

A Thermodynamic Investigation into the Stabilization of Poly(dA)·[poly(dT)]₂ Triple Helical DNA by Various Divalent Metal Ions

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Received June 16, 2009, Accepted September 29, 2009

Effects of representative group II and transition metal ions on the stability of the poly(dA)·[poly(dT)]₂ triplex were investigated by the van't Hoff plot constructed from a thermal melting curve. The transition, poly(dA)·[poly(dT)]₂ → poly(dA)·poly(dT) + poly(dT), was non-spontaneous with a positive Gibbs free energy, endothermic ($\Delta H^\circ > 0$), and had a favorable entropy change ($\Delta S^\circ > 0$), as seen from the negative slope and positive y-intercept in the van't Hoff plot. Therefore, the transition is driven by entropy change. The Mg²⁺ ion was the most effective at stabilization of the triplex, with the effect decreasing in the order of Mg²⁺ > Ca²⁺ > Sr²⁺ > Ba²⁺. A similar stabilization effect was found for the duplex to single strand transition: poly(dA)·poly(dT) + poly(dT) → poly(dA) + 2poly(dT), with a larger positive free energy. The transition metal ions, namely Ni²⁺, Cu²⁺, and Zn²⁺, did not exhibit any effect on triplex stabilization, while showing little effect on duplex stabilization. The different effects on triplex stabilization between group II metal ions and the transition metal ions may be attributed to their difference in binding to DNA; transition metals are known to coordinate with DNA components, including phosphate groups, while group II metal ions conceivably bind DNA *via* electrostatic interactions. The Cd²⁺ ion was an exception, effectively stabilizing the triplex and melting temperature of the third strand dissociation was higher than that observed in the presence of Mg²⁺, even though it is in the same group with Zn²⁺. The detailed behavior of the Cd²⁺ ion is currently under investigation.

Key Words: Triplex DNA, Divalent ions, Thermodynamics, Enthalpy, Entropy

Introduction

Since the first triple helical nucleic acids were reported in 1957,¹ in which a 2 : 1 ratio of poly(U) and poly(A) was shown to form a stable complex, they have been widely studied for their high selectivity of the third strand, for recognizing a single site in large duplex DNA, and for providing potentials for biological and therapeutic applications that include inhibition of gene expression and design of artificial nucleases.² The discovery of an intramolecular triplex (H-DNA) within a supercoiled plasmid highlighted the biological importance of the triple helical DNA.³⁻⁵ In the case of DNA, poly(dA)·poly(dT) and poly(dG)·poly(dC) can form a triple helix with poly(dT) and poly(dC)⁺, respectively, in the presence of divalent Mg²⁺ ions.¹ In the triple helix, the third poly(dT) and poly(dC)⁺ strand locate in the major groove of the template duplex and form Hoogsteen type hydrogen bonds with the template polypyrimidine strand. In general, the third Hoogsteen base-paired strand is less strongly bound to the duplex than the two Watson-Crick bound strands of the corresponding duplex.

The presence of divalent ions, particularly Mg²⁺, is crucial in the structure and biological activity of biomolecules, including nucleic acids and various proteins. Especially in the stabilization of the triplex nucleic acids, the presence of the Mg²⁺ ion is essential. The role of the Mg²⁺ ion in the stabilization of the triplex RNA has been investigated.^{4,5} It was proposed that RNA stabilization arises from two energetically distinct modes of Mg²⁺ binding, namely diffuse- and site-binding.⁵ The Mg²⁺ ions are attracted electrostatically to the strong anionic field surrounding the RNA in a diffusive

binding mode, while they bind at the specifically arranged electronegative ligands. However, the role of the Mg²⁺ ions in the stabilization of the nucleic acids has not been fully elucidated. The divalent metal ions belonging to the *d*-block, such as Co²⁺, Ni²⁺, and Zn²⁺, exhibited contrasting interactions with DNA.⁶ For instance, the Zn²⁺ ion is believed to coordinate with negatively charged phosphate groups at low or intermediate concentrations while inducing aggregation of DNA or formation of M-DNA at a high concentration.⁷

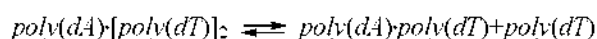
In order to understand the role of divalent ions, including Mg²⁺, in the stabilization of triplex DNA, the thermodynamic aspects of the concentration-dependent thermal melting profile of poly(dA)·[poly(dT)]₂ triplex DNA, in the presence of various alkaline-earth metals (Mg²⁺, Ca²⁺, Sr²⁺, Ba²⁺) and transition metal ions (Ni²⁺, Cu²⁺, Zn²⁺, Cd²⁺) were investigated.

