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Synthesis of Double-Headed 2-5A-Antisense Chimeras and Their Ability to Activate Human RNase L

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Abstract—The synthesis of a novel 2-5A-antisense chimera having two molecules of a 2-5A tetramer at the 5′-terminus of the antisense moiety with a 2-(hydroxymethyl)-1,3-propanediol linker is described. The ability of the synthesized 2-5A antisense chimeras to activate RNase L was estimated by monitoring the cleavage of a target RNA by the activated RNase L. The double-headed 2-5A-antisense chimera linked with two molecules of a butanediol linker more efficiently cleaved the target RNA as compared with the single-headed 2-5A-antisense chimera and the double-headed 2-5A-antisense chimera linked with a molecule of the butanediol linker.

A small oligoadenylate containing unique 2′,5′-phosphodiester bonds, known as 2-5A, plays a key role in mediating the antiviral effect of interferon.[1,2] RNase L, an enzyme found in many eukaryotic cells, is allosterically activated by 2-5A. The activated RNase L cleaves single-stranded RNAs preferentially on the 3′-side of UpNp.[3]

Recently, an oligonucleotide modified with a 5′-monophosphorylated 2-5A tetramer has been applied to antisense studies.[2,4] The antisense moiety of the 2-5A-antisense chimera sequence-specifically binds to mRNA. The 2-5A moiety activates RNase L, and then the activated RNase L cleaves mRNA. Thus, even if a duplex between an antisense oligonucleotide and mRNA is not a substrate of RNase H, the 2-5A-antisense chimera can irreversibly inhibit the translation of mRNA. Quite recently, we have also reported the synthesis of 2-5A-antisense chimeras modified with a hydroxyethyl group at the 5′-phosphate and/or containing 8-methyladenosine at the 2-5A moiety.[5] The 2-5A-antisense chimera with the hydroxyethyl group was more resistant to hydrolysis by alkaline phosphatase than that without the hydroxyethyl group. Furthermore, we showed that the 2-5A-antisense chimera modified with the hydroxyethyl group and containing 8-methyladenosine is more efficient in human RNase L activation ability than that without the hydroxyethyl group and 8-methyladenosine. However, it also turned out that those 2-5A-antisense chimeras were 40–80-fold less potent than the parent 2-5A tetramer, itself, in RNase L activation ability.[5]

It is known that RNase L is activated by binding with a 2-5A molecule followed by dimerization.[6,7] Although the mechanism underlying the difference in potencies of the 2-5A-antisense chimeras and the 2-5A tetramers in RNase L activation has not yet been investigated, it may involve steric interference by the antisense moiety in the interaction of the 2-5A moiety and the enzyme, or in the process of the enzyme dimerization. To resolve these problems, we designed a novel 2-5A-antisense chimera having two molecules of the 2-5A tetramer at the 5′-terminus of the antisense moiety with a 2-(hydroxymethyl)-1,3-propanediol linker (Fig. 1). Here, we report a synthesis of the double-headed 2-5A-antisense chimeras and their ability to activate recombinant human RNase L.

A single-headed 2-5A-antisense chimera, ASC **1**, and the double-headed 2-5A-antisense chimeras, ASC **2** and **3**, were synthesized with a DNA/RNA synthesizer using phosphoramidite units **4**–**7**[8] (Fig. 2). The phosphoramidite **7** was synthesized as follows: 2-(Hydroxymethyl)-1,3-propanediol was treated with 2.1 equivalents of DMTrCl in pyridine to give 1,3-*O*-bis(dimethoxytrityl)-2-hydroxymethyl-1,3-propanediol in 50% yield. The bis-DMTr derivative was treated with 2-cyanoethyl tetraisopropylphosphorodiamidite in the

