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Divalent metal ion effect on helix–coil transition of high molecular weight DNA in neutral and alkaline solutions

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ABSTRACT

Effect of Mg^{2+}, Ca^{2+}, Ni^{2+} and Cd^{2+} ions on parameters of DNA helix–coil transition in sodium cacodylate (pH 6.5), Tris (pH 8.5) and sodium tetraborate (pH 9.0) buffers have been studied by differential UV–visible spectroscopy and by thermal denaturation. Anomalous behavior of the melting temperature T_m and the melting interval ΔT in the presence of $MgCl_2$ was observed in Tris, but not in cacodylate or tetraborate buffers. Changes in the buffer type and pH did not influence T_m and ΔT dependence on Ca^{2+} and Cd^{2+} concentrations. Decrease of the T_m and ΔT of DNA in the presence of Ni^{2+} and Cd^{2+} was caused by preferential ion interaction with N7 of guanine. This type of interaction was also found for Mg^{2+} in Tris buffer. The anomalous decrease in the T_m and ΔT values was connected to formation of complexes between metal ions and Tris molecules. Transition of DNA single-stranded regions into a compact form with the effective radius of the particles of 300 ± 100 Å was induced by Mg^{2+} ions in Tris buffer.

1. Introduction

Rakitin et al. [1] have shown in 2001 that metallized form of DNA (M-DNA) discovered in 1993 [2] possesses a metallic conductivity. This finding originated extensive studies of the thermodynamic properties of DNA and RNA M-forms as well as favorable conditions for their formation due to emerged possibility to use these macromolecules in nanoelectronic devices (transistors, electron conductors, switches and memory devices) and nanobiosensors [1–7].

It has been previously found that M-forms of DNA and RNA consisting of canonical bases are formed in the presence of Zn^{2+}, Ni^{2+} and Co^{2+} ions at alkaline pH (8.5–9.0) [1,2]. Unlike in neutral solutions (for example, see [8–13]), thermodynamic properties of DNA metal complexes at pH > 7 have been studied very little [2,4–6,14]. In particular, it was revealed that M-form has higher thermal stability than B or A forms of polynucleotides [2,5]. The nature of this effect remains unclear. Indeed, in addition to the formation of intramolecular inter-chain bonds (G)N1-Mt^{2+}-N3(C) and (A)N1-Mt^{2+}-N3(T) within the DNA base pairs (which are the reason of M-form formation [2]), Zn^{2+}, Ni^{2+} and Co^{2+} must also interact with oxygen atoms of DNA phosphate groups. At low/intermediate metal ion concentrations such an interaction would increase DNA thermal stability by reducing the repulsive negative charge on the phosphates. On the other hand, binding of these metals to N7 of purines and formation of inner-sphere complexes has to result in the opposite effect and destabilize DNA [10–12]. Finally, DNA thermal stability can be influenced by such factors as a decrease of the proton concentration in a solution upon moving to alkaline pH values and the resulting change of the buffer solution content.

All the above factors, influencing the DNA thermal stability, can be studied individually by monitoring the metal ion concentration dependence of DNA helix–coil transition under conditions when each of the factors is dominant. The obtained data are necessary for quantitative evaluation of changes of the DNA thermodynamic parameters during its transition to metallized form.

Therefore, the aim of the present work was to study the DNA thermal stability in solutions at neutral and alkaline pH in the presence of different concentrations of alkali-earth (Mg^{2+}, Ca^{2+}) and transition (Ni^{2+}, Cd^{2+}) metals.

2. Materials and methods

Sodium salt of salmon sperm DNA with molecular weight $(4–6) \times 10^6$ Da as well as sodium cacodylate $Na[CH_3]_2AsO_2{\cdot}3H_2O$ (pH 6.5 and 7.0) and Tris buffers $C_4H_{11}O_3N$ (pH 8.5 and 9.0) were purchased from Serva, Germany. DNA contained less then 0.5% of RNA and protein impurities and was used as supplied. Salts of $MgCl_2{\cdot}6H_2O$, $CaCl_2{\cdot}6H_2O$, $NiCl_2{\cdot}6H_2O$, $CdCl_2{\cdot}0.5H_2O$, NaCl (chemi-

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Table 1
DNA helix–coil transition temperature (T_m) in the absence of Mt^{2+}.

