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*Nataliia Shtemenko [a, b], Oleksandr Holichenko [a], Olexandr Shtemenko [a]*

# COMPARATIVE INVESTIGATION OF INTERACTION OF THE DIRHENIUM(III) CLUSTER COMPOUND WITH DIFFERENT NUCLEOTIDE CONTENT DNAs

**[a] Ukrainian State University of Chemical Technology, Dnipro, Ukraine**
**[b] Oles Honchar Dnipro National University, Dnipro, Ukraine**

Comparative investigation of interaction of bis-dimethylsulfoxide-cis-tetrachlorodi-μ-pivalatodirhenium(III) (I) with two eukaryotic DNAs with different nucleotide content was performed by the method of UV-titration. Eukariotic deoxyribonucleic acids from calf thymus (CT DNA, 40% GC) and from herring sperm (HS DNA, 44% GC) were the matter of investigation. We observed an increase in the absorption of both DNAs with increasing the concentration of added solutions of I. Electronic absorption spectra traces of both examples of DNA exhibited pronounced hyperchromism in the presence of increasing amounts of I. The calculated binding constants were $K_{bCT\ DNA}$=2221 l/mol and $K_{bHS\ DNA}$= =3238 l/mol. Hyperchromicity of the complexes DNA-I was different for the investigated DNAs (14.413% and 81.943% for CT DNA and HS DNA, respectively). The conclusions was made that addition of I in low concentrations to DNA led to formation of complexes DNA-I with following unwinding or unfolding of the helix that did not reach 50% of unfolding. At high concentrations of I, the preferable interaction took place with guanine; interaction of I with guanine nucleotides led to significant (more than 50%) of unfolding of guanine rich sequences. I was sensitive to the nucleotide content of DNA in high concentrations and strongly reacted with guanine reach sequences with selective and mighty unfolding abilities.

**Keywords:** dirhenium(III) complex, quadruple bond, DNA, binding constant, hyperchromism.

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## *Introduction*

Synthesis, X-ray crystal structure, in vivo tumor growth suppression and nephroprotective activity of bis-dimethylsulfoxide-cis-tetrachlorodi-μ-pivalatodirhenium(III), cis-$Re_2[(CH_3)_3CCOO]_2Cl_4 \cdot 2(CH_3)_2SO$ (I) was previously shown [1]. The interactions of I with calf thymus DNA (CT DNA) were also investigated by electronic absorption titrations, ΔT melting and viscosity measurements, which indicated that compound I interacted relatively strong with the DNA (Kb $2.2 \cdot 10^3$ $M^{-1}$), most likely by forming covalently bound complexes. In that study the interaction of I with plasmid DNA was investigated by electrophoresis mobility shift assays, and was assigned as kinking and unwinding of super coiled bacterial DNA; moreover, DNA cleavage by I was enhanced in the presence of redox active species. The *in vivo* antitumor activity of I was considerable and was accompanied by significant elimination of red blood cells and kidney damages. In combination with cisplatin, I (combined Re–Pt antitumor system) led to suppression of tumor growth or complete tumor elimination. The antihemolytic and nephroprotective abilities of I only or as a part of the Re–Pt antitumor system were established. Further our works devoted to elucidation of the possible mechanism of anticancer and DNA-binding activity of the dirhenium(III) cluster compounds [1–4] brought a lot of new information. For example, some dirhenium(III) clusters were shown to be mighty G4 quadruplex (guanine-rich non-canonical DNAs) binders [4], that on the one hand opens an explanation of the anticancer properties of these compounds and on the other hand underlines

importance of the further investigations of the selective DNA–rhenium clusters interactions.

From this point of view, comparative investigation of interaction of I with different nucleotide content DNAs, that is the aim of the present work, is topical and could bring some new data to the item described above.

