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DNA binding of novel organogermanium sesquioxides with anthraquinone or naphthalene moiety*

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Abstract: ***Objective*** To study the interaction of newly synthesized Ge132 derivatives with DNA and determine the anticancer mechanism of the compounds. ***Methods*** The affinity and mode of interaction of four novel organogermanium sesquioxides with calf thymus DNA (CT-DNA) and two synthetic oligonucleotides, 22-mers poly (dA·dT) and 22-mers poly(dG·dC), were investigated by a combination of absorption spectroscopy, fluorometric technique, viscosity, and DNA thermal denaturalization. ***Results*** The compounds had moderate affinity with CT-DNA and two synthetic oligonucleotides, 22-mers poly(dA·dT) and 22-mers poly(dG·dC), with binding constants on the order of $10^3 \sim 10^5 M^{-1}$ in intercalative mode, and showed different preference for duplexes upon the different groups introduced to organogermanium sesquioxides. ***Conclusion*** The newly synthesized Ge132 derivatives can intercalate into DNA. The results suggest that the same methyl group in different types of compounds plays different role for the interaction of related compounds with DNA, and they may offer a new important guidance for the synthesis and mechanism on anticancer active organogermanium compounds.

Key words: Organogermanium sesquioxide; DNA binding; Absorption spectroscopy; Viscosity; Thermal denaturalization

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有机锗蒽醌酰胺和有机锗萘酚酯倍半氧化物与 DNA 的相互作用

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摘 要 目的 研究新合成的有机锗化合物与 DNA 的相互作用,为从分子水平上揭示它们的抗癌作用机理提供资料,为进一步设计合成更为有效的抗癌药物提供理论指导。**方法** 综合应用紫外-可见分光光度法(UV-Vis)、热变性(Melting Temperature Studies)、荧光光谱变化(Fluorescence Titration Experiments)、黏度测定(Viscosity Measurements)等方法,研究了 4 种化合物与 3 种 DNA,既小牛胸腺 DNA(ct-DNA)、22-mers poly (dA·dT) 和 22-mers poly(dG·dC)的相互作用。**结果** 4 种化合物与 CT-DNA 结合后,4 种化合物的 UV-Vis 均发生了不同程度的减色效应和红移现象,预示着 4 种化合物可能通过插入方式与 DNA 发生作用。由化合物与 ct—DNA、22mer poly(dA·dT) 和 22mer poly (dG·dC)作用的热变性曲线可以看出,3 种 DNA 的热变性温度 Tm 均有提高,意味着药物与 DNA 的插入结合。发现 4 药物小分子对 CT-DNA 黏度均有影响,随着药物浓度的增加,DNA 溶液的黏度也随之增大。3 种 DNA 均能使 4 种化合物的荧光发生淬灭,通过计算得出相应的结合常数,属于中等强度的结合,结合常数介于 $10^3 \sim 10^5 M^{-1}$ 之间。**结论** 药物与 DNA 作用后,药物的 UV-Vis 变化、DNA 的热变性温度和黏度的变化均说明 4 种有机锗化合物是以插入结合方式与 DNA 发生作用的。以上研究结果说明,新型结构的有机锗化合物各功能基团之间产生了协同作用,它们均能与 DNA 发生明显的相互作用,并且很可能改变原有的有机锗倍半氧化物的抗癌作用机理,产生直接细胞毒作用,因而提高了整体化合物的抗癌活性。对进一步合成高效低毒的有机锗抗癌药物、揭示它们的作用机理具有重要的参考价值。

关键词 有机锗半氧化物;DNA 结合;吸收光谱;黏度;热变性

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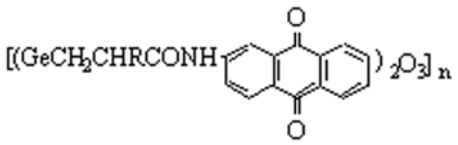
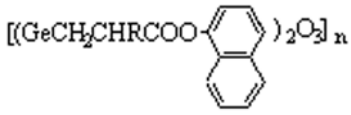
1 Introduction

