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Melting of complexes of DNA-*cis*-DDP in acidic environment

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Abbreviation: *cis*-DDP, *cis*-diamminedichlorplatinum (II)

Abstract

The peculiarities of helix-coil transition of DNA at complex-formation with *cis*-diamminedichlorplatinum (II) (*cis*-DDP) have been investigated in acidic environment by UV absorbance technique. It is shown that observed features of behavior ΔT (pH) and T_m (pH) DNA at pH 2.8-3.0 are possible to explain by formation in DNA of pseudo-ring structures at covalent linking of *cis*-DDP with DNA.

Keywords: DNA-*cis*-DDP complexes; melting interval; melting temperature; nucleotide protonation; pH depend melting

Introduction

Antitumor compound *cis*-diamminedichlorplatinum (II) (*cis*-DDP) has successfully been applied in the chemotherapy of tumors (Eastman, 1985; Sherman and Lippard, 1987; Alink *et al.*, 1991; Boogard and Redijk, 1991; Coluccia *et al.*, 1995). Numerous experiments have shown that this compound forms covalent linkage with N7 of guanine (Eastman, 1985; Sherman and Lippard, 1987; Reedijk, 1991) and more than 95% of the bound *cis*-DDP forms bifunctional adducts with DNA. Bound *cis*-DDP was associated with guanines of the same strand in sequences such as d(GpG) (65%), d(GpXpG) (7%), and later at d(ApG) (23%) with saturation. Approximately 1% of *cis*-DDP interacts with guanines of various DNA strands. About 5% of general platination corresponds to monofunctional linkage with guanine base. The concrete type of *cis*-DDP binding to DNA depends on concentration of platinum complexes. At low concentration of *cis*-DDP, ($r_b < 10^{-3}$), the molecule of a platinum complex at first binds to DNA in monodentate mode, and then with increasing of concentration, this binding turns into bidentate. ($r_b < 10^{-3}$, where r_b is relative concentration of the platinum complexes-$r_b = C_{cis\text{-}DDP}/C_{base\ pairs}$).

All mentioned types of covalent linkage of *cis*-DDP essentially change DNA structure and consequencially, influence on DNA melting behaviour. Also, the linkage of *cis*-DDP with DNA was shown to induce deformation of a double helix. At the levels of $r_b > 10^{-3}$, breakages of single-and even double-stranded DNA were observed (Eastman, 1985; Butour and Jonson, 1986; Reedijk, 1991; Malinge and Leng, 1999). The completely unexpected behavior was noted at low concentrations of *cis*-DDP where the tertiary structure of DNA is significantly changed at concentration of *cis*-DDP $10^{-5} < r_b < 10^{-3}$ (Haroutiunian *et al.*, 1997, 1998). At these concentrations *cis*-DDP is linking to guanines of different strands that are at significant distance from each other (a several hundred base pairs) thus forming pseudo-ring structures from linear DNA.

There have been numerous reports on the study of *cis*-DDP-DNA complex-formation *in vivo*. Eastman (1985) and Sherman and Lippard (1987) have shown that *cis*-DDP is covalently linked with DNA *in vivo* resulting in the inhibition cell proliferation. During the cell division, there appears to be a significant jump of acidification in relation with the ionization nucleotide bases. Such changes of pH strongly do induce an affect on DNA thermostability (Suchorukov and Shvartsburg, 1985) and likely affects interaction of anti-tumor compound *cis*-DDP with DNA, especially, when all possible centers of ionization are already engaged by any ligand (for example *cis*-DDP).

The analysis of ΔT (pH) widening dependence shows, that a strict decreasing of DNA melting temperature took place at extremes of pH environment (pH < 4.5 and pH > 9). Earlier study of Akhrem *et al.* (1989) and Lando *et al.* (1981, 1994) explain such behavior of ΔT (pH) by distinctions in the mechanism of DNA nitrogen bases ionization, where AT-pairs are ionized only in melted condition, and GC-pair both in melted and in helical.

To sum up it is possible to expect that in conditions of extreme values of environmental pH occurring at low concentrations of *cis*-DDP the alterations of secondary and tertiary structure of DNA will be differ from ones obtained for pure DNA.

