Stereochemically Pure Acridine-modified DNA for Site-selective Activation and Scission of RNA

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Two phosphoramidite monomers bearing acridine, which differ in the configuration at the branching point of the linker, have been synthesized and incorporated in DNA. In site-selective RNA scission by Lu(III), the DNA derived from L-threoninol linker is 2.5 times as effective as that from D-threoninol, confirming the importance of appropriate stereochemistry for efficient RNA activation.

To date, various conjugates of oligonucleotides with functional molecules (e.g., fluorescent dyes, intercalating agents, metal complexes, and others) have been prepared because of their attractive potentials for biological and biochemical applications.[1] Although functional molecules have been often tethered to either the terminus of main chain of oligonucleotide or the nucleoside, the introduction of them to the main chain within DNA strand has been also attracting broad interest.[2] In this main-chain modification, stereochemistry of the central carbon atom tethering the functional molecules is crucially important, since it governs both the position and orientation of the molecules in helically structured nucleic acids.[3] Nevertheless, little is yet known about the preparation of stereochemically pure DNA conjugates and the effect of this stereochemistry on their functions.

Recently, we found that acridine-bearing DNA is effective for site-selective RNA scission.[4,5] When these conjugates form heteroduplexes with complementary RNA, the phosphodiester linkages of RNA opposite to the acridine are efficiently activated, and selectively and rapidly hydrolyzed by metal ions such as lanthanide(III), Zn(II), and Mn(II). Acid catalysis by the ring nitrogen of acridine is one of the keys for this RNA activation.[4b,6] The conjugates used there were prepared by using 1,3-propanediol linker and involved a chiral central carbon. Accordingly, the reaction system was a mixture of two diastereomers, in which the acridine initially heads toward two different directions. This situation is unfavorable for detailed analysis of the mechanism of RNA activation and also for optimal catalytic activity. Here, we solve this problem by synthesizing two stereochemically pure acridine-DNA conjugates (**1-D** and **1-L**). The effect of chirality of the central carbon atom on the RNA activation is quantitatively analyzed.

The phosphoramidite monomers for the stereochemically pure introduction of acridine residues were synthesized from D- or L-threoninols in 6 steps, and incorporated in the middle of the 36-mer DNA with standard phosphoramidite chemistry.[7] All the oligonucleotides in Figure 1 were purified by PAGE and RP-HPLC, and fully characterized by MALDI-TOF MS. Site-selective RNA cleavage reactions were performed at pH 8.0 and 37 °C for 4 h, and analyzed by PAGE under denaturing conditions.[8]

In the absence of any oligonucleotide additives, the substrate RNA_1 was randomly cleaved by Lu(III) (lane 1 in Figure 2). The conversion for the cleavage at each phosphodiester was ca. 1.5%. When DNA_1-L (derived from L-threoninol) was added to the system, however, the RNA_1 was site-selectively and efficiently cleaved at the target site U-19 (lane 3: Note that this site is just in front of the acridine in the RNA_1/DNA_1-L heteroduplex). The cleavage yield at the 5′-phosphate of U-19 (the lower band) was 11.0%.[9] In comparison with the phosphodiester linkage in the single-stranded substrate in lane 1, the target linkage is 7.3 fold activated by the acridine in DNA_1-L. Quite interestingly, this RNA-activating ability is greater than that of its diastereomeric isomer DNA_1-D. When the combination of DNA_1-D and Lu(III) was used for the reaction, the acceleration with respect to lane 1 was only 2.9 fold (lane 2). The RNA-activating ability is notably dependent on the configuration at the central carbon atom.

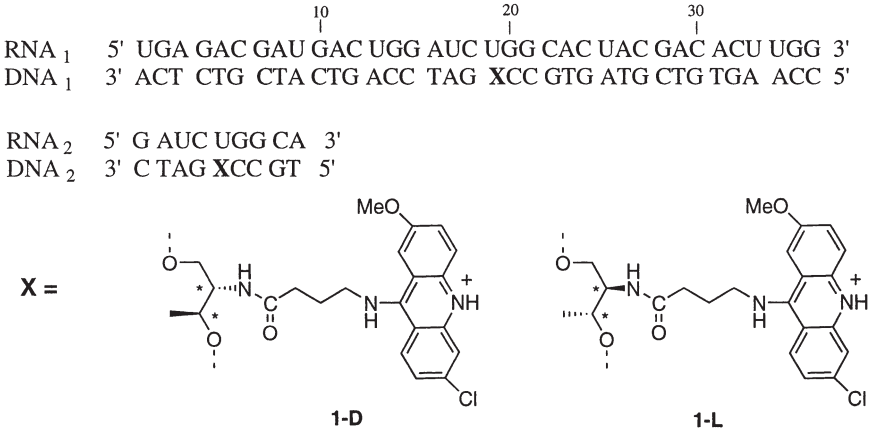

Figure 1. Structures of the substrate RNA and the DNA oligomers used in the present study.

