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The interaction of quinoline-organogermanium sesquioxides with DNA*

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Abstract: ***Objective*** To study the interaction of two novel quinoline-organogermanium sesquioxides with calf thymus DNA(CT-DNA) and two synthetic oligonucleotides, d(AT)22 and d(GC)22, and reveal the anticancer mechanism of the organogermanium compounds. ***Methods*** The interaction was investigated by absorption spectroscopy, DNA thermal denaturalization, viscosity, and fluorometric titration method. The binding constants were calculated from fluorescence-titration data by nonlinear least-squares analysis. ***Results*** The new compounds could interact with DNA by intercalation. The binding constants of the compounds with CT-DNA and the synthetic oligonucleotides were found to be on the order of 104-105 L/mol. ***Conclusion*** DNA may be the primary effect target of the quinoline-organogermanium sesquioxides. These results provided the important information for the design and synthesis of new types of organogermanium compounds with stronger anticancer activity.

Keywords: Organogermanium sesquioxide; DNA; Interaction; Intercalation; Binding constant
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喹啉有机锗倍半氧化物与 DNA 的相互作用

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摘　要　目的　研究 2 种喹啉有机锗倍半氧化物与小牛胸腺 DNA(CT－DNA)和 2 种人工合成的寡聚核苷酸(22 聚 AT 碱基对 $d(AT)_{22}$ 和 22 聚 GC 碱基对 $d(GC)_{22}$ 的相互作用,以便揭示化合物的抗癌作用机制。**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 2 种喹啉有机锗倍半氧化物与 3 种 DNA 的相互作用。**结果**　有机锗化合物与 CT-DNA 结合后,它们的 UV－Vis 光谱均发生了不同程度的减色效应和红移现象,相对荧光强度显著增强;化合物能使 DNA 的热变温度(Tm)提高、黏度增强;通过荧光滴定数据计算得到相应的结合常数(10^4-10^5 L/mol)。**结论**　喹啉有机锗倍半氧化物能够与 DNA 以插入方式结合,喹啉基团与有机锗的协同作用增强了抗癌活性,DNA 可能是其作用靶点。这些结果对进一步合成高效低毒的有机锗抗癌药物、揭示它们的抗癌作用机制具有重要的参考价值。

关键词　有机锗化合物;DNA;相互作用;插入结合;结合常数

Introduction

Because of the broad anticancer spectrum, relatively low toxicities and side effects, and organogermanium sesquioxides have become important area for the design and synthesis of anticancer drugs[1]. To improve the anticancer activity further, many scientists have synthesized different kinds of organogermanium compounds[2-5]. Among them, Ge-132 and its derivatives have been specially paid attention to due to their

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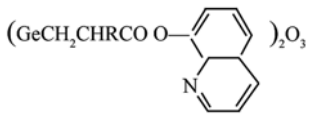
low toxicities and strong anticancer activities. Research results[6-9] indicated that some organogermanium sesquioxides showed stronger anticancer activities than Ge-132 itself.

Although a lot of organogermanium compounds are reported to possess strong anticancer activities, the anticancer mechanism of the compounds has not been clearly identified until now, and the effect target still remains to be elucidated in molecular level. In previous paper, we reported the synthesis of two novel Ge-132 derivatives substituted in carboxyl group with quinoline moiety and found that they displayed strong cytotoxic activities against PC-3M cell line[10] (compounds 1 and 2, as shown in Fig. 1), the introduction of planar quinoline moiety to the parent compound (Ge-132) could enhances the cytotoxic activity greatly. In this paper, we studied the affinity and mode of interaction of two quinoline-organogermanium compounds 1 and 2 with calf thymus DNA (CT-DNA) and two synthetic oligonucleotides, d(AT)$_{22}$ and d(GC)$_{22}$ duplex DNA. The results indicated that the compounds interacted with DNA by intercalation.



1: R=H; 2, R=CH_3

Fig. 1 Structure of compound 1 and 2

Materials and Methods

Materials

Calf thymus DNA (CT-DNA), d(AT)$_{22}$, and d(GC)$_{22}$ were purchased from Amersham Pharmacia Biotech (Piscataway, USA). Compound 1 and 2 were synthesized by the method described previously[10]. BPE buffer (pH = 7.0) consisting of 6 m mol/L Na_2HPO_4, 2 m mol/L NaH_2PO_4 and 1 m mol/L Na_2EDTA was prepared with twice distilled water. All the experiments were carried out in the BPE buffer. CT-DNA was used as natural DNA with roughly the same ratio of AT and GC base pair. Two synthetic oligonucleotides, d(AT)$_{22}$ and d(GC)$_{22}$ duplex DNA, were selected to determine the preference of interaction of compounds. The preparation and the concentration determination of three kinds of DNA were carried out by the methods described in previous paper[11].

