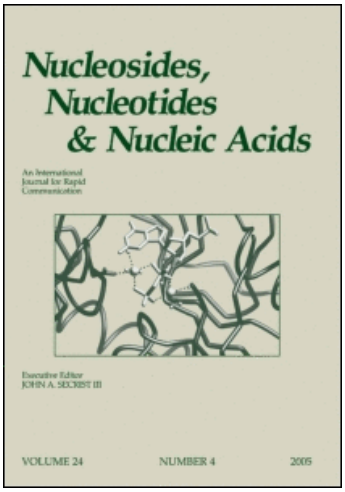

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THE SYNTHESIS OF 2-5A ANTISENSE CHIMERAS WITH VARIOUS NON-NUCLEOSIDE COMPONENTS.

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Abstract : We have synthesized a series of 2-5A chimeras in which the nature of the oligoadenylate-antisense linkage and the length of the 2',5'-oligoadenylate were varied. In addition, a branched linker was introduced to relocate the 2',5'-oligoadenylate with respect to the antisense domain.

The 2-5A system is a natural defense mechanism which mediates some of the antiviral effects of interferon[1-2]. In its natural form, the small and unique 2',5'-phosphodiester bond-linked oligoadenylate referred to as 2-5A (p_n5'A2'(p5'A2')$_m$p5'A) plays a key role in the anti-encephalmyocarditis virus action of interferon. 2-5A is biosynthesized by 2-A synthetase from ATP after the synthetase is activated by double-stranded RNA presumably arising from initial viral replication. The synthesized 2-5A in turn activates a 2-5A-dependent endonuclease (RNase L) which is capable of degrading viral mRNA as well as cellular mRNA and rRNA. On the other hand, antisense oligonucleotides hold considerable promise both as research tools for inhibiting gene expression and as exogenous agents for the treatment of a myriad of human diseases. Recently, we have developed[2-4] a novel and effective approach to the specific and targeted cleavage of RNA by combining the extreme specificity of the antisense concept with the potent RNA-degrading activity of the 2-5A-dependent endonuclease RNase L. With the new approach, we demonstrated the specific ablation of the PKR mRNA in HeLa cells with no effect on β-actin mRNA[5] .

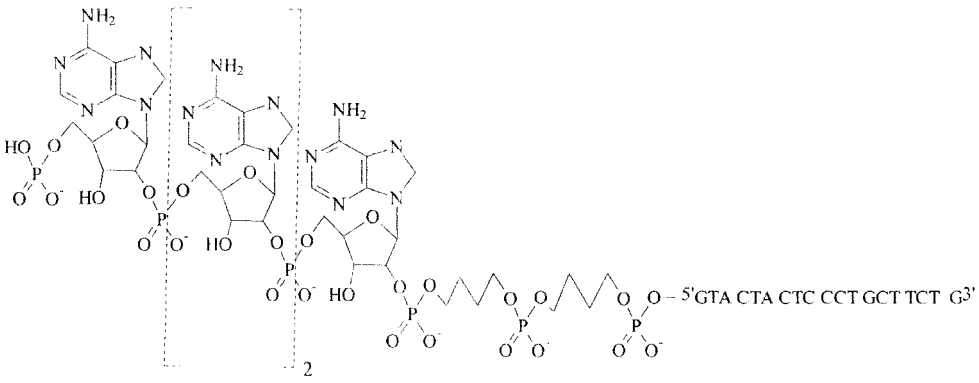


FIG 1. Structure of 2-5A antisense chimera

To direct 2-5A-dependent RNase to cleave unique RNA sequences, a 2',5'-oligoadenylate tetramer, p5'A2'p5'A2'p5'A2'p5'A, was covalently linked to an antisense oligonucleotide through a non nucleotide linker to form a chimeric molecule, known as 2-5A antisense chimera (Fig. 1).

When the 2-5A antisense chimera molecule was first designed, the linker was considered as an important part because it provides a necessary physical distance between the 2'-5'A tetramer and antisense oligonucleotide. Two butanediol were first selected as the linker because of its simplicity and feasibility. Since then, many efforts have been focused on choosing the correct target with most effective sequence of the antisense oligonucleotide. Several other techniques including phosphorthioate have also been employed in order to increase the stability of the chimera. However, the structure activity relationship of the linker in the chimeric molecule still remains as one of the most interesting research areas. In this paper, we have synthesized a series of chimera in which the nature of the linkage and the length of 2'-5'-oligoadenylate were varied as listed in table 1. Chimeras 1- 4 bear no linker between the 2'-5'-oligoadenylate and antisense oligonucleotide while the length of the 2'-5'-oligoadenylate varies from octamer to trimer. Chimera 5 remains the original design where the linker is two butanediols. Chimera 6 takes 2-tetrahydrofuran as the linker, whereas chimera 7 introduces an long chain of polyether 18 as a linker. Instead having a linear structure as conventional antisense oligomers do, chimera 8 adopts a branched structure where the 2'-5'-oligoadenylate is

TABLE 1

1 p5'A2'p5'A2'p5'A2'p5'A2'p5'A2'p5'A2'p5'A2'p5'A2'p—5'GTA CTA CTC CCT GCT TCT G3'

2 p5'A2'p5'A2'p5'A2'p5'A2'p5'A2'p5'A2'p————5'GTA CTA CTC CCT GCT TCT G3'

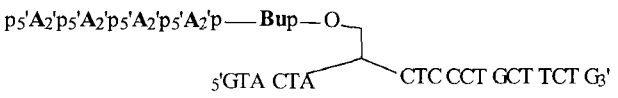
3. p5'A2'p5'A2'p5'A2'p5'A2'p————5'GTA CTA CTC CCT GCT TCT G3'

4. p5'A2'p5'A2'p5'A2'p————5'GTA CTA CTC CCT GCT TCT G3'

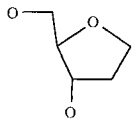
5. p5'A2'p5'A2'p5'A2'p5'A2'p———(BupBup)————5'GTA CTA CTC CCT GCT TCT G3'

6. p5'A2'p5'A2'p5'A2'p5'A2'p———(dSpacer)$_2$p————5'GTA CTA CTC CCT GCT TCT G3'

7. p5'A2'p5'A2'p5'A2'p5'A2'p———(Spacer 18)p————5'GTA CTA CTC CCT GCT TCT G3'

8. 

Bu = $OCH_2CH_2CH_2CH_2O$ Spacer 18 = $O(CH_2CH_2O)_5CH_2CH_2O$

dSpacer = 

attached in the middle of the antisense oligonucleotide through a branched linker. This branched structure enables us to study the position activity relationship of the chimera.

The activities of above chimeras were tested against RNA-dependent protein kinase (PKR) mRNA with the presence of purified, recombinant human RNase L in cell free system. The preliminary results suggested that the property and the length of the linker greatly influence the activity of the chimera. Chimera 5, which is the original design of the chimera with two butanediol as the linker still provides the best activity among all the molecules. On the other hand, chimera 7, which has the longest linker polyether 18, produces the less activity than others. Without decrease the activity, branched chimera 8 introduced a different degradation pattern as predicated. However, further experiment is need to confirm these results and determine the SAR of the linker.

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