***Materials and methods***

Bis-dimethylsulfoxide-cis-tetrachlorodi-μ-pivalatodirhenium(III), cis-$Re_2[(CH_3)_3CCOO]_2Cl_4 \cdot 2(CH_3)_2SO$ (I) was synthesized according to procedure described elsewhere [1]. Eukariotic deoxyribonucleic acids from calf thymus (CT DNA) and from herring sperm (HS DNA) were purchased from Sigma (USA). Content of guanine-cytosine (GC) pairs of nucleotides was 40% in CT DNA and 44% in HS DNA according to Sigma certificates.

*DNA binding titration experiments and constant measurements*

Absorption measurements were performed on a Hewlett-Packard diode array spectrophotometer (HP 8453). The buffered DNA solutions that were used exhibited a ratio of 1.8:1 for the absorptions at 260 and 280 nm, indicating that the DNA was sufficiently free from proteins. Binding titration experiments were performed according to ref. [1]. The DNA binding constant, $K_b$ ($M^{-1}$), was obtained by ыtting the titration data according to the following equation

$$1/(A-A_0)=1/A_0+1/[K_b \times A_0 \times C(complex)],$$

after plotting $1/(A-A_0)$ vs. 1/C(complex) dependence and recording the slope and intercept of the resulting curve, where $A_0$ and A are the absorption values before and after adding the complex, respectively, and C (complex, mol/l) is the concentration of the added complex.

*Estimation of hyperchromism*

Hyperchromism (H) was calculated according to the following formula

$$H=[(A_{bound}-A_{free})/A_{bound}] \times 100\%,$$

where $A_{free}$ is the absorbance of free DNA, and $A_{bound}$ is the absorbance of DNA bound by complex compound [5].

***Results and discussion***

GC-content (or guanine-cytosine content) is the percentage of nitrogenous bases in a DNA or RNA molecule that are either guanine (G) or cytosine (C) and indicates the proportion of G and C bases in the whole DNA molecule. Each GC base pair is held together by three hydrogen bonds G≡C, while AT (adenine-thymine) base pairs are held together by two hydrogen bonds A=T. GC-pairs are more stable than AC; however, the hydrogen bonds themselves do not have a particularly significant impact on molecular stability, which is instead caused mainly by interactions of base stacking [6]. There are some data about dependences of thermostability [6,7] or intensity of autolysis of DNA [8] on their GC-content.

Structural formula of I is presented in Fig. 1.

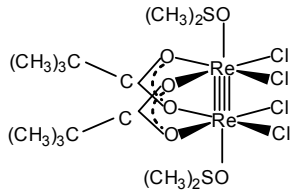


Fig. 1. Structure of cis-$Re_2[(CH_3)_3CCOO]_2Cl_4 \cdot 2DMSO$

The choice of the compound I for realization of the determined goal of investigation is grounded on several following points:

(1) anticancer activity *in vivo*, described in the introduction section;

(2) rather strong interaction with CT DNA;

(3) stability of I in aquatic solutions: our data showed that by comparison of stability of dirhenium(III) clusters, we established the following dependences [9]: we observed that with increasing the length of alkyl group in cis-dicarboxylates and with increasing the branching of alkyl groups, the rate of hydrolysis decreased; we concluded that this dependence was connected with an increase in positive induction effect of carboxylic acid residue and, as a consequence with strengthening of the quadruple bond Re–Re. Hence, I was approximately in twice more stable as compared, for example, with diacetate, dipropionate or dibutyrate complexes;

4) recent experiments with non-canonical DNAs showed that I interacted selectively with this guanine-rich sequences which is very important for anticancer interventions. Thus, investigation of interaction of the cluster dirhenium(III) compounds with different DNAs may confirm their ability to selectively recognize different nucleotide differences. Changes in the infrared micro-spectroscopic characteristics of CT DNA and HS DNA caused by cationic elements and proteins were shown in details elsewhere [7].