NaCl (M)	Solution #	Buffer[a]	pH_{T_o} [b]	T_m (°C)	pH_{T_m} [b]
2.5×10^{-2}	1	Cacodilic	7.0	76.3	6.9
	2	Tris	9.0	74.6	8.0
5×10^{-3}	3	Cacodilic	6.5	68.8	6.4
	4	Cacodilic	7.0	68.5	6.9
	5	Tris	8.5	69.7	7.6
	6	Tetraborate	9.0	71.2	8.8

[a] Concentration of cacodilic and Tris buffers was 10^{-2} M, concentration of tetraborate buffer was 5×10^{-3} M.

[b] pH_{T_o} and pH_{T_m} are the solution pH values at room temperature (T_o = 23 °C) and at DNA melting temperature (T_m), respectively. The values for pH_{T_m} have been determined according to [14,15] and were $\gamma = -d(pH)/dT = -0.002$ units pH/K for cacodilic; $\gamma = -0.004$ units pH/K for tetraborate and $\gamma = -0.02$ units pH/K for Tris buffer. In the DNA melting temperature interval, the pH of the solution increased by 0.02, 0.04 and 0.2 pH units in cacodilic, tetraborate and Tris buffers, correspondingly.

cal grade) and sodium tetraborate buffer $Na_2B_4O_7{\cdot}10H_2O$ (pH 9.0) were obtained from Reachim, Russia.

Tris buffer is known to have a strong dependence of its pH on temperature [14]. However, it was used in the present work because it is commonly utilized for studies of biomolecules at alkaline pH [5,14]. Particularly, Tris was used by Wood et al. [5], who described the most detailed conditions of DNA and RNA metallization.

Chlorides of divalent metals were added only to solutions #3, #5 and #6 (see Table 1). The necessary concentration of the alkaline-earth metal ions was obtained by adding the appropriate amount of the 0.1 M $MtCl_2$ buffered stock solution into the DNA solution prepared in the same buffer. For preparation of the stock solutions of transition metals (0.03 M Mt^{2+}) triple-distilled water (pH 6.7) was used instead of buffer to avoid the formation of hydroxides (including insoluble ones) [16]. Acidity of the DNA solutions in Tris remained unchanged upon $MtCl_2$ addition up to 2×10^{-3} M for Mg^{2+} and Ca^{2+} and 7×10^{-4} M in the case of Ni^{2+} and Cd^{2+} ions.

The concentration of Mt^{2+} ($[Mt^{2+}]$) chlorides was initially determined by weight and then controlled by the trilonometric titration. The DNA phosphorous concentration (P) was $(2–3)\times10^{-5}$ M as determined by the molar extinction coefficient at 260 nm [15]. The pH of the solutions was determined by a pH-meter pH-340 (Russia) at room temperature with the error of ±0.03 pH units.

2.1. Differential UV and visible spectroscopy

Differential spectra (DS) $\Delta A(\nu)$ were measured at room temperature $T_o = 23 \pm 2$ °C with M40 spectrophotometer (Carl Zeiss, Jena, Germany). The four-cuvette scheme was utilized. Cuvette #1 containing DNA and cuvette #2 with the buffer solution were placed in the reference channel of the spectrophotometer. Cuvette #3 with the identical to cuvette #1 DNA solution and cuvette #4 with the identical to cuvette #2 buffer were placed in the sample channel. DS were measured after addition of the same $MtCl_2$ amount into cuvettes #2 and #3. To compensate for the DNA dilution in cuvette #3, the appropriate amount of the buffer was added to cuvette #1. To obtain equilibrium spectra, the solutions in the cuvettes were incubated at room temperature (T_o) for 120 min after the metal chloride addition. Our test measurements have shown that the equilibrium was basically reached already after 60 min of incubation. All DS were normalized to DNA concentration as follows:

$$\Delta\varepsilon(\nu) = \frac{\Delta A(\nu)}{P}. \quad (1)$$

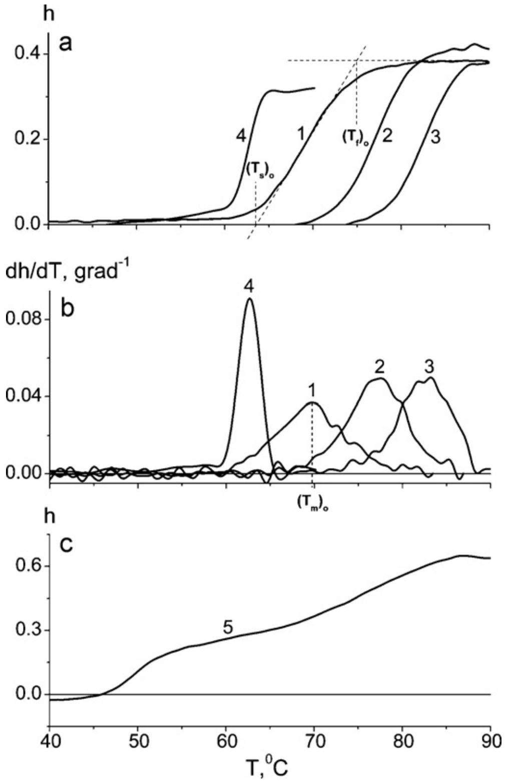

Fig. 1. Typical DNA melting curves (a and c) and their temperature derivatives (b) in solutions containing $MgCl_2$ and $CaCl_2$ (Tris, pH_{T_o} 8.5). (1) $[Mt^{2+}] = 0$; (2) 10^{-4} M Ca^{2+}; (3) 2×10^{-4} M Mg^{2+}; (4) 4×10^{-4} M Mg^{2+}; and (5) 5×10^{-4} M Mg^{2+}.

2.2. Thermal denaturation

DNA melting curves were measured with UV-vis spectrophotometer (Carl Zeiss, Jena, Germany) as a heat-induced hyperchromicity in the maximum of DNA absorption (ν = 38,500 cm^{-1} or λ = 260 nm) as a function of temperature $h(T)$:

$$h(T) = \frac{\Delta A(T)}{A_{T_o}}, \quad (2)$$

where $\Delta A(T)$ is a change in the optical density of DNA solution upon heating, and A_{T_o} is its optical density at the room temperature.

The measurements were carried out by the double-cuvette scheme: identical solutions of DNA with $MtCl_2$ were placed in cuvettes #1 and #3 in the reference and sample channels of the spectrophotometer, respectively. After 120 min of the preliminary incubation of both cuvettes at room temperature (T_o) the background cuvette was thermostated at $T = T_o \pm 0.5$ °C, while the sample cuvette was slowly heated at a rate of 0.25 °C/min to ensure the thermodynamic equilibrium of the heated solution at all temperatures. The solution temperature was determined with an error not exceeding 0.5 °C. The dispersion among values of DNA melting temperature (T_m) determined in the repeated measurements performed under similar conditions was 1.5 °C. Parameters of the helix–coil transition as well as the differential spectra were computed by connected to the spectrophotometer personal computers. T_m was determined as the temperature corresponding to the maximum of the function of derivative $\alpha = dh(T)/dT$ on temperature (Fig. 1). Determined in such a way T_m value corresponded within 1 °C to the temperature at which 50% of polymer units transit into an unordered state. The interval of the helix–coil transition was determined as follows:

$$\Delta T = T_f - T_s, \quad (3)$$