Liquid crystalline microphases of DNA molecules complexed with compounds of platinum(II)

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The formation of liquid crystalline microphases (0.3 M $NaClO_4$, and 120 and 170 mg PEG/ml) from low-M_r DNA (salmon sperm) complexed with *cis* and *trans* dichlorodiamine-platinum(II) was investigated. It was shown that the amplitude of the negative band in the CD spectrum, characteristic of a liquid crystalline microphase of DNA, decreased upon complexing with platinum compounds. It was estimated that the influence of *cis* Pt(II) on the optical properties of liquid crystalline microphase of DNA molecules strongly differed from the effect of *trans* Pt(II); the phenomenon did not depend on [PEG]. The reasons of the decrease of the negative band in the CD spectra of the DNA liquid crystalline microphases are discussed.

Liquid crystalline microphase of DNA *Platinum(II) complex* *Secondary structure of DNA*

1. INTRODUCTION

The molecules of double-stranded nucleic acid of low-M_r are known to form liquid crystalline microphases in water–salt solutions containing poly(ethyleneglycol) (PEG) [1,2]. A peculiarity of the liquid crystalline microphases of DNA is the presence of intense bands in the circular dichroism (CD) [3,4] or optical rotation dispersion [5] spectra in the absorption region of nitrogen bases (λ_{max} ~260 nm). Following theoretical conceptions, high optical activity of liquid crystalline microphases can result either from the helical twist of neighbouring DNA molecules (in this case a cholesteric liquid crystal is formed from rigid linear DNA molecules) or specific orientation of nitrogen bases in the DNA molecules with respect to a director of microphase (in this case the formation of a cholesteric liquid crystal from the DNA molecules is not required for high optical activity).

In order to elucidate the connection between the orientation of DNA nitrogen bases and the optical properties of liquid crystalline microphases, the study of the spectra of microphases formed in PEG-containing solutions from DNA molecules that were modified by protonation, methylation or complex formation with different antibiotics was initiated in [6–8]. It is shown that methylation and protonation of nitrogen bases leads to a sharp diminishing of the amplitude of the negative band in the CD spectrum whereas the binding of antibiotics to DNA is accompanied by differences in the character changes of the CD spectra. It is also noticed that the mode of packing of DNA molecules in a liquid crystalline microphase probably remains constant and does not depend on peculiarities of the secondary structure of DNA [9].

The present work was undertaken with the purpose to develop an approach for the study of relations between the orientation of the DNA nitrogen bases and peculiarities of the circular dichroism spectra of liquid crystalline microphases and to examine the formation of microphases from DNA molecules complexed with *cis* and *trans* isomers of dichlorodiamineplatinum(II) (*cis* Pt(II) and *trans* Pt(II)). Our interest in platinum compounds was predetermined not only by different space

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geometry of these isomers but also by dissimilar biological activities [10–12].

2. MATERIALS AND METHODS

The salmon sperm DNA (SKTB, Novosibirsk) was used after additional purification. After ultrasonic depolymerization of high-M_r DNA (4°C; 50 s; 22 kHz; desintegrator UZDN-4, USSR) the preparation of low-M_r DNA was obtained. The M_r-value of the DNA was estimated by gel electrophoresis (0.6% agarose). The concentration of DNA was determined by the absorption of solutions (λ = 260 nm, ϵ_p = 6600).

The *cis* Pt(II) preparation (Institute of Biophysics, Czechoslovak Academy of Sciences), the *trans* Pt(II) preparation (Institute of General and Inorganic Chemistry, USSR Academy of Sciences) and PEG preparation of M_r 4000 (Loba Chemie) were used without purification.

The DNA, PEG, *cis* Pt(II) and *trans* Pt(II) solutions were prepared using 0.3 M $NaClO_4$ (pH 5.8) as solvent. The solutions of platinum compounds were used following technique [13] not earlier than 24 h after preparing them. The concentration of platinum was estimated by the plasma-induced emission spectroscopy using Jobin-Yvon 38 emission spectrometer [14].

The DNA–platinum complexes were prepared by mixing the DNA and *cis* Pt(II (or *trans* Pt(II)) solutions; the reaction mixtures were stored within 3 days at 37°C in the dark as suggested in [13]. The concentration of DNA in solution was constant (~3 × 10^{-5} M nucleotides), the concentration of platinum varied. Value r (i.e., the ratio of the molar concentration of platinum compounds to the molar concentration of nucleotides in solution) was varied from 0.005 to 1.

The liquid crystalline microphase was formed by intense mixing of solutions of DNA (or that of the DNA–Pt(II) complex) with PEG-containing solutions (3 min; Chirana mixer). The concentration of condensed DNA molecules in PEG-containing solutions was estimated in all the cases by sedimentation in a low-speed centrifuge K 23 (6000 rev./min, 60 min, 4°C). The absorption spectra were registered by a Specord M 40 spectrophotometer; the CD spectra by a Jobin-Yvon Mark III dichrograph; the measurements being taken 24 h after the microphase was prepared (1-cm cells were used).

