Orientation of ends of G-quadruplex structure investigated with end-extended oligonucleotides

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ABSTRACT

The human telomere terminus can adopt the structure of a G-quadruplex. This structure has become an attractive target for anticancer drugs, because it effectively inhibits telomerase activity. In this study, we investigated the orientation of both 5’ and 3’ ends of the stable G-quadruplex structure. To verify the orientation, we designed end-extended G-quadruplex forming oligonucleotides. We carried out gel electrophoresis and the NMR analysis and found that the ends of the stable G-quadruplex structure are located on opposite faces of each of the quadruplexes.

INTRODUCTION

The formation and stabilization of the DNA G-quadruplex in the human telomeric sequence have been shown to inhibit the activity of telomerase, a cancer-specific reverse transcriptase that is activated in 80-90% of tumors⁵. Thus, the telomeric DNA G-quadruplex has been considered as an attractive target for cancer therapeutic intervention. Structural information on the human telomeric DNA G-quadruplex is necessary for the structure-based rational drug design. Patel and co-workers proposed four possible G-quadruplex topology² (Form a-d in Figure 1). NMR structural studies have been reported on two G-quadruplex structures (Form a and Form b)³,⁴, however, the other two G-quadruplex structures (Form c and Form d) have not been determined yet.

To verify the formation of these structures, we designed end-extended oligonucleotides and examined their electrophoretic mobility using the native PAGE analysis (Figure 1). We found that Form a and Form b are the stable G-quadruplex structures, in which the both ends of the folding structures point in opposite direction. NMR analysis of the oligonucleotides was consistent with the PAGE analysis.

RESULTS AND DISCUSSION

To distinguish four different G-quadruplex structures suggested by Patel and co-workers, we extended both ends of oligonucleotides (Figure 1). Because these extended tails are complementary to each other, the apparent molecular masses are different based on the orientation of the tails.

First, we examined native PAGE analysis to evaluate the mobility of d[TATATA(GGGTTA)₂GGTATATA] (extended tail sequences are underlined). We observed a relatively broad band for this oligonucleotide, which implies the existence of the rapid exchange between multiple G-quadruplex conformers.

Figure 1. Schematic structure of end-extended oligonucleotides

Figure 2. Native PAGE analysis of oligonucleotides. Lanes 1-5 contain ODN1 and ODN1a-1d, respectively.
Table 1 The sequence of oligonucleotides (\(^{13}G\) = 8-bromoguanine)

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence</th>
</tr>
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<tbody>
<tr>
<td>ODN1</td>
<td>5'-TATATA(^{13}G)GGTTA(^{13}G)GGTTA(^{13}G)GGTTA(^{13}G)GGTTATATA-3'</td>
</tr>
<tr>
<td>ODN1a</td>
<td>5'-TATATA(^{13}G)GGTTA(^{13}G)GGTTA(^{13}G)GGTTA(^{13}G)GGTTATATA-3'</td>
</tr>
<tr>
<td>ODN1b</td>
<td>5'-TATATA(^{13}G)GGTTA(^{13}G)GGTTA(^{13}G)GGTTA(^{13}G)GGTTATATA-3'</td>
</tr>
<tr>
<td>ODN1c</td>
<td>5'-TATATA(^{13}G)GGTTA(^{13}G)GGTTA(^{13}G)GGTTA(^{13}G)GGTTATATA-3'</td>
</tr>
<tr>
<td>ODN1d</td>
<td>5'-TATATA(^{13}G)GGTTA(^{13}G)GGTTA(^{13}G)GGTTA(^{13}G)GGTTATATA-3'</td>
</tr>
<tr>
<td>ODN2a</td>
<td>5'-A(^{13}G)GGTTA(^{13}G)GGTTA(^{13}G)GGTTA(^{13}G)GG-GG-3'</td>
</tr>
<tr>
<td>ODN2b</td>
<td>5'-A(^{13}G)GGTTA(^{13}G)GGTTA(^{13}G)GGTTA(^{13}G)GG-GG-3'</td>
</tr>
<tr>
<td>ODN2c</td>
<td>5'-A(^{13}G)GGTTA(^{13}G)GGTTA(^{13}G)GGTTA(^{13}G)GG-GG-3'</td>
</tr>
<tr>
<td>ODN2d</td>
<td>5'-A(^{13}G)GGTTA(^{13}G)GGTTA(^{13}G)GGTTA(^{13}G)GG-GG-3'</td>
</tr>
</tbody>
</table>

To limit the structural diversity, we introduced 8-bromoguanines (\(^{13}G\)) in oligonucleotides. Since the \(^{13}G\) favors a syn conformation, the introduction of \(^{13}G\) in syn conformation of G-quadruplex is known to stabilize the certain conformation. Thus, we prepared various \(^{13}G\)-containing oligonucleotides: ODN1a-d are assumed to stabilize the Form a-d, respectively (Table 1). The results of native-PAGE analysis of ODN1a-d indicate that two major bands were present for each oligonucleotide (Figure 2). Slower migrating bands are predominant in ODN1a and ODN1b, while faster migrating bands are predominant in ODN1c and ODN1d. Because both ends of ODN1c and ODN1d point in the same orientation, the folding structures of ODN1c and ODN1d are more compact than those of ODN1a and ODN1b. The PAGE analysis of ODN1 having consensus sequence of ODN1a-d showed that faster mobility species are predominant in ODN1, which implies that the ends of the stable G-quadruplex structure are located on opposite faces of each of the G-quadruplex.

Since we have already determined the detailed structure of ODN2b, we then examined the structure of other oligonucleotides, ODN2a,c,d (Figure 3). Observation of distinct twelve imino proton signals of ODN2a as in the case of ODN2b suggests the formation of a single species of stable Form a G-quadruplex. In contrast, imino proton resonances of ODN2c and ODN2d gave more complicated signals, which implies that Form c and d are unstable and there is an existence of exchange between multiple species of G-quadruplexes. The results are consistent with native-PAGE experiments.

CONCLUSION

The orientation of the ends in the G-quadruplex structure was investigated using native PAGE and NMR analyses. The end-extended \(^{13}G\)-containing oligonucleotides showed different mobility based on the G-quadruplex structures. We found that the ends of the stable G-quadruplex structure are located on opposite faces of each of the G-quadruplexes. This may provide important information for understanding the telomere structure and the development of telomere G-quadruplex-binding molecules as telomerase inhibitors.

REFERENCES


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