Methods

UV-Visible spectrophotometer (JASCO V-550) equipped with a ETC-505T thermoelectric temperature controller, fluorescence spectroscopy (FP-6500), and DNA thermal denaturalization were used to study the interaction of two quinoline-organogermanium compounds 1 and 2 with CT-DNA and oligonucleotides. Absorption spectra were recorded at room temperature when the concentrations of the compounds (1 and 2) were kept constant while changing the concentration of DNA. DNA blank solution in the same nucleotide concentration interacted with compounds was prepared and used as a reference during measuring the absorption spectra. The DNA thermal denaturalization (melting) were carried out in 20 μmol/L DNA and 20 μmol/L drug (c_{drug}/c_{DNA} = 1/1) at 260 nm, and the heating rate was 1 ℃/min in the temperature range from10℃ to 100 ℃.

Viscosity measurements were carried out on a Ubbelohde type viscometer at room temperature in BPE buffer. The CT-DNA was selected and the concentration was kept constant (300 μmol/L), and the compound concentration was increased gradually from 15 μmol/L to 90 μmol/L. Flow time was measured with a digital stopwatch.

Fluorescence titration were recorded on Hitachi F-4600 fluorospectro photometerby fixing the concentration of the organogermanium compound constant (10 μmol/L). While the DNA concentrations were kept changing from 0.01 μmol/L to 10 mmol/L. The excitation and emission wavelength of compound 1 and 2 in fluorescence spectra were 409 nm and 474 nm, respectively. The binding constants were obtained through the nonlinear least squares analysis of the fluorescence titration[11].

All the experiments were carried out three times, and the results was the mean.

Results

Absorption Spectra and Melting Temperature

Measurements

Uv absorption was used to study the interaction of the compounds with DNA. In Fig. 2 the Uv-visible absorption spectra of compounds 1 and 2 in the absence and presence of CT-DNA were shown. The hypochromic and bathochromic effects of the spectrum could be seen by the addition of CT-DNA to compound 1 and 2. The results reflected the interaction of compounds with DNA. When the concentration ratio of DNA to compound was from 0 to 30, the absorption bands of compounds 1 and 2 at about 239 nm exhibited hypochromism of about 19% and 10%, respectively. And they appeared about 5 nm bathochromic shift.

The melting curves of $d(GC)_{22}$ in the absence and presence of compounds 1 and 2 were shown in Fig. 3. The variations of the melting temperature (T_m) of CT-DNA with helix-to-coil transition and the two oligonucleotides were presented in Table 1. The melting temperatures of three kinds of DNA were increased obviously with the addition of compounds, especially in the case of $d(GC)_{22}$.

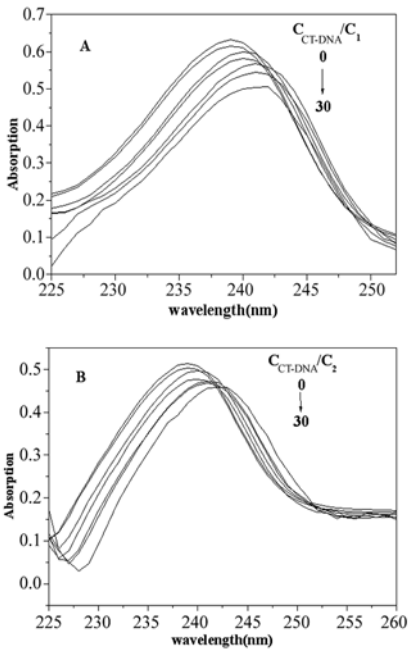
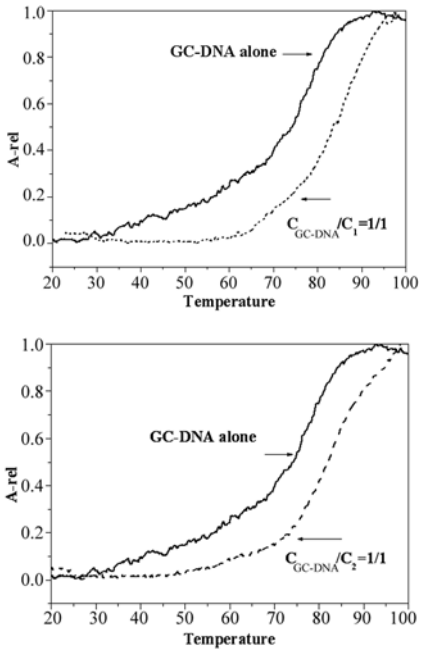