Experiment

Formation and dissociation of the poly(dA)·[poly(dT)]₂ triplex. Poly(dA) and poly(dT) were purchased from Sigma and dissolved in a 5.0 mM cacodylate buffer solution, pH 7.0, containing 10 mM NaCl. The triplex poly(dA)·[poly(dT)]₂ was obtained by heating a 2 : 1 molar ratio of poly(dT) and poly(dA) in the presence of the desired concentration of the metal ion, followed by overnight annealing at room temperature. The concentrations of polynucleotide were measured spectrophotometrically using the extinction coefficients of $\epsilon_{257nm} = 8600 \text{ M}^{-1}\text{cm}^{-1}$ and $\epsilon_{264nm} = 8520 \text{ M}^{-1}\text{cm}^{-1}$ for poly(dA) and poly(dT), respectively. The thermal melting profile was

recorded by following the absorbance at 260 nm, with increasing temperature on a Cary 100 UV-visible spectrophotometer. The temperature was increased by an increment of 0.2 °C and was equilibrated for a minute at each temperature. The metal ions for stabilizing the duplex and triplex were purchased from Sigma as bis-chloride forms and were dissolved in the buffer solution.

Thermal melting profile and thermodynamic analysis. The thermal melting profile for the triplex shows two, well resolved transitions, the one at low temperature, corresponding to the dissociation of the Hoogsteen base-paired third poly(dT) strand, and that at higher temperatures, reflecting the dissociation of the template double strand. The first transition that occurs at the lower temperature can be denoted by:



$$\alpha = \frac{A_{260\text{nm}}(\text{duplex} + \text{single stranded}) - A_{260\text{nm}}(T)}{A_{260\text{nm}}(\text{duplex} + \text{single stranded}) - A_{260\text{nm}}(\text{triplex})}$$

where $A_{260\text{nm}}(T)$ is the absorbance at a given temperature, α is the fraction of the third strand. The concentration of the duplex and single strand are the same, being $(1-\alpha)\frac{C_t}{2}$, and that of the triplex is $\frac{\alpha C_t}{2}$, where C_t is the total concentration of poly(dA)·poly(dT) and poly(dT) available to form triplex poly(dA)·[poly(dT)]₂. Thus, the observed equilibrium constant is given by:⁸

$$K_{eq} = \frac{[D][S]}{[T]} = \frac{(1-\alpha)^2 C_t}{2\alpha} \quad (1)$$

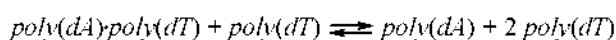
The temperature-dependent equilibrium constant is related to the thermodynamic parameters, ΔH° and ΔS° , through the well-known van't Hoff equation:

$$\ln K_{eq} = -\frac{\Delta H^\circ}{R} \left(\frac{1}{T} \right) + \frac{\Delta S^\circ}{R} \quad (2)$$

where R is the gas constant (1.987 cal·mol⁻¹·K⁻¹) and T is the absolute temperature. The Gibb's free energy for the transition can easily be calculated from the equation:

$$\Delta G^\circ = -RT(\ln K_{eq}) = \Delta H^\circ - T\Delta S^\circ \quad (3)$$

The second transition that occurs at higher temperature corresponds to the transition from the double stranded poly(dA)·poly(dT) and poly(dT) to the three single-stranded polynucleotides:



In this case, the equilibrium constant is given by:⁸

$$K_{eq} = \frac{2(1-\alpha)^2 C_t}{3\alpha} \quad (4)$$

Results and Discussion

Melting profile and thermodynamic parameters in the presence of Mg²⁺ ions. The temperature-dependent absorbance change of the poly(dA)·[poly(dT)]₂ triplex at 260 nm in the presence of various concentrations of Mg²⁺ ion is depicted in Fig. 1(a). As the temperature increases, the dissociation of the triplex poly(dA)·[poly(dT)]₂ exhibited two transitions, except for at the lowest Mg²⁺ ion concentration (40 μM). Therefore, it is clear that the Mg²⁺ ion concentration required to stabilize the 30 μM nucleobase concentration (or 10 μM base triplet) of the poly(dA)·[poly(dT)]₂ triplex is above 40 μM. When the Mg²⁺ ion concentration reached 80 μM, the melting profile started to exhibit two transitions. The melting temperatures of both transitions that corresponded to the transition from triplex to duplex, plus one single strand (T_{m1}), and that from duplex to two single-stranded DNAs (T_{m2}), increased as the Mg²⁺ ion concentration increased. Increase in the melting temperature in T_{m1} is more effective than T_{m2} . The melting temperature can be seen more clearly when the change in absorbance per change in the temperature, a differential of absorbance relative to the temperature, is plotted with respect to the temperature (Fig. 1b). As can be seen clearly, T_{m1} increases as the Mg²⁺ ion concentration increases, being 37.9 °C at 80 μM Mg²⁺ and 53.2 °C at 200 μM, while the values of T_{m2} were observed at 65.3 and 69.8 °C at the same Mg²⁺ concentrations (Table 1). From the slope and y-intercept of the van't Hoff plot (Fig. 2),

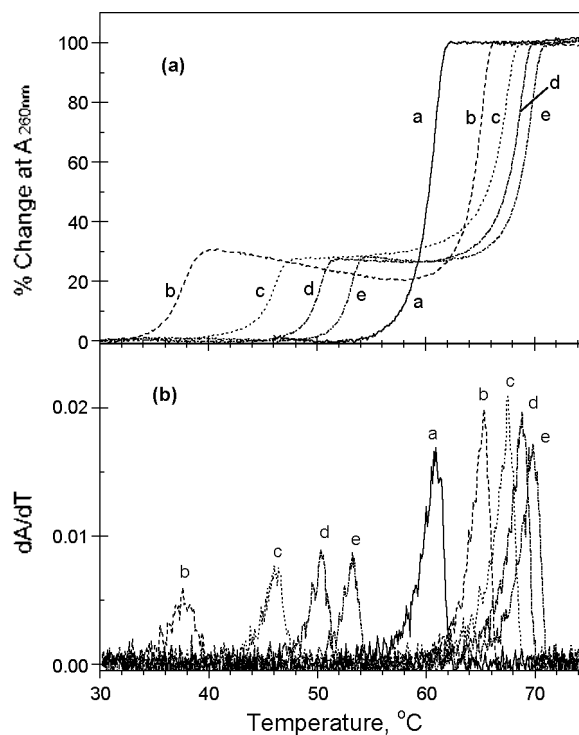


Figure 1. Panel a: Thermal melting curve of the poly(dA)·[poly(dT)]₂ triplex in the presence of various Mg²⁺ ion concentrations. From curve a to curve e, the Mg²⁺ concentration was 40, 80, 120, 160, and 200 μM. Panel b: Differential form of the thermal melting curve of the same data. The dA/dT values were plotted with respect to temperature.

the entropy and enthalpy change for the dissociation of the triplex and duplex can be calculated. The values are summarized in Table 1. However, it has to be noted that the thermodynamic values calculated here from the observed absorbance by the conventional spectrophotometer may not be sufficiently accurate (may contain some degree of error) because the temperature range adopted in this work is very narrow (typically 2~3 degrees), and the change in enthalpy was assumed to be constant at the given temperature range. The latter limitation can be seen particularly in the dissociation of the duplex, as the van't Hoff plot deviated somewhat from a straight line. Nevertheless, thermodynamic parameters obtained from these measurements may provide some clues for the metal ion-DNA interaction. As seen in Fig. 2, the slope of the van't Hoff plot for both transitions is negative and the y-intercept are positive.

Table 1. Thermodynamic parameters for the transition: poly(dA)·[poly(dT)]₂ ⇌ poly(dA)·poly(dT) + poly(dT) ⇌ poly(dA) + 2poly(dT), in the presence of various concentrations of Mg²⁺ ions. ΔG was calculated from average enthalpy and entropy change.

