Quantitative studies of DNA binding with trans complexes of Pt^{II} and Pd^{II} featuring tetrazolylacetic acids and their derivatives as ligands*

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The interaction of calf thymus DNA (CT DNA) with *trans*-$[PtCl_2L_2]$ and *trans*-$[PdCl_2L_2]$ complexes (L — 5-methyl-1*H*-tetrazol-1-ylacetic acid and its ethyl, butyl, and isobutyl esters; ethyl esters of 2-R-2*H*-tetrazol-5-ylacetic acids, R = Bu^t, CH_2CH_2OH) have been quantitatively studied by a spectrophotometric method. The binding constants (K_b) of the tetrazole-containing coordination compounds with CT DNA at pH 7.2 and 298 K are in the range of $(3.67—5.93) \cdot 10^5$ mol L^{-1} for the Pd^{II} complexes and $(7.82—13.30) \cdot 10^5$ mol L^{-1} for and the Pt^{II} complexes, respectively. The K_b values, as well as negative values of ΔG_b (−(31.7—34.9) kJ mol^{-1}) indicate the effective binding between the studied complexes and CT DNA.

Key words: Pt^{II} and Pd^{II} complexes, tetrazoles, tetrazolylacetic acids, DNA binding constants.

A number of platinum complexes (cisplatin, carboplatin, nedaplatin, oxaliplatin, *etc.*) are generally accepted drugs, which are widely used for treatment of oncological diseases.[1] In recent years there has been an increasing number of publications on directed synthesis and studies of structural features, physical and chemical properties, and biological activity of complexes with nitrogen-containing heterocyclic fragments as ligands.[2,3] Tetrazole-containing complexes of platinum group metals are considered as promising objects of medicinal chemistry.[4] It is known that the introduction of a tetrazolyl fragment into a molecule of a biologically active substrate often improves its efficacy, increases the action duration, and decreases the acute toxicity.[5—7]

Among the various tetrazole derivatives, particular attention should be paid to tetrazolylacetic acids, as these could be considered as analogues of natural amino acids, where the amino group is substituted by the tetrazolyl moiety. Coordination compounds of transition metal ions with such ligands can have optimal solubility, lipophilicity, and pronounced biological activity as well as participate in various intermolecular interactions.[8] Thus, in several cell lines, *trans*-$[PtCl_2$(ethyl 2-*tert*-butyl-2*H*-tetrazol-5-ylacetate$)_2]$ complex exhibited cytostatic activity comparable to cisplatin: IC_{50} values in HT-29, MCF-7, MDA-MB-231, and RC-124 cell lines were, respectively, equal to 14.2, 5.8, 11.02, and 5.86 μmol L^{-1}.[9,10] We have earlier synthesized and characterized a series of coordination compounds of Pt^{II} and Pd^{II} with tetrazolylacetic acids and their derivatives.[9—11] Despite the obvious potential benefits of the studies related to methods of synthesis of these coordination compounds, their structure, and biological properties, there is still a lack of sufficient research in this area.

Various tetrazolylacetic acid derivatives containing a carboxymethyl group at 1- and 5-positions of the tetrazole ring were selected as model compounds for our study. These ligands form stable complexes with platinum group metal ions, which can be considered as potential cytostatics.[10]

DNA molecules are known to be the main biological target for antitumor drugs based on coordination compounds of platinum group metals.[3,12—14] According to our earlier experimental and theoretical data, the Pt^{II} and Pd^{II} complexes with tetrazolylacetic acids and their esters as ligands interact with DNA, and most likely the interaction occurs *via* minor groove binding mechanism.[10] Nevertheless, no quantitative studies of the efficiency of such interactions had yet been carried out.

In the present work, the interaction of *trans*-$[PtCl_2L_2]$ and *trans*-$[PdCl_2L_2]$ complexes (L — 5-methyl-1*H*-tetrazol-1-ylacetic acid and its ethyl, butyl, and isobutyl esters (**1—5**); ethyl esters of 2-R-2*H*-tetrazol-5-ylacetic acids, R = Bu^t, CH_2CH_2OH (**6—9**)) with the calf thymus DNA (CT DNA) have been quantitatively studied by means of UV spectroscopy. The values of binding constants (K_b) and changes in binding free energy (ΔG_b) were determined.

