Further evidence for redox activation of the plasmid – dirhenium(III) complexes interactions

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The DNA-interactions in vitro are still necessary investigations for determination of the possible anticancer properties of the compounds, candidates for application in cancer therapy. The aim of the present work was to realize if the interaction of cis-dicarboxylates of dirhenium(III), with pivalato- (I), isobutirato- (II) and adamantyl- (III) ligands cleaves the plasmid in the same manner and what is the influence of the ligands on this process. For experiments we used the prokaryotic plasmid which is good model to analyze DNA-cleaving ability of different substances that exists in supercoiled conformation and turns to nicked and linear forms. It was shown that gradual conversion of the supercoiled Form I to a mixture of supercoiled (Form I) and nicked (Form II) DNA takes place and increasing amounts of Form II are produced with higher concentrations of I–III under increasing of concentration, that showed the DNA -cleaving abilities of all investigated dirhenium complexes. This process was taking place with different intensity in the range I > II > III, that demonstrates the influence of the organic radical on the cleaving activity of the dirhenium(III) complexes. Under hydrogen peroxide conditions, I and II showed close results, demonstrating more intensive process of cleaving, including formation of the linear plasmid (Form III) under higher concentration, witnessing about redox-activation of the DNA-cleaving reaction. Cleaving activity of III was approximately the same in all experiments, that was demonstrated only by decreasing of the supercoiled form I and increasing of the nicked form II of the plasmid and by absolutely absence of the linear form III of the plasmid. The electrophoresis mobility shift assays showed that rhenium cluster compounds have nuclease activity and confirmed that natural DNA may be their target in the living cells. The conclusion was made that the mechanism of DNA-cleavage reaction of the dirhenium(III) complexes is multiple in which the electron donating (withdrawing) effects of the ligands and catalytic activity of the metal core should be taken in consideration.

Key words: dirhenium(III) clusters, plasmid, DNA-cleavage, redox-activation.

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Introduction

Earlier using the method of UV-titration we have shown that the rhenium(III) quadruple bonding compounds interacted with supercoiled Calf Thymus DNA (CT DNA) (Paramonova et al., 2016; Polokhina et al., 2016) and that the mechanism of the interaction was redox-activated. Also, bis-dimethylsulfoxide-cis-tetrachlorodi-µ-pivalatodirhenium(III) cis–Re2((CH3)3CCOO)2Cl4•2DMSO (I) was shown to cleave plasmid DNA by electrophoretic mobility shift assay (Shtemenko et al., 2013) in different manner depending from the redox state of the medium. The DNA-interactions in vitro are still necessary investigations for determination of the possible anticancer properties of the compounds, candidates for application in cancer therapy (Ismail et al., 2019). It is known, that anticancer properties of the dirhenium(III) dicarboxylates in vivo depended on the length of the alkyl ligands (Leus et al., 2012).

Thus, the aim of the present work was to realize if the interaction of other two dicarboxylates of dirhenium(III), with shorter (isobutirato-) and longer, bulkier (adamantyl-) ligands cleave the plasmid in the same manner and what is the influence of the ligands on this process.
Materials and methods

**Materials.**
Cis–Re$_2$(I-C$_3$H$_7$COO)$_2$Cl$_4$•2DMSO bis-dimethylsulfoxide-cis-tetrachlorodi-μ-isobutyramidendium(III) (II) and cis–Re$_2$(C$_{10}$H$_{15}$COO)$_2$Cl$_4$•2DMSO bis-dimethylsulfoxide-cis-tetrachlorodi-μ-adamantylcarboxylatodirhenium(III) (III) were synthesized according to (Golichenko, Shtemenko, 2006, 2015). Supercoiled plasmid pUC18 and DNA ladder were purchased from Fermentas, Life Science (USA).

**Electrophoretic Mobility Shift Assays.**
The mobility assays were performed according to (Keck, Lippard, 1992; Mitra et al., 1997) by using aliquots (10 µl) of native supercoiled plasmid pUC18 (20 µg/ml) and increasing concentrations of complex (0 to 80 µM). Electrophoresis experiments were carried out using 1 % agarose gel and buffer solution (40 mM Tris-acetate and 1 mM EDTA, pH 8.2). The applied voltage was 250 V and the gels were run for a period of 20 min. After electrophoresis, the gels were stained with 0.5 mg/L ethidium bromide and imaged under UV light. The efficiency of the DNA cleavage was measured by determining the ability of the complex to form open circular or nicked circular DNA from its supercoiled forms. The same assays were also carried out in the presence of H$_2$O$_2$ (experiment b) and 2-mercaptoethanol (experiment c) (25 µM). Gel image analysis was made with the help of free LabImage 1D – Software for 1D Gels and Western Blots (https://www.kapelanbio.com/products/).

