Clear distinction of pyrimidine bases on the complementary strand by fluorescence change of novel fluorescent nucleosides

Kazuo Tanaka, Akimitsu Okamoto and Isao Saito
Department of Synthetic Chemistry and Biological Chemistry, Faculty of Engineering, Kyoto University and SORST, Japan Science and Technology Corporation, Kyoto 606-8501, Japan

ABSTRACT

New fluorescent nucleosides, methoxybenzodeazaadenine ($^{MDA}$) and methoxybenzodeazainosine ($^{MDI}$) which can sharply distinguish between C and T bases, respectively. The hybridization of an ODN probe containing $^{MDA}$ and $^{MDI}$ with a target DNA facilitates the judgment with the fluorescence spectra of the type of pyrimidine bases located at a specific site on the target DNA. The $^{MDA}$- and $^{MDI}$ containing ODN are very effective probes for pyrimidine SNP typing.

RESULTS and DISCUSSION

Synthesis of ODNs containing $^{MDA}$ and $^{MDI}$

The synthesis of $^{MDA}$ and incorporation into DNA were previously reported.6

The synthesis of $^{MDI}$ nucleoside was readily achieved in two steps from 4-chloro-1H-pyrimido[4,5-b]indole as shown in Scheme 1.7 The nucleoside 4 was converted to phosphoramidite 5 for use in a DNA synthesizer. The ODNs synthesized are summarized in Table 1.
Scheme 1. Synthesis of MD\textsuperscript{I} phosphoramidite unit.\textsuperscript{a}

\textsuperscript{a} Reagents and conditions: (a) sodium hydride, acetonitrile, 2, 82%; (b) 6 N NaOHaq, 94%; (c) 4,4'-dimethoxytrityl chloride, pyridine, 91%; (d) 2-cyanoethyl tetraisopropylphosphoramidite, tetrazole, acetonitrile, quant.

Table 1. Oligodeoxynucleotides (ODNs) used in this study

<table>
<thead>
<tr>
<th>Sequences</th>
<th>ODN(MDA)</th>
<th>5'-d(CGCAAT\textsuperscript{MD}ATAACGC)-3'</th>
</tr>
</thead>
<tbody>
<tr>
<td>ODN(MDI)</td>
<td>5'-d(CGCAAT\textsuperscript{MDI}ATAACGC)-3'</td>
<td></td>
</tr>
<tr>
<td>ODN(T)</td>
<td>5'-d(GCGTTATATTGCC)-3'</td>
<td></td>
</tr>
<tr>
<td>ODN(C)</td>
<td>5'-d(GCGTTACATTGCC)-3'</td>
<td></td>
</tr>
</tbody>
</table>

The fluorescence spectra of ODNs

We measured the fluorescence spectra of ODNs containing MDA and MD\textsuperscript{I} duplexes (Figure 1). The fluorescence behaviors of ODNs were strongly dependent on the pyrimidine bases opposite MDA or MD\textsuperscript{I}. The fluorescence spectra of ODN(MDA)/ODN(C) had a strong fluorescence peak at 420 nm, whereas the fluorescence of the ODN(MDA)/ODN(T) duplex was almost completely quenched. In contrast, The fluorescence spectra of ODN(MDI)/ODN(T) had a strong fluorescence peak at 400 nm, whereas the fluorescence of the ODN(MDI)/ODN(C) duplex was quenched.

Figure 1. (a) Fluorescent spectra of 25 \(\mu\text{M} ODN(MDA)\) hybridized with 25 \(\mu\text{M} ODN(T)\) or ODN(C) (50 mM sodium phosphate, 0.1 M sodium chloride, pH 7.0, r.t.). Excitation was at 330 nm. (b) Fluorescent spectra of 25 \(\mu\text{M} ODN(MDI)\) hybridized with 25 \(\mu\text{M} ODN(T)\) or ODN(C) (50 mM sodium phosphate, 0.1 M sodium chloride, pH 7.0, r.t.). Excitation was at 320 nm.

In summary, we have devised a simple method for the detection of single nucleotide alteration by exploiting novel BDF probes.

REFERENCES