Of the physiologically active organogermanium compounds, Ge-132 and Ge-132 derivatives or analogs have been specially paid attention due to their relatively low toxicities and diversely biological activities[1-3]. In fact, much recent work has concentrated on Ge-132 derivatives or analogs rather than the parent compound. Ge-132 derivative are considered to be formed through the replacement of one of the ethyl hydrogens by an organic group, or replacement of the hydroxyl in carboxyl group by forming ester or amide[2,4]. Ge-132 analog are the organogermanium sesquioxide containing Ge-O groups. It has been found that some of Ge-132 derivatives show stronger antitumor activities than Ge-132 itself[5-8]. Research results[6] indicate that hybrid compounds of organogermanium sesquioxides and bioactive moiety could improved the anticancer activities to a significant degree. Encouraged by these promising results, we synthesized four novel Ge-132 derivatives substituted in carboxyl group with anthraquinone or naphthalene moiety, 1, 2, 3, and 4, and also studied the cytotoxic activities against K562 cell line[9]. The results showed that the introduction of planar aromatic naphthalene or anthraquinone moiety to the parent compound, Ge-132, enhanced the cytotoxic activity greatly. However, the anticancer mechanism of the compounds still remains to be elucidated. In the present work we investigated the affinity and mode of interaction of the organogermanium sesquioxides and calf thymus DNA and two synthetic oligonucleotides, 22-mers poly(dA · dT) and 22-mers poly(dG · dC), by a combination of absorption spectroscopy, fluorometric technique, viscosity, and DNA thermal denaturalization. The newly synthesized Ge132 derivatives can intercalate into DNA, and the binding of the compounds to DNA was found to be modest, with binding constants on the order of $10^3 \sim 10^5 M^{-1}$ determined for their binding to calf thymus DNA and the synthetic oligonucleotides, respectively.



1: R=H; **2**: R=CH_3



3: R=H; **4**: R=CH_3

2 Experimental Section

All experiments on the interaction of compounds with DNA were carried in BPE buffer consisting of 6mmol/L Na_2HPO_4, 2mmol/L NaH_2PO_4, 1mmol/L EDTA, pH7.0. Three kinds of DNA, calf thymus DNA (CT-DNA), 22-mers poly (dA · dT) and 22-mers poly (dG · dC), were used to examine the interaction between the compounds and DNA. UV-Vis spectrophotometer (JASCO V-550) equipped with a ETC-505T thermoelectric temperature controller; fluorescence spectroscopy (FP-6500) and DNA melting were used to study the compounds binding to CT-DNA, $d(AT)_{22}$ $d(AT)_{22}$ and $d(GC)_{22}$ $d(GC)_{22}$ duplex DNA, respectively. Self-complementary, AT or GC duplex DNA was prepared by heating the oligomers to 95℃ for 3 min, and slowly cooling to room temperature, and then equilibrating for 48 h at 4℃ before use. DNA solution viscosity in the absence or presence of the compounds was measured three times at each binding ratio at 25℃.

Absorption spectroscopy and fluorescence titration data were recorded at room temperature by keeping the concentration of the drug constant while varying the nucleic acid concentration. Fluorescences were measured at an excitation wavelength of 320nm and at an emission wavelength of 550nm for 1 and 2, at an excitation wavelength of 294nm and at an emission wavelength of 456nm for 3 and 4. Fluorescence titration data were fit directly to get binding constants, using a fitting

function incorporated into the program Fit All (MTR Software, Toronto) described as ref[10] and[11].

Viscosity experiments were carried out on a Cannon-Manning Semi-Micro viscometer at 25℃ in BPE buffer. Flow time was measured with a digital stopwatch, each sample was measured three times and the average flow time was calculated. The CT-DNA concentration was kept constant (300μmol/L in base pair) in all the samples, but the drug concentration was increased from 15μmol/L to 90μM. Data are presented as $(\eta/\eta^0)^{1/3}$ versus the ratio of drug concentration to DNA concentration, where η was the viscosity of DNA in the presence of drug and η^0 was the viscosity of DNA alone. The values of η and η^0 were calculated by the expression (t-tb) / tb, where t is the observed flow time and tb is the flow time of buffer alone.

