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Linker chain effect of ferrocenylnaphthalene diimide derivatives on a tetraplex DNA binding

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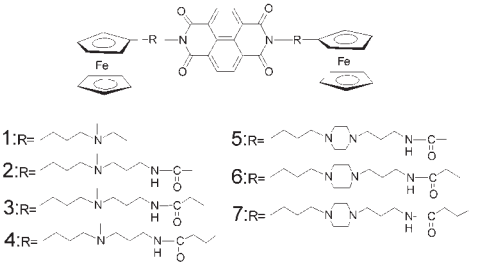
ABSTRACT

Spectrophotometric binding studies of a series of the naphthalene diimide derivatives, 1 - 7, carrying different chains with a human telomere oligonucleotide, $d(TTAGG)_4$ was carried out in 0.1 M AcOK-AcOH buffer (pH 5.6) and 0.1 M KCl. Under this condition, this DNA could exist as the mixture of two-type tetraplex structures and these derivatives could bind to this DNA with strong affinity of 10^6 M^{-1}. The effect of the linker chain is not so large in those binding affinity, but the ligand 5 having piperazine skeleton in the linker chain had relative higher affinity for this tetraplex DNA than other derivatives. Large hypochromic effect of these derivatives upon binding to the tetraplex DNA suggested that the binding mode of these derivatives might contribute the stacking interaction between the naphthalene diimide and guanine tetraplex planes.

INTRODUCTION

Human telomere DNA has a repeat sequence of d(TTAGGG) spreading to several kilo bases and exists in the termini of the each chromosomal DNAs. Four repeating units of d(TTAGGG) can form the tetraplex structure in the presence of metal cation such as sodium or potassium cation although the precise structure of the tetraplex is not clear now.[1] Recent investigations of the small ligands which can bind to human telomere tetraplex DNA have focused on new anticancer agent. The binding of these ligands with the tetraplex DNA can cause the stabilization enough to inhibit the access of telomerase to elongate the telomeric repeat sequence. Several kinds of the ligands such as teromestatine,[2] PIPER,[3] or TMPyP[4] have been reported in this cause.

In the previously paper, we have been developing the ferrocenylnaphthalene diimide **1** (Scheme 1) as a threading intercalator aiming for the development of the double stranded DNA specific ligand and recently we found that **1** could bind to tetraplex DNA with larger affinity than to double stranded DNA.[5] To improve its binding affinity for tetraplex DNA, we synthesized a series of the naphthalene diimide ligands, **1 - 7,** carrying the different linker chain between ferrocene and naphthalene diimide parts as shown in Scheme 1 and carried out in their binding experiments of with the synthetic human telomere oligonucleotide, $d(TTAGGG)_4$.



Scheme 1. Chemical structure of ferrocenylnaphthalene diimide, **1 - 7**.

EXPERIMENTAL

Materials

Human telomere oligonucleotide, $d(TTAGGG)_4$, was custom-synthesized by Genenet Co. (Fukuoka, Japan). Circular dichroism (CD) spectra of $d(TTAGGG)_4$ in 0.1 M AcOK-AcOH buffer (pH5.6) containing 0.1 M KCl suggested to the existence of the mixture of propeller-type parallel and mixed-type hybrid structure.[1] Ferrocenylnaphthalene diimide derivatives **1 - 7** was synthesized as described previously.[6, 7]

Equilibrium binding study

Spectrophotometric titration of **1 - 7** with varied amount of

Table 1 The binding parameters of ferrocenylnaphthalene diimide derivatives, **1-7**, with $d(TTAGGG)_4$.[a]

Ferrocenylnaphthalene diimide	1	2	3	4	5	6	7
Binding constant, $10^{-6}K/M^{-1}$	1.17±0.19	0.72±0.09	n.d.	n.d.	1.52±0.14	0.85±0.07	n.d.
n value	(3.4)	(4.4)	n.d.	n.d.	(4.2)	(3.7)	n.d.

