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Thermodynamic parameters of DNA condensed by hexamminecobalt (III)

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Abstract:

Thermodynamic parameters of DNA melting are studied in wide rage of hexamminecobalt(III) concentrations, including the concentration, when DNA is in condensed form. Increase of thermostability was observed at [Co]/[P] rates from 0 to 0.3. DNA melting ethalpy did not change in this interval and equaled to 50±5 J/g. Then it rapidly dropped down to 31±3 J/g at [Co]/[P] = 0.35. The magnitudes of the melting range width (ΔT_m) were 8-9 times less than those of the solutions that did not contain hexamminecobalt(III) equaled to 1.4-1.5^oC. In addition to the major peak at 81.6^oC, a new peak appeared during melting of condensed DNA at 90^oC, which we consider as manifestation of the condensate melting process.

Keywords*: hexamminecobalt(III); DNA condensation; DNA melting; DNA transition temperature; DNA melting range*

Abbreviation: Co, hexamminecobalt(III), differential scanning calorimety (DSC)

1. Introduction

The study of physical properties of condensed DNA is interesting for both fundamental and practical reasons. DNA in cells exists in condensed state and regulation of this state is a part of the cell cycle [1]. In the spermatozoid and viruses, the distance between DNA surfaces in the condensate is almost equal to those in the solid bodies [2 – 4]. Study of condensed DNA is of a great importance in development of gene therapy [5]. The process of condensation has been already studied during several decades [6-8], but many questions about mechanisms of DNA condensation and the properties of condensed DNA remain unanswered. For example, there is no consensus about the forces responsible for the condensation process.

DNA is a negatively heavily charged molecule. Neutralization of about 90% of negative phosphate groups is a necessary step for DNA condensation [8]. One of the methods to achieve DNA condensation in diluted solutions is adding of cations with charges equal or more than 3+ [9]. On the other hand, electrostatic interactions are not the only ones responsible for creation of DNA condensates. For instance, cobalt(III) sepulchrate is more efficient condensing agent than hexamminecobalt(III), in spite of the fact that the charges of these compounds are the same [10].

In general, condensed DNA has either toroidal, or rod-like structure [4, 11]. The size of the toroid depends on experimental conditions [12, 13]. DNA condensed by hexamminecobalt(III) is packed in a hexagonal lattice with DNA interaxial separation equal to 28 Å [4]. This means that in case of B DNA, polymer surface separation is 8 Å. Creation of condensate with such close separation of macromolecules should be accompanied with certain thermal effect. Despite the fact that DNA condensation is of a great interest of scientists working in many fields of life sciences, the works concerning energetics of binding of multivalent cations with DNA are relatively few. There have been found two binding modes at DNA-hexamminecobalt(III) interaction leading to formation of the condensates [9,14]. The first mode was ascribed to neutralization of two-thirds of negative charges of DNA. The second one was connected to endothermic heat of condensate formation. Also, it was shown that both the first binding mode and the second condensation mode were affected: increasing concentration of NaCl weakened binding and delayed the condensation.

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The goal of the present work was to study thermal characteristics of condensed DNA denaturation. In this paper, we present the results of study of thermodynamic parameters of DNA melting in diluted solutions and condensate obtained by differential scanning calorimety (DSC).

2. Materials and methods

Heat capacity was measured by DSC (DACM-4A), with the cell working volume of 0.46 ml and the operating temperature range from -10 to 150^{o}C. DNA concentration was 0.5mg/mL except when otherwise appointed. The heating rate was 2 K·min^{-1}. The specific Cp values were calculated using the value of the partial volume of the native DNA (0.54 ml/g). Melting range width (ΔT_m) was calculated at the halfwidth of the melting peak. Na-DNA of calf thymus and hexamminecobalt trichloride ($Co[NH_3]_6Cl_3$) were obtained from Sigma. Both were used without further purification. The [Co]/[P] ratios in our experiments were 0.05, 0.1, 0.15, 0.2, 0.25, 0.3 and 0.35. The prepared samples were incubated during 24 hours at 4^{o}C. The concentrations of DNA and hexamminecobalt(III) were determined by spectrophotometer. DNA-hexamminecobalt(III) complex melting has been studied in HEPES buffer (pH 7.4) at different Na^+ concentrations (5, 10, 20 mM). The exact concentration of DNA ([Co]/[P]=0.35) was measured by absorption of the supernatant after the solution was centrifuged at 8000*g* during 20 min. There was no light scattering taking place in the supernatants, what indicates that there was no condensed DNA in the solutions. The scattering was tested at 320nm. DNA concentrations in the supernatant were in the range 0.12 – 0.13 mg/ml.

