Thermodynamics of DNA Condensation Caused by Mn^{2+} Binding

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Interaction between Mn^{2+} ion and the two forms of DNA duplex (supercoiled and linearized pUC119 DNA) in solution has been examined by isothermal titration calorimetry. Although DNA condensation reaction heat was observed at 323 K, this was not the case at 298 K. DNA condensation was entropically driven and supercoiled DNA was found to be more susceptive. The enthalpy of DNA condensation is estimated 0.42 kJ/mol for both DNA forms. Conversely, the entropy of DNA condensation was 0.13 kJ/mol K for supercoiled DNA, and 0.12 kJ/mol K for linearized DNA. The difference of entropy is attributable to their DNA conformation.

Key words thermodynamics; DNA condensation; isothermal titration calorimetry; supercoiled DNA; Mn^{2+} binding

The condensation of DNA is an important process for virus packaging, chromosome formation and gene transformation. The DNA is compacted in length by a factor of as much as 10000 when accommodated in a living cell. *In vitro* condensation is of interest due to its biological importance. The condensation is provoked by multivalent cations, such as cobalt hexamine[1] or spermidine,[2] in an aqueous solution. In general, its morphology is toroidal, a doughnut-like shape, but sometimes sphere or rodlike. According to X-ray study, DNA helices form a hexagonal array within the toroidal structure.[3]

DNA condensation occurs when its charge is neutralized by cations of +3 or higher in an aqueous solution, although +2 ions can serve in solution of a lower dielectric constant. This implies that the major contribution to the free energy of condensation is electrostatic. However, Ma and Bloomfield have shown that Mn^{2+} can produce toroidal condensates of supercoiled plasmid DNA, but not of linearized plasmid.[4] In their report, they described as follows: "The mechanism of condensation of supercoiled DNA by Mn^{2+} appears to be quite different from that believed to underlie the more extensively studied condensation induced by higher valence cations." It motivated us to study this work.

Matulis *et al.* have studied the thermodynamics of binding of the trivalent cations cobalt(III) hexamine and spermidine to plasmid DNA *via* isothermal calorimetry.[5] Their results showed that there were two processes attributed to cation binding and consecutive DNA condensation. Both processes, the binding and condensation reactions, were entropically driven and enthalpically opposed. Here we describe the thermodynamics of the condensation caused by Mn^{2+}.

We determined the thermodynamic parameters (Gibbs free energy, enthalpy, and entropy) of two processes, Mn^{2+} binding to DNA and DNA condensation, using isothermal titration calorimetry. The parameter of Mn^{2+} binding to DNA was also determined by equilibrium dialysis study.

Experimental

Sample Preparation *Escherichia coli* strain JM109, with pUC119 plasmid DNA, was kindly provided by associate professor Shunsuke Iwanami (Tomakomai National College of Technology). Bacteria were grown in an Luria-Bertani (LB) medium containing ampicillin at 310 K. Plasmid pUC119 DNA (3162 bp) was isolated using a QIAGEN Plasmid Purification Kit. Supercoiled pUC119 plasmid DNA was also purified using a phenol extraction procedure and ethanol precipitation, before the solution was subsequently dissolved in 10 mM HEPES buffer (pH 7.0).

Linearized pUC119 plasmid DNA was prepared by *Hind* III, with a reaction time of 3 h at 310 K, before the enzyme was then denatured and removed through a phenol extraction procedure. Finally, the linearized DNA duplex was precipitated in ethanol, and then dissolved in 10 mM HEPES buffer (pH 7.0).

$MnCl_2$ was purchased from Wako Pure Chemical Ind., Ltd. 10 mM $MnCl_2$ solution was prepared in 10 mM HEPES buffer (pH 7.0).

Isothermal Titration Calorimetry The titration was carried out using a Microcal MCS calorimeter. Supercoiled or linearized plasmid DNA solution in a 10 mM HEPES buffer (pH 7.0) was placed in the calorimeter cell. The concentration of the DNA (bp) in the cell was 0.1 mM at the beginning of titration. 10 mM $MnCl_2$ solution was injected with a 250 μl injection syringe. The injection volume was 6.744 μl and the injection was repeated 22 times at 10 min intervals. The heat of dilution of the $MnCl_2$ was determined by the use of a 10 mM HEPES buffer with no DNA, and by injecting an equivalent volume of $MnCl_2$ solution for the same times as above.

