the CD spectrum of poly(A)·poly(U) with **3** is larger and shifted to long wavelength (270 nm) than that of poly(A)·poly(U) only. This tendency implies that the transition of poly(A)·poly(U) from double to single-strand occurred when the zinc(II) complex **3** was added (ref.7). Moreover, spectrum (b) is quite similar to spectrum (c), which is a spectrum of poly(U) in

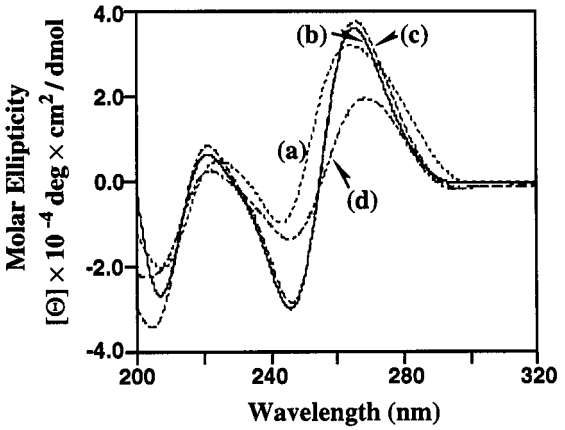


Fig.3 CD spectra of (a) poly(A)·poly(U)(commercially available),
(b) poly(A)·poly(U) in the presence of 40 μM **3**,
(c) Spectrum of poly(U) in the presence of 40 μM **3** combined (*Note a*) with the spectrum of poly(A),
(d) Spectrum of poly(A) in the presence of 40 μM **3** combined (*Note a*) with the spectrum of poly(U),
at 25 °C and pH 7.6 (5 mM Tris-HCl buffer, 10 mM NaCl) with 1.0 cm cell. The concentration of each polymer was kept constant at 50 μM(P).

the presence of 40 μM **3** combined (*Note a*) with the the spectrum of poly(A), although spectrum (d) was quite different from (b). This fact strongly suggests that the transition of poly(A)·poly(U) double-strand to single-strand is promoted by the zinc(II) cyclen complex and that after the transition the zinc(II) complex binds to poly(U) under these conditions.

Dyes (e.g. ethidium bromide, acridine orange) bound to double-stranded DNA induce CD bands in visible region (>300 nm). However, in the above experiments no induced CD was observed.

Effects of Zn^{II}-cyclen complexes (1, 3, 6, and 7) on melting temperature of poly(dG)·poly(dC)

To study base-pair selectivity, the effects of Zn^{II}-cyclen complexes (**1**, **3**, **6**, and **7**) on the T_m value of poly(dG)·poly(dC) were examined. Thermal melting experiments for poly(dG)·poly(dC) were carried out in 50 %(v/v) aqueous formamide solution (pH 7.6, 2.5 mM Tris-HCl buffer, 5 mM NaCl) because the melting temperature of poly(dG)·poly(dC) is too high (> 95 °C) under the same condition for poly(A)·poly(U) and poly(dA)·poly(dT) (ref.8). The concentration of all compounds tested was 50 μM. The results were summarized in Table 1.

TABLE 1. Effects of Additives on Melting Temperature of Poly(dG)·poly(dC)[a]

compounds	T_m	ΔT_m
none	73.5	—
Zn^{II}-cyclen (**1**)	81.5	+ 8.0
naphthalene-pendant Zn^{II}-cyclen (**7**)	80.0	+ 6.5
acridine-pendant Zn^{II}-cyclen (**3**)	> 85.0 [b]	> +11.5 [b]
cyclen (**10**)	76.5	+ 3.0
acridine-pendant cyclen (**9**)	77.0	+ 3.5

[a] The concentrations of poly(dG)·poly(dC), the cyclens, and the related compounds were 100 μM(P), 50 μM, and 50 μM, respectively, where r = 1.0 (r = [compound]/[base-pair]).
[b] No transition was observed in A_{270} (25 - 95 °C).

Note a: mean of spectra

No destabilization effects of zinc(II) complexes on poly(dG)·poly(dC) duplex were observed by thermal denaturation experiments as shown in Table 1. All of the compounds stabilized the double-stranded poly(dG)·poly(dC), presumably due to their interactions with phosphate anions as cations, outside-binding reagents, or intercalators. Among the cyclen complexes tested, the acridine-pendant Zn^{II}-cyclen **3** showed the largest stabilization effect for the duplex poly(dG)·poly(dC), probably through the strong binding of **3** with guanine (through N_7 coordination and the π–π stacking) of poly(dG)·poly(dC).