**Results and discussion.**
To study cleavage ability of metal-containing substances and their cleavage pathway, an assay is widely used, which employs a special type of DNA, a plasmid DNA (Wang et al., 2015; Kettenman et al., 2018; Kadoya et al., 2019). Single strand DNA cleavage (SSC) is the process, in which one scission is introduced in one of the two DNA strands in one cleavage event. Double strand DNA cleavage (DSC) denotes the process, in which two scissions are introduced to each of the strands of the DNA double helix in a single cleavage event. Although both pathways introduce double strand breaks ultimately, it is important to note that these are two inherently different processes. The pathway that is responsible for the induction of strand breaks is of more interest, as the direct double strand cleavage pathway is considered to be responsible for the cytotoxicity of the initiating substances to cancer cells.

Typically, the quaternary structure of plasmids is superhelical, which means that the double stranded DNA forms a compact folded structure. When one of the DNA strands is broken, a swivel, i.e. a free rotation site in the complementary strand opposite to the break, relieves the strain in the twisted molecule. This causes the supercoiled structure to relax into an uncoiled form, which is referred to as nicked DNA. When both strands of the DNA are cleaved opposite to each other, linear DNA is observed. Supercoiled DNA is often referred to as form I, nicked DNA as form II and linear DNA as form III. These forms are good resolved by electrophoresis (Sears et al., 2013; Kadoya et al., 2019).

We used these good described phenomena to investigate cleaving activity of the investigated substances II and III (Fig. 1, 2) and to compare the results with previously obtained data for the substance I.

The intensity of the bands obtained by scanning procedure is presented on the Fig. 3.

As it is clear from obtained results, the gradual conversion of the supercoiled Form I to a mixture of supercoiled (Form I) and nicked (Form II) DNA takes place and increasing amounts of Form II are produced with higher concentrations of I–III under increasing of concentration that showed the DNA-cleaving abilities of all investigated dirhenium complexes (Experiment a). This process was taking place with different intensity in the range I > II > III, that demonstrates the influence of the organic radical on the cleaving activity of the dirhenium(III) complexes. This range coincides with the binding constants, obtained from the UV-titration experiments (Paramonova et al., 2016; Polokhina et al., 2016).

Under hydrogen peroxide conditions (Experiment b) I and II showed close results, demonstrating more intensive process of cleaving, including formation of the linear plasmid (Form III) under higher concentration, witnessing about redox-activation of the DNA-cleaving reaction. Cleaving activity of III was approximately the same in Experiments a–c, that was demonstrated only by decreasing of the supercoiled form I and increasing of the nicked form II of the plasmid and by absolutely absence of the linear form III of the plasmid.
Fig. 1. Ethidium stained agarose gel electrophoresis depicting pUC18 mobility and effects (a) in the presence of increasing concentrations of II; (b) pUC18 with II in the presence of hydrogen peroxide; (c) pUC18 with II in the presence of mercaptoethanol. Lane Pa – untreated plasmid, Pb – plasmid with hydrogen peroxide; Pc – plasmid with mercaptoethanol; lanes 1: 10 µM; 2: 20 µM; 3: 40 µM; 4: 80 µM (concentration of II); S – standard

In the presence of mercaptoethanol (Experiment c), the activation of the cleaving abilities of I–III are also evident but no Form III is observed as compared to the activity of I–II only. The presence of the slowest moving bands in Fig. 1, 2 (slower than Form II) indicates formation of high molecular weight adducts that may be explained by the ability of unwound plasmids to polymerize (Vilfan et al., 2006) or to form unwound DNA-I intra-strand adducts similar to cisplatin and dirhodium compounds (Dunham et al., 2005).