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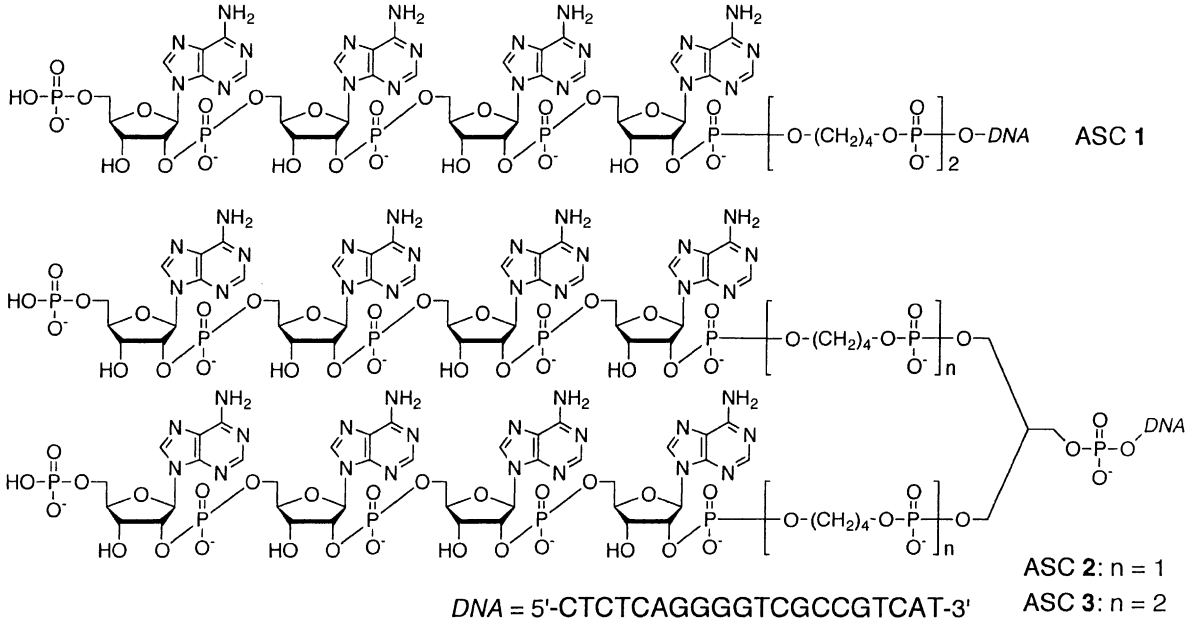

Figure 1. Structures of 2-5A-antisense chimeras.

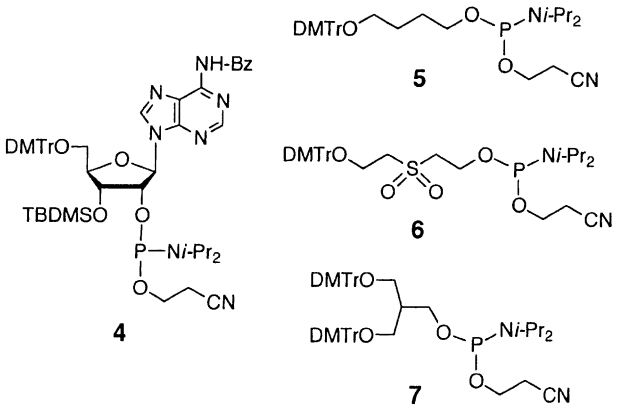

Figure 2. Structures of phosphoramidite units.

presence of tetrazole in CH_3CN to afford the phosphoramidite **7**[10] in 76% yield. Deprotection of synthesized 2-5A-antisense chimeras was performed according to the reported procedure.[5] The obtained 2-5A-antisense chimeras were purified by reversed-phase HPLC or denaturing 20% polyacrylamide gel electrophoresis (PAGE) to give ASC **1**, **2** and **3** in 24, 5, and 3 OD units at 260 nm starting from 1 μmol scale, respectively. These ASCs were analyzed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF/MS), and the observed molecular weights supported their structures.[11]