Equilibrium Dialysis Study Equilibrium dialysis was performed using Dispo-Equilibrium Dialyzer™ (Harvard Bioscience). Supercoiled and linearized plasmid DNA solution and $MnCl_2$ solution were prepared in a 10 mM HEPES buffer (pH 7.0). $MnCl_2$ concentrations were adjusted to 1, 0.8, 0.6, and 0.4 times to DNA concentration. 75 μl of 0.5 mM DNA was put into one cell, and 75 μl $MnCl_2$ solution into the other. Subsequently, these dialyzers were shaken in a water bath shaker (Taitec Personal-11) at 298 K. After 48 h, the $MnCl_2$ solutions were extracted and measured using an atomic absorption photometer (Varian USA SpectrAA-400). Based on these data, the binding ratios were calculated and a Scatchard plot was prepared.

Results

Binding of Mn^{2+} to DNA at 298 K In Figs. 1 and 2, the experimental results of an isothermal titration calorimetry of supercoiled and linearized pUC119 plasmid DNA with Mn^{2+} is shown, respectively. Integrations, with respect to the time of the heat and standard calorimetric data regression analysis, are shown in Fig. 3. This figure shows that the reaction is endothermic and that the cations bind to the linearized DNA in a single stage. Using Microcal Origin software in the standard way, the data were fitted. The fitting parameters used were as follows: the number of Mn^{2+} bound per DNA base pairs (n_1*) was 0.912 ($\pm$0.011), the association constant (K_1) was 1.26×10^5 ($\pm9.2\times10^3$) M^{-1}, and the enthalpy change (ΔH_1) was 2.85 ($\pm$0.042) kJ/mol. Using these values, the free energy and the entropy change were calculated *via* the following equation:

$$\Delta G = -RT\ln K$$

$$\Delta S = (\Delta H - \Delta G)/T$$

Consequently, the free energy (ΔG_1) was -29.1 kJ/mol and the entropy (ΔS_1) was 0.107 kJ/mol K. These indicate

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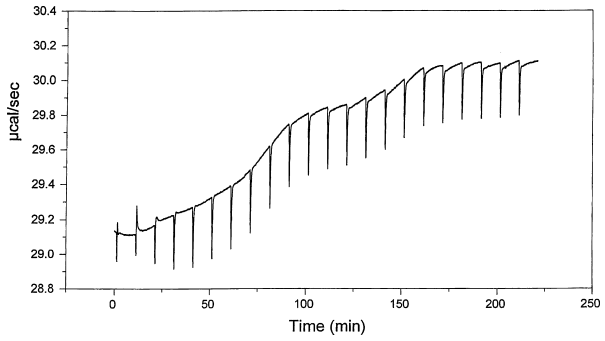

Fig. 1. Raw Data for the Titration of Mn^{2+} into Supercoiled DNA at 298 K

The data was obtained by setting 6.744 μl of a 10 mM $MnCl_2$ to be added every 10 min up to a total of 22 injections to supercoiled DNA in cell at 298 K.

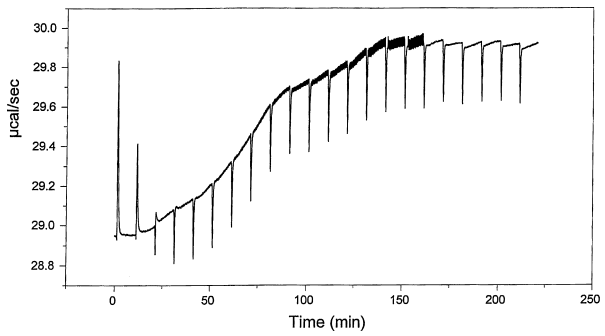

Fig. 2. Raw Data for the Titration of Mn^{2+} into Linearized DNA at 298 K

The measurement condition was the same as that described in the Fig. 1 legend, except the DNA sample.