3 Results and Discussion

Absorption Spectra and Melting Temperature Measurements

Addition of ct-DNA to 1 and 2 induced strong hypochromic and bathochromic effects of the absorption spectrum. The absorption bands at about 275 and 265nm exhibited hypochromism of about 33% and 42%, and bathochromic shift of 7nm and 20nm, respectively, reflecting strong interaction between the drug chromophores and the DNA bases. Hypochromic and bathochromic effects were also observed in 3 and 4, with 18% and 20.4% hypochromicity, 4 and 2nm red shifts, respectively. The results suggested an intimate association of the four compounds with ct-DNA and it was also likely that these compounds bind to the helix by intercalation. Figure 1 showed the absorption spectra of 1 and 3 in the absence and in the presence of ct-DNA recorded in BPE buffer.

Thermal denaturation measurements of DNA were further performed to obtain a qualitative evaluation of the DNA binding affinity of these compounds. Support for the intercalation comes from thermal denaturation measurements as it has been established that the stacking of intercalation into the nucleic base pairs can stabilize the double helix and thereby increase the melting points of DNA. Three DNA of different base pair composition[CT-DNA (which contains roughly equal proportions of A • T and G • C base pairs), 22-mers poly(dA • dT), and 22-mers poly(dG • dC)] were used. The variations of the melting temperature (T_m) of helix-to-coil transition of ct-DNA and the two polynucleotides were presented in Table 1. It could be seen from the Table 1 that ct-DNA melted at 66.7℃ in the absence of any drug under the experimental condition, the Tm of CT-DNA was increased by 2.6, 2.1, 6.3, and 4.3℃ in the presence of 1, 2, 3, and 4, respectively. For the 22-mers poly (dG • dC) and 22-mers poly(dA • dT), the melting temperatures in the presence of the four compounds were also increased by 2～7℃.

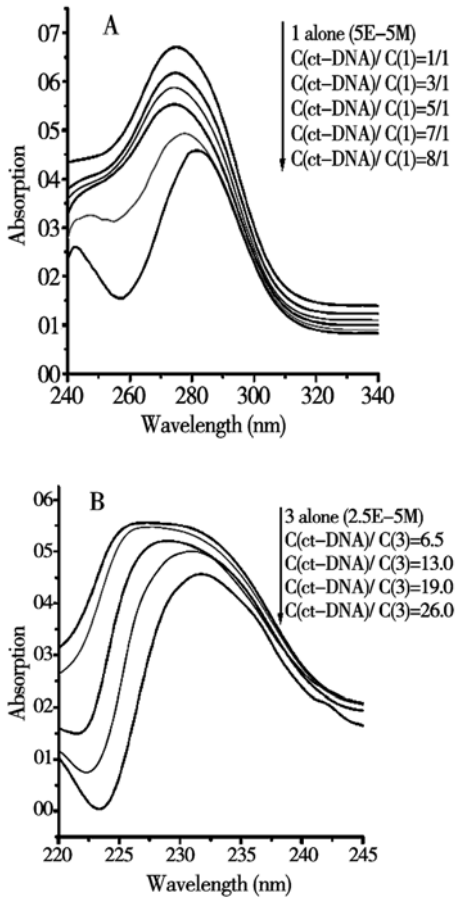


Figure 1. Absorption spectra of 1 and 3 (50 μmol/L for 1 and 25 μmol/L for 3) with increasing concentration of CT-DNA. Panel A for 1, B for 3

Table 1 UV Melting temperature (Tm) of CT-DNA, 22-mers poly (dA · dT), and 22-mers poly (dG · dC) in the absence and in the presence of the Compounds 1, 2, 3, and 4.

DNA	T_m*				
	1	2	3	4	DNA alone
CT-DNA	69.3	68.8	73.0	71.0	66.7
22-mers poly(dA · dT)	39.0	39.7	40.5	39.4	37.0
22-mers poly(dG · dC)	81.4	82.6	83.6	82.1	76.5

Tm measurements were performed in BPE buffer (pH 7.1) using 20μM DNA and 10μM drug ($C_{drug}/C_{DNA} = 1/2$) in 500μl quartz cell at 260nm with a heating rate of 1 ℃/min. Each drug was tested in triplicate, and the results were averaged.