The introduction of transition metal complexes into DNA solutions can lead to changes in the band shape of the electronic absorbance spectra due to the DNA interaction with the coordination compounds.[3,12,13,15]

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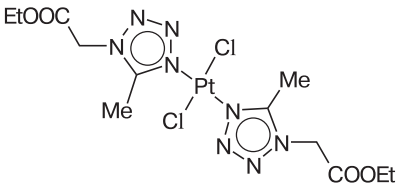

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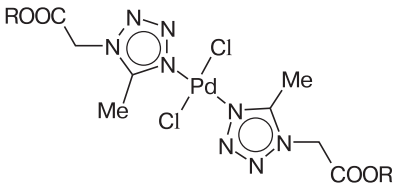

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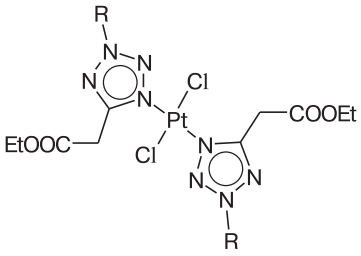
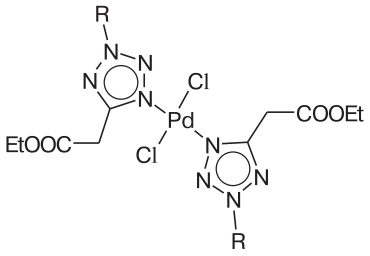


6, **7**



8, **9**

R = H (**2**), Et (**3**), Bu (**4**), Bu^{i} (**5**), Bu^{t} (**6**, **8**), CH_2CH_2OH (**7**, **9**)

The nature of these changes strongly depends on the interaction type. Thus, the decrease in the optical density of solutions is observed in case of intercalation. This decrease usually causes a batho- or hypsochromic shift of a spectral band. At the same time, groove binding can lead to a hyperchromic effect.[12,13]

UV absorption spectra of aqueous DNA has a characteristic peak with maximum at 260±3 nm. In the present study, the changes in the absorption band of CT DNA were used to monitor the interaction of complexes **1**—**9** with a double helix. The absorption spectra of CT DNA in a buffer solution (50 m*M* NaCl/50 m*M* tris-(hydroxymethyl)aminomethane—HCl; pH 7.2) at various concentrations of CT DNA and constant concentration of complex **3** are shown in Fig. 1. Similar spectra were obtained for all the compounds **1**—**9**. The spectral data was further used to determine binding constants (K_b) of complexes with CT DNA. Changes in the spectral behavior of solutions of CT DNA mixtures with complexes at various concentrations can be most clearly detected when analyzing the calculated and normalized absorption spectra (Fig. 2), displaying a pronounced bathochromic shift ($\Delta\nu = 442$ cm^{-1}). According to the known literature data, such a shift indicates the existing association between the investigated metal complexes and the biopolymer.[12] The association can be quantitatively described by the binding constants (K_b).[12]

A number of publications[12,16—18] describe methods for calculation of K_b values based on the spectral data. Using these approaches, the K_b values for metal complexes **1**—**9** were derived from Eq.:

$$[DNA]/(\varepsilon_a - \varepsilon_f) = [DNA]/(\varepsilon_b - \varepsilon_f) + 1/(K_b(\varepsilon_b - \varepsilon_f)),$$

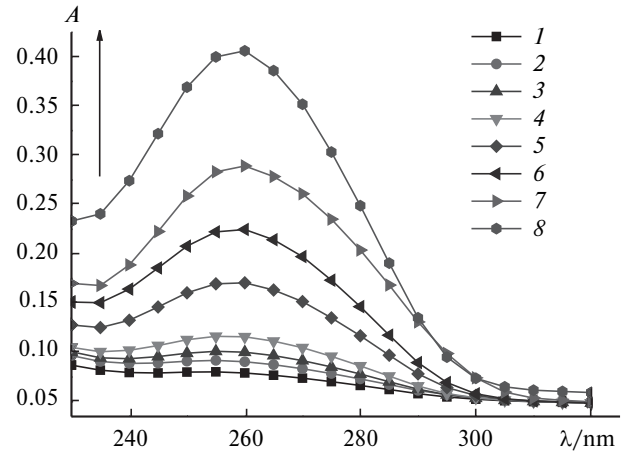


Fig. 1. UV absorption spectra of CT DNA at its various concentrations (1.36, 2.27, 3.20, 4.55, 6.82, 9.10, 18.2, and 27.3 μmol L^{-1}) in the presence of complex **3** (4.55 μmol L^{-1}) in a buffer solution (50 m*M* NaCl/50 m*M* Tris-(hydroxymethyl)aminomethane—HCl (pH 7.2)) at stoichiometric ratios r = [DNA] : [complex] = = 0.3 (*1*), 0.5 (*2*), 0.7 (*3*), 1.0 (*4*), 2.0 (*5*), 3.0 (*6*), 4.0 (*7*), and 6.0 (*8*).