Fig. 2　Uv spectra of compounds 1(A) and 2(B) in the absence and presence of CT-DNA



A-rel = $(A-A_i)/(A_f-A_i)$,

A-rel was the relative absorbance ratio, A was the observed absorbance, A_i was initial absorbance, and A_f was final absorbance

Table 1　The melting temperatures (T_m) of CT-DNA, $d(AT)_{22}$ and $d(GC)_{22}$ in the absence and presence of compound

Fig. 3　The melting curves of $d(GC)_{22}$

DNA or bound DNA	Melting point, T_m/℃
CT-DNA	66.2
CT-DNA-1	72.1
CT-DNA-2	70.7
$d(AT)_{22}$	37.0
$d(AT)_{22}$-1	40.1
$d(AT)_{22}$-2	40.4
$d(GC)_{22}$	76.5
$d(GC)_{22}$-1	85.2
$d(GC)_{22}$-2	83.1

Viscosity

Fig. 4 showed the viscosity titration curves of CT-DNA in the presence of compound 1 and 2 at 25.0℃ in BPE buffer. As comparison, ethidium bromide (EB) was used. The viscosity of the DNA sample increased obviously with the addition of the compounds. The values of m were between 0.85 and 1.0, which was only a little lower than that of EB.

Fluorescence Titration Studies

With the addition of DNA to the compound, the

relative fluorescence intensity was increased gradually (Fig. 5). The binding constants (K) of the compounds with CT-DNA and the two synthetic oligonucleotides, $d(AT)_{22}$ and $d(GC)_{22}$, were shown in Table 2.

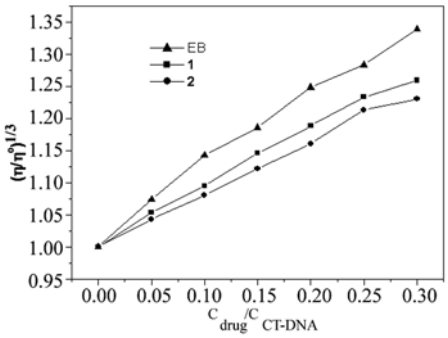


Fig. 4　Viscosity titration curves of CT-DNA in the presence of compound 1, 2, and EB at 25℃ in BPE buffer

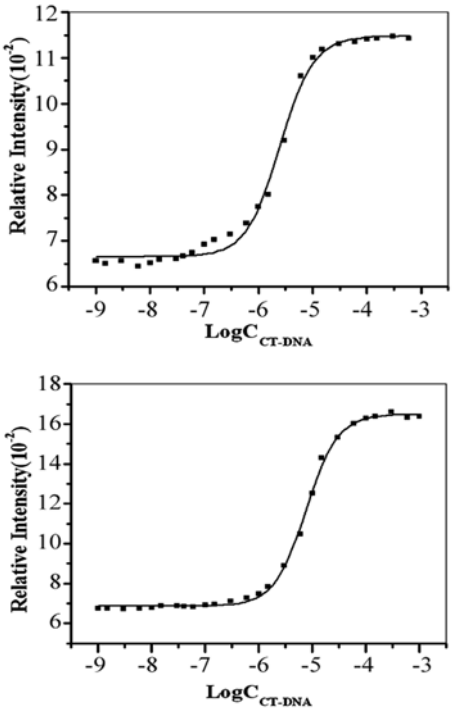


Fig. 5　Fluorescence titration curves for the interaction of compound 1 (A) and 2 (B) with CT-DNA

Table 2　DNA binding constants of compound 1 and 2

DNA	K (L/mol ×10⁴)	
	1	2
CT DNA	64.5	13.2
$d(AT)_{22}$	2.71	17.5
$d(GC)_{22}$	21.7	16.7

Discussion

It is well known that the hypochromism and bathrochromic shift in Uv-visible spectra are thought to be the evidence of intercalation[12-14]. In our study, the interaction of the compounds with DNA caused a marked hypochromism and bathrochromic shift of 5 nm, showing a strong interaction between drug chromophores and DNA bases. These results suggested that the compounds might bind to DNA helix by intercalation.