The mechanism of the SSC and DSC of plasmids was in detail studied for bleomycin (BLM), an iron-containing natural nuclease (Decker et al., 2006). The experimental and computational studies presented here on the reaction coordinate of activated BLM strongly support the low-spin (BLM)FeIII-OOH...
complex as the active oxidizing species and a direct hydrogen-atom abstraction as the reaction mechanism for the initial attack on DNA. The mechanism for double-strand cleavage by a single BLM molecule would then involve the Fe$^{IV}$ intermediate generated by the first reaction as the active species for the initial second H-atom abstraction reaction damaging the second DNA strand. A hydroperoxo species of the dicopper(II) was also identified as the active species and spectroscopically identified in the reaction of the complex with plasmids (Kadoya et al., 2019). Analogical investigations for the dirhenium(III) complexes are absent, but acceleration of the nuclease reaction by hydrogen peroxide shown by us makes possible to think that the same hydroperoxo species may play definite role in the investigating processes with rhenium substances.

It is well-known that the microenvironment in the cancer cells is significantly different from that in normal cells (Zhou et al., 2014) and the concentration of reactive oxygen species (ROS) including H$_2$O$_2$ is relatively higher in cancer cells than in normal cells (Nogueira, Hay, 2013), that is mainly caused by mitochondrial malfunction (Aykin-Burns et al., 2009). Thus, redox activating of the process of cleavage by H$_2$O$_2$ shown by us in the Experiment b, is of great value as could work in cancer cells but not so extensively in normal cells and this phenomena could be used to reduce the side-effects of anticancer drugs.

Fig. 3. Intensity of bands of different forms of plasmid pUC 18 under influence of I, III, III in % to intensity of Pa, Pb, Pc accordingly
The influence of ligands surrounding the active center of metal-containing artificial nucleases was investigated on the copper–dipicolamine complexes (Wang et al., 2015). It was shown that replacement of a hydrogen atom of the dipicoxyl ligand with a benzyl group enhanced the efficiency both of SSC and DSC cleavage; also introducing an electron donating group resulted in further increase in efficiency, whereas the presence of electron withdrawing group reduced the efficiency; the introducing of different ROS-inhibitors decreased the intensity of cleavage. In our experiments I (pivalic derivative) and II (isobutiric derivative) are homologues, I has two longer alkyl ligands, that we may note that they are more electron donating and thus agree with previously made conclusions about possible formation of the intermediate complex for which electron donation is a stabilization factor. As for III: recently we have shown that some dirhenium complexes with diadamantyl ligands had catalase-like activity (Shamelashvili et al., 2016); thus, III could act as inhibitor of ROS-formation in the nuclease event. Nuclease activity of the dirhenium(III) clusters to our mind is of great interest and requires additional deep investigations.

Conclusions
The electrophoresis mobility shift assays showed that compounds I–III have nuclease activity and confirmed that natural DNA may be their target in the living cells. The redox-activation of DNA cleavage, observed in the electrophoresis experiments, may explain the anticancer activity of dirhenium(III) compounds as well as the positive effects of combined therapy, that includes their simultaneous introduction together with cisplatin. The present study and our other recent reports showed the dependence of the DNA-cleaving activity of dirhenium clusters on the nature of the ligands in the coordination sphere of the Re₆⁺ clusters. The obtained data showed the multiple mechanism of DNA-cleavage reaction of the dirhenium(III) complexes in which the electron donating (withdrawing) effects of the ligands and catalytic activity of the metal core should be taken in consideration.

