# DIFFERENTIAL SCANNING CALORIMETRY OF DNA – METAL COMPLEXES

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**SUMMARY**

*Differential scanning calorimetry (DSC) has been used to investigate the helix-coil transition of deoxyribonucleic acid - DNA in the presence of chloride salts of $Ca^{2+}$ and $Mg^{2+}$. Thermal denaturation of DNA was observed in a wide range of metal ion concentrations. DNA from chicken erythrocytes was employed as target for metal interaction in aqueous solutions. The dependence of the melting temperature $T_m$ of DNA, the width of the DNA melting curve $\Delta T$, and the enthalpy of the helix-coil transition of DNA on the molar ratio $[Me^{2+}]/[PO_2^-]$ have been determined. The thermal stability of DNA depends not only on the type of ion but also on the value of its concentration and on the nature of solvent. It was shown that the thermal stability of DNA increases at low $[Me^{2+}]/[PO_2^-]$ ratios and the melting temperature passes through the maximum. With the further increase of the metal ions concentration the melting temperature changes very little. At high ion concentrations the melting temperature decreases.*

***Keywords**: DNA macromolecules, metal ions, differential scanning calorimetry*

## 1. INTRODUCTION

Our objective in this paper is to characterize thermodynamic changes that DNA undergoes upon thermal melting in the presence of divalent alkaline earths $Ca^{2+}$ and $Mg^{2+}$. Studies on DNA interaction with metal ions are of great interest owing to the crucial role which metal ions play in functioning of the genetic apparatus *in vivo.* Along with water, metal ions stabilize the structure of nucleic acids, control the equilibrium between different forms of secondary and tertiary structures, possess antitumour activity, and take part in processes of DNA transcription and replication [1]. These effects depend not only on the type of metal, but also on the value of their effective concentration [2]. It is also important to note that the interaction of metal ions with DNA can serve as a simple model for its binding to more complex ligands, including many drugs, mainly antibiotics, which are in ionic form and bind to DNA by cationic mechanism.

DNA – cation metal interactions and their effects on DNA structure have been investigated using a variety of techniques, including sedimentation equilibrium measurements, circular dichroism spectroscopy, UV-visible spectrophotometry, vibrational and NMR spectroscopy. From experiments of UV-visible spectroscopy and circular dichroism it was concluded that alkaline earths interact primarily with DNA phosphates, stabilizing the double helix through reduced charge repulsion of its complementary strands. Raman studies showed that $Ca^{2+}$ and $Mg^{2+}$ interact with the ionic phosphates and show little interaction with bases [3] (see also citations therein). There is some evidence that the interaction of alkaline earth ions with bases is realized indirectly. For example, they enhance base stacking, probably by reducing electrostatic repulsion along the DNA chain [4].

Despite the enormous number of publications devoted to this problem, many questions have still not been fully resolved. In particular, complexes of ions of divalent and polyvalent metals have been insufficiently studied at low ionic strengths and the theoretical interpretation of the results is not clear. In this connection it is of particular interest to study the helix-to-coil transition for which adequate model theories have been suggested [5,6].

In this work we investigate the effect of calcium and magnesium ions on the parameters of the helix-to-coil transition of DNA at low $Na^+$ concentrations. It was studied by differential scanning calorimetry (DSC), enabling us to observe the complex nature of the process and evaluate the temperature and enthalpy of the transition at different concentrations of the metal ions.

## 2. MATERIALS AND METHODS

All solutions were prepared with deionized water. Chloride salts of divalent cations $Ca^{2+}$ and $Mg^{2+}$ were obtained from Ubichem and Lachema, respectively, and used without further purification. The buffer solution used in this study was $0{,}5.10^{-3}$M Tris-HCl (pH=7). DNA from chicken erythrocytes was purchased from Reanal. The molecular weight was $10^7$ Dalton and the protein content less than 5%. The concentration of the DNA stock solution was about $2 mg \cdot ml^{-1}$ ($6{,}5 \cdot 10^{-3}$ mol of phosphoric acid residues); this was controlled by measurements of the absorption spectrum at 260nm taking $\varepsilon$ = 6 500 $M^{-1} \cdot cm^{-1}$ at this wavelength. For the UV-absorption measurements, with the use of the spectrophotometer Shimadzu UV-2401, the DNA concentration was about just one twentieth of the concentration of the DNA stock solution. The complexes of DNA with bivalent metal ions were formed by mixing the DNA stock solution and metal solutions of different

concentrations in the ratio 1:1. So, the values of divalent metal cation to phosphate molar ratios [$Me^{2+}/PO_2^-$] were in the range from 0.05 (0,0625) to 30 (30) for $Ca^{2+}$ ($Mg^{2+}$) cations.

