Synthesis and biological properties of DNA-sugar conjugates

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ABSTRACT
In order to improve the pharmacological properties of antisense and antigen oligonucleotides, oligodeoxynucleotides conjugated with amino sugars at the 5'-terminus were synthesized by solid phase fragment condensation (SPFC) method. The obtained DNA-sugar conjugates were evaluated in their chemical and biological activities to show that 5'-end modification of oligonucleotides with sugars enhanced the thermal stability of the hybrid duplex with complementary DNA, the resistance against nuclease digestion and the membrane permeability of the conjugates.

RESULTS AND DISCUSSIONS

Synthesis of DNA-Sugar Conjugates by Solid Phase Fragment Condensation

Synthesis of DNA-sugar conjugates were achieved as shown in Scheme 1. DNA fragment assembled on CPG support by automated DNA synthesizer was modified at 5'-terminus with carbonyldimidazole (CDI), ethylenediamine and hexamethylene-1,6- disocyanate, successively on solid phase. With the isocyanato derivative, amino sugars, D-glucosamine and D-galactosamine, were coupled at room temperature, respectively. Amino sugars were subjected to the reaction without any protective groups on their hydroxyl groups. After treatment with aqueous ammonia at 50 °C for 6h, the products were purified on RPHPLC to give a desired product as a pure form. The yields of C1 and C2 were determined based on the absorbance at 260 nm to be 28.6 % and 13.4 %, respectively.

Hybridization Properties of DNA-Sugar Conjugates

Hybridization properties of the conjugates C1 and C2 were investigated by UV melting study and surface plasmon resonance (SPR) analysis.

UV melting study at 260 nm showed that both C1 and C2 could form hybrid duplex with complementary DNA with higher affinity than natural double stranded DNA. (Table) It is notable that the melting temperature of the duplex of C1 and its complementary DNA strand was higher than natural dsDNA by 10.0 °C and that of C2 by 5.8 °C in the absence of Mg²⁺.

INTRODUCTION

Inhibition of a specific gene by antisense and antigen oligonucleotides has been intensively studied and have attracted much attention from a medical point of view. Although chemical modifications on oligonucleotides have given them a number of advantages for their practical use, it is true that further improved properties of the drugs are required for the antisense and the antigen therapeutic method to be well established in a true sense of the word. In the present study, we investigated on the synthesis of DNA-sugar conjugates by solid phase fragment condensation and the biological properties of them in order to improve the pharmacological properties of them by conjugation of oligonucleotides with functional biomolecules.
ODMT

1) 3% TCA

2) ImCOIm (50 eq.), CH3CN, r.t., 30 min
3) NH2CH2CH2NH2 (20 eq.), r.t., 1 h

OCN-(CH2)6NCO (50 eq.), CH3CN, r.t., 2 h

Sugar-NH2 (10 eq.), r.t., 12 h

1) conc NH3, 55°C, 6 h
2) RPHPLC purification

N1: 5'-AGAGAGAGAGAAA-3'
N2: 5'-TTTTTTCTCTCTCTCT-3'

<table>
<thead>
<tr>
<th>Hybrids</th>
<th>Tm/ΔTm (°C)</th>
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<tbody>
<tr>
<td>N1/N2</td>
<td>47.0/-41.0/-</td>
</tr>
<tr>
<td>N2/C1</td>
<td>49.8/+2.8</td>
</tr>
<tr>
<td>N1/C2</td>
<td>48.5/+1.5</td>
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</tbody>
</table>

Conditions: 50 mM Tris buffer, pH 7.0, [NaCl] = 100 mM, [OligoDNA] = 3mM, [Mg2+] = 20 mM or none

SPR study was performed on BIACORE X for C2. Biotin labeled oligonucleotide (5'-AGAGAGAGAGAAA-3') was immobilized on Streptavidin modified tip, and SPR spectrogram was recorded for the C2 solution at various concentrations (0.25 - 2.5 μM) at 20 °C. Binding constant Ks was determined as 1.29 x 107. (Ks = 4.07 x 104, kd = 3.15 x 103)

Resistance of DNA-Sugar Conjugates against Nuclease Digestion

Nuclease resistance of the conjugates C1 and C2 were investigated with DNase 1 (data not shown). The results showed that modification of oligonucleotides with sugars at the 5'-end enhanced the stability of the oligonucleotide against the endonuclease digestion.

Cellular Uptake of DNA-Sugar Conjugates

Analyses by a fluorescence microscope and a flow cytometry revealed that cellular uptake of DNA-sugar conjugate C1 and C2 revealed that membrane permeability of the conjugates were highly enhanced (data not shown).

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REFERENCES