

Research Article

Probing the Effects on the Thermal Stability of Salmon Sperm DNA in the Presence of Metal Cations by Measuring DNA Melting Temperatures

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Abstract

Thermal melting experiments were performed using salmon sperm DNA and metal cations in buffered solution over a temperature range of 20-90 degrees Celsius. The DNA melting temperature (T_m) was determined for DNA in the presence and absence of several metal cations Bi^{3+} , Cd^{2+} , Co^{2+} , Cu^{2+} , Au^{3+} , Ni^{2+} , Mg^{2+} , Mn^{2+} , Hg^{2+} , Pd^{2+} , Pt^{2+} , and U^{6+} at pH 7.4 by measuring the absorbance of each solution at 260 nm as a function of temperature. Our results demonstrate enhanced thermal stability of salmon sperm DNA in the presence of Au^{3+} , Cu^{2+} , Pt^{2+} , Mn^{2+} , Pd^{2+} , Mg^{2+} , and U^{6+} , at their most effective concentrations, and resulting thermal instability of salmon sperm DNA in the presence of Bi^{3+} , Cd^{2+} , Co^{2+} , Ni^{2+} , Hg^{2+} , and at high concentrations of metal cations in solution. Metal ionic size, concentration, and charge appeared to be important factors in conferring thermal stability on salmon sperm DNA in these studies.

Introduction and Background

When a DNA solution is heated, the double-stranded DNA unwinds, and the hydrogen bonds that hold the two strands together weaken and finally break. The process of breaking a double-stranded DNA into single strands is known as DNA melting. The temperature at which the DNA strands are half denatured, meaning half double-stranded, half single-stranded, is called the Melting Temperature (T_m) [1]. The amount of strand separation, or melting, is measured by the absorbance of the DNA solution at 260 nm. Nucleic acids absorb light at this wavelength because of the electronic structure in their bases, but when two strands of DNA come together, the close proximity of the bases in the two strands quenches some of this absorbance. DNA denatures when it is heated in solution and the double helix dissociates into single strands [2]. When the two strands separate, this quenching disappears and the absorbance rises 30%-40%. This is called Hyperchromicity [2]. The hyperchromicity is the effect of stacked bases in a double helix absorbing more ultraviolet light as the double-stranded DNA unfolds. Practically, the T_m is defined as the temperature in degrees

Celsius at which 50% of all molecules of a given DNA sequence are hybridized into a double strand, and 50% are present as single strands [Figure 1]. The T_m can happen at various temperatures. It has been shown that divalent cations can make the DNA double helix more stable [3-5]. Size, charge, concentration of metal cations has been found to be important in affecting the T_m of DNA [6]. The more stable metal cations have been reported to confer stability on the DNA, reflected in a higher the T_m . The dependence of ionic strength, cation radius and charge, and salt concentration on the melting temperature of DNA has been studied for more than 40 years and continues to be a current research topic [7-10]. The chemistry and biochemistry of nucleic acids found in DNA lend itself to studies of metal cation interactions with DNA. Previous research has provided a model that describes DNA aggregation and denaturation during heating in the presence of divalent metal cations in which the following sequence of events occur:

1. The cations initially interact with the DNA at phosphate and/or base sites, resulting in proton displacement.
2. A combination of metal-base interactions and heating disrupts

the base pairing within the DNA duplex. This allows divalent metals and protons to bind to additional sites on the DNA bases during the aggregation/melting process.

3. Strands whose bases have swung open upon disruption are linked to neighboring strands by metal ion bridges.
4. Near the midpoint of the melting transition, thermal energy breaks up the aggregate.
5. Finally, all cross-links break, resulting in single-stranded DNA complexed with metal ions [1].

