Interaction of Platinum Compounds PtenCl₂ and [Pten Cl SO(CH₃)₂] NO₃ with DNA Molecule in Solution

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Abstract. It was demonstrated that the replacement of chlorine ion on dimethyl sulfoxide (DMSO) in the first coordination sphere of platinum changes the result of compound interaction with DNA. DMSO molecule blocks one of the active centers of the platinum compound participating in the formation of coordination bonds with macromolecule, and DNA does not substitute DMSO molecule in the platinum first coordination sphere.

Keywords: calf thymus DNA; platinum compounds; dimethyl sulfoxide; circular dichroism; hydrodynamic properties.

1. Introduction

Some platinum compounds show cytostatic activity against different tumours. The first anticancer drug successfully used in clinical practice till now [1] is cis-diamminedichloroplatinum (II) (cis-DDP) that shows high anticancer activity compared to its trans-isomer. Despite a wide clinical use of cis-DDP its disadvantages [2] call for a search of its effective substitutes among coordination platinum compounds [3]. Cis-DDP is administered intravenously. While transported to nuclear DNA the drug may interact with various compounds, for example sulfur-containing proteins [4].

We have evaluated DNA interaction in a solution with platinum compound [Pten Cl SO(CH₃)₂] NO₃, will be denoted further as Pten (DMSO). The comparison of DNA interaction with Pten (DMSO) and PtenCl₂ (denoted further as Pten) was done. Pten is an analog of cis-DDP that contains ethylenediamine as bidentate ligand in the first coordination sphere of platinum instead of two ammonia molecules. DMSO has high ability to pass through biological membranes. It is used as a solvent for many medications [5], including platinum-based drugs. The task of the research was to clarify the role of DMSO in the process of the formation of a DNA complex with a platinum compound.
2. Experimental

2.1. Materials

The molecular mass of calf thymus DNA (Sigma Aldrich) \( M = 9 \times 10^6 \) g/mol was determined with DNA intrinsic viscosity \([\eta]\) in 0.15 M NaCl. Platinum compounds have been synthesized by Dr. Spevak V. N. at St. Petersburg Technological Institute. For the evaluation of DNA complexes with platinum compounds, we have used the methods that allow monitoring DNA conformation at the level of its secondary and tertiary structure. The measurements were made at 21°C.

2.2. Flow Birefringence

Birefringence values \( \Delta n \) of DNA solutions were determined using an original device with a half-shade elliptical equalizer. It was shown earlier that the form-effect in measured DNA optical anisotropy is negligible. In this case the measured birefringence value allows determining the optical anisotropy of DNA statistical segment \((\alpha_1, \alpha_2)\) (for Kuhn model of macromolecule) [6].

\[
\frac{(\Delta n/g)}{(\eta_r-1)\eta_0} = \frac{[n]}{[\eta]} \sim (\gamma_1 - \gamma_2) \sim (\alpha_1 - \alpha_2),
\]

where \( \eta_r \) is the relative viscosity of DNA solution, \( \eta_0 \) is the viscosity of the solvent, \([n]/[\eta]\) is the relation of the DNA dynamic optical constant to the intrinsic viscosity value. The \( (\Delta n/g) \) value or \([n]/[\eta]\) ratio are proportional to the optical anisotropy of DNA \((\gamma_1 - \gamma_2)\) where \( \gamma_1 \) and \( \gamma_2 \) are its polarizabilities along the main axes (for a molecular ellipsoid model). In the absence of a form-effect \((\gamma_1 - \gamma_2) \sim (\alpha_1 - \alpha_2)\),

where \((\alpha_1 - \alpha_2)\) is the optical anisotropy of Kuhn’s segment. \((\alpha_1 - \alpha_2) = S\Delta\beta\) (\(\Delta\beta\) is base pair optical anisotropy, \( S\) - the number of monomers (base pairs) in a statistical segment). For high molecular DNA \( S = 2a/l \) (\( a \) is DNA persistent length, \( l \) is a distance between nearest base pairs along DNA axis).

