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 2-5A tetramer at the 5'-terminus of the antisense moiety with an ethylene glycol 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. It was found that 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.

INTRODUCTION
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). 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 UpN. Recently, an oligonucleotide modified with a 5'-monophosphorylated 2-5A tetramer has been applied to antisense studies (1). The antisense moiety of a 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 a 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 (2). 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 activates human RNase L as efficiently as that without the hydroxyethyl group and 8-methyladenosine. However, it was also revealed that those 2-5A-antisense chimeras were 40-80 fold less potent than the parent 2-5A tetramers in the ability to activate RNase L. It is known that RNase L is activated by binding with a 2-5A molecule and then dimerizing (1). Although the mechanism underlying the difference of potencies of the 2-5A-antisense chimeras and the 2-5A tetramers in activating RNase L 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 on the enzyme dimerization. To overcome 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 an ethylene glycol linker (Fig. 1). In this paper, we report a synthesis of the double-headed 2-5A-antisense chimeras and the ability of those to activate recombinant human RNase L.

RESULTS AND DISCUSSION
The double-headed 2-5A-antisense chimeras, ASDNA 2 and ASDNA 3, and a single-headed 2-5A-antisense chimera, ASDNA 1, were synthesized by the phosphoramidite method with a DNA/RNA synthesizer. The ability of the synthesized 2-5A antisense chimeras to activate RNase L was estimated by monitoring the cleavage of a target RNA 4, 5'[(CCCCCCCCCCCUUCCCCCCC)]3', by the activated RNase L. Recombinant human RNase L was expressed in E. coli and purified according to the reported procedure with a
Figure 1. Structures of 2-5A-antisense chimeras.

Figure 2. Polyacrylamide gel electrophoresis of 5'-32P-labeled RNA 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).

Figure 3. Percentages of intact RNAs. ASDNA 1 (●), ASDNA 2 (●), and ASDNA 3 (▲).

slight modification (3). The RNA (100 nM) labeled at the 5'-end with 32P was incubated with the enzyme (240 nM) that had been pre-incubated with the 2-5A-antisense chimeras (500 nM). The reactions were analyzed by polyacrylamide gel electrophoresis under denaturing conditions (Fig. 2). It was found that the double-headed 2-5A-antisense chimera, ASDNA 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, ASDNA 1, and the double-headed 2-5A-antisense chimera, ASDNA 3, linked with a molecule of the butanediol linker.

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