These studies characterized and compared the structural and thermodynamic changes that DNA undergoes upon thermal melting in the presence of the divalent alkaline earths Sr^{2+} , Ba^{2+} , Mg^{2+} , and Ca^{2+} , and transition metals Mn^{2+} , Co^{2+} , Ni^{2+} , and Cd^{2+} using a fixed metal ion concentration and calf thymus DNA at pH=6.4. Earlier studies examined the effects of Na^+ and Mg^{2+} ions on the helix-coil transition of DNA [5,11]. Other research has demonstrated, using a limited number of metal ions, the effect of metal ions of varying sizes and concentrations on DNA melting temperature [6]. We wished to probe the relationship of metal ionic size, concentration, and charge on the melting of salmon sperm DNA in the presence of an expanded and diverse set of metal ion cations.

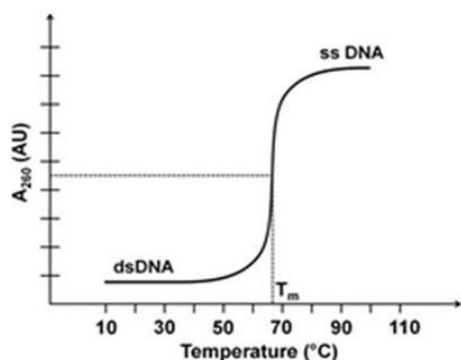


Figure 1: The T_m is the temperature at which half of the DNA unfolds. For the sample indicated above, the $T_m=67.0^\circ\text{C}$.

Materials and Methods

All chemicals were obtained from Sigma-Aldrich Chemical Co. and all metal chloride salts and uranyl acetate ($\text{C}_4\text{H}_6\text{O}_6\text{U}\cdot 3\text{H}_2\text{O}$) were used as received from Fisher Scientific Co. HEPES and cacodylate buffers (pH 7.4) were prepared from 2-[4-(2-Hydroxyethyl)Piperazin-1-Yl] Ethanesulfonic Acid (HEPES) and Sodium Cacodylate Trihydrate. Deoxyribonucleic Acid (DNA), sodium salt from salmon testes, was obtained from Sigma Chemical Co. and prepared by dissolving 5.0 mg/mL in buffer. DNA concentration was determined by UV absorbance at 260 nm. The DNA purchased from Sigma had a %G-C content of 41.2% and had a molecular mass of approximately 1.3×10^6 Da (about 2,000 base

pairs). All buffers were prepared using de-ionized water. To prepare each DNA metal complex, DNA was dissolved to 5% w/w in a solution containing 50-250 mM metal chloride +5 mM sodium cacodylate (pH=7.40) or 5 mM HEPES (pH=7.40) buffer. Phosphate buffers were not used in order to eliminate additional phosphate into the sample solutions and to prevent precipitation of the insoluble metal phosphate complexes. Solutions were maintained at 4°C overnight prior to use.

These solution conditions were used for all experiments described herein. A standard pH meter was used to measure pH with an accuracy of ± 0.01 pH unit at 25°C . The pH meter was calibrated using pH=7.00 and pH=10.00 standard buffers obtained from Aldrich Chemical Co. A thermistor was used to measure the temperature of each sample with an accuracy of $\pm 0.1^\circ\text{C}$. A Hewlett-Packard programmable temperature-controlled Peltier sample cell was used for heating each sample. Matched quartz cuvettes (1.0 cm) were used for each sample. All spectro photometric measurements were made using a Hewlett-Packard Model 8453 diode array UV-visible spectrophotometer. Absorbance at 260 nm and temperature were recorded for each sample from 20.0 - 90.0°C . Full spectra from 220-750 nm were recorded for each sample. Melting temperatures (T_m) were determined as described in the Introduction by determining the temperature at which 50% of the DNA is unfolded from double-stranded to single-stranded DNA using graphs of Absorbance at 260 nm versus Temperature ($^\circ\text{C}$).

Results

Our results are shown in Table 1 and 2 and in Figures 1-5. Table 1 summarizes the results of T_m measurements for all of the metal cations plus DNA melts as well as for DNA melts conducted in the absence of metal cations in this study. The first DNA melting experiments with Pt^{2+} and DNA in HEPES buffer at pH 7.4 showed a biphasic melting curve, with $T_{m1}=58.0^\circ\text{C}$ and $T_{m2}=78.0^\circ\text{C}$ at a Pt^{2+} concentration of 50 mM [Figure 2]. Cacodylate buffer (pH 7.4) was found to be the most optimal buffer to use in our studies, as it did not coordinate to metal ions studied, did not add additional phosphate to the solutions, and it was used in previous studies with DNA and metal ions [1]. For each metal ion studied, a metal ion concentration of 200 mM was used to compare observed the T_m for each metal ion-DNA solution in cacodylate buffer, pH 7.4.