The DNA melting was further used to study the DNA binding mode of the two compounds with DNA. Stacking the small molecule into the nucleic base pairs in DNA can stabilize the double helix and thereby increase the melting points of DNA[15]. It can be seen from Table 1 that under the experimental condition, CT-DNA melted at 66.2 ℃ in the absence of any drug. the T_m' s of CT-DNA in the presence of compound 1 and 2 were increased by 5.9, and 4.3 ℃, respectively. For $d(AT)_{22}$ and $d(GC)_{22}$, the melting temperatures in the presence of the compounds were also increased by 3-9 ℃. All these results provided support for the intercalation.

Viscosity experiments were carried out to confirm the interaction mode between the two compounds and DNA. When a small molecule binds DNA along DNA groove, only little changes in viscosity are observed. However, intercalation means that small molecule interacts with DNA between base pairs, this process causes increase of the DNA contour length[13,15]. The slope m values depend on the DNA-binding mode. The classical intercalators, such as ethidium bromide (EB), the m values are between 0.8 and 1.5[16]. In our study, the viscosity of CT-DNA solution increased gradually with the addition of the compounds, and the values of m were between 0.85 and 1.0 (as shown in Fig. 5), the two compounds increased the length of CT-DNA, resulting in an increased viscosity. Although the values of m were little lower than that of EB, the results were enough to suggest that they bind to DNA by intercalation.

UV-Visible absorption, melting temperatures, and

viscosity data show unambiguously that the new compounds do interact with DNA by the mode of intercalation.

Relative fluorescence intensities of the compounds when bound to DNA were increased (as shown in Fig. 5). The binding affinities were obtained by using the fluorescence data of the quinoline-organogermanium compounds[11], the binding abilities of the two compounds to DNA were found to be modest with binding constants on the order of 10^4-10^5 L/mol. The methyl substitution effect was observed in the binding studies. For compound 1 without methyl group in the linking chain (Fig. 1), the binding constant was 6.45×10^5 L/mol, which was 5-fold larger than that of compound 2 with the methyl group (1.32×10^5 L/mol). The results demonstrated that the replacement of the methyl group in the linking chain facilitated the compound binding to DNA. Compound 1 showed strong GC sequence preference. Its GC duplex binding affinity, 2.17×10^5 L/mol, is 8.5-fold stronger than binding to AT duplex (2.71×10^4 L/mol). For compound 2, however, equal binding affinity was found for GC (1.75×10^5 L/mol) and AT (1.67×10^5 L/mol) duplex DNA, indicating that the methyl group in the linking chain not only influenced their DNA binding affinity but also modulated their DNA sequence specificity.

The Uv-visible absorption and red shift of the quinoline-organogermanium compounds in the presence of DNA were decreasd, and the melting temperatures and relative viscosity of DNA in the presence of the compounds were increased. It indicated that the quinoline-organogermanium compounds bound to DNA by intercalation. The methyl substitution effect of these two compounds was observed in DNA binding studies. The methyl group in the linking chain between germanium and the quinoline moiety affected compound-DNA binding affinity, and modulated their DNA sequence selectivity. These results provided new insights into the design of the incorporation of anticancer agent Ge132 into pharmacophore moiety targeting specific DNA sequences.

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(≥110min)可以造成心肌炎症反应和缺血 - 再灌注损伤,这些因素对 AF 的发生亦有促进作用。左房扩大可导致心肌电重构及不应期离散度增加,此时心肌细胞由于电活动的不均一性而容易产生折返径路[11],引发 AF。

总之,在临床工作中对于术后 ICU 停留时间较长、桥血管数量较多、高龄(≥70 岁)、体外循环时间过长(≥110min)、左房内径增大(≥35mm)的患者必须予以足够的重视,在保证治疗质量的同时应尽量减少相关因素的影响并做好相应保护措施,尽可能降低术后新发 AF 的概率。

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