T ⇌ D + S				
[Mg ²⁺] μM	T _{m1} °C	ΔH Kcal·mol ⁻¹	ΔS cal·mol ⁻¹ ·deg ⁻¹	ΔG (25 °C) Kcal·mol ⁻¹
40	-	-	-	-
80	37.9	82.5 ± 1.1	259.7 ± 3.6	5.1
120	46.2	102.2 ± 2.2	314.5 ± 6.9	8.4
160	50.3	132.7 ± 3.5	404.6 ± 10.9	12.1
200	53.2	148.7 ± 1.1	449.9 ± 3.6	14.6
D + S ⇌ 3S				
[Mg ²⁺] μM	T _{m2} °C	ΔH Kcal·mol ⁻¹	ΔS cal·mol ⁻¹ ·deg ⁻¹	ΔG (25 °C) Kcal·mol ⁻¹
40	60.8	110.1 ± 3.0	324.1 ± 9.1	13.5
80	65.3	114.7 ± 5.7	334.2 ± 16.6	15.1
120	67.5	87.1 ± 4.4	250.6 ± 13.0	13.0
160	68.8	86.8 ± 4.6	248.6 ± 13.4	12.7
200	69.8	94.2 ± 4.4	269.3 ± 13.0	13.9

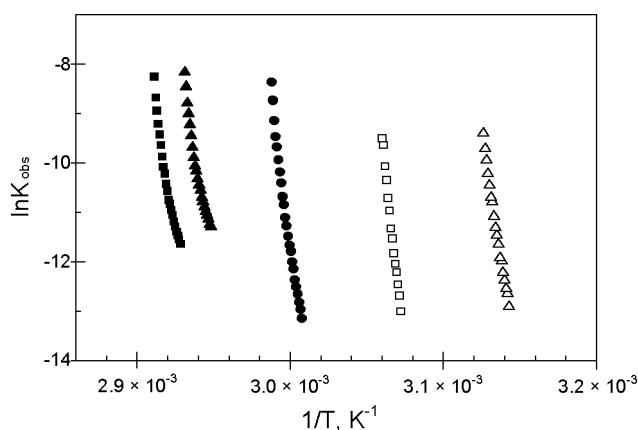


Figure 2. Chosen examples of the van't Hoff plot for the triplex to duplex transition (open symbols) and duplex to single strand transition (closed symbols) in the presence of 40 μM (circles), 120 μM (triangles), and 200 μM (squares) Mg²⁺.

suggesting that both transitions are endothermic and entropically favorable. The thermodynamic parameters obtained from the slope and y-intercept are summarized in Table 1. In the case of the triplex to duplex plus single-stranded transition, the unfavorable enthalpy showed the tendency to increase, being 82.5 Kcal·mol⁻¹ in the presence of 80 μM Mg²⁺ and 148.7 Kcal·mol⁻¹ at 200 μM, suggesting that in the presence of higher concentrations of the Mg²⁺ ions, the Hoogsteen paired third poly(dT) strand is more stable. In the range of the concentration investigated in this work, no saturation of Mg²⁺ ions was found, implying that not all the added Mg²⁺ participates the third strand stabilization. The entropy change increases with increasing Mg²⁺ ion concentration. Considering that increases in entropy change reflect the degree of disorder of the system, the triplex at a low Mg²⁺ ion concentration may be more flexible because the degree of disorder of the product, duplex plus single stranded poly(dT), is conceivably similar.