[a]Experiments were conducted in 0.1 AcOK-AcOH buffer (pH5.6) containing 0.1 NaCl.

d(TTAGGG)$_4$ was carried out in 0.1 M AcOK-AcOH buffer (pH5.6) containing 0.1 M KCl, and an absorption changes at 384 nm based on **1** – **7** were monitored. The binding affinities for tetraplex DNA were determined from the data obtained by Scatchard analysis: $\nu/L=K(n-\nu)$ (1), where ν is the moles of ligand bound per DNA molecule, and K is observed binding constant, and n is the maximum number of ligand bound per one DNA molecule.[4]

RESULTS AND DISCUSSION

All of derivatives **1** - **7** showed the absorption maximum at 384 nm as transition moment based on the ferrocenylnaphthalene diimide skeleton in 10 mM MES (pH 6.2) and 1 mM EDTA. Upon addition of calf thymus DNA as a double stranded DNA in this solution, large hypochromic and small red shifts were observed in this buffered solution based on the threading type intercalation. The binding parameters of these derivatives with calf thymus DNA were calculated from McGhee & von Hippel-type Scatchard equation[8] and showed that the binding constants with 10^5 M^{-1} order (Data not shown) of these derivatives were varied depending on the kinds of their linker chains. This finding is in agreement with the results described previously.[5-7]

In the next step, the binding studies of these derivatives were carried out with d(TTAGGG)$_4$. Absorption maximum of these derivatives at 384 nm showed also the large hypochromic shift and small red shift same as a case of calf thymus DNA in the buffered solution of 0.1 M AcOK-AcOH (pH 5.6) containing 0.1 M KCl. Figure 1 shows that the typical absorption change of 10 μM **5** after titration of 150 μM d(TTAGGG)$_4$. This shows that all of these derivatives can bind to the tetraplex DNA even in such a high salt condition and this binding behaviour might contain the stacking interaction between the naphthalene diimide and guanine tetraplex planes. Spectrophotometric titration experiments of **1**, **2**, **5**, and **6** were succeeded, whereas the same experiments for **3**, **4**, and **7** were not succeeded because of the precipitation formation. However, this observation also suggested the binding possibility of these derivatives with this DNA. Table 1 summarized the binding parameters, K and n values calculated by equation (1) of Scatchard analysis. These results suggested that these derivatives can bind to tetraplex structure with 10-times higher binding affinity than that for double stranded one and four molecules of ferrocenylnaphthalene diimide derivatives were bound to one molecule of (TTAGGG)$_4$. Binding constants of ferrocenylnaphthalene diimide derivatives with (TTAGGG)$_4$ showed the similar values. However, the slightly improved binding affinity was observed within the error in the case of the ferrocenylnaphthalene diimide havening the piperazine chains. This might be contributing the some anchoring effect of the substituents of these derivatives in the complex with the tetraplex DNA. Preliminary study of *in vitro* telomerase inhibition suggested that these ligands could inhibit the telomerase elongation reaction.

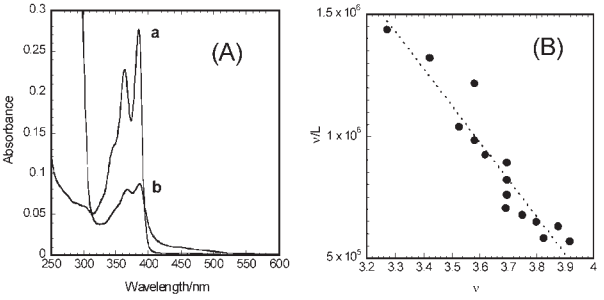


Fig. 1 (A) Absorption spectra of 10 μM ferrocenynaphthalene diimide **5** in the absence (a) or presence of 1 μM (TTAGGG)$_4$ (b) in 0.1 M AcOK-AcOH (pH5.6) containing 0.1 M KCl. (B) Scatchard plot for **5** with (TTAGGG)$_4$ under same as the condition of (A).

CONCLUSION

Series of the ferrocenylnaphthalene diimide derivatives **1** - **7** could strongly bind to the tetraplex structure of (TTAGGG)$_4$ with high salt condition. The binding ratio of these derivatives to the tetraplex DNA suggested that four these derivatives could bind to one human tetraplex DNA.

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