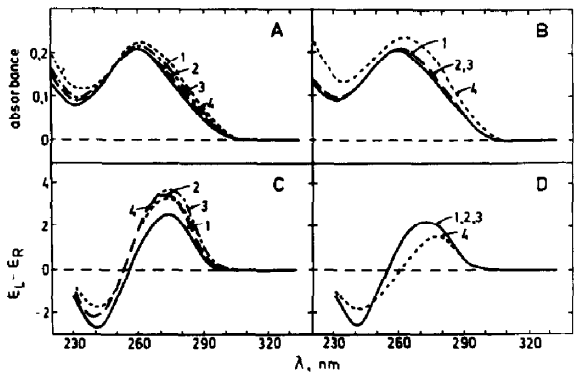


Fig.1. The absorption and the CD spectra of the linear DNA molecules complexed with *cis* Pt(II) (A and C, respectively) and *trans* Pt(II) (B and D) (0.3 M $NaClO_4$). r: 1–0; 2–0.05; 3–0.1; 4–0.5.

3. RESULTS

Fig.1A illustrates the absorption spectra of DNA after binding with *cis* Pt(II); fig.1B shows the same for *trans* Pt(II). The CD spectra that correspond to the DNA–*cis* Pt(II) and DNA–*trans* Pt(II) complexes are presented in fig.1C,D. One can see that although the DNA absorption spectra change upon binding with the platinum compounds, these changes are not specific. It should be noted that at a high concentration of Pt ($r \geq 0.5$) a 10% hyperchromic effect was observed, the absorption maximum being shifted from 258.5 to 262 nm. The changes of the CD spectra upon DNA-binding with Pt(II) are more specific. Particularly in the CD spectrum of the DNA molecules complexed with *cis* Pt(II) (fig.1C) we observed an increase of the positive band ($\lambda \sim 275$ nm) with an increase of r from 0.005 to 0.1, whereas the further increasing of r involved the diminishing of the amplitude of the band. The amplitude of the negative band ($\lambda \sim 240$ nm) decreased with increasing r-values. In the CD spectrum of the DNA molecules complexed with *trans* Pt(II) (fig.1D) we noticed a decrease of the amplitude of the bands at 275 and 240 nm while r was increasing.

Fig.2 (A,B) compares the CD spectra of liquid crystalline microphases from free DNA with the CD spectra of microphases formed from DNA molecules complexed with *cis* Pt(II) and *trans*

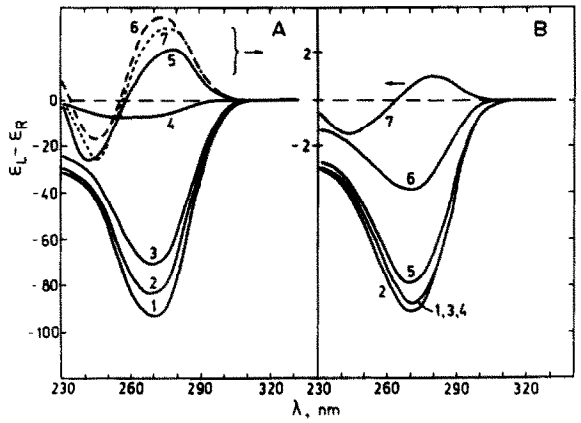


Fig.2. The CD spectra of microphases formed from DNA–*cis* Pt(II) (A) and DNA–*trans* Pt(II) (B) complexes (120 mg PEG/ml; 0.3 M $NaClO_4$). *r*: 1–0; 2–0.005; 3–0.01; 4–0.02; 5–0.03; 6–0.1; 7–0.5.

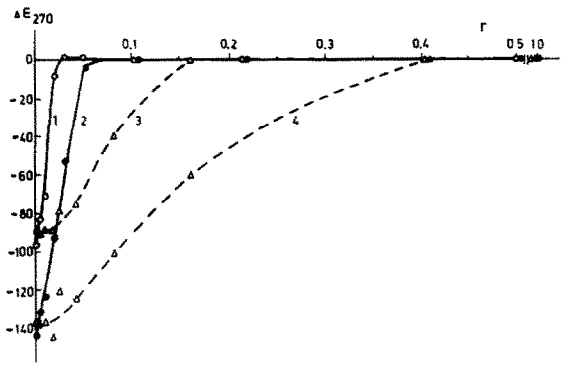


Fig.3. The dependence of the amplitude of the band in the CD spectra of microphases formed from DNA–*cis* Pt(II) (curves 1,2) and DNA–*trans* Pt(II) (curves 3,4) complexes *vs r*; (1,3) 120 mg PEG/ml; (2,4) 170 mg PEG/ml.

Pt(II). One can see that the DNA microphase forming in the PEG-containing (120 mg/ml) solution has an intense negative band (see curve 1 in fig.2A,B). In the CD spectra of the liquid crystalline microphase of DNA molecules complexed with *cis* Pt(II), the amplitude of the negative band (λ ~ 270 nm) diminishes as platinum binds (*r*, 0.005–0.02) to DNA (curves 2–4, fig.2A). The attention is drawn to the fact that at a definite level of binding ($r \geqslant 0.03$, curves 5–7, fig.2A) the CD spectra of the microphases that are formed from DNA molecules complexed with *cis* Pt(II) do not differ in their shapes from the CD spectrum typical for linear non-condensed DNA molecules.