Carroll and co-workers reported that initial cleavage of a synthetic RNA **8**, 5′-r($C_{11}U_2C_7$)-3′, occurs on the 3′-side of r($C_{11}U_2$) to yield an r(C_{11}UpUp) fragment with a 3′-phosphate, and a second cleavage occurs on the 3′-side of r(C_{11}U) to give an r(C_{11}Up) fragment with a higher enzyme concentration or longer incubation time.[3] Thus, the ability of ASCs to activate

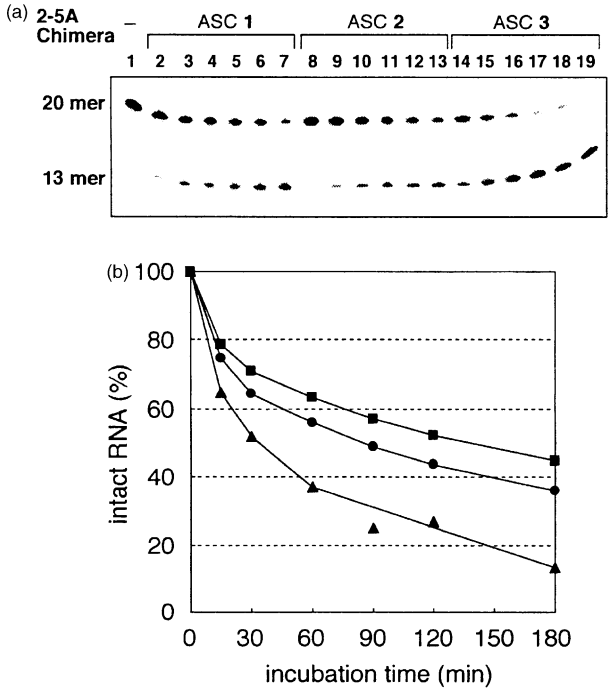

Figure 3. (a) Polyacrylamide gel electrophoresis of 5′-^{32}P-labeled RNA **8** hydrolyzed by recombinant human RNase L activated with 2-5A-antisense chimeras. RNAs were incubated with RNase L for 15 min (lanes 2, 8, and 14), 30 min (lanes 3, 9, and 15), 60 min (lanes 4, 10, and 15), 90 min (lanes 5, 11, and 17), 120 min (lanes 6, 12, and 19), and 180 min (lanes 7, 13, and 19). (b) Percentages of intact RNAs. ASC **1** (●), ASC **2** (■), and ASC **3** (▲).

RNase L was estimated by monitoring the cleavage of a synthetic RNA **8**, 5′-r($C_{11}U_2C_7$)-3′, by the activated RNase L. Recombinant human RNase L was expressed in *Escherichia coli* and purified according to the reported procedure.[12] The RNA (100 nM) labeled at the 5′-end with ^{32}P was incubated with the enzyme

(240 nM) that had been pre-incubated with the 2-5A-antisense chimeras (500 nM).[13] The reactions were analyzed by 20% PAGE under denaturing conditions (Fig. 3a). The densities of radioactive bands on the gel were determined with a bio-imaging analyzer. As shown in Figure 3b, the half-lives of the target RNA **8** were 87, 136 and 34 min for ASC **1**, **2** and **3**, respectively. Thus, it was found that ASC **3** more efficiently cleaved the target RNA as compared with ASC **1** and **2**.

In conclusion, we have synthesized novel 2-5A-antisense chimeras, ASC **2** and **3**, having two molecules of a 2-5A tetramer at the 5′-terminus of their antisense moieties. The double-headed 2-5A-antisense chimera, ASC **2**, linked with two molecules of the butanediol linker more efficiently cleaved the target RNA as compared with the single-headed 2-5A-antisense chimera, ASC **1**, and the double-headed 2-5A-antisense chimera, ASC **3**, linked with a molecule of the butanediol linker. Thus, ASC **3** will be a candidate for a novel antisense molecule.

Acknowledgements

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