Figure 3 depicts the results, which showed six metal cations (Au^{3+} , Cu^{2+} , Pt^{2+} , Mn^{2+} , Pd^{2+} , Mg^{2+}) enhanced the thermal stability of DNA, as evidenced by $T_m > 72.4^\circ\text{C}$, compared to a T_m of 72.4°C for DNA alone. The other six metal cations (Bi^{3+} , Cd^{2+} , Co^{2+} , Ni^{2+} , Hg^{2+} , U^{6+}) when combined with DNA in cacodylate buffer at pH 7.4, resulted in solutions with $T_m < 72.4^\circ\text{C}$, indicating a destabilized DNA. Concentration dependent melting studies for DNA in the presence of Pt^{2+} and U^{6+} was conducted and can be seen in

Table 1 and Figure 4. The highest T_m (82.7°C) was obtained in the presence of DNA with Pd²⁺ in cacodylate buffer, pH 7.4, while the lowest T_m measured was the Bi³⁺ - DNA solution, T_m = 38.5°C.

Metal Ion	T_m (°C)	Metal Ion, mM	Difference in T_m (°C)
DNA	72.4		
DNA	54		HEPES buffer, pH=7.4
AuDNA	78.5	200	6.1
BiDNA	38.5	200	-33.9
CdDNA	54	200	-20
CoDNA	67.8	200	-4.6
CuDNA	76	200	0.6
HgDNA	51.8	200	-20.6
MgDNA	76.4	200	4
MnDNA	77.2	200	4.8
NiDNA	64.5	200	-7.9
PdDNA	82.7	200	10.3
PtDNA	87.1	50	14.7
PtDNA	85.4	100	13
PtDNA	84	150	11.6
PtDNA	81.2	200	8.8
PtDNA	49.9	250	-22.5
PtDNA	58.0,78.0	50	+24.0HEPES buffer, pH=7.4
UDNA	82.7	50	10.3
UDNA	78.1	100	4.7
UDNA	66.8	150	-5.6
UDNA	46.5 2	200	-25.9
UDNA	42.4	250	-30

Table 1: Data for the melting of salmon sperm DNA in the presence of metal cations^a.

Metal Ion	T_m (°C)	Ionic Radius, pm ^b
DNA	72.4	
DNA	54	HEPES buffer, pH=7.4
AuDNA	78.5	84
BiDNA	38.5	105
CdDNA	54	95
CoDNA	67.8	65
CuDNA	76	73
HgDNA	51.8	119
MgDNA	76.4	86
MnDNA	77.2	73
NiDNA	64.5	69

PdDNA	82.7	87
PtDNA	87.1	80
PtDNA	78	80 HEPES buffer, pH=7.4
UDNA	82.7	87

Table 2: Ionic radii (pm) of metal ions and T_m (°C) at the most effective M^{n+} concentrations for maximizing T_m via melting of salmon sperm DNA in the presence of metal cations^a.

a. Samples contained salmon sperm DNA, 5.0 mM cacodylate buffer pH=7.40. Metal cation solutions were prepared from the corresponding metal salts and were dissolved in 5 mM sodium cacodylate (pH 7.40) buffer.