In the transition from duplex poly(dA)·poly(dT) plus poly(dT) to three single-stranded DNAs, as in the one poly(dA) and 2 poly(dT)s case, the values of the enthalpy and entropy and their dependency on Mg²⁺ concentration is clearly different from those observed for the triplex-duplex transition. The enthalpy change for the duplex to single-stranded DNA is similar or larger compared to that of the triplex to duplex transition at low Mg²⁺ concentrations (110.1 Kcal·mol⁻¹ in 40 μM Mg²⁺) while it is significantly lower at the Mg²⁺ concentration of 120 μM or above (Table 1, 94.2 Kcal·mol⁻¹ in 200 μM Mg²⁺). Although it is unclear, more Mg²⁺ ions are associated with the phosphate groups of the template duplex at a high Mg²⁺ ion concentration, which may affect the stability of the duplex. Entropy changes exhibited similar behavior, being favorable at low Mg²⁺ concentrations, when 40 or 80 μM of Mg²⁺ ions were present, and were higher compared to that in the presence of Mg²⁺ ion concentration higher than 120 μM. The Gibb's free energy for the transition from poly(dA)·poly(dT) to poly(dA) and poly(dT) are independent of the Mg²⁺ concentration, ~13 Kcal·mol⁻¹ at 25 °C. Therefore, the overall transition is endothermic. In contrast, the transition from triplex to duplex, dissociation of the third strand, exhibited 5.1 Kcal·mol⁻¹ at 25 °C in the presence of 80 μM of Mg²⁺ ion. This value increased upon increasing the Mg²⁺ ion concentration. Therefore, it seems to be clear that the Mg²⁺ ions contributing to the stabilization of the triples is not saturated, even at 200 μM. It is further conclusive that the binding site of the Mg²⁺ ion that stabilizes the triplex is different from that for the duplex.

Effect of other alkali-earth metal ions on triplex stabilization. Effects on the stabilization of triplex poly(dA)·[poly(dT)]₂ and duplex poly(dA)·poly(dT) by other alkali-earth divalent ions are compared to Mg²⁺ ions in Fig. 3, at low (80 μM) and high (160 μM) ion concentrations. Similarly, with Mg²⁺ ions, none of the Ca²⁺, Sr²⁺, or Ba²⁺ ions showed any effect on third strand stabilization at 40 μM. The appearance of T_{m1} in the presence of 80 μM of Ca²⁺ ions is less pronounced compared to that in the presence of the same concentration of Mg²⁺ ions; in Fig. 3(a), the maximum in the dA/dT value is significantly smaller, suggesting that the hypochromicity of the third strand formed by the presence of Ca²⁺ ions is clearly smaller than that in the presence of Mg²⁺ ions. In the case of Sr²⁺ and Ba²⁺, the

T_{m1} was not observed, even at 80 μM , suggesting that the ions in the higher periods are far less effective. The T_{m2} corresponding to the dissociation of the duplex poly(dA)-poly(dT) was similar for Mg^{2+} , Ca^{2+} , and Sr^{2+} ions, being 65.3, 64.8, and 64.3 $^{\circ}\text{C}$, respectively. The hypochromicities are also similar, suggesting that the effects in the stabilization of duplex poly(dA)-poly(dT) by these ions are similar, which is in contrast with the third strand case. The T_{m2} in the presence of Ba^{2+} ions is somewhat lower, being 62.4 $^{\circ}\text{C}$, and the hypochromicity is also lower.

At a concentration of 160 μM (Fig. 3b), all ions from group 2 exhibited two T_m values, ensuring the occurrence of two transitions, triplex to duplex and duplex to single strand DNA. However, the effects were different. As was discussed in the 80 μM case, the effect in stabilization of the duplex (T_{m2}) is similar for Mg^{2+} , Ca^{2+} , and Sr^{2+} ions, being 68.8, 68.3, and 68.3 $^{\circ}\text{C}$, respectively, and slightly higher than those at 80 μM . The hypochromicity also appeared to be similar. The Ba^{2+} ion was the least effective ($T_{m2} = 66.6$ $^{\circ}\text{C}$), with the lowest hypochromicity, as observed at 80 μM . The observed T_{m1} increased with increasing period, the order being: Mg^{2+} (50.3 $^{\circ}\text{C}$) > Ca^{2+} (49.5 $^{\circ}\text{C}$) > Sr^{2+} (42.7 $^{\circ}\text{C}$) > Ba^{2+} (39.9 $^{\circ}\text{C}$). The tendency of decreasing the stabilization effect with increasing period suggests that the size of the ion may play an important role in triplex stabilization. The Mg^{2+} ion may fit in a certain site to interact with the third poly(dT) strand in the triplex. Conversely, the Ba^{2+} ion is possibly too large to fit in the same site. Thermody-

