Searching study for the suitable ferrocynlnaphthalene diimide derivative in the electrochemical telomerase assay

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ABSTRACT

Interaction of ferrocynlnaphthalene diimide 1 with the tetraplex oligonucleotide, AGGG(TTAGGG)₉, which is a part of human telomere sequence, was studied in 0.1 M AcONa-AcOH (pH 5.5) containing 0.1 M NaCl coupled with 22-meric single and double stranded oligonucleotides using spectrophotometric titration experiment to search for the more suitable tetraplex DNA-binding ligand to achieve the electrochemical telomerase assay. CD spectra and polyacrylamide gel electrophoresis revealed that this tetraplex oligonucleotide kept to the single conformational structure of basket type G-quadruplex under these conditions. Scatchard analysis showed that 1 can bind to the tetraplex oligonucleotide with the binding constant of 10³ M⁻¹ order, which was the highest value among the other oligonucleotides. Binding number of 1 for these oligonucleotides were ca. 2, 11, and 3 for tetraplex, double stranded, and single stranded oligonucleotides, respectively. These values were reasonable when considering with the binding mode of 1 as a threading intercalator.

INTRODUCTION

Some of threading intercalators are known to have a high affinity for tetraplex DNA and have been studied in viewpoint of new anticancer drug focused on telomerase inhibitor.¹ Recently, we found out that ferrocynlnaphthalene diimide 1 could bind to tetraplex DNA at high potassium ion concentration and applied to electrochemical telomerase assay coupled with TS (telomerase substrate)-primer-immobilized electrode.² When sample solution had a telomerase activity, TS-primer on the electrode was elongated to form the telomeric repeat sequence and 1 could bind to the tetraplex DNA formed on the electrode to generate electrochemical signal of 1 depending on the telomerase activity. We also measured the binding affinities of the ferrocynlnaphthalene diimide derivatives carrying different linker chain for human telomere oligonucleotide of d(TTAGGG).³ However, the structure of this oligonucleotide is not clear in the aqueous solution adopted there. In this paper, we studied the binding affinity of 1 for AGGG(TTAGGG)₉, which is known to hold precise structure under 0.1 M NaCl conditions.⁴ Furthermore, the binding affinities of 1 for single and double stranded 22-meric oligonucleotide were studied under the same conditions.

Scheme 1 Structure of ferrocynlnaphthalene diimide 1

MATERIALS AND METHODS

Materials. Oligonucleotide carrying the part of human telomere sequence [dDNA, AGGG(TTAGGG)₉], single stranded oligonucleotide (ssDNA, 5’-CAC CCT ACA CAC CTT CAT CAC C-3’), or double stranded oligonucleotide between 5’-CAC CCT ACA CAC CTT CAT CAC C-3’ and its complementary sequence (dsDNA) was custom-synthesized by Genenet Co. (Fukuoka, Japan). 1 was synthesized by the procedure reported previously.⁵

Binding study. Spectrophotometric titration of 1 with varied amount of oligonucleotides was carried out in 0.1 M AcONa-AcOH (pH 5.5) containing 0.1 M NaCl, and absorption changes at 384 nm based on 1 were monitored. The binding affinities for oligonucleotides were determined from the data obtained by Scatchard analysis: v/v=–K(n-v) (1), where v is the moles of 1 bound per DNA molecules, and K is observed binding constant, and n is the maximum number of 1 bound per one DNA molecule.⁶

RESULTS AND DISCUSSION

Circular dichroism (CD) spectra of 1 with dDNA in 0.1 M AcONa-AcOH (pH 5.5) containing 0.1 M NaCl, suggested to form an intramolecular antiparallel “basket-type” G-quadruplex. Polyacrylamide gel electrophoresis showed the
existence of a single conformational structure. Melting curve measurement of dsDNA showed its single double stranded structure under the experimental conditions. Ferrocenylnaphthalene diimide 1 showed the absorption maximum at 384 nm in 0.1 M AcONa–AcOH buffer (pH 5.5) containing 0.1 M NaCl. Upon addition of these oligonucleotides, large hypochromic and small red shifts were observed in the buffered solution containing 1. Figure 1 showed the absorption change of 5 μM 1 after titration of tDNA. Spectrophotometric titration experiment of 1 with ssDNA or dsDNA showed similar titration curves as in Fig. 1. The behavior in the case of dsDNA is in agreement with that of calf thymus DNA as natural double stranded DNA and suggested that 1 could bind to dsDNA through threading intercalation. On the other hand, similar behavior was observed in the case of ssDNA. This is reasonable from the fact that naphthalene diimide can bind to single stranded DNA through the stacking interaction between aromatic planes of naphthalene diimide and nucleic-acid base.

The binding affinities of 1 in each case were calculated from the equation (1) and summarized in Table 1. Binding number, which can estimate the number of 1 bound to one oligonucleotide, was also showed in Table 1. Although the binding affinity of 1 for dsDNA is slightly higher than that for ssDNA, binding numbers is quite different for each DNA. About 11 molecules of 1 could bind to one 22-meric double stranded oligonucleotide of dsDNA. This is good agreement with the expected number according to the estimation of nearest-neighbour exclusion principle in the intercalation process. 1 could bind to ssDNA, but the number of the bound 1 was less than three. This suggested that 1 could bind to dsDNA through threading intercalation and the complex was stabilized in the case of dsDNA and supported our idea that 1 can be used for the discrimination of dsDNA from ssDNA.

The binding constant of 1 for tetrplex DNA was 10-times or 5-times larger than that for ssDNA or dsDNA, respectively. This result shows the highest affinity of 1 for tDNA and supported the data reported previously.7 Binding number in Table 1 also showed that two molecules of 1 were bound to one tDNA. This is reasonable when considering with the fact that threading intercalator bound to tetrplex DNA through the stacking with upper and/or lower G-quartet plane.6

**CONCLUSION**

To search for the more suitable ferrocenylnaphthalene diimide derivatives carrying high affinity for tetrplex DNA, we settled the experimental conditions where tetrplex DNA has single conformational structure. Under these conditions, we could estimate the binding affinity quantitatively by using oligonucleotide carrying single structural conformation and 1 could bind to tetrplex DNA with higher affinity than to single and double stranded DNAs. This result suggested that series of ferrocenylnaphthalene diimide derivatives synthesized could be evaluated quantitatively under the conditions studied in this experiment.

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**REFERENCES**

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**Table 1 Binding parameters of 1 with oligonucleotides having the different structures.**

<table>
<thead>
<tr>
<th>Oligonucleotide used in this experiment</th>
<th>$10^{9}$K/M $^1$</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>ssDNA: 5'-CAC CCT ACA CAC CTT CAT CAC C</td>
<td>1.8</td>
<td>2.8</td>
</tr>
<tr>
<td>dsDNA: 5'-CAC CCT ACA CAC CTT CAT CAC C</td>
<td>3.1</td>
<td>10.7</td>
</tr>
<tr>
<td>3'-GTT GGA TGT GTG GAA GTA GTG G</td>
<td></td>
<td></td>
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<tr>
<td>tDNA: 5'-AGG GTT AGG GTT AGG GTT AGG G</td>
<td>15.0</td>
<td>1.7</td>
</tr>
</tbody>
</table>

$^1$Experiments were carried out in 0.1 M AcONa-AcOH (pH 5.5), 0.1 M NaCl, 25°C.