b. <https://www.webelements.com/>

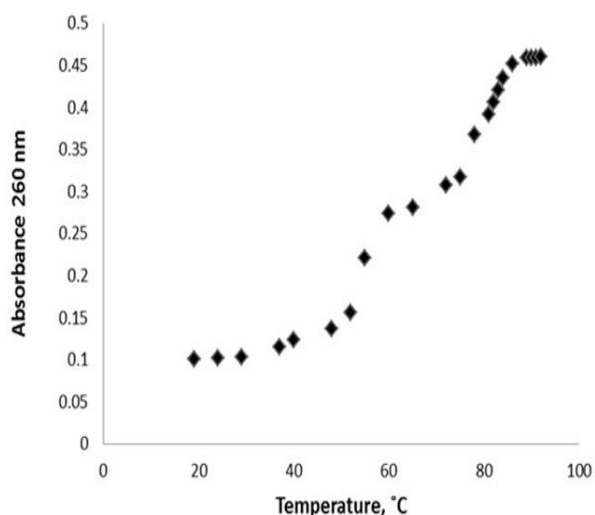


Figure 2: The biphasic melting temperature curve for 50 mM Pt²⁺ and salmon sperm DNA from 20.0-90.0°C in 5 mM HEPES buffer, pH=7.4.

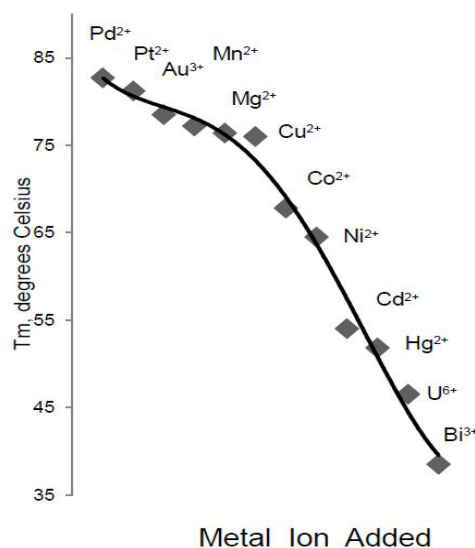


Figure 3: Salmon sperm DNA T_m versus M^{n+} (200 mM) added, 5 mM cacodylate buffer, pH 7.4.

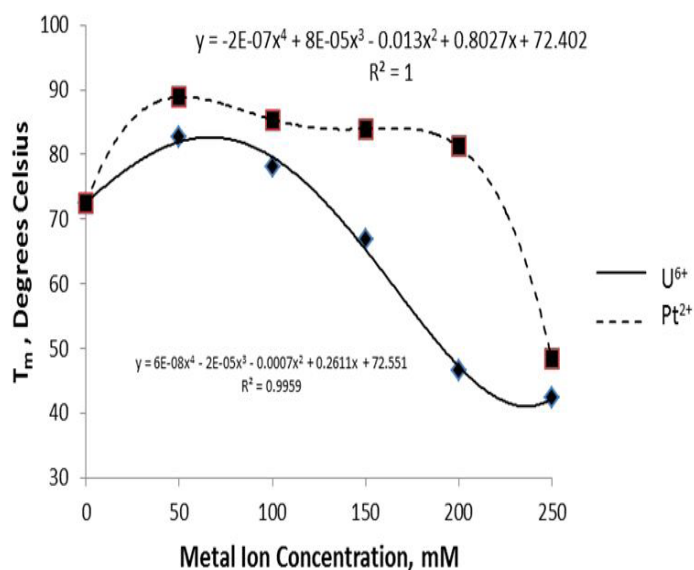


Figure 4: Concentration dependence of DNA T_m for added Pt^{2+} and U^{6+} ; data in Table 1.

In order to examine the relationship between T_m of each metal ion-DNA solution and the metal ionic radius, a graph of T_m versus metal ionic radius at the most effective metal ion concentration is seen in Figure 5, with data in Table 2. Results in Figure 5 and Table 1 show that Pt^{2+} -DNA solutions, at 50 mM Pt^{2+} concentration, exhibit a maximum T_m for all solutions studied. Thermal stability of metal ion-DNA solutions is maximum for metal ionic radii from 70-90 pm [Figure5].