amic parameters in the triplex and duplex equilibrium, as well as that of the duplex and single strand, are in similar ranges in the accuracy of the method adopted in this work, whose limitation was discussed in the previous section. For all ions, the ΔH° values were positive for both the T_{m1} and T_{m2} transitions (endothermic transition), while ΔS° values were also positive (favorable). The measured values of the thermodynamic parameters (data not shown) fall into similar ranges with similar behavior in the Mg^{2+} ion case. For instance, the enthalpy change corresponding to the triplex to duplex transition at various ion concentrations was approximately 80 ~ 100 $\text{Kcal}\cdot\text{mol}^{-1}$ for Ca^{2+} ion, 70 ~ 130 $\text{Kcal}\cdot\text{mol}^{-1}$ for Sr^{2+} ion, and 60 ~ 90 $\text{Kcal}\cdot\text{mol}^{-1}$ for Ba^{2+} ion, with Ba^{2+} being the lowest. This corresponds to the dissociation of the duplex at 80 ~ 100, 90 ~ 140, and 90 ~ 120 $\text{Kcal}\cdot\text{mol}^{-1}$, respectively, for Ca^{2+} , Sr^{2+} , and Ba^{2+} ions. The entropy changes also fall into the similar range. The Gibbs free energy at 25 $^{\circ}\text{C}$ for the triplex to duplex transition was 4.5, 5.3, 9.5, and 9.6 $\text{Kcal}\cdot\text{mol}^{-1}$, respectively, in the presence of 80, 120, 160, and 200 μM of Ca^{2+} ion, while it was 13.7 ± 2.0 $\text{Kcal}\cdot\text{mol}^{-1}$, being independent of the ion concentration for all Ca^{2+} concentrations. Other divalent ions from alkali-earth elements, namely Sr^{2+} and Ba^{2+} , exhibited a similar tendency (data not shown), except for at an ion concentration of 80 μM , at which no triplex stabilization was observed.

Effect of transition metal ions in triplex stabilization. The thermal transition curve in the presence of various divalent transition metal ions, including Ni^{2+} , Zn^{2+} , and Cu^{2+} , are com-

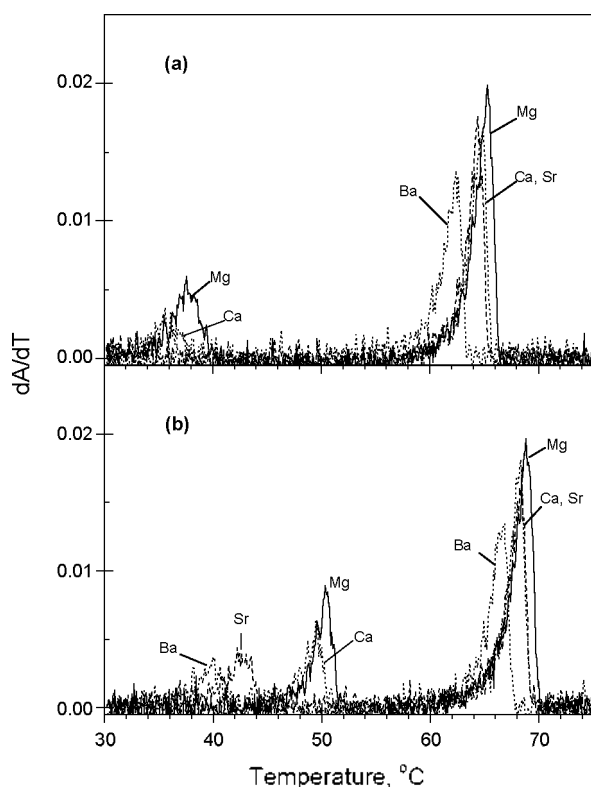


Figure 3. The dA/dT plot of the dissociation of the poly(dA)-[poly(dT)]₂ triplex in the presence of various divalent ions from group II elements. Panel a: 80 μM and panel b: 160 μM .