2.3. Viscometry

The value of relative viscosity \( \eta_r \) of the DNA solutions was measured with Zimm-Crothers type viscometer [7]. The range of velocity gradients used was \( g \equiv (0.5 \pm 2) \) sec\(^{-1}\). The DNA intrinsic viscosity value determined by an extrapolation of the dependence of the reduced viscosity \((\eta_r - 1)/C\) on DNA concentration to \( C_{DNA} = 0 \) is related to the conformation parameters of a macromolecule by the equation:

\[
[n] = \Phi(\epsilon)(\frac{R_T^2}{M})^{3/2} \alpha^3
\]

where \( M \) is the DNA molecular mass, \( \Phi(\epsilon) \) is the Flory parameter that depends on the solvent quality and bending rigidity of the macromolecule [8], \( \alpha \) is the linear swelling coefficient for DNA molecule (ratio of the mean square distances between the ends of the real and the ideal chain in a solution):

\[
\alpha = \left(\frac{R_T^2}{k_B}\right)^{1/2}
\]
2.4. Spectroscopy

The DNA concentration in the solution was determined from the difference of the absorption at \( \lambda = 270 \) and 290 nm following hydrolysis of DNA solution with 6% HClO\(_4\) during 15 min at 100\(^\circ\) C [9]. The DNA nativity was monitored through the value of hyperchromic effect after the denaturation of a macromolecule. SF-26 spectrophotometer (Russia) was used. Circular dichroism (CD) spectra of DNA were recorded with Jobin-Yvon Mark-IV autodichrograph (France).

3. Discussion of results

DNA CD spectra in complexes with Pten and the result of their processing are shown at Fig. 1.

![Fig. 1: CD spectra of DNA at different concentrations of Pten in 0.005 M NaCl (A) and the result of their processing: wavelength (1) and maximum of positive band amplitude (2). C\(_{\text{DNA}}\) = 1.5 \times 10^{-5} M (bp).](image)

The injection of DMSO to the first coordination sphere of Pten instead one atom of chlorine significantly changes the binding of this compound to DNA molecule. The changes in DNA CD spectra as a result of Pten (DMSO) complexation with DNA differ from the changes observed after Pten (or cis-DDP) binding to DNA, DNA CD spectra are almost identical for complexes with Pten and cis-DDP (one can see the shift of positive maximum and the increase of positive band amplitude with the shift of a zero point). In contrast to observed changes, Pten (DMSO) binding to DNA causes only a decreases in positive band amplitude in DNA CD spectra without shift of maximum at any \( C_{\text{Pt}} \). Probably, the presence of DMSO in the first coordination sphere of platinum inhibits the formation of bidentate complexes responsible indicating due to the increase of amplitude of positive band and the shift of its maximum in CD spectra after DNA complexation with cis-DDP [10]. We have studied the competition of Pten (DMSO) and cis-DDP compounds for the binding site on a macromolecule. The tests have shown that after the formation of DNA-Pten (DMSO) complexes the further formation of DNA-cis-DDP complexes is not observed. At the same time, DNA-cis-DDP complexes prevent further Pten (DMSO) binding to DNA [11]. Therefore, these two platinum compounds ((Pten(DMSO) and cis-DDP) interact with DNA at the same position. Since the main binding position for cis-DDP is N7 of guanine in a major groove, we suppose that Pten (DMSO) also interacts with DNA at this site. This assumption has been confirmed in experiments with DNA protonation after complexation with Pten (DMSO). Our experiments were limited in acid region by pH values at which the distraction of the secondary structure of DNA was started (we checked DNA secondary structure by measured hyperchromism at 260 nm). So in our experiment only double stranded DNA was used. It was shown
that in 0.005 M NaCl at pH < 4.7 the bases in double-helical DNA become protonated. DNA protonation is associated with a typical change in the DNA CD spectra [11]. These changes in DNA CD spectra are not observed after DNA complexation with Pten (DMSO) at lower pH values. It proves that DNA-Pten(DMSO) complexation blocks the proton acceptor group on DNA and prevents DNA protonation. Since the double-helical DNA protonation starts at N7 guanine position, the obtained data prove that Pten (DMSO) binds to DNA at the same site.

The study of DNA hydrodynamic properties after complexation with Pten and Pten (DMSO) has shown that the concentration dependences of reduced viscosity and reduced birefringence at determination of intrinsic viscosity and dynamic optical constant value respectively do not depend on the free platinum solution concentration, which confirms the implementation of the energetically strong binding of platinum with DNA [10]. Such complex is formed after solution incubation for more than 8 hours at $t = 4 \degree C$.