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References
Aykin-Burns N., Ahmad I.M., Zhu Y. et al. (2009). Increased levels of superoxide and H₂O₂ mediate the differential susceptibility of cancer cells versus normal cells to glucose deprivation. Biochem. J., 418, 29–37. https://doi.org/10.1042/BJ20081258
Decker A., Chow M.S., Kemsley J.N. et al. (2006). Direct hydrogen-atom abstraction by activated bleomycin: an experimental and computational study. J. Am. Chem. Soc., 128, 4719–4733. https://doi.org/10.1021/ja057378n
Dunham S.U., Chifotides H.T., Mikulski S. et al. (2005). Covalent binding and interstrand cross-linking of duplex DNA by dirhodium(II,II) carboxylate compounds. Biochemistry, 44, 996–1003. https://doi.org/10.1021/bi0486637
Golichenko A.A., Shtemenko A.V. (2006). Cluster rhenium(III) complexes with adamanantarcarboxylic acids: synthesis and properties. Russ. J. of Coord. Chem., 32, 242–249. https://doi.org/10.1134/S1070328406040038
Golichenko A.A., Shtemenko A.V. (2015). Crystal structure of di-μ-isobutyrate-κ₄O₃:O₄-bis[cis-dichlorido(dimethysulfoxide-κS)rhenium(III)]. Acta Cryst. Section E: Cryst. Com., E71, 1219–1221. https://doi.org/10.1107/S2056989015017429
Ismail M.B., Booyseen I.N., Akerman M.P. (2019). DNA interaction studies of rhenium compounds with Schiff base chelates encompassing biologically relevant moieties. Nucleosides, Nucleotides and Nucleic Acids, 38(12), 950–971. https://doi.org/10.1080/15257770.2019.1639058
Kadoya Y., Fukui K., Hata M. et al. (2019). Oxidative DNA cleavage, formation of μ-1,1-hydperoxo species and cytotoxicity of dicopper(II) complex supported by a p-cresol-derived amide-tether ligand. Inorganic Chemistry, 58(21), 14294–14298. https://doi.org/10.1021/acs.inorgchem.9b02093
Keck M.V., Lippard S.J. (1992). Unwinding of supercoiled DNA by platinum ethidium and related complexes. J. Am. Chem. Soc., 114, 3386–3390. https://doi.org/10.1021/ja00035a033
Kettenmann S.D., Louka F.R., Marine E. et al. (2018). Efficient artificial nucleases for mediating DNA cleavage based on tuning the steric effect in the pyridyl derivatives of tripod tetraamine-cobalt(II) complexes. Eur. J. Inorg. Chem., 2018, 2322–2338. https://doi.org/10.1002/ejic.201800276
Додаткові докази редокс-активації взаємодії плазміда – комплекси диренію(ІІІ)

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Leus І.В., Shamelashvili K.Л., Skorik О.Д. et al. (2012). Antioxidant and antitumor activity of dirhenium dicarboxylates in animals with Guerin carcinoma. Ukr. Biochim. Zh., 84(3), 87–96. (In Ukrainian)

Mitra K., Kim W., Daniels J.S., Gates K.S. (1997). Oxidative DNA cleavage by the antitumor antibiotic leimarnycin and simple 1,2-dithiolan-3-one 1-oxides: evidence for thiol-dependent conversion of molecular oxygen to DNA-cleaving oxygen radicals mediated by polysulfides. J. Am. Chem. Soc., 119, 11691–11692. https://doi.org/10.1021/ja971359z

Nogueira V., Hay N. (2013). Molecular pathways: reactive oxygen species homeostasis in cancer cells and implications for cancer therapy. Clin. Cancer Res., 19, 4309–4314. https://doi.org/10.1158/1078-0432.CCR-12-1424

Paramonova K., Golichenko A., Babyb S. et al. (2016). The interaction of DNA with cluster rhenium compounds of different structural types. World of Med. and Biol., 56(2), 140–144.

Polokhina K., Golichenko A., Babyb S. et al. (2016). Investigation of the interaction of cluster compounds of rhenium with biological active ligands with supercoiled DNA by electronic spectroscopy. Visnyk of Lviv University, Biol. series, 72, 15–24.

Sears R.B., Joyce L.E., Ojami M. et al. (2013). Photoinduced ligand exchange and DNA binding of cis-[Ru(phpy)(phen)(CH3CN)2]+ with long wavelength visible light. J. Inorg. Biochem., 121, 77–87. https://doi.org/10.1016/j.jinorgbio.2012.12.003

Shamelashvili K.L., Shtetemenko N.I., Leus І.V. et al. (2016). Changes in oxidative stress intensity in blood of tumor-bearing rats following different modes of administration of rhenium-platinum system. Ukr. Biochem. Journal, 88(4), 29–39. https://doi.org/10.15407/ubj88.04.029

Shtemenko N.I., Chifotides H.T., Domasevitch K.V. et al. (2013). Synthesis, X-ray structure, interactions with DNA, remarkable in vivo tumor growth suppression and neoprotective activity of cis-tetrachloro-dipivalato dirhenium(III). J. of Inorg. Biochem., 129, 127–134. https://doi.org/10.1016/j.jinorgbio.2013.09.001

Vilfan I.D., Conwell C.C., Sarkar T., Hud N.V. (2006). Time study of DNA condensate morphology: implications regarding the nucleation, growth, and equilibrium populations of toroids and rods. Biochemistry, 45, 8174–8183. https://doi.org/10.1021/bi060396c

Wang W., Lee Y.A., Kim G. et al. (2015). Oxidative DNA cleavage by Cu(II) complexes: effect of periphery substituent groups. J. Inorg. Biochem., 153, 143–149. https://doi.org/10.1016/j.jinorgbio.2015.07.015