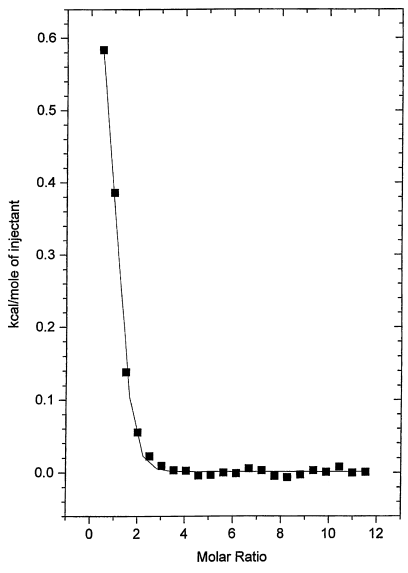

Fig. 3. Integration Data for the Titration of Mn^{2+} into Linearized DNA at 298 K

The plots result from the integration of raw data of Fig. 2 with respect to time and subtracting the dilution heat of Mn^{2+}. The solid line shows a theoretical curve, based on the assumption that $n_1^*=0.912$, $K_1=1.26\times10^5\,\mathrm{M}^{-1}$, and $\Delta H_1=2.85$ kJ.

that the Mn^{2+} binding to DNA is entropically driven.

However, the reaction is less endothermic for supercoiled DNA (see Fig. 1). To confirm whether the effect is due to the decrease of the binding constant of Mn^{2+} to DNA, we performed equilibrium dialysis studies (Fig. 4). The line shown

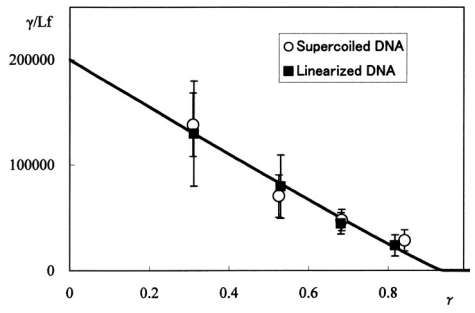

Fig. 4. A Scatchard Plot of Mn^{2+} Bound to Supercoiled and Linearized DNA at 298 K

Plots are the average of 6 independent experiments. The solid line shows a theoretical curve, based on the assumption that $n_1^*=1.1$, and $K_1=2.0\times10^5\,\mathrm{M}^{-1}$.

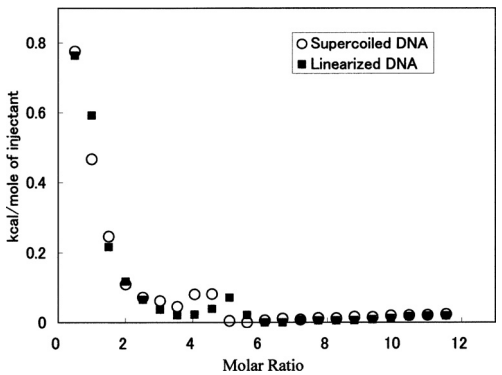

Fig. 5. Calorimetric Titration Curves of $MnCl_2$ Binding to Supercoiled and Linearized DNA at 323 K

The measurement conditions were the same as that described in the Fig. 1 legend except for temperature.

in Fig. 4 is the result of fitting the data to the excluded site model.[6] For both supercoiled and linearized DNA, the same association constant and binding site with Mn^{2+} were determined. The fitting parameters were as follows: the number of Mn^{2+} bound per DNA base pairs (n_1^*) was 1.1 and the association constant (K_1) was $2.0\times10^5\,\mathrm{M}^{-1}$. These values are in positive correlation to the calorimetry data for linearized DNA.

Binding of Mn^{2+} to DNA at 323 K The thermodynamic profile at 323 K differs from that of at 298 K (Fig. 5), which should be due to DNA condensation: it is known that Mn^{2+} causes DNA condensation at a high temperature.[7] The reaction arising from DNA condensation is estimated to be endothermic. Assuming that Mn^{2+} binds DNA with similar affinity, the thermodynamic parameters of DNA condensation can be obtained using a model of two sets of independent binding sites.[5] This assumption is plausible because the first stage of the titration curve of linearized DNA at 323 K is equivalent to that of the same at 298 K (see Table 1). The thermodynamic parameters of DNA condensation were as follows: the stoichiometry (n_2^*) was 4.5, the association constant (K_2) was $1.0\times10^7\,\mathrm{M}^{-1}$, and the enthalpy change (ΔH_2) was 0.42 kJ/mol for supercoiled DNA while $n_2^*=5.0$, $K_2=1.0\times10^6\,\mathrm{M}^{-1}$, and $\Delta H_2=0.42$ kJ/mol for linearized DNA. The free energy (ΔG_2), calculated using this association constant, was -43 kJ/mol and the entropy (ΔS_2) was 0.13 kJ/mol K for supercoiled DNA while $\Delta G_2=-37$ kJ and $\Delta S_2=0.12$ kJ/mol K for linearized DNA (Table 2).