3. Results and Discussion

Discrete condensed particles such as toroides can not be found at high concentrations of DNA needed for DSC studies. As the fundamental intermolecular energetic factors responsible for condensation are probably the same regardless DNA concentration [9], we found it reasonable to carry out measurements with solutions suitable for DSC studies.

3.1 Temperature dependence

The temperature dependence of partial heat capacity of calf thymus DNA for different concentrations of Na^+ ions and hexamminecobalt(III) is given in Fig.1. Typical calorimetric melting curves of calf thymus DNA (Fig.1) show complex, but reproducible patterns of individually distinguishable melting peaks. As shown in [15], the fine structure of calorimetric melting curve is far from accidental one and reflects the relative population of the cooperative sequences along the calf thymus DNA. This sequence reveals a large variety of thermal stabilities and the superposition of the individual sequences results in the envelope of the complete melting curve.

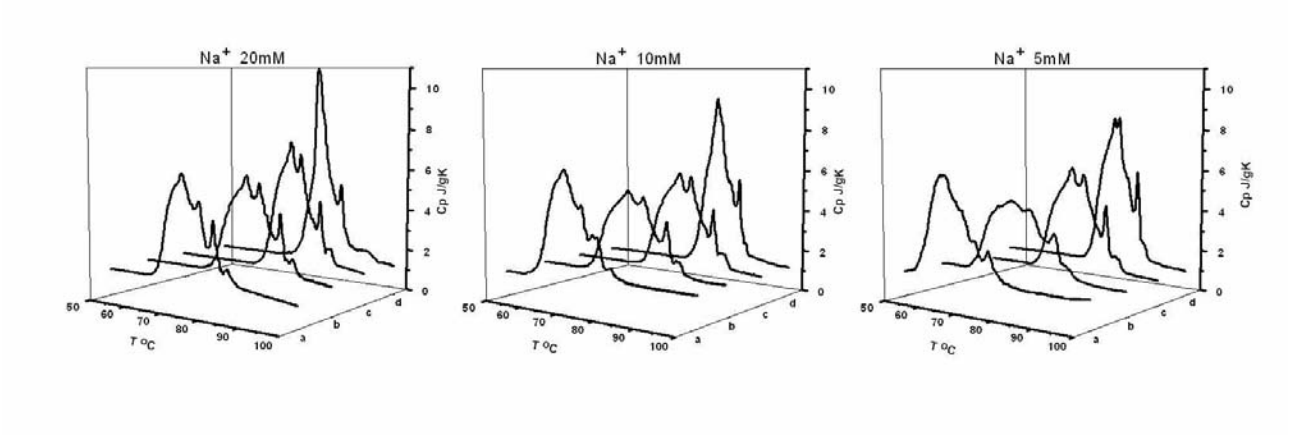

Fig. 1.
DNA melting curves at different hexamminecobalt(III) concentrations.
a-[Co]/[P] =0.0; b-[Co]/[P] =0.1; c-[Co]/[P] =0.2; d-[Co]/[P]=0.3

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The presence of repetitive short homogeneous sequences such as *satellite* DNAs, which turn out to be related to the individual peaks sticking out from a broad melting of calf thymus DNA, is clearly demonstrated in Fig.1. The locations of the peak on the heat capacity vs. temperature curve depend on GC content of the given satellite fraction and the melting temperature increases with increase of GC content.

In the case, when [Co]/[P]=0.35, the DNA precipitation takes place. It is impossible to calculate the dependence of heat capacity on temperature and the melting enthalpy. So, there are presented only thermograms (Fig. 2).