Materials and Methods

Ultra-pure calf thymus DNA (RNA < 0.1%, protein < 0.1%, molecular weight 3×10^7 Da) and *cis*-DDP (Sigma) were used. Concentration of *cis*-DDP was $10^{-5} < r_b < 10^{-3}$. The experiments were carried out in the following solution 10^{-2} M $NaClO_4$+10^{-3} M NaCl. Ionic strength (μ) of [Na^+] was equal to 0.01 (Lando *et al.*, 1994). The melting of DNA and its complexes with *cis*-DDP carried out on spectrophotometer PYE Unicam-SP 8-100 (England) (Vardevanyan *et al.*, 2001). Speed of scanning of temperature was 0.25°C/min. The size of points corresponds to average meaning of 5-6 measurements.

Results and Discussion

The phenomenon of DNA deprotonation in the *cis*-DDP solution was partially investigated by Malahti and Natarajan (1998). In the cis-DDP solution, the increasing environmental pH facilitates the process of the nitrogen bases deprotonation when some binding centers of nucleotides are already engaged by ligand molecule. As a result, the values of pK of groups capable deprotonate both in AT- and in GC-pairs decreases (for example the values of pK for GC-pairs in helical sites, decrease from 10.7 to 9.5). The phenomenon of DNA protonation in *cis*-DDP solutions, has not so far been investigated.

Figure 1 (A and B) shows dependencies of melting temperature (T_m (pH)) and temperature range (ΔT (pH)) of Calf Thymus DNA at concentration of *cis*-DDP $10^{-2} < r_b < 10^{-5}$. As it is visible from the given Figures in presence of *cis*-DDP the process of DNA protonation occurs more effectively. Hence the initial solution of DNA without *cis*-DDP was prepared in buffer with ionic strength [Na^+] = 0.01 (instead of 0.1, as was in work (Lando *et al.*, 1981)). Also it was necessary to expect the decrease of melting temperature in a case of DNA without *cis*-DDP and temperature range of melting begins at higher pH. In presence of *cis*-DDP at concentration of platinum complexes $r_b = 10^{-5}$, the melting temperature of DNA increases by more than 4°C.

As indicated in the Table I, strong alterations of parameters T_m and ΔT of DNA take place at concentration of *cis*-platinum $r_b = 10^{-5}$. The melting temperature of calf thymus DNA increases almost 4°C. It is necessary to note, that the used concentration of a compound is so small (1-2 molecules of *cis*-DDP for 10,000 pairs of nucleotides) that it is impossible to explain such significant alterations of melting parameters by known theories of DNA-ligand melting. It is possible to explain such a behavior of transition parameters, proceeded from a possibility of pseudo-ring structure formation of DNA, occurred by linking of linear molecule of DNA at its platination.

As shown in Figure 1 (A and B), protonation of DNA in presence of *cis*-DDP becomes more effective. It is particularly strongly shown at concentration $r_b = 10^{-5}$. It is appropriate to note that specific minimum for melting temperature range, observable on curves T (pH) for DNA itself at pH = 2.8 ~ 3.0, is shifted to values of pH = 5.5. Melting temperature moves to higher values of pH (decreasing of T_m begins from pH 6.0, instead of pH 4.0).

As described earlier, the presence of minimum on ΔT (pH) curve can be explained by protonation of AT-pairs in melted form and GC pairs both in melted and helical forms. Such a character of mechanisms of nitrogen bases protonation results in continuous decrease of T_{AT} at decreasing of pH, and since the certain meaning of pH T_{GC} satiation. As a result (as