Calorimetric measurements were carried out with differential scanning calorimeter DASM-4. It is known that DSC is accomplished by "scanning up" in the temperature and measuring the difference in the heat generated in a sample and the reference cell. This heat difference is related to the conformational energy in DNA sample. One milliliter of the DNA-metal sample was loaded into its appropriate reservoir. The same solution without DNA was used in the reference cell. Both the sample and reference solutions were scanned from 15 °C to 98 °C, at the rate of 1°C/min. The accuracy volume of each one was 0.455ml. The constant external pressure of 180 kPa was applied to the sample and reference solutions. The integrated enthalpy $\Delta H(T)$, used to estimate the extent of DNA melting, was obtained by integrating the measured heat capacity relative to the reference solution in the absence of DNA ($\Delta C_p$) with respect to the temperature. $\Delta H_{cal}$ was obtained from the total area under $\Delta C_p$ versus $T$ curves. As the transition temperature $T_m$ we took the value of the temperature at the maximum of the melting curve. The width of the transition was defined as the halfwidth of the peak (i.e. the width at the half height). The van't Hoff enthalpy was obtained from the relation

$$\Delta H_{vH} = 4RT_m^2\left[\Delta C_p(T_m)/\Delta H_{cal}\right], \qquad (1)$$

where $R$ is the gas constant and $\Delta C_p(T_m)$ is the measured value of the heat capacity at $T_m$ relative to the reference solution in the absence of DNA. Note that for helix-to-coil transition of DNA, $C_p$(coil) - $C_p$(helix) = 0 [3].

$$\Delta H_{cal} = \frac{P_2}{P_1}\frac{s\Delta T}{vm}\ , \qquad (2)$$

where $P_1$ and $P_2$ are the areas under the calibrating and experimental curves, respectively, $s$ = 25 μW is the power applied to the cells during the calibrating measurements, $m$ is the mass of the measured sample, $\Delta T$ is the temperature interval of the calibrating peak, and $v$ is the scanning rate.

## 3. RESULTS AND DISCUSSION

The results of the heat capacity scans of DNA-metal complexes are summarized in Tables 1 and 2, which show that both of the cation complexes melt at higher temperatures than DNA without metal. This implies that these cations stabilize the DNA structure by reducing the charge repulsion between the phosphates on each of the DNA strands. Figures 1 and 2 show the most characteristic temperature dependences of the heat capacity for DNA complexes with $Mg^{2+}$ and $Ca^{2+}$ ions, respectively.

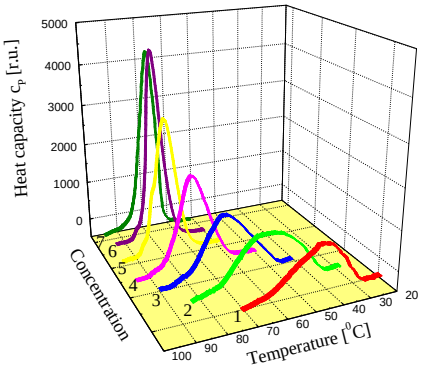


**Fig. 1** The temperature dependence of the heat capacity for DNA complex with $Mg^{2+}$. The molar ratio [$Mg^{2+}/PO_2^-$] for DSC melting curves is: 1-0, 2-0.0625, 3-0.125, 4-0.25, 5-1.25, 6-4.0, 7-30.0.

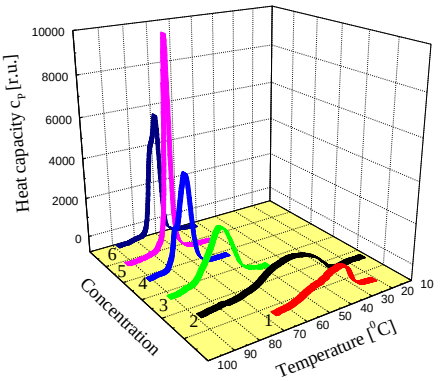


**Fig. 2** The temperature dependence of the heat capacity for DNA complex with $Ca^{2+}$. The molar ratio [$Ca^{2+}/PO_2^-$] for DSC melting curves is: 1-0, 2-0.05, 3-0.25, 4-0.75, 5-2.5, 6-30,0.