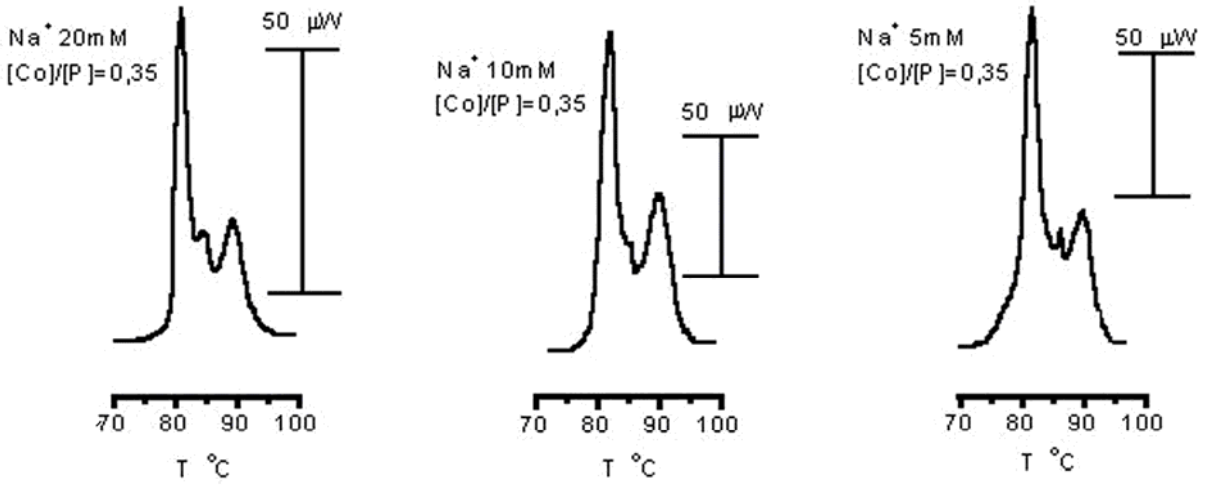

Fig. 2.
DSC scans for DNA.

Changes of calorimetric melting curves of DNA at increase of hexamminecobalt(III) concentration, indicate the interaction between DNA and $Co(NH_3)_6^{3+}$. Analyzing thermodynamic parameters of DNA-Co(III) complex melting, such as complex melting enthalpy (ΔH_m) and temperature (T_m), and melting range width (ΔT_m) are very important for obtaining of new information on the mechanisms of hexamminecobalt(III) binding with DNA.

3.2 DNA melting temperature

DNA melting temperature substantially depends on hexamminecobalt(III) concentration. Namely, increase of $Co(NH_3)_6^{3+}$ concentration results in increase of melting temperature, what is an indicator of increase of DNA thermostability (Fig.3).

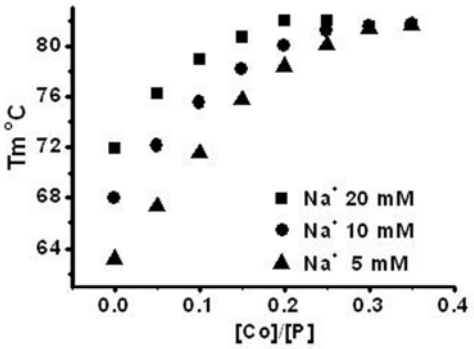

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Fig. 3.
Dependence of DNA melting temperature on hexamminecobalt(III) concentration.

DNA melting temperature at Na^+ concentrations of 5, 10 and 20 mM is 81.3±0.1°C, 81.5±0.1°C and 81.5±0.1°C, respectively at [Co]/[P] =0.3. The temperature values corresponding to the curves' maxima of condensed DNA melting, at [Co]/[P]=0.35 stay the same within the limits of experimental error for different content of Na^+ and are equal to 81.5±0.1°C (at $[Na^+]$=5mM), 81.7±0.1°C (at $[Na^+]$=10 mM) and 81.6±0.1°C (at $[Na^+]$=20mM) (Fig. 3). Based on such coincidence of temperature values we can assume that Na^+ ions do not take part in DNA thermostability at [Co]/[P] rates from 0.3 to 0.35. This fact is connected with replacement of Na^+ ions at DNA surface by hexamminecobalt(III) [16]. To elucidate the reasons of change of DNA thermostability and melting range width caused by interaction with hexamminecobalt(III), it is necessary to know the sites where hexamminecobalt(III) binds to DNA [17-22]. Scatchard analysis has shown that there exist two binding constants ($K_1=1.4\cdot10^5$ M^{-1} and $K_2=9.2\cdot10^4$ M^{-1}) [18], indicating different affinities of DNA to hexamminecobalt(III) complexation. The authors suggested that the weaker interaction is the one between hexamminecobalt(III) and phosphorus groups, whereas the stronger one is due to binding of hexamminecobalt(III) in DNA major groove. Theoretical and experimental studies regarding different oligonucleotides containing GpG sites has shown that hexamminecobalt(III) forms hydrogen bonds with O6 and N7 of guanine in major grove through NH_3 groups [17]. Particular attention needs to be paid to GpGpG regions. The binding appeared to be very specific. One amine primarily interacts with N7 of 5'-guanine, but also with the O6 atoms. One of the other two amines forms a hydrogen bond with N7 atom of the second guanine and the other one to the O6. The forth amine is connected with 3'-guanine, N7 and O6 atoms through water bridge. Such DNA-hexamminecobalt(III) complexation should result in increase of DNA thermostability. The increase of DNA thermostability is caused by nonspecific interaction of hexamminecobalt(III), with phosphate groups screening their negative charges.