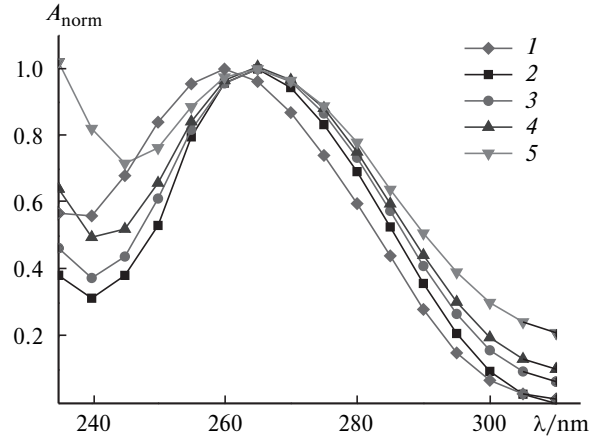


Fig. 2. Calculated and normalized CT DNA absorption spectra in the presence of complex **3** ($A_{norm} = [A_{observed} - A_{bind}] \cdot [A_{max}]^{-1}$) at stoichiometric ratios r = [DNA] : [complex] = 0 (*1*), 1 (*2*), 2 (*3*), 4 (*4*), and 6 (*5*).

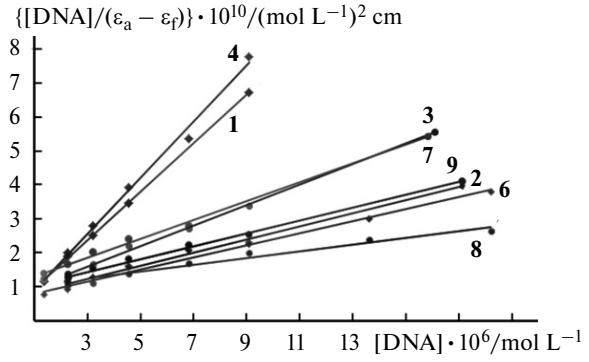


Fig. 3. Linear dependence of [DNA]/($\varepsilon_a - \varepsilon_f$) on [DNA] for complexes **1—9** ($r > 0.98$ in all cases).

where [DNA] is DNA concentration; K_b is binding constant; ε_a is observed molar extinction coefficient ($A_{observed}$/[M]); ε_f and ε_b are, respectively, molar extinction coefficients of metal complex (M) non-bound and completely bound to DNA. As can be seen from Fig. 3, the dependence of [DNA]/($\varepsilon_a - \varepsilon_f$) on [DNA] for complex **3** is linear and has a high coefficient of linear regression. The linearity of this dependence is observed for complexes **1**, **2**, **4—9** as well.

The values of binding constants and changes in binding free energy of metal complexes with CT DNA ($\Delta G_b = -RT\ln K_b$) are given in Table 1.

The obtained K_b values for tetrazole-containing complexes of platinum(II) and palladium(II) indicate a strong interaction between these complexes and CT DNA. At the same time, platinum(II) complexes (**1**, **6**, and **7**) exhibit a somewhat higher affinity for binding to DNA than palladium(II) complexes (**2—5**, **8**, **9**). This corresponds to the data earlier obtained for platinum(II) and palladium(II) coordination compounds with other heterocyclic ligands.[19] The values of K_b and ΔG_b indicate possible spontaneous formation of stable associates of metal complexes **1—9** with DNA.[20] In view of the above, complexes **1—9** can be considered as potential cytostatics acting *via* mechanism of binding to a DNA molecule.

Table 1. Values of binding constants (K_b) and changes in the binding free energy of complexes **1—9** with CT DNA (ΔG_b)

Compound	K_b/L mol^{-1}	ΔG_b/kJ mol^{-1}
1	$1.33 \cdot 10^6$	−34.9
2	$4.64 \cdot 10^5$	−32.3
3	$5.68 \cdot 10^5$	−32.8
4	$5.02 \cdot 10^5$	−32.5
5	$4.80 \cdot 10^5$	−32.4
6	$7.82 \cdot 10^5$	−33.6
7	$8.82 \cdot 10^5$	−33.9
8	$3.67 \cdot 10^5$	−31.7
9	$5.93 \cdot 10^5$	−32.9

Experimental

Tetrazole-containing complexes **1—9** were prepared and characterized according to the previously described procedure.[10,11] The commercially available CT DNA (Sigma) was used in the study. UV absorption spectra of solutions of complexes **1—9** and CT DNA in the range of 220—320 nm were recorded on Shimadzu UV 2401 PC and Shimadzu UV-1800 spectrophotometers using quartz cuvette (l = 1 cm). The DNA concentration was determined by Spirin's method, *i.e.* from the difference in the absorbance of its hydrolyzed solutions in 4% $HClO_4$ at 270 and 290 nm.[21] The stock solution of CT DNA was stored at 278 K and used for four days from the date of preparation. The working solutions were obtained by mixing the solutions of CT DNA and corresponding complexes at room temperature. For all the studied metal complexes and working solutions of DNA, the validity of Lambert—Bouguer—Beer law was verified in the concentration range of 1.36—27.3 μmol L^{-1} for DNA solutions and 4.55—36.4 μmol L^{-1} for solutions of complexes, respectively. The spectral experiments were carried out in a buffer solution of 50 m*M* tris-(hydroxymethyl)aminomethane—HCl (Tris—HCl) (pH 7.2)/50 m*M* NaCl.

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