This result shows that destabilization effects by Zn^{II}-cyclen complexes are selective for A-U and dA-dT base pairs.

Fluorescence studies on interaction of acridine-pendant Zn^{II}-cyclen 3 with double-stranded RNA/DNA

To further investigate interaction of these zinc(II) complexes with double-stranded RNA/DNA, fluorescence studies were carried out using acridine-pendant Zn^{II}-cyclen **3**. The fluorescence spectra of **3** and its ligand **9** in the presence of polynucleotides are shown in Fig.4. Decrease of the fluorescence intensities were observed in the presence of 20 μM(P) poly(A)·poly(U) and poly(dA)·poly(dT) (Fig.4a), which support the earlier conclusion from the T_m results that the duplex poly(A)·poly(U) and poly(dA)·poly(dT) dissociate into single strands as a consequence of **3** binding to N(3)-deprotonated uracil or thymine in poly(U) or poly(dT). The **3**-poly(U) and -poly(dT) interactions decreases the fluorescence intensities (ref.3). However, when **3** interacts with 20 μM(P) poly(dG)·poly(dC), the fluorescence intensities increased due to intercalation and/or hydrophobic interaction of the acridine moiety with GC base pairs in double-stranded poly(dG)·poly(dC).

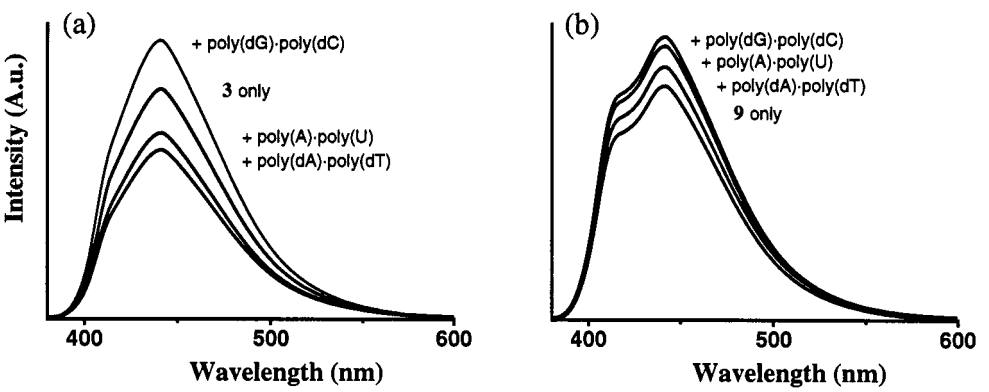
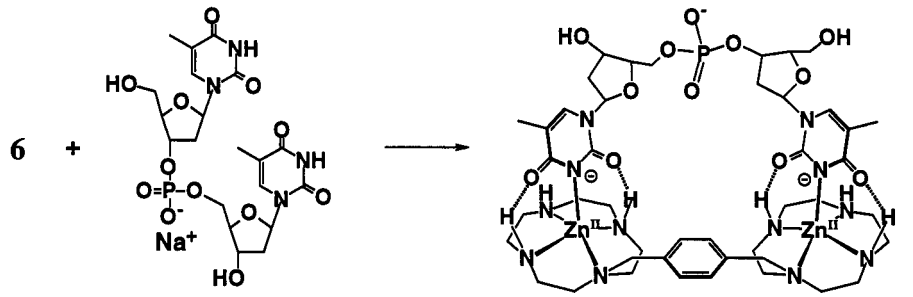


Fig.4 Fluorescence spectra of (a) acridine-pendant Zn^{II}-cyclen **3** (10 μM), and (b) acridine-pendant cyclen **9** (10 μM) in the absence and the presence of polynucleotides (20 μM(P)) in Tris-HCl buffer at 25 °C and pH 7.6 (I = 0.10 ($NaNO_3$)).

An increase of the fluorescence intensities was observed in the presence of poly(dG)·poly(dC), poly(A)·poly(U) and poly(dA)·poly(dT) with diprotonated ligand **9** (Fig.4b). This shows that the fluorescence intensities are increased when intercalators such as acridine interact with double strands.