3.3 DNA melting enthalpy

Increase of hexamminecobalt(III) concentration did not change DNA melting enthalpy. Within the limits of experimental error and at [Co]/[P] = 0-0.3 its value coincides with that of the one not containing $Co(NH_3)_6^{3+}$ (ΔH_m=50±5 J/g, at Na^+ 5, 10 and 20mM). In order to get data on the melting enthalpy at [Co]/[P]=0.35 the sample was centrifuged and the supernatant was studied out. The result showed dropping of ΔH_m down to 30±3J/g (Fig. 4).

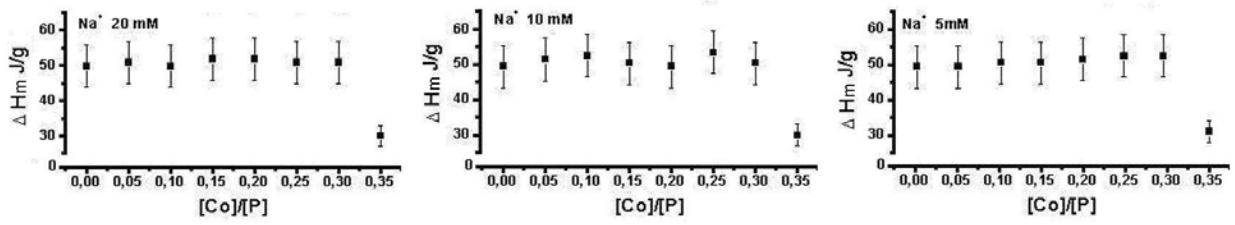

Fig. 4.
Dependence of DNA melting enthalpy on hexamminecobalt(III) concentration.

The melting enthalpy of DNA is determined by disruption of hydrogen bonds between the bases, stacking interactions and destruction of the hydration water structure. Hydration water is mainly located in DNA grooves [23] and by the phosphate groups [24].

It was shown in work [25] that 70-80% of melting enthalpy is stipulated by destruction of DNA hydration water structure in diluted solutions. As it was already mentioned,

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hexamminecobalt(III) interacts with DNA in grooves and with phosphate groups, what is to lead destruction of the hydrated water.

It can be assumed that enthalpy decrease was caused by dehydration of DNA, which was proved by the increase of the solution volume at DNA-hexamminecobalt(III) interaction [26]. This interaction is driven entropically [9, 14], what is another evidence for DNA dehydration, as entropic process is accompanied by release of water molecules from DNA surface.

3.4 DNA melting range width

The profile of DNA melting curve for given nucleotide sequences, is determined by the difference between thermostabilitiies of A-T and G-C pairs. This difference is reflected in DNA melting range width. The difference of thermostability between A-T and G-C pairs depends on environmental conditions and by their variation, it is possible to achieve the state, when thermostabilities of A-T and G-C pairs coincide with each other [27]. At this, melting interval is narrowed, what is observed at DNA-hexamminecobalt(III) interaction.

DNA melting range width (ΔT_m) changes at increase of hexamminecobalt(III) concentration. Namely, its value increases at first and then decreases. Finally, at [Co]/[P] =0.35, the magnitudes of ΔT_m is 8-9 times less than those of the solutions that do not contain hexamminecobalt(III), these values are equal to 1.4°C (5, 10 mM Na^+), and 1.5°C (20 mM Na^+) (Fig. 5).