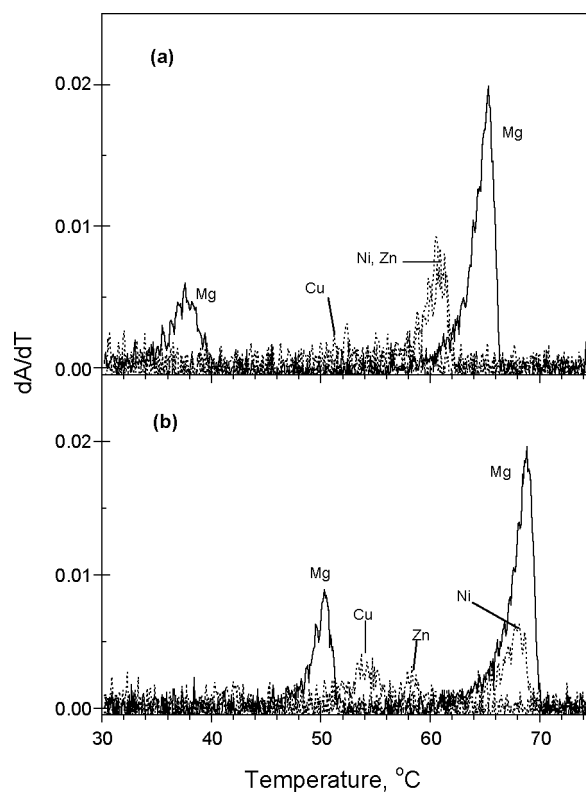


Figure 4. The dA/dT plot of the dissociation of the poly(dA)-[poly(dT)]₂ triplex in the presence of various divalent transition metal ions. Those obtained in the presence of the Mg^{2+} ion (solid curve) are inserted for comparison. Panel a: 80 μM and panel b: 160 μM .

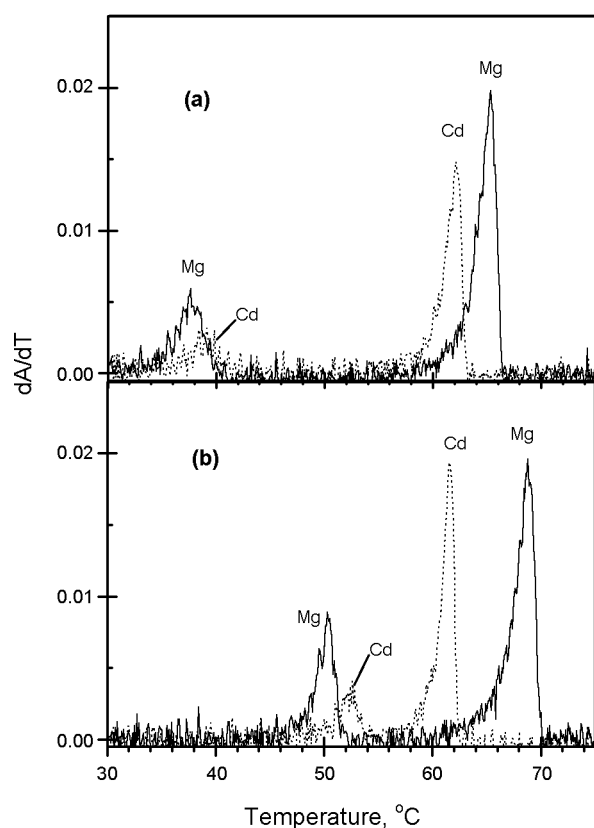


Figure 5. The dA/dT plot of the dissociation of the poly(dA)·[poly(dT)]₂ triplex in the presence of Cd²⁺ and Mg²⁺ ions. Panel a: 80 μM and panel b: 160 μM.

pared with the Mg²⁺ ion in Fig. 4. Surprisingly, even at 80 μM, the Cu²⁺ ion contributes neither triplex nor duplex stabilization, while Ni²⁺ and Zn²⁺ produced one band in the dA/dT versus temperature plot. The single band observed in the plot may be attributed to the dissociation of the duplex, implying none of these ions capable of stabilizing the poly(dA)·[poly(dT)]₂ triplex. At higher concentrations (160 μM), the presence of ions produced one band in the dA/dT versus temperature plot for all ions. The temperature at the maximum was highest for the Ni²⁺ ion and lowest for the Cu²⁺ ion and between T_{m1} and T_{m2} observed for the Mg²⁺ ion. Although the exact description for the observed difference in behavior between the divalent ions from the alkali-earth metal and that from the transition is not clear, the difference in electron configuration of these two groups of ions may play a certain role. The electron configurations of the divalent ions from group II resemble the inert gases, while those of the transition metal possess electrons belonging to the d -orbitals. Thus, it is conceivable that the divalent ions from group II, namely Mg²⁺, Ca²⁺, Sr²⁺, and Ba²⁺, may make contact with certain components of DNA via electrostatic interactions, while those from transition metals, including Ni²⁺, Zn²⁺, and Cu²⁺, coordinate with the DNA components, particularly with the negatively charged phosphate groups.⁷