Zhou D., Shao L., Spitz D.R. (2014). Reactive oxygen species in normal and tumor stem cells. Adv. Cancer Res., 122, 1–67. https://doi.org/10.1016/B978-0-12-420117-0.00001-3

Дорадчеві докази редокс-активації взаємодії плазміда – комплекси диренію(ІІІ)

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Дослідження взаємодії з ДНК in vitro є необхідним для визначення можливих протипухлинних властивостей сполук – кандидатів на застосування у терапії раку. Мета цієї роботи полягає у тому, щоб зрозуміти, чи однааково розщеплюють плазміду цис-дикарбоксилати диренію(ІІІ) з півалатними (І), ізобутіратними (ІІ) та адамантикарбоксилатими (ІІІ) лігандами, і чи впливають ліганди на цей процес. Для експериментів ми використовували прокаріотичну плазміду, яка є придатною моделлю для аналізу здатності розщеплювати ДНК різними речовинами та існує у суперспіралізованій конформації і перетворюється у деспіралізовану та лінійну форми. Було показано, що відбувається поступове перетворення суперспіралізованої форми I у суперспіралізовану і деспіралізовану форму II при підвищенні концентрації, що свідчить про вплив органічного радикала на нуклеазну активність комплексів диренію(ІІІ). За наявності гідроген пероксиду І та ІІ показали близькі результати, демонструючи більш інтенсивний процес розщеплення, який включає утворення лінійної плазміди (форма III) при більш високій концентрації оцінкуваної концентрації плазміди, що показує здатність всіх досліджених діреневих комплексів розщеплювати ДНК. Цей процес відбувається з різною інтенсивністю в ряду І > ІІ > ІІІ, що свідчить про вплив органічного радикала на нуклеазну активність комплексів диренію(ІІІ). За наявності гідроген пероксиду І та ІІ показали близькі результати, демонструючи більш інтенсивний процес розщеплення, який включає утворення лінійної плазміди (форма III) при більш високій концентрації, що свідчить про редокс-активацію реакції розщеплення ДНК. Розщеплююча активність III була приблизно однаковою у всіх експериментів, що було продемонстровано зменшенням суперспіралізованої форми ІІ, які щоб відбувається редокс-активація реакції розщеплення ДНК. Розщеплююча активність ІІІ була приблизно однаковою у всіх експериментів, що було продемонстровано зменшенням суперспіралізованої форми ІІ та збільшенням деспіралізованої форми ІІІ та аміну повною відсутністю конформації плазміди. Аналіз зсуву рухливості при електрофорезі показав, що класичні сполуки ренію володіють нуклеазною активністю, і підтвердив, що природна ДНК може бути їх мішенью в живих клітинах. Був зроблений висновок про те, що механізм реакції розщеплення ДНК комплексами диренію(ІІІ) є багаторівневим, при цьому слід брати до уваги електронодонорні ефекти лігандів і каталітичну активність кластерного диреневого ядра.

Ключові слова: кластери диренію, плазміда, розщеплення ДНК, редокс-активація.

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**Дополнительные доказательства редокс-активации взаимодействий плазмиды – комплексы дирения(III)**

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Изучение взаимодействия с ДНК in vitro всё ещё является необходимым исследованием для определения возможных противоопухолевых свойств соединений – кандидатов на применение в терапии рака. Цель настоящей работы состояла в том, чтобы понять, одинаково ли расщепляют плазмиду цис-дикарбоксилаты дирения(III) с пивалатными (I), изобутиратными (II) и адамантилкарбоксилатными (III) лигандами, и какое влияние оказывают лиганды на этот процесс. Для экспериментов мы использовали прокариотическую плазмиду, которая является хорошей моделью для анализа способности расщеплять ДНК различными веществами, существует в суперспирализованной конфигурации и превращается в деспирализованные и линейные формы.

Было показано, что происходит постепенное превращение суперспирализованной формы I в смесь ДНК со суперспирализованной (форма I) и деспирализованной (форма II) плазмидами, а также полным отсутствием линейной формы III плазмиды. Анализ сдвига подвижности при электрофорезе показал, что кластерные соединения рения обладают нуклеазной активностью.

**Ключевые слова:** кластеры дирения(III), плазмида, ДНК-расщепление, редокс-активация.

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