According to the procedures described in the section «Materials and methods», the interaction between CT DNA, HS DNA and I was investigated in the zone 220–270 nm. The results are presented

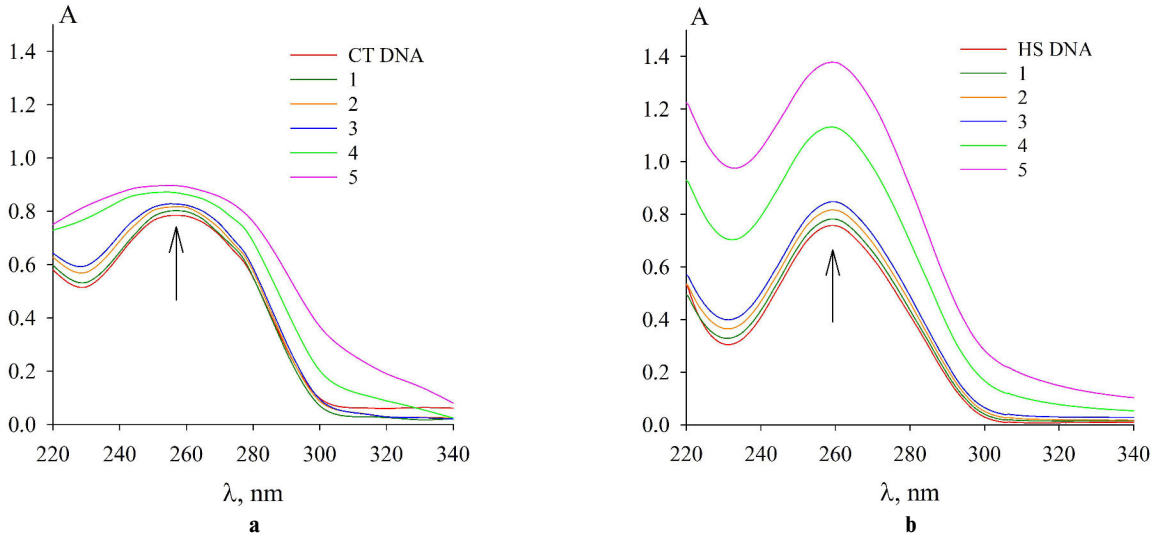


Fig. 2. UV-Vis titration spectra in the region 220−340 nm of CT DNA (a) and HS DNA (b) (1 μM, constant concentration) with Re(III) cluster compound (0−20 μM) in cacodylate buffer: (1) − 1 μM complex; (2) −2 μM complex; (3) − 3 μM complex; (4) − 10 μM complex; and (5) − 20 μM complex

**Absorption in the area 260 nm (A) and calculated hyperchromism (H,%) of CT DNA and HS DNA upon addition of I**

| Volume of added I (μl) | A | H,% | Volume of added **I** (μl) | A | H,% |
|---|---|---|---|---|---|
| CT DNA | | | HS DNA | | |
| – | 0.784 | – | – | 0.7576 | – |
| 1 | 0.801 | 2.168 | 1 | 0.7821 | 3.234 |
| 2 | 0.816 | 4.082 | 2 | 0.8167 | 7.801 |
| 3 | 0.828 | 5.612 | 3 | 0.8477 | 11.893 |
| 10 | 0.872 | 11.224 | 10 | 1.1318 | 49.393 |
| 20 | 0.897 | 14.413 | 20 | 1.3784 | 81.943 |

in Table and in Fig. 2.

According to the obtained data, we observe an increase in the absorption of both DNAs with an increase in the volume of added solutions of I, which was predictable. The arrows in Fig. 2 show the intensity changes corresponding to an increase of I concentration. Electronic absorption spectra traces of both examples of DNAs exhibited pronounced hyperchromism in the presence of increasing amounts of I.

