Multipyrne tandem probes for detection of C677T polymorphism in MTHFR gene

Svetlana A. Kholodar*, Daria S. Novopashina, Mariya I. Meschaninova, Alexander A. Lomzov and Alya G. Venyaminova

Institute of Chemical Biology and Fundamental Medicine SB RAS, 8 Lavrentiev ave., Novosibirsk 630090, Russia

ABSTRACT

We designed tandems of oligo(2'-O-methylribonucleotides) conjugates containing two bispyrene (5'-bisPyr and 3'-bisPyr) groups on their junction for detection of C677T polymorphism in the methylenetetrahydrofolate reductase gene (MTHFR). The potential of SNP detection with multipyrne tandems of oligo(2'-O-methylribonucleotides) was demonstrated.

INTRODUCTION

Previously we synthesized and tested the tandems of two bispyrene conjugates of oligo(2'-O-methylribonucleotides) as fluorescent probes for SNP detection.1 The present work is devoted to the detailed investigation of tandem probes targeted to the C677T polymorphism in methylenetetrahydrofolate reductase (MTHFR) gene (fragment 651-691nt). The effect of the tandem disposition relatively to SNP on mismatch discrimination efficacy was analyzed.

RESULTS AND DISCUSSION

Oligo(2'-O-methylribonucleotides) have a number of advantages as oligonucleotide probes including endonuclease resistance and sensitivity to mismatches.2 Bispyrene conjugates of oligo(2'-O-methylribonucleotides) bearing fluorophore at 5’- or 3’-terminus were designed as components of tandem (Fig.1). We used “inverted” thymidine as a 3’-terminal modification of 5’-pyrene conjugates to prevent its elongation by DNA polymerase during amplification. We designed two types of tandem of 5’- and 3’-bispyprene conjugates of oligo(2'-O-methylribonucleotides) which varied in disposition of tandem components relatively to SNP (Fig.1). The mismatch was situated in position 4 for the tandem 1 and in position 1 for the tandem 2. Two components of tandem are assembled by the target DNA, and pyrene residues form the intermolecular excited dimer (excimer).1

![Fig 1: Multipyrne tandem for detection of C677T polymorphism.](https://example.com/fig1)

![Fig 2: Stability of 5’-bispyrene component bisPyr-ggccccgaucaucacuaT(A) and control non-modified oligo(2'-O-methylribonucleotide) ggcggaacaucaucacucua(B) in a culture medium (IMDM) containing 10% fetal calf serum at 37°C. Probes were taken after 5, 10 and 30 min, 1, 3 and 6 h and 1 day of incubation (lanes 1-7).](https://example.com/fig2)

The chemical and biological stability is a general prerequisite for application of fluorescent probes in vitro and in vivo. The nucleolytic stability of all components in culture medium with serum was demonstrated. As example, introduction of 5'-bisPyr group and "inverted" thymidine in tandem component lead to noticeable increase of its stability (Fig.2). Moreover all components of tandems were completely stable in conditions corresponding to PCR: temperature up to 96°C, Tris-HCl (pH 8.9 at 25 °C) buffer, 2 hours (data not shown).

The 41-mer oligodeoxyribonucleotides corresponding to the region 651-691 nt of MTHFR gene were used as targets. The sufficient differences between Tm of fully complementary duplexes and Tm of duplexes containing mismatch were observed (Table 1). Melting temperatures of tandem 2 duplexes were lower than the melting temperatures of tandem 1 duplexes.
Fig. 3 The fluorescence emission spectra of tandem duplexes with the fully complementary DNA (curve 1) and mismatch DNA (curve 2). Spectra were recorded at 25°C at the excitation wavelength 345 nm in 16 mM Tris-HCl (pH 8.9) buffer containing 67 mM (NH₄)₂SO₄, 3.6 mM MgCl₂ and 0.05% Tween 20; [oligonucleotide] = 200 nM.

Table 1 The melting temperature of tandem duplexes⁵ and mismatch discrimination efficacy

<table>
<thead>
<tr>
<th>Duplex</th>
<th>Tm (°C)</th>
<th>ΔTm (°C)</th>
<th>I∞/I₀</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tandem 1/DNA (N=G)</td>
<td>64.6</td>
<td>-</td>
<td>2.7</td>
</tr>
<tr>
<td>Tandem 1/mmDNA (N=A)</td>
<td>48.1</td>
<td>16.5</td>
<td>3.3</td>
</tr>
<tr>
<td>Tandem 2/DNA (N=G)</td>
<td>54.3</td>
<td>-</td>
<td>2.8</td>
</tr>
<tr>
<td>Tandem 2/mmDNA (N=A)</td>
<td>39.1</td>
<td>15.2</td>
<td>4.3</td>
</tr>
</tbody>
</table>

*Tm have been obtained by fitting procedure of UV-melting curves; Tm (°C) - melting temperature; ΔTm (°C) - difference between Tm for match and mismatch duplexes (16 mM Tris-HCl (pH 8.9) buffer containing 67 mM (NH₄)₂SO₄, 3.6 mM MgCl₂; [tandem]=1·10⁻⁷ M, [target]=1·10⁻⁸ M).

Fig. 4 First derivatives of melting curves based on excimer fluorescence of duplexes 1 – Tandem 2-DNA and 2 – Tandem 2/mmDNA. Thermal dependence of fluorescence was recorded in 16 mM Tris-HCl (pH 8.9 at 25°C) buffer containing 67 mM (NH₄)₂SO₄, 3.6 mM MgCl₂ and 0.05% Tween 20 at the excitation wavelength 345 nm; temperature at 480 nm; [oligonucleotide]=200 nM.

The difference between the intensity of excimer fluorescence in match and mismatch duplexes was more pronounced in the case of tandem 2 (Fig.3). The monomeric fluorescence was the same for both types of DNA target. The ratio of the intensity of eximer fluorescence to the intensity of monomeric fluorescence (I∞/I₀) can be used for determination of SNP (Table 1).

The changes of eximer fluorescence intensity during tandem duplex denaturation permitted us to use the melting curve analysis for determination of duplex thermal stability and SNP detection (Fig.4). The potential of SNP detection with tandems of pyrene-labeled oligo(2'-O-methylribonucleotides) by duplex melting curve analysis based on excimer fluorescence registration was demonstrated.

CONCLUSION

Our results are permitted to conclude that designed multipyrene tandem constructions on the base of oligo(2'-O-methylribonucleotides) possessing high binding affinity to DNA, high nucleolytic resistance and sensitivity of fluorescence to the mismatch are perspective instruments of molecular biotechnology and may be used as fluorescent probes for SNP detection.

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*Corresponding author. E-mail: danov@nibo.ch.nsc.ru