As can be seen, at low ion concentrations [$Mg^{2+}/PO_2^-$] = 0.0625 and [$Ca^{2+}/PO_2^-$] = 0.05, the width of the melting curve ($\Delta T$) increases relative to the width characteristic for DNA without divalent ions. At these concentrations we observed a considerable growth in the transition temperature $T_m$. These results have been attributed to the fact that at low metal/DNA phosphate ratio, partial denaturation releases bound cations, which become free to bind at other duplex sites. This results in increased stability of the unmelted regions and creates a broader melting transition as we observe for DNA in the absence of metal [3]. The $\Delta H_{vH}$ values at these concentrations of divalent cations decrease. It is due to elevating of cooperativity of the helix-to-coil transition. We suppose that small initial amounts of ions bounded to DNA phosphates locally strongly change the DNA conformation but

| $Mg^{2+}/P$ | $T_m$ [$^0$C] | $\Delta H_{v.H}$ [kJ.mol$^{-1}$] | $\Delta H_{cal}$ [kJ.mol$^{-1}$] | $\Delta T$ [$^0$C] | $T_S$ [$^0$C] | $T_F$ [$^0$C] |
|---|---|---|---|---|---|---|
| 0,0 | 46,30 | 1,72 | 10,32 | 20,03 | 33,63 | 71,66 |
| 0,0625 | 65,08 | 1,54 | 13,93 | 25,30 | 40,49 | 84,42 |
| 0,125 | 71,95 | 2,18 | 13,06 | 17,88 | 51,22 | 89,18 |
| 0,25 | 78,15 | 3,46 | 12,54 | 11,15 | 63,88 | 91,91 |
| 1,0 | 82,60 | 4,55 | 13,93 | 8,15 | 70,09 | 94,41 |
| 1,5 | 83,13 | 4,90 | 14,13 | 7,90 | 71,24 | 94,14 |
| 1,8 | 83,18 | 5,10 | 14,62 | 8,05 | 72,00 | 96,12 |
| 2,5 | 83,68 | 5,29 | 14,20 | 7,37 | 71,40 | 94,33 |
| 3,0 | 83,30 | 5,79 | 14,82 | 6,65 | 70,84 | 93,29 |
| 4,0 | 83,08 | 6,39 | 14,76 | 5,90 | 72,72 | 93,88 |
| 5,0 | 81,28 | 5,70 | 14,53 | 6,93 | 70,57 | 92,65 |
| 10,0 | 81,30 | 5,70 | 14,54 | 6,88 | 72,33 | 93,88 |
| 20,0 | 81,23 | 5,44 | 14,96 | 7,50 | 71,15 | 93,67 |
| 30,0 | 80,65 | 5,63 | 15,28 | 7,10 | 71,63 | 94,23 |

**Tab. 1** Thermodynamic and melting parameters for the helix-to-coil transition of the DNA complex with $Mg^{2+}$. $T_S$ and $T_F$ are the initial and final temperature of the DNA transition, respectively.

| $Ca^{2+}/P$ | $T_m$ [$^0$C] | $\Delta H_{v.H}$ [kJ.mol$^{-1}$] | $\Delta H_{cal}$ [kJ.mol$^{-1}$] | $\Delta T$ [$^0$C] | $T_S$ [$^0$C] | $T_F$ [$^0$C] |
|---|---|---|---|---|---|---|
| 0,0 | 46,28 | 1,82 | 10,47 | 18,79 | 37,52 | 85,50 |
| 0,05 | 67,00 | 1,42 | 15,02 | 19,54 | 49,06 | 92,03 |
| 0,125 | 75,68 | 1,72 | 14,22 | 11,15 | 61,37 | 91,28 |
| 0,25 | 78,23 | 2,66 | 13,07 | 8,87 | 71,33 | 91,07 |
| 0,375 | 79,53 | 5,77 | 13,57 | 6,94 | 65,84 | 91,25 |
| 0,5 | 79,80 | 5,80 | 14,24 | 6,55 | 65,57 | 90,56 |
| 0,625 | 79,98 | 6,07 | 14,40 | 6,36 | 66,70 | 89,20 |
| 0,667 | 80,65 | 6,46 | 14,54 | 6,11 | 68,51 | 89,61 |
| 0,75 | 81,28 | 6,40 | 14,86 | 6,02 | 66,05 | 88,02 |
| 0,833 | 80,84 | 6,57 | 14,92 | 5,99 | 68,73 | 89,79 |
| 1,0 | 81,10 | 7,26 | 15,05 | 5,48 | 68,52 | 88,10 |
| 1,25 | 80,18 | 7,47 | 15,09 | 4,81 | 67,32 | 87,44 |
| 1,5 | 79,45 | 7,71 | 15,07 | 4,64 | 67,09 | 86,14 |
| 2,0 | 79,33 | 7,82 | 14,90 | 4,44 | 68,27 | 87,08 |
| 2,5 | 77,55 | 14,24 | 14,91 | 2,43 | 66,03 | 84,65 |
| 5,0 | 77,78 | 13,63 | 14,84 | 2,62 | 72,85 | 86,25 |
| 10,0 | 77,15 | 11,83 | 14,87 | 3,05 | 72,33 | 85,90 |
| 15,0 | 76,74 | 8,74 | 15,77 | 4,58 | 70,64 | 86,77 |
| 20,0 | 76,53 | 8,12 | 16,01 | 5,13 | 70,08 | 87,79 |
| 30,0 | 76,00 | 7,80 | 15,94 | 5,20 | 69,23 | 87,89 |

**Tab. 2** Thermodynamic and melting parameters for the helix-to-coil transition of the DNA complex with $Ca^{2+}$. $T_S$ and $T_F$ are the initial and final temperature of the DNA transition, respectively.

still are not able to support the regulary DNA structure and the high cooperativity of DNA transition. When the concentration of cations increases, $0{,}0625 \le [Mg^{2+}/PO_2^-] \le 0{,}25$ and $0{,}05 \le [Ca^{2+}/PO_2^-] \le 0{,}375$, the transition range of the macromolecules narrows. Melting temperature increases and $\Delta T$ decreases. It depends on the fact that the stability of DNA regions enriched with the GC pairs increases more slowly than the stability of the regions enriched with AT pairs. With the further increase of the ion concentration, namely at $[Mg^{2+}/PO_2^-] = 1{,}5$ and $[Ca^{2+}/PO_2^-] = 0{,}6$, we observe satellite peaks enriched with the GC pairs. The values of the temperature of the main and satellite peaks at higher metal concentrations draw closer, which corresponds to narrowing of the general melting range. In these conditions, the potential for metal interaction at available duplex sites is

diminished during partial denaturation and potential for transition broadening is thereby reduced [3]. At the ratios $[Mg^{2+}/PO_2^-] = 4$, $[Ca^{2+}/PO_2^-] = 2.5$ we can see (Tables 1 and 2) the minimum of the melting range. At the regions of the highest divalent cation concentrations in DNA solutions, $5 \leq Me^{2+}/PO_2^-] \leq 30$, the melting parameters $T_m$ and $\Delta T$ change very little. This give evidence on finishing of the processes connected with the change of stability and cooperativity of the DNA-metal complex. From our experiments we can conclude that $Ca^{2+}$ and $Mg^{2+}$ interact primarily with the ionic phosphates and show little interaction with the bases (the region of metal concentrations where $T_m$ decreases). Alkaline earth metal ions do bind to DNA bases, or at least influence them indirectly. For example, they enhance base stacking, probably by reducing electrostatic repulsion along the DNA chain.

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**BIOGRAPHIES**

Jana Tóthová was born on August 6, 1965. She graduated in 1988 at the Faculty of Science, P.J. Šafárik University in Košice, in Physics, specialization Biophysics and Chemical Physics. Since 1988 she is working at the Department of Biophysics of the same faculty. Her scientific interests are in the field of molecular and cellular biophysics.

Valéria Havrilová was born on June 9, 1978. In 1997-2002 she studied Biophysics and Chemical Physics at the Department of Biophysics of the Faculty of Science, P.J. Šafárik University in Košice. She has defended the diploma thesis on the theme "Study of the thermodynamic properties of biopolymers".

Pavol Jasem was born on June 6, 1950. In 1974 he graduated in Physics at the Faculty of Mathematics and Physics, Charles University in Prague. In 1975 he defended the rigorous work at the same faculty and obtained the title RNDr. The PhD. thesis he defended in 1982 at the Physico-Technical Institute of Low Temperature Physics of the USSR Academy of Sciences. That work was focused on the study of low-molecular amines on the structure and properties of DNA. In 1988 he became Associate Professor at the Faculty of Mathematics and Physics, Comenius University in Bratislava. His scientific interests are in the field of molecular biophysics, particularly in the physics of biopolymers.