Viscosity To further clarify the interactions between the compounds and DNA, viscosity measurements were carried out, for hydrodynamic data provide perhaps the most critical test for intercalative binding in the absence of X-ray crystallographic or NMR structural data[12]. If a compound binds in a DNA groove, without intercalating, only modest changes in viscosity are generally observed, since this has little effect on the effective length of the polymer. Intercalation, on the other hand, proceeds by unwinding the double helix to accommodate a compound that becomes inserted and stacked between the base pairs. This process results in an effective increase in the DNA contour length[13]. When the relative increase in contour length, $(\eta/\eta_0)^{1/3}$, versus the ratio of drug concentration to DNA concentration, r, is plotted, the slope m of this plot has different values depending on the DNA-binding affinity of the intercalator. Representative intercalators such as ethidium bromide (EB), proflavine, and aminoacridines usually have values of m between 0.8 and 1.5[14-15]. The data of viscosity measurements showed that the representative intercalators, EB, had value of m about 1.16, and the values of m of the four compounds were between 0.85～1.0, relatively little lower than that of the 'classic' intercalating agent, EB. Evidently, the four novel compounds increased the length of CT-DNA, resulting in an increased viscosity, which suggested that they bound to DNA by intercalation.

These viscosity data, along with UV-Vis absorption and melting results, indicated unambiguously that the new compounds did behave as intercalators.

Fluorescence Titration Studies

We exploited intrinsic fluorescence of the four compounds to evaluate the strength of the interaction of the drug with the three kinds of DNA. Addition of DNA to the four compounds result in the fluorescence decrease, and this proper is very useful for accurate determination of the DNA binding affinities. Titration data are fit directly to get binding constants[10-11]. The binding constants (K_{app}) of the compounds with ct-DNA and two synthetic oligonucleotides, 22-mers poly (dA · dT) and, were presented in Table 2.

The binding of the novel compounds to DNA was found to be modest (in comparison to classical intercalators), with binding constants on the order of $10^3 \sim 10^5 M^{-1}$. Generally, the compounds with naphthalene moiety (3 and 4) bound DNA more strongly than the compounds with anthraquinone moiety (1 and 2), exception for the binding of 2 with 22-mers poly(dG · dC) with a Kapp of $1.03 \times 10^{-4} M^{-1}$. It can also be seen from Table 2 that the compound 1 and 2 had preference for binding to AT-rich duplexes, whereas the compound 3 and 4 had a little preference for binding to GC-rich duplexes.

Table 2 The DNA binding constants of compounds 1, 2, 3, and 4

Compd	$K_{app} \times 10^{-4} M^{-1}$		
	CT-DNA	22-mers poly(dA · dT)	22-mers poly(dG · dC)
1	0.55	1.62	1.03
2	0.54	10.3	5.26
3	9.51	22.9	27.2
4	2.28	5.08	7.97

Of the compounds 3 and 4, 3 bound DNA stronger than 4 (about the 4 times higher of the K_{app}), indicating that the exist of methyl group was unfavourable to the interaction of the naphthalene compounds (3 and 4) with DNA. In contrast, for anthraquinone compounds, 1 and 2, the exist of methyl group enhanced the binding affinities of the compound with DNA (about 5 times difference), except ct-DNA. The results suggested that the same methyl group in different types of compounds played different role for the interaction of related compounds with DNA.

The compounds had moderate affinity with ct-DNA and two synthetic oligonucleotides, 22-mers poly(dA · dT) and 22-mers poly(dG · dC), with binding constants on the order of $10^3 \sim 10^5 M^{-1}$ in intercalative mode, and showed different preference for duplexes upon the different groups introduced to organogermanium sesquioxides. The results may offer a new important guidance for the synthesis and mechanism on anticancer active organogermanium compounds.

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