In the CD spectra of the liquid crystalline microphases of the DNA–*trans* Pt(II) complexes (curves 2–7, fig.2B) one can observe a decrease of the amplitude of the negative band (λ ~ 270 nm) as *trans* Pt(II) binds to the DNA.

If the microphases are prepared at a higher concentration of PEG (170 mg/ml) the alteration of the amplitude of the negative band in the CD spectra of the microphases with the increase of the platinum concentration is similar in character to the alteration observed above.

Fig.3 illustrates the changes in amplitude of the negative bands ($\Delta\epsilon_{270}$) in the CD spectra of microphases formed from DNA molecules bound into complexes with *cis* Pt(II) and *trans* Pt(II) depending on the content of Pt(II) in the solutions. It is seen that the amplitude of the intense negative band typical for the liquid crystalline microphase of free DNA diminishes as platinum binds to DNA. It should also be noted that the value of *r* at which the negative band is absent depends on the PEG concentration. In particular, the liquid crystalline microphase formed from the DNA–*cis* Pt(II) complexes at 120 mg PEG/ml does not have a negative band in the CD spectra at $r > 0.02$ (curve 1, fig.3), and at $r > 0.05$ in the case of microphase formed at 170 mg PEG/ml (curve 2, fig.3). For the microphases formed from DNA–*trans* Pt(II) complexes the value of *r* at which the negative band is absent in the CD spectrum exceeds significantly a similar value of *r* for the DNA–*cis* Pt(II) complexes. For microphases formed from DNA–*trans* Pt(II) complexes at 120 mg PEG/ml the negative band in the CD spectrum is absent at $r \geqslant 0.16$ (curve 3, fig.3), and at 170 mg PEG/ml it is absent and at $r \geqslant 0.4$ (curve 4, fig.3).

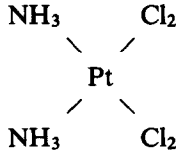
Thus the data obtained indicate that the optical properties of a liquid crystalline microphase of DNA can serve as a criterion that allows to differentiate the properties of the DNA–platinum complexes.

4. DISCUSSION

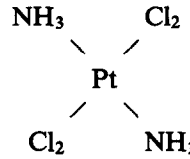
The changes in the absorption and in the CD spectra of linear DNA molecules upon formation of complexes with both *cis* Pt(II) and *trans* Pt(II) coincide with the data obtained in [15,16], which

prove that the binding of platinum compounds to DNA is accompanied by a local destabilization of the DNA secondary structure. This appears to be the result of interaction of platinum with nitrogen bases. The dissimilar character of changes of the CD spectra of linear DNA molecules complexed with *cis* Pt(II) and *trans* Pt(II) is attributed to the different types of the complexes formed [16,17].

The disappearance of the negative band in the CD spectra of microphases formed from DNA molecules complexed with platinum compounds can be reasoned as follows. Firstly, the decrease of the amplitude of the negative band may reflect the fact that the complexing of the DNA molecules is accompanied by the diminishing of the concentration of the DNA molecules, which are capable of forming a liquid crystalline microphase. However, sedimentation of microphases formed from complexes of DNA with platinum compounds in a low-speed centrifuge demonstrates a nearly constant concentration of precipitated DNA molecules. Therefore, this explanation is not valid. Secondly, the decrease of the negative band in the CD spectra of microphases of DNA–Pt(II) complexes can reflect the fact that interaction of Pt(II) with nitrogen bases is accompanied by the alteration of the DNA secondary structure. Indeed, according to data in [15–19], *cis* Pt(II) and *trans* Pt(II) when interacted with DNA nitrogen bases may induce disturbances in the stacking interaction of bases and in hydrogen bonding between guanine and cytosine residues; they may also form links between two nucleotide residues in one or in both DNA strands. As a result of such intrastrand cross-linking, sites appear whose structures are dissimilar to those of native DNA. A different influence of *cis* Pt(II) and *trans* Pt(II) on intensity of the band in the CD spectra of the DNA microphases, which correlates with differences in biological activity [10–12], is obviously predetermined by the fact that *cis* Pt(II) interacts with nitrogen bases more effectively than *trans* Pt(II). As in [17] it can be thought that the *cis* isomer (with geometrical configuration



and a 3.2 Å distance between the Cl-atoms which equals that between nitrogen bases) forms both intra- and interstrand links. In the case of the *trans* isomer (with geometric configuration



and a 4.6 Å distance between the Cl-atoms) the formation of links is less probable.

Thus, the data obtained prove that in PEG-containing solutions (120 and 170 mg/ml) the formation of a liquid crystalline microphase from DNA molecules with an altered secondary structure is accompanied by a decrease of the optical activity of the microphases. It is presently being solved if the helical ordering of the neighboring DNA molecules in microphase in PEG-containing solutions disappears; i.e., instead of the cholesteric liquid crystal, a crystal of a nematic type is formed.

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