The 1:1 complexation between 6 and d(TpT)

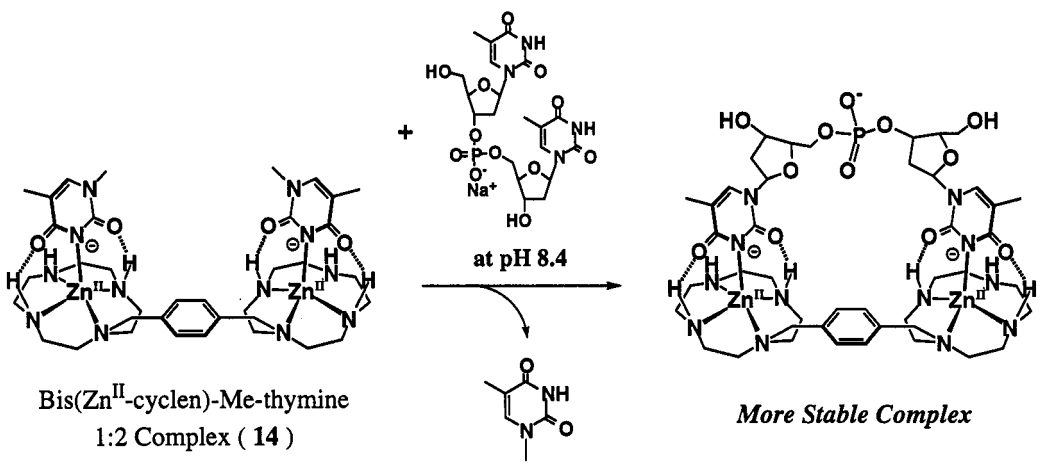
Among these zinc(II) complexes, **6** was most effective for the dissociation of poly(A)·poly(U). Bis(Zn^{II}-cyclen) bridged by para-xylyl group **6** was designed as a host molecule that would bind to two neighboring uracils (or thymines) in a 1:2 fashion. We isolated a 1:1 complex **13** which consists of **6**



d(TpT), Na^+ **13** $\cdot 2ClO_4 \cdot Na \cdot 3H_2O$

and d(TpT), and a 1:2 complex **14** which consists of **6** and 2 equimolar 1-methylthymine. Their compositions were confirmed by ^1H NMR and elemental analysis.

To compare affinities of **6** for monomeric thymine base and d(TpT), a competition experiment between 1-methylthymine and d(TpT) was carried out by ^1H NMR. When the solution of the isolated complex **14** (10 mM) in D_2O (50 mM TAPS buffer (pD 8.4), I = 0.1 ($NaNO_3$), 35 °C) was added to d(TpT) sodium salt (1.0 eq based on **6**), it was observed that most of **6** interacted with d(TpT) to form complex **13**. This change indicates that d(TpT) (bidentate ligand) can displace 1-methylthymine (monodentate) on bis(Zn^{II}-cyclen) **6**, to form the more stable complex **13**. Thus, dimeric zinc(II) complex **6** has a stronger binding ability to poly(dT) and poly(U) than **1**, and is more effective in dissociating double-stranded poly(A)·poly(U) than the monomeric zinc(II) complexes **1**, **3**, and **7**.



Bis(Zn^{II}-cyclen)-Me-thymine
1:2 Complex (**14**)

More Stable Complex

SUMMARY AND CONCLUSION

A study of Zn^{II}-cyclen derivatives binding to thymine and uracil bases in double-stranded poly(A)·poly(U), poly(dA)·poly(dT) by means of thermal denaturation experiments, CD and UV spectral measurements has been made. Comparison of T_m values in the presence of various concentrations of these zinc(II) complexes has disclosed their strong abilities to *destabilize* the double-stranded poly(A)·poly(U) and poly(dA)·poly(dT). The UV and CD studies revealed that they exclusively bind to N(3)-deprotonated uracil (and thymine) bases in the polynucleotides. The destabilization effect by these zinc(II) complexes were competitively hindered by the barbiturate anion which also binds to Zn^{II}-cyclen

derivatives (ref.6). Base-pair selectivity in the polynucleotides was further investigated by thermal denaturation experiments. The zinc(II) complexes showed no destabilization effect on poly(dG)·poly(dC) in 50 % aqueous formamide solution. These results support the proposal that these zinc(II) complexes recognize and bind to neutral thymine and uracil bases even in the presence of phosphate anions and break hydrogen-bonded A-T and A-U base pairs of double-stranded poly(dA)·poly(dT) and poly(A)·poly(U). Such complexes was of use for site selective melting of AT rich regions which are often seen in transcription regulatory domains. The present findings on the interaction between nucleobase receptors and double-stranded polynucleotides will have important implications for nucleic acid chemistry as well as medicinal chemistry.

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