The DNA band at ~260 nm arises from the $\pi-\pi^*$ transitions of the nucleic acid bases. Changes in the intensity of this characteristic band reflect the corresponding structural modifications of DNA, which include changes in stacking, disruption of the hydrogen bonds between complementary strands, covalent binding of the DNA bases, intercalation of aromatic rings and others. Earlier, we proposed that there occurs the covalent binding of I with concomitant unwinding of the DNA strands with outcome of nucleic acids from stacking interactions [1]. These events are the most plausible from our point of view, but other mechanisms could be possible too.

In the first points of the experiment ((2)−(4) in Table and (1)−(3) in Fig. 2,a,b), an increase in the intensity of absorption of both DNAs are practically equal. However, on the last points ((5) and (6) in Table and (4) and (5) in Fig. 2,a,b), the absorption of complexes DNA−I of HS DNA is by 1.32 and 1.54 times greater than complexes DNA-I of CT-DNA. The calculated binding constants are $K_{bCT\ DNA}$=2221 l/mol and $K_{bHS\ DNA}$=3238 l/mol, the difference is in 1.46 times. The values of hyperchromism in these points for HS DNA are by 4.46 and 5.68 greater than for CT DNA, correspondingly. All these data witness about possibility of I to react selectively with DNA depending from its nucleotide content in the region of high concentrations of I.

The influence of nucleotide composition of DNAs on the absorption in the UV area 260 nm under interaction with different metal ions was studied [10,11]. It was shown that intensity of

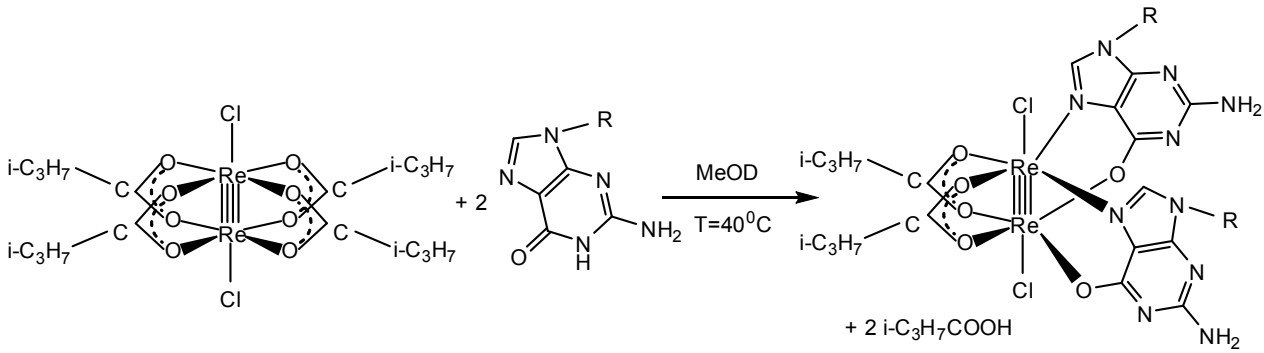


Fig. 3. Reaction between $Re_2(i\text{-}C_3H_7COO)_4Cl_2$ and alkyl (R) – guanine, R=Me, Et [15]

absorption of complex CT DNA–$Zn^{2+}$ was lower than HS DNA–$Zn^{2+}$ practically by 1.5 times. That was explained by guanine–metal ion interactions, which are much more intensive than adenine-metal ion. This fact was explained by electron-donating properties of nucleic bases and GC pairs [12,13].

Nucleic acids absorb light at 260 nm because of the electronic structure in their bases, but when two strands of DNA come together, the close proximity of the bases in the two strands quenches some of this absorbance. When the two strands separate, this quenching disappears and the absorbance rises by 30–40% usually when 50% of the DNA are separated. This is called hyperchromicity [14]. The hyperchromicity is the effect of stacked bases in a double helix absorbing more ultraviolet light as the double-stranded DNA unfolds. In our experiments, CT DNA does not reach the level of 50% unfolding in the 5 and 6 points under influence of I, but HS DNA that is richer only on 4% of GC pairs, was unfolded in the 5 point of the experiment (49%) and at the 6 point the hyperchromicity reached 81% that was not usual for known examples of titration of any DNA [14]. Hence, dirhenium(III) cluster compounds were not only sensitive to the nucleotide content of DNA, but strongly react with guanine reach sequences with selective and mighty unfolding abilities.