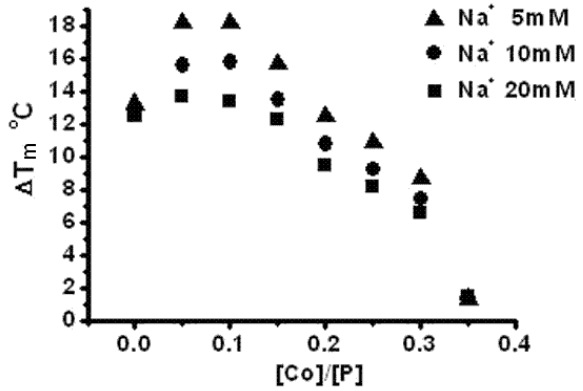

Fig. 5.
Dependence of DNA melting range width on hexamminecobalt(III) concentration.

Changes of DNA melting range width can be explained by the difference between thermostabilities of A-T and G-C pairs. Since DNA melting range width gets broader at low concentrations of examminecobalt(III) ([Co]/[P] from 0.05 to 0.1) (Fig. 5), one can assume that G-C rich sites of DNA get more thermostable than A-T ones. Narrowing of DNA melting range width at high concentrations of hexamminecobalt(III) ([Co]/[P] from 0.15 to 0.35) shows that thermostabilities of A-T and G-C pairs get closer to each other.

Increase of DNA melting range width at low concentrations of hexamminecobalt(III) indicates that hexamminecobalt(III) preferably links to GC- rich areas and after saturation of those they start to interact with the phosphate groups of AT-rich areas.

The melting range width ΔT_m of DNA–hexamminecobalt(III) complex is different at different concentrations of Na^+ ions. At 5, 10 and 20mM concentrations of Na^+, the corresponding values of ΔT_m were 13.2°C, 12.8°C and 12.5°C, when ([Co]/[P]=0). The melting range width reaches its maximum at the rates within [Co]/[P]= 0.05-0.1 and equals to 18.2 (at 5mM of Na^+), 15.8(at 10mM of Na^+) and 13.7(at 20mM of Na^+). Hence it follow that high concentrations of Na^+ blocks penetration of hexamminecobalt(III) into DNA grooves to interact with GC-rich areas and it interacts with the phosphate groups of A-T pairs.

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3.5 supernatant melting curves

The peaks of melting curves overlap each other at increase of hexamminecobalt(III) concentration (Fig. 1). At [Co]/[P] = 0.35, there appears a new peak at 90°C (Fig. 2). Peaks with maximum at T_m=81.6°C correspond to the heat required for destruction of the double strands. In order to find out the origin of the new peak we centrifuged the sample. Spectroscopic investigation has revealed that there is no light scattering taking place in the supernatant, what indicates that there is no condensed DNA in it (Fig. 6). The peak at 90°C did not appear in the melting curve of the supernatant (Fig. 7).

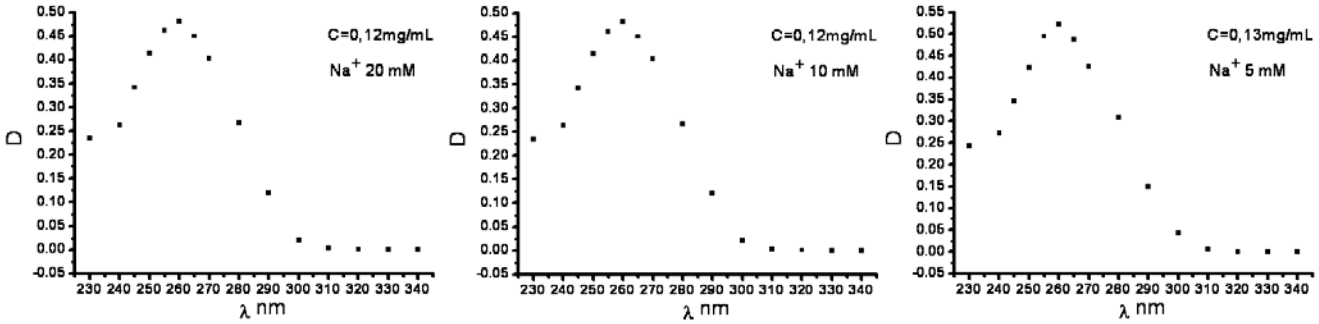

Fig. 6.
Supernatants UV spectra at [Co]/[P]=0.35.