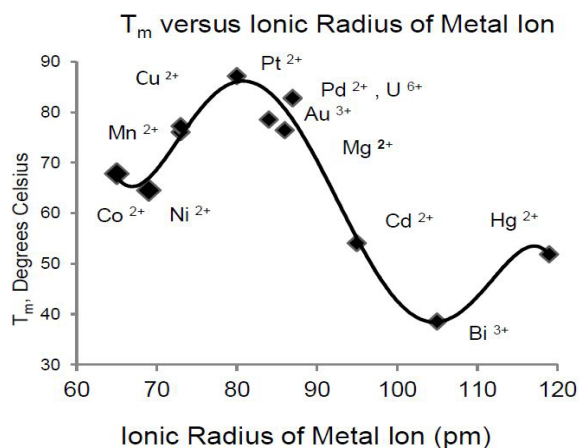


Figure 5: The DNA melting temperature (T_m) as a function of metal ion radius for metal ions added to DNA at the most effective metal ion concentration.

Discussion and Conclusions

The investigation of the thermal melting behavior of each of twelve metal ions with salmon sperm DNA has demonstrated the

following:

1. At low concentrations (50 mM) of Pt^{2+} ion, Pt^{2+} -DNA adducts exhibit biphasic melting curves in HEPES buffer at pH 7.4, with an increase in T_m by 24.0°C when compared to that of DNA alone. Above 100 mM Pt^{2+} and U^{6+} concentrations, the observed T_m for each metal ion-DNA adduct was much lower than that of DNA alone [Figure 4, Table 1].
2. Keeping the concentration of all metal ions constant (200 mM) in constant buffer solution with DNA present, Pd^{2+} -DNA adducts exhibited the highest increase in T_m (+10.3°C), conferring thermal stability on DNA.
3. At the most effective metal ion concentrations in which each metal ion-DNA adduct exhibited the highest T_m , Pt^{2+} -DNA adducts exhibited the highest T_m of 87.1°C, with an increase of 14.7°C in T_m when compared to DNA alone ($T_m = 72.4°C$).
4. At the most effective metal ion concentrations of metal ion-DNA adducts, metal ions with ionic radii from 70-90 pm were most effective at maximizing T_m , thereby stabilizing DNA. Metal ions with ionic radii less than 70 pm and greater than 90 pm, resulted in metal ion-DNA adducts with the lowest observed T_m , indicating destabilization of the metal ion-DNA complex. A discussion and summary of each of these findings is given separately for each statement.

Biphasic Thermal Melting Curve for Pt^{2+} -DNA Adduct

The biphasic thermal melting curve for the Pt^{2+} -DNA adduct depicted in Figure 2 was seen only at low Pt^{2+} concentration (50 mM). Pt^{2+} complexes, particularly square planar complexes such as cis-platin, have been demonstrated to have potent anti-cancer activity. X-ray crystallographic analysis [12,13] of the complex formed between cis-plat in and DNA showed coordination of the Pt^{2+} ion with N7 of guanine bases in the DNA helix. Our results suggest that such a biphasic DNA unfolding process arises from a substantial increase in stability (T_m) of remaining native DNA during denaturation. This increase in stability derives from free energy of metal ion becoming more negative due to the release of high affinity metal ion (Pt^{2+}) by unfolding DNA. This proposed tendency for biphasic denaturation is greatest at low concentrations of Pt^{2+} where greatest increases in stability occur. Biphasic unfolding arising from such Pt^{2+} redistribution results from denaturation of different kinds of DNA molecules, metal ion-poor and metal-ion-rich species, and not from sequential unfolding of domains within the same molecule. A similar proposal was advanced in the description of biphasic denaturation of human albumin due to ligand redistribution during protein unfolding [14]. Only Pt^{2+} -DNA adducts at low Pt^{2+} concentrations exhibited biphasic behavior in these studies.

