Development of DNA-intercalator-polyamine multi-conjugate bearing the ability of the sequence-specific RNA hydrolysis

Tomohisa Moriguchi, Tomoyuki Ohike and Kazuo Shinozuka

Department of Chemistry, Gunma University, 1-5-1 Tenjincho, Kiryu, Gunma 376-8515, Japan

ABSTRACT

A novel anthraquinone-trisamine modified DNA conjugate was developed. The trisamine-bearing 2,7-disubstituted anthraquinone derivative was introduced into the central position of the DNA oligomer. The ability of the sequence-specific RNA cleavage of this novel modified DNA was investigated.

INTRODUCTION

It has been known that no natural sequence-specific RNase is reported up to date, therefore, the development of the artificial RNases are valuable for the molecular biology and the sequence-specific regulation of the gene expression. Previously, we reported that the novel intercalator-polyamine conjugate, which mimicked the active site of the RNA hydrolysis enzyme, RNase A, constructed by acridine as an intercalator with a primary amine and an imidazole moiety as a general acid-base catalyst, showed the hydrolysis of the RNA molecule. Therefore, these results prompted us to study the development of the artificial sequence-specific RNase containing the polyamine moiety as an RNA cleavable moiety. Previously, we reported the novel artificial DNA that contained the deoxyuridine derivatives bearing the tris(2-aminoethyl)amine moiety at the C-5 position of the uracil base in strand of the canonical thymidine at the central position of the DNA. When the sequence specific cleavage of the complementary RNA was attempted by this modified DNA, the specific cleavage of the target RNA was observed, however, the cleavage position of the target RNA was not the complementary site of the deoxyuridine derivative, but the single-stranded position. These results were concluded that the RNA cleavage moiety seemed to be absent near the favourable position of the effective RNA cleavage. Next, in order to locate the RNA cleavage moiety more tightly near the favourable position for the effective RNA cleavage, we introduced the intercalator molecule with the polyamine moiety to the modified DNA molecule to develop the excellent RNA cleavage molecules.

In this report, we develop the novel DNA-intercalator-polyamine multi-conjugate having the ability of the sequence-specific RNA hydrolysis. The disubstituted anthraquinone moiety was used as an intercalator in this study, and this anthraquinone moiety was introduced into the central position of the modified DNA by the connection with the non-nucleoside backbone, 2,2-bis(hydroxymethyl)propionic acid, instead of the corresponding nucleoside. The ability of the sequence-specific cleavage of the target RNA 21 mer was also evaluated.

RESULTS AND DISCUSSION

The 2,7-disubstituted anthraquinone derivative was used as a result of the modelling of the complex of the anthraquinone moiety in the DNA/RNA hetero duplex in this study. The starting material, 2,7-dichloroanthraquinone (1), which was prepared from anthraquinone-2,7-disulphonic acid di-sodium salt, was allowed to react with the linker diamine derivative, 1,3-diaminopropane to give the corresponding mono-substituted derivative (2). The non-nucleoside backbone, 2,2-bis(hydroxymethyl)propionic acid, was condensed with the monoamine derivative in the presence of the condensing reagent, DMT-MM, to give the desired product (3) in a good yield. When this reaction was carried out in the presence of DCC as a condensing reagent, the desired product (3) was given in a relatively low yield. The polyamine moiety was introduced into the 7-position of the anthraquinone moiety, and the primary amine groups were protected with trifluoroacetyl group by the reaction with the excess amounts of ethyl trifluoroacetate. The amine-

Fig. 1 Sequence and structure of the modified DNA and target RNA in this study.
protected diol derivative (5) was converted to the corresponding phosphoramidite derivative (6) by the sequential dimethoxytritylation and phosphitylation by the standard procedures.

The introduction of this phosphoramidite derivative into the modified DNA was carried out by the modified procedure, that the coupling periods of the anthraquinone phosphoramidite was extended to 360 s and the concentration of the MeCN solution of the phosphoramidite derivative was changed to 0.3 M. After the synthesis of the modified DNA by the automated DNA synthesizer, the standard deprotection and purification procedures were carried out to give the desired modified DNA GK-2038 in 21.8% isolated yield.

Next, in order to establish the interaction of the anthraquinone moiety into DNA duplex, the thermal stability of the duplex with the complementary DNA was measured (Table 1). The thermal stability of the duplex containing anthraquinone moiety (GK-2038) showed the lower thermal stability than the corresponding full match duplex (GK-1041). However, when compared with the 1 base-deleted duplex (GK-1042), the slight stabilization was observed, therefore, the interaction of the anthraquinone moiety into the duplex DNA seemed to be occurred. This prediction was also supported by the observation of the hyperchromic shift of the anthraquinone moiety (around 550 nm) on UV-visible absorption spectra measurement.

### Table 1: Thermal stability of the duplex DNAs*.

<table>
<thead>
<tr>
<th></th>
<th>Tm (°C)</th>
<th>ΔTm (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GK-1041</td>
<td>50.6</td>
<td></td>
</tr>
<tr>
<td>GK-1042</td>
<td>40.6</td>
<td>-10.0</td>
</tr>
<tr>
<td>GK-2038</td>
<td>43.3</td>
<td>-7.2</td>
</tr>
</tbody>
</table>

*2.5 μM each DNA, 10 mM sodium phosphate, 100 mM NaCl (pH 7.4).

From the confirmation of the interaction of the anthraquinone moiety into duplex DNA, the target RNA cleavage experiment by GK-2038 was carried out. The site-specific cleavage of the target RNA was observed by the HPLC analysis. Moreover, the confirmation of the cleavage site of the target RNA was confirmed by the gel electrophoresis studies.

### REFERENCES