Hence, one can assume that the new peak represents the heat of destruction of condensed DNA. In other words, DNA double helix unwinding takes place while the single strand condensate still exists. As a result, we conclude that DNA double strand melts at 81.6°C, but the links between single strands disrupt at 90°C. According to these data and taking to the account that hexamminecobalt(III) and DNA phosphate groups are strongly hydrated and interaxial distance between condensed DNA molecules is 28 Å [4], one can suppose that there are structured water molecules originated within the condensate, destruction of which is reflected in the enthalpy represented by the second peak.

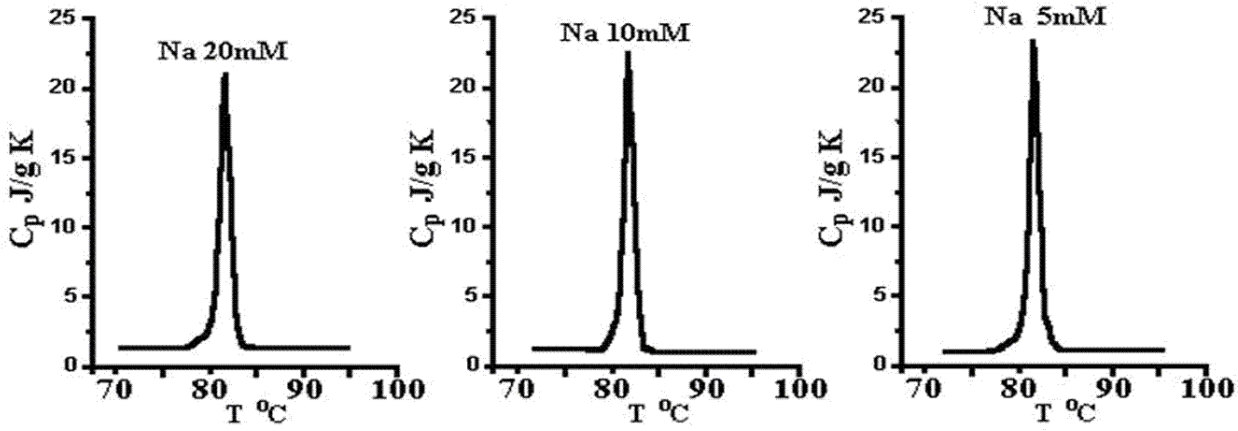

Fig. 7.
DNA supernatant melting curves at [Co]/[P]=0.35.

4. Conclusions

The results of our study showed that DNA melting enthalpy (50±5 J/g) stayed the same in the presence of hexamminecobalt(III) at ratios of [Co]/[P] from 0.0 to 0.3, while at [Co]/[P]=0.35 it dropped down to 31±3 J/g. As 70-80% of DNA melting enthalpy is stipulated by destruction of its

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hydration water structure, dehydration of DNA at DNA-hexamminecobalt(III) interaction can be considered as a cause of this decrease of enthalpy.

DNA-hexamminecobalt(III) interaction leads to increase of DNA melting temperature. Content of Na^+ ions affect DNA thermostability at ratios of [Co]/[P] from 0.0 to 0.3 and has no effect at [Co]/[P] from 0.3 to 0.35. Which is an indication of replacement of Na^+ ions at DNA surface by hexamminecobalt(III).

Dependence of DNA melting range width (ΔT_m) on concentration of hexamminecobalt(III) is not monotonous. Its initial increase at low concentrations of hexamminecobalt(III) is accompanied by sharp decrease even to the values 8-9 times less than that of DNA without hexamminecobalt(III) at [Co]/[P] = 0.35, when DNA condensation takes place.

After melting of condensed by hexamminecobalt(III) DNA double strands (at 81.6^{o}C), the new peak was found at 90^{o}C. We consider the later as the condensate melting process. Hence, we assume that melting of the double helix takes place at first, while the single strands remain in condensed state till the temperature reaches 90^{o}C.

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