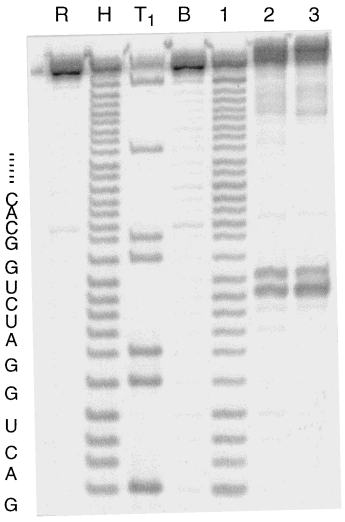

Figure 2. Site-selective RNA scission by Lu(III) in the presence of stereochemically pure acridine-modified DNA. Lane 1, Lu(III) only; lane 2, DNA_1-D; lane 3, DNA_1-L. At pH 8.0 and 37 °C for 4 h; $[RNA_1] = 5\,\mu mol\,dm^{-3}$; $[DNA] = 10\,\mu mol\,dm^{-3}$; $[Lu(III)] = 100\,\mu mol\,dm^{-3}$; [Tris-HCl] = $10\,mmol\,dm^{-3}$; $[NaCl] = 200\,mmol\,dm^{-3}$. R, RNA_1 only; H, alkaline hydrolysis; T_1, RNase T_1 digestion; B, control reaction in buffer solution.

In contrast with this large dependence of RNA-activating ability on the stereochemistry, the T_m values of the heteroduplexes of these two diastereomeric isomers were almost the same (the T_m of DNA_2-L/RNA_2 was 38.0 °C, and that of DNA_2-D/RNA_2 was 37.5 °C).[10] Thus, the possibility that the L-isomer binds to the RNA more strongly than does D-isomer and this factor promotes the RNA activation by the former is unlikely. Both **1-D** and **1-L** have rather flexible propane-derived side chains. Accordingly, their acridines can take similar positions in the heteroduplexes by strong electrostatic and stacking interactions, although the side-chain of the D-isomer first takes a wrong direction for the intercalation. Assumedly, the large difference of RNA-activating ability is ascribed to subtle difference in the orientation of the acridine that determines the efficiency of acid catalysis. In a proposed mechanism, the proton on the ring-nitrogen of the acridine in the L-isomer straight heads for the leaving O5′ atom in the transition state, whereas the acridine in the D-isomer is oriented less favorably (Figure 3).

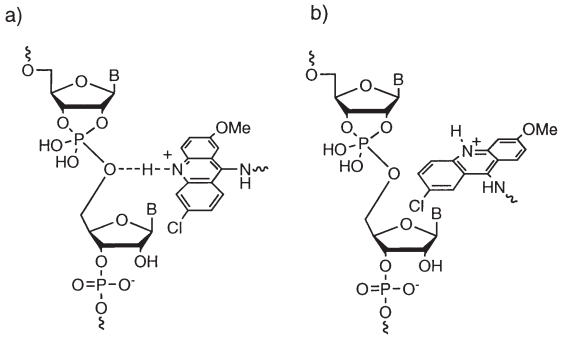

Figure 3. Proposed orientation of the acridines in L-isomer (a) and D-isomer (b) in the transition state for the RNA hydrolysis.

In conclusion, two kinds of stereochemically pure acridine-DNA conjugates were successfully prepared, and the difference of configuration at only one carbon atom results in large difference of RNA-activating ability of the acridine.

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References and Notes

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7 D-Threoninol (or L-threoninol) was first coupled with N-Fmoc-protected 4-amino-*n*-butyric acid, and then the primary alcohol in the adduct was protected by dimethoxytrityl group. After the Fmoc group was removed, the product was reacted with 6-chloro-9-(*p*-chlorophenoxy)-2-methoxyacridine. Finally, the resulting 9-amino-6-chloro-2-methoxyacridine derivative was reacted with 2-cyanoethyl tetraisopropyl phosphorodiamidite to obtain the phosphoramidite monomer for **1-D** (or **1-L**). The phosphoramidites in anhydrous acetonitrile ($0.1\,mol\,dm^{-3}$) was used for the DNA synthesis.

8 For the cleavage reactions, 2 equiv. of DNA was used in order to minimize RNA that is free from binding with the DNA and to facilitate quantitative analyses. The cleavage is pseudo-first order reaction around this reaction time.

9 Selective cleavages occur also at the 3′-phosphate of U-19 (the upper band; 2.5% yield). However, this cleavage also occurs when unmodified 1,3-propanediol linker is introduced into DNA in place of **1-L** (or **1-D**), and is not much activated by the acridine modification (see ref. 4b).

10 For experimental convenience, the middle part of RNA_1 (RNA_2 in Figure 1) was used together with the corresponding DNA. Conditions: $[RNA] = [DNA] = 1\,\mu mol\,dm^{-3}$, [Tris-HCl] = $10\,mmol\,dm^{-3}$, $[NaCl] = 200\,mmol\,dm^{-3}$. The heating rate was 1.0 °C/min.

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