It was shown that cis-DDP, Pten and Pten (DMSO) form a coordination bonds to DNA. Indeed, the study of the dependences of the intrinsic viscosity and optical anisotropy values on CPt in a solution for DNA complexes with Pten and cis-DDP (Fig. 2 (a), curves 1, 3, Fig. 2 (b), curves 1, 4) show a considerable similarity of DNA binding with these compounds. At the same time, substantial difference in Pten and Pten (DMSO) binding with DNA can be seen at evaluation of optical anisotropy of these complexes. Figure 2 (a) shows that with the growth of cis-DDP and Pten concentration in the solution the DNA optical anisotropy value ($\gamma_1 - \gamma_2$) decreases, whereas in case of growth of $C_{\text{Pt}}$ (DMSO) in the solution there is an increase (up to $C_{\text{Pt}} < 1 \times 10^{-5}$ M) and then a decrease of ($\gamma_1 - \gamma_2$) which is stopped at $C_{\text{Pt}} > 6 \times 10^{-5}$ M. We can suppose that for Pten (DMSO) at $C_{\text{Pt}} > 6 \times 10^{-5}$ M all possible DNA binding sites are filled out. Since at this range of $C_{\text{Pt}}$ the ($\gamma_1 - \gamma_2$) values and intrinsic viscosity [$\eta$] of DNA are the same for cis-DDP, Pten, Pten (DMSO). The difference in binding of Pten (DMSO) and Pten (and accordingly cis-DDP) does not show the difference in the degree of binding, but in the final structure of the formed DNA-Pt complexes. This assumption is confirmed by viscometry data (Fig. 2 (b)).

**Fig. 2:** (a) Relative change of optical anisotropy of DNA in complex with: 1 – Pten; 2 – Pten (DMSO); 3 – cis-DDP; 4 – trance-DDP in a 0.005 M NaCl solution. (b) Relative change of intrinsic viscosity of DNA in complex with: 1 – Pten; 2 – Pten (DMSO); 3 – Pten with 1 M DMSO injected in the solution; 4 – cis-DDP in a 0.005 M NaCl solution.

The analysis of optical anisotropy dependence on $C_{\text{Pt}}$ shows (Fig. 2 (a)) that the result of DNA complexation with Pten (DMSO) is closer to DNA-trans-DDP complexes that are usually monodentate [12]. Indeed, the addition of Pten (DMSO) at small $C_{\text{Pt}}$ to DNA solution leads to an increase of ($\gamma_1 - \gamma_2$) value (Fig. 2 (a), curve 2) which is also observed for DNA-trans-DDP complexes (Fig. 2 (a), curve 4). DNA binding with Pten and cis-DDP is associated with the decrease of DNA optical anisotropy (Fig. 2 (a), curve 3). At the same time, the DNA intrinsic viscosity is gradually reduced for all compounds (Fig. 2 (b)). There is also a resemblance in DNA CD spectra changes after complex formation with Pten and Pten (DMSO).

After the addition of DMSO to the solution during DNA-Pten system preparation (final DMSO concentration was 1 M) the dependence of the DNA intrinsic viscosity on the platinum concentration...
was the same as for DNA-Pten complex without DMSO (Fig. 2 (b)). Thus, the presence of free DMSO molecules in DNA solution does not affect the DNA-Pten interaction. The study of DNA interaction with cis- and trans-DDP in the presence of free DMSO was carried out by the CD method. It was also confirmed the absence of DMSO effect on the result of the complex formation. These data prove that free DMSO molecules in our experiments cannot substitute water molecules (or chlorine atoms) in the first coordination sphere of platinum. Equal change of DNA molecule volume with the increase of Pten and Pten (DMSO) concentration in the solution indicates that the amounts of platinum bound with DNA in both cases are approximately equal. The range of Pten and Pten (DMSO) concentrations (> $6 \times 10^{-5}$ M) at which the possible DNA binding sites are filled corresponds to the ratio $C_{Pt}/C_{DNA} > 0.5$ (5 platinum atoms per 10 DNA bases), which is approximately equal to the relevant assessment of cis-DDP (the maximal degree of cis-DDP binding that does not cause the destruction of DNA secondary structure, $C_{Pt}/C_{DNA} = 0.4$, $C_{DNA}$ is expressed in moles of DNA base pairs). The difference is in the manner of binding of these two platinum compounds with DNA molecule. We presume that Pten (DMSO) forms one coordination bond with N7 guanine, whereas Pten forms two bonds.

4. Conclusion

The obtained data suggest that DMSO molecule blocks one of the active centers of the platinum complex participating in the formation of coordination bonds. Our experiments have shown that DNA nitrogen group (N7 guanine) is the convenient binding site for platinum compounds. DMSO in the first coordination sphere of platinum in Pten(DMSO) prevents the formation of coordination bond with this position.

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