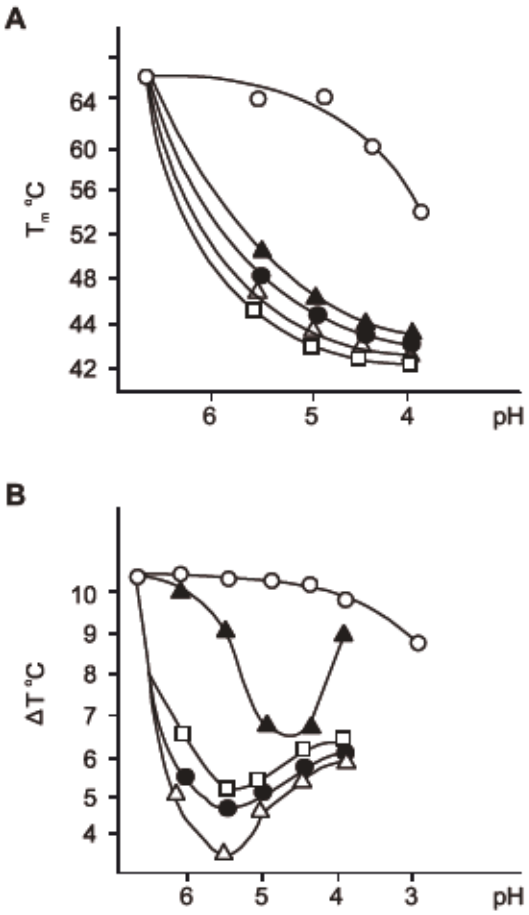

Figure 1. A. Dependence of melting temperature of calf thymus DNA on pH of environment at various concentration of *cis*-DDP: (○)-pure DNA, (▲)-$r_b = 10^{-5}$; (●)-$r_b = 10^{-4}$; (□)-$r_b = 10^{-3}$; (△)-$r_b = 10^{-2}$. B. Dependence of melting interval of calf thymus DNA on pH of environment at various concentration of *cis*-DDP: (○)-pure DNA, (▲)-$r_b = 10^{-5}$; (●)-$r_b = 10^{-4}$; (□)-$r_b = 10^{-3}$; (△)-$r_b = 10^{-2}$.

Table 1. Melting temperature (T_m) and melting interval (ΔT) for complexes of *cis*-DDP with DNA of different GC-composition at various concentration of cis-platinum (r_b).

C, Mol	r_b	Calf thymus		Cl.Perfringens		M.Luteus	
		T_m	ΔT	T_m	ΔT	T_m	ΔT
0	0	65.5	9.8	60.0	5.8	78.8	7.0
1×10^{-9}	1.0×10^{-5}	65.8	10.0	60.1	6.2	69.7	20.0
5×10^{-9}	5.8×10^{-5}	69.1	10.3	60.0	6.3	69.9	19.8
1×10^{-8}	1.0×10^{-4}	70.7	10.6	59.7	6.0	-	-
5×10^{-8}	5.9×10^{-4}	68.3	10.5	60.8	6.1	66.1	16.0
1×10^{-7}	7.0×10^{-4}	67.8	11.3	58.2	6.2	-	-
5×10^{-7}	6.0×10^{-3}	66.4	10.8	58.0	6.4	66.3	17.1
1×10^{-6}	8.0×10^{-3}	66.0	10.3	55.0	6.7	-	-
5×10^{-6}	6.0×10^{-2}	60.3	9.0	51.8	8.5	62.0	28.8
1×10^{-5}	1.0×10^{-1}	52.0	12.7	43.8	10.9	-	-
5×10^{-5}	5.8×10^{-1}	50.5	13.0	45.2	12.8	50.8	20.8

it follows from the Frank-Kamenetskii's formula $\Delta T = K (T_{GC}-T_{AT})$ for heterogeneous block DNA (Berestetskaya *et al.*, 1974)), ΔT at some levels should pass through a minimum.

With respect to our results, the formation of pseudo-ring structures of DNA, (and therefore strong changes of tertiary structure of macromolecule) couldn't avoid affecting the mechanisms of nitrogen base ionization. The point is that, melting of linear and circular DNA with the same GC-content should essentially differ from each other. In particular, Belincev and Gagua, (1989) have observed that DNA from *E. coli* at ionic strength equal to 0.01 T_m and ΔT of the ring circular form differ from the ones for linear at 20°C and 15°C, correspondingly. For circular closed DNA the physics of helix-coil transition essentially differs from its linear analogue (from energetic point of view the melting of circular DNA connected with its topological features, occurs at higher temperatures than for linear polymer in the same conditions). Therefore the protonation of melted AT-pairs in circular DNA becomes more difficult, while the protonation of helical GC-pairs becomes more preferable. The saturation of parameter T_{GC} takes place at higher pH. As a result, as it follows from the formula (1), the minimum on ΔT (pH) should be shifted to higher pH, as is really observed. It is similarly possible to explain also the character of dependence T_m (pH).

To explain the effect of increasing of minimum on ΔT (pH) widening with the increase of *cis*-DDP concentration, it can be reasoned that some mechanisms of DNA platination are possibly depending on concentration of cis-DDP (Butour and Jonson, 1986, Coste *et al.*, 1999, Malinge and Leng, 1999). By increasing of concentration of *cis*-DDP the mechanisms of complex-formation vary, too. And, the simultaneous existence of several mechanisms of DNA-*cis*-DDP interaction is possible. One of the basic types of complex-formation is inter-strand bidentate interaction of *cis*-DDP with DNA. Such type of interactions result in additional tension along DNA chain. The energy of these tensions is great enough and, may result in break of DNA strands. The breaks occur inside the rings, lead to the decreasing of ratio of circular structure of DNA and hence increase the concentration of a linear fraction and restoration of peculiarities corresponding to a linear form of DNA.

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