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SYNTHESIS AND PROPERTIES OF 2'-5' and 3'-5' LINKED α-OLIGORIBOADENYLATES

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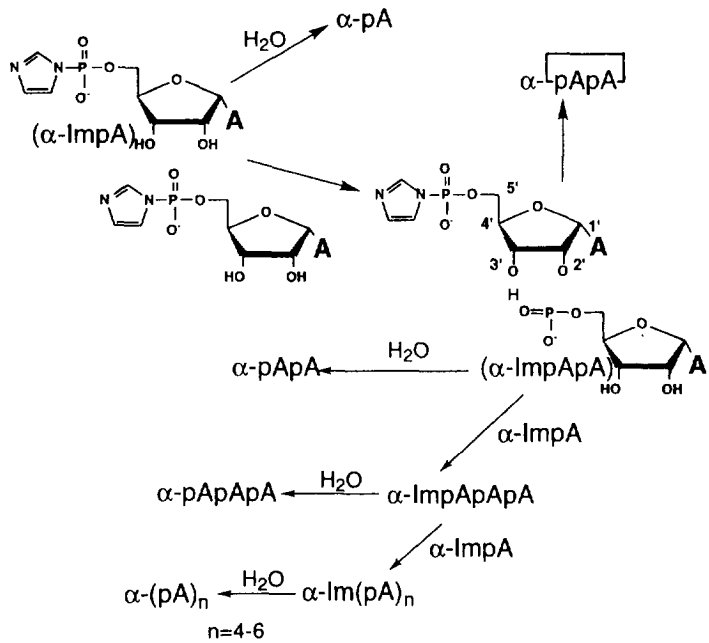
Abstract: Uranyl ion-catalyzed oligomerization of α-adenosine 5'-phosphorimidazolide in neutral aqueous solution yielded 2'-5' and 3'-5' linked α-oligoriboadenylates. Spectroscopic properties of α-oligoriboadenylates were studied by NMR and CD. α-Oligoriboadenylates with 2'-5' linkage showed strong resistance against nucleases, but weak binding ability to RNase L.

2'-5' Linked oligoriboadenylates of the general formula, pppA2'