The T_m of Metal Ion-DNA Adducts at Constant Metal Ion Concentration

Figure 3 shows the decreasing relative thermal stability (decreasing T_m) of metal ion- DNA adducts at constant 200 mM metal ion concentration in DNA solutions (5 mM cacodylate buffer, pH 7.4). This trend in decreasing T_m of metal ion-DNA adducts follows the order of $\text{Pd}^{2+} > \text{Pt}^{2+} > \text{Au}^{3+} > \text{Mn}^{2+} > \text{Mg}^{2+} > \text{Cu}^{2+} > \text{Co}^{2+} > \text{Ni}^{2+} > \text{Cd}^{2+} > \text{Hg}^{2+} > \text{U}^{6+} > \text{Bi}^{3+}$ and follows with the decreasing ability of these metal ions to bind to DNA via the guanine bases [12,13]. Inversely, the trend in increasing T_m of the metal ion-DNA adducts follows the order $\text{Bi}^{3+} < \text{U}^{6+} < \text{Hg}^{2+} < \text{Cd}^{2+} < \text{Ni}^{2+} < \text{Co}^{2+} < \text{Cu}^{2+} < \text{Mg}^{2+} < \text{Mn}^{2+} < \text{Au}^{3+} < \text{Pt}^{2+} < \text{Pd}^{2+}$, and correlates with the decreasing ability of these metal ions to serve as known DNA intercalators/disruptors [12,13,15-17]. It has been shown that Bi^{3+} , which adopts a trigonal planar geometry, when combined with dyes such as planar methylene blue, intercalates with DNA [15]. Our research suggests that metal ions which are more likely to bind to DNA via the guanine bases [12,13] increase the thermal stability and T_m of the metal ion-DNA adducts, while metal ions which are more likely to intercalate with DNA, decrease the stability of the metal ion-DNA adducts and lower the T_m relative to that of DNA alone [15]. In summary, at constant metal ion concentration (200mM), the T_m measured in this series of metal ion-DNA adducts varies directly with the propensity of the metal ion to bind to DNA, and varies inversely with the ability of the metal ion to intercalate with DNA.

The T_m at Most Effective Metal Ion Concentration of Metal Ion-DNA Adducts

The most effective metal ion concentrations in which each metal ion-DNA adduct exhibited the highest T_m varied slightly in the metal ion series studied. Table 1 show that only Pd^{2+} , Pt^{2+} , and U^{6+} conferred maximum thermal stability on DNA at metal ion concentrations less than 200 mM. The size of the metal cation of these ions may be the major contributor to this effect.

Correlation Analysis of Maximum T_m with Metal Ionic Radius

Thermal stability of metal ion-DNA solutions is maximum for metal ionic radii from 70-90 pm [Figure 5], and is minimized for metal ionic radii less than 70 pm and greater than 90 pm. The T_m at the most effective metal ion concentration, that is, the concentration at which the metal ion conferred the maximum stability on the metal ion-DNA adduct (highest T_m) correlated with the metal ionic radius of 70-90 pm. This range of ionic radii is significant as it is in the range of the known metal ions such as square planar Pt^{2+} and Pd^{2+} , square planar Cu^{2+} , square planar Au^{3+} (iso-electronic with Pt^{2+}), and six- coordinate U^{6+} and Mn^{2+} which are of sufficient size, charge and metal-ligand geometry to bind to two

consecutive N7s of Guanine (G) bases in DNA [12,13]. It is proposed that metal ions with ionic radii less than 70 pm and greater than 90 pm, are not of the appropriate size, charge, geometry and coordination number to coordinate, bond, or intercalate to DNA [13]. Bi^{3+} - DNA adducts in our studies displayed the lowest T_m of all complexes studied ($T_m = 38.5^\circ\text{C}$, -33.9°C lower than DNA alone under the same buffer and pH conditions). It may be that both Bi^{3+} and Hg^{2+} intercalate in our DNA studies; further research is in progress with these cations.

Comparison of Salmon Testes DNA Studies with Calf Thymus DNA Studies

The method described here for the use of NaDNA isolated from salmon testes is applicable to tissues extremely rich in this substance, such as thymus, spermatozoa, and prepared nuclei. It is generally agreed that mammalian cells (as, for example, calf thymus...) “are capable of yielding high molecular weight DNA from cells, with very little protein present to decrease purity” [18]. Previous studies examining the binding of a group of eight metal ions to calf thymus DNA showed similar trends in DNA melting behavior, although the studies were conducted at $\text{pH} = 6.4$, and constant metal ion concentration [1]. There was no attempt to systematically probe the effects of metal ionic size, concentration, and charge on conferring thermal stability on DNA in these previous studies as was done in this report.

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