Although the Cd²⁺ ion belongs also to the transition metal ions, and is in the same group as Zn²⁺, it exhibited exceptional effects in stabilization of both the poly(dA)·[poly(dT)]₂

Table 2. Thermodynamic parameters for the transition: poly(dA)·[poly(dT)]₂ ⇌ poly(dA)·poly(dT) + poly(dT) ⇌ poly(dA) + 2poly(dT), in the presence of various concentrations of Cd²⁺ ions. ΔG was calculated from average enthalpy and entropy change.

T ⇌ D + S				
[Cd ²⁺] μM	T_{m1} °C	ΔH Kcal·mol ⁻¹	ΔS cal·mol ⁻¹ ·deg ⁻¹	ΔG (25 °C) Kcal·mol ⁻¹
40	-	-	-	-
80	39.0	74.3 ± 1.6	232.0 ± 3.8	5.1
120	48.6	108.2 ± 2.2	320.2 ± 5.7	12.7
160	52.4	110.6 ± 1.2	333.7 ± 3.1	11.1
200	54.8	113.4 ± 3.8	339.7 ± 11.2	12.1
D + S ⇌ 3S				
[Cd ²⁺] μM	T_{m2} °C	ΔH Kcal·mol ⁻¹	ΔS cal·mol ⁻¹ ·deg ⁻¹	ΔG (25 °C) Kcal·mol ⁻¹
40	58.0	136.2 ± 4.8	406.1 ± 13.8	15.1
80	62.1	107.9 ± 5.4	316.6 ± 15.8	13.5
120	61.5	106.7 ± 4.7	313.0 ± 13.5	13.4
160	62.1	103.8 ± 4.2	314.6 ± 12.6	10.0
200	61.0	113.0 ± 5.5	333.2 ± 16.1	13.7

triplex and the poly(dA)·poly(dT) duplex. As shown in Fig. 5 and Table 2, T_{m1} was higher than that induced by the Mg²⁺ ion, being 39.0 and 52.4 °C at concentrations of 80 and 160 μM, respectively. However, T_{m2} is lower at all concentrations. The hypochromism for triplex dissociation is lower for Cd²⁺ than for Mg²⁺ at all concentrations, while that for duplex dissociation by Cd²⁺ is lower at low ion concentrations and the same at high ion concentrations compared to that observed for the Mg²⁺ ion. The thermodynamic parameters for the triplex to duplex and duplex to single strand transitions at various Cd²⁺ concentrations are summarized in Table 2. As observed for Mg²⁺, the enthalpy changes appear to be unfavorable (endothermic), while entropy changes were favorable for both transitions. The values were in a similar range with the Mg²⁺ ion. However, some differences in the thermal melting of the triplex were also observed: the strong concentration dependency in enthalpy changes observed for the Mg²⁺ ion was less pronounced for the Cd²⁺ ions. Indeed, the enthalpy change corresponding to the third strand dissociation in the presence of the Cd²⁺ ion is independent of the concentration, except for the lowest concentration (80 μM). The magnitude of the Gibbs free energy was nearly 12 Kcal·mol⁻¹ at 25 °C, 2 ~ 3 times larger compared to Mg²⁺. Proper description for this observation awaits further investigation.

Conclusions

The presence of divalent metal ions originating from group II, namely Mg²⁺, Ca²⁺, Sr²⁺, and Ba²⁺, stabilize the poly(dA)·[poly(dT)]₂ triplex and poly(dA)·poly(dT) duplex. The positive Gibbs free energy for both dissociations from triplex to duplex and duplex to single strand were established, indicating that the dissociations are non-spontaneous and driven by favorable entropy changes. Nevertheless, the divalent trans-

ition metal ions, namely Ni^{2+} , Zn^{2+} , and Cu^{2+} , were unable to stabilize the triplex and were far less effective in duplex stabilization compared to the Mg^{2+} ion. The Cd^{2+} ion, also a transition metal ion with the same electron configuration as Zn^{2+} , was an exception and was more effective in triplex stabilization and less effective in duplex stabilization compared to the Mg^{2+} ion.

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