In our previous work, we confirmed the binding of two 9-methylguanine (or 9-ethytlguanine) and 9-methyladenine (or 9-ethyladenine) bases per dirhenium unit in a bidentate fashion in equatorial positions via sites N7/O6 and N1/N6, respectively, with concomitant nucleophilic substitution of two carboxylate groups to form a single isomer [15] (Fig. 3).

In that work, the reaction was studied with the dirhenium(III) tetracarboxylate, representative of another structural type of dirhenium(III) clusters. But the same or analogical reaction may take place with dicarboxylates of dirhenium(III) due to their mightier activities in reactions of nucleophilic substitution [1].

***Conclusions***

Taking altogether, it is possible to propose the next consequences of the events: addition of I in low concentrations to DNA lead to formation of complexes DNA–I with following unwinding or unfolding of the helix that does not reach 50% of unfolding; under high concentrations of I the preferable interaction takes place with guanine; interaction of I with guanine nucleotides lead to significant (more than 50%) of unfolding of guanine rich sequences. The obtained data may be useful in the search for selective guanine-rich DNA binders, which are perspective potential anticancer and antiviral substances.

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## ПОРІВНЯЛЬНЕ ДОСЛІДЖЕННЯ ВЗАЄМОДІЇ КЛАСТЕРНОЇ СПОЛУКИ ДИРЕНІЮ(III) З ДНК РІЗНОГО ВМІСТУ НУКЛЕОТИДІВ

***Н.І Штеменко, О.А. Голіченко, О.В. Штеменко***

Методом УФ-титрування проведено порівняльне дослідження взаємодії біс-диметилсульфоксид-цис-тетрахлороди-m-півалатодиренію(III) (**I**) з двома еукаріотичними ДНК з різним вмістом нуклеотидів. Об'єктом дослідження були еукаріотичні дезоксирибонуклеїнові кислоти з тимусу теляти (CT DNA, 40% GC) та із сперми оселедця (HS DNA, 44% GC). Спостерігалося збільшення максимуму поглинання для обох ДНК із збільшенням концентрації доданих розчинів **I**; електронні спектри поглинання обох зразків ДНК демонстрували виражений гіперхромізм у присутності зростаючих кількостей **I**; розраховані константи зв'язування склали $Kb_{CT\ DNA}$=2221 л/моль та $Kb_{HS\ DNA}$=3238 л/моль; гіперхромність комплексів DNA-**I** була різною для досліджених ДНК (14,413% для CT DNA і 81,943% для HS DNA). Зроблено висновки, що додавання низьких концентрацій **I** до ДНК призводить до утворення комплексів ДНК-**I** з подальшим розкручуванням або розгортанням спіралі, що не досягає 50% розгортання; при високих концентраціях **I** переважаючою є взаємодія з гуаніном; взаємодія **I** з гуаніновими нуклеотидами приводить до значного (більше 50%) розгортання гуаніновмісних послідовностей ДНК; **I** чутливий до нуклеотидного складу ДНК у високих концентраціях і сильно реагує з гуаніновмісними послідовностями ДНК з вибірковою та потужною здатністю до розгортання.

**Ключові слова**: комплекс диренію(III), почверний зв'язок, ДНК, константа зв'язування, гіперхромізм.

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***N. Shtemenko [a, b, *], O. Holichenko [a], O. Shtemenko [a]***

**[a] Ukrainian State University of Chemical Technology, Dnipro, Ukraine**

**[b] Oles Honchar Dnipro National University, Dnipro, Ukraine**

**[*] e-mail: n.shtemenko@i.ua**

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