Table 1. Thermodynamic Parameters of Mn^{2+} Binding to Plasmid DNA

	n_1^*	K_1 (M^{-1})	ΔH_1 (kJ)	ΔG_1 (kJ)	$T\Delta S_1$ (kJ)	ΔS_1 (kJ/K)
Supercoiled (298 K)	1.18	1.03×10^5	1.36	−29.6	31.0	0.104
Linearized (298 K)	0.912	1.26×10^5	2.85	−29.1	32.0	0.107
Supercoiled (323 K)	0.782	3.46×10^4	5.24	−28.1	33.3	0.103
Linearized (323 K)	1.03	1.12×10^5	3.73	−31.2	34.9	0.108
Dialysis (298 K)	1.1	2.0×10^5		−30		

Table 2. Thermodynamic Parameters of DNA Condensation

	n_2^*	K_2 (M^{-1})	ΔH_2 (kJ)	ΔG_2 (kJ)	$T\Delta S_2$ (kJ)	ΔS_2 (kJ/K)
Supercoiled (323 K)	4.5	1.0×10^7	0.42	−43	43	0.13
Linearized (323 K)	5.0	1.0×10^6	0.42	−37	37	0.12

Discussion

In the present experiments, we found that DNA condensation was induced more effectively when using supercoiled DNA rather than in its linearized form. It is known that Mn^{2+} can produce toroidal condensates of supercoiled plasmid DNA, but not of linearized DNA at room temperature.[4] However, our results show that no condensation reaction heat is observed at 298 K. This difference might arise from the fact that uranyl acetate used in Ma's experiment might promote DNA condensation. Wildom and Baldwin reported that in the absence of uranyl acetate stain, the samples were not found to have toroidal structure in electron microscopy.[1] Alternatively it might arise based on a difference of [Mn^{2+}]/[DNA], which Ma's experiment condition was [Mn^{2+}]/[DNA]=1×10^5. However, DNA condensates induced by Mn^{2+} in solution differ only slightly for both DNA forms in our atomic force microscopy (AFM) observations (data not shown). The midpoint of the condensation reaction at 323 K was [Mn^{2+}]/[DNA]=4.5 for supercoiled DNA, while [Mn^{2+}]/[DNA]=5.0 for linearized DNA. Vibrational circular dichroism spectroscopy of calf thymus DNA·Mn^{2+} complex at 328 K shows that DNA condensation is induced at [Mn^{2+}]/[DNA]=4.8.[8] The enthalpy change (ΔH_2) was 0.42 kJ/mol for both DNA forms. The free energies (ΔG_2) were −43 kJ/mol and −37 kJ/mol, respectively, leading to entropies (ΔS_2) of 0.13 and 0.12 kJ/mol K (Table 2). These values are very similar to that induced by cobalt(III) hexamine, namely $\Delta G=-38.0$ kJ/mol and $\Delta S=0.148$ kJ/mol K at 323 K.[5] The difference of ΔS_2 between the supercoiled and linearized forms results from their conformation. *i.e.* the entropy of the supercoiled form is lower than that of linearized DNA. ΔS_2 contains ΔS of the DNA conformation change and ΔS of the release of structured water from DNA.[7] The sign of ΔS of the former is negative and that of the latter is positive. Consequently, ΔS of the release of structured water from DNA is dominant and equivalent to both DNA forms because Mn^{2+} ion binds both DNA forms equally. When the entropy of the supercoiled form is lower than linearized DNA, ΔS of the DNA conformation change is supercoiled form<linearized form. Taking account of the knowledge mentioned above, it can't be stated positively that "The mechanism of condensation of supercoiled DNA by Mn^{2+} appears to be quite different from that believed to underlie the more extensively studied condensation induced by higher valence cations."

When standard calorimetric data regression analysis was performed for supercoiled DNA at 298 K, the initial injection data was ignored (see Fig. 1). In some literature, the initial injection data was excepted because it would be incorrect.[9] However, the tendency toward decreased heat reaction at initial injection for supercoiled DNA at 298 K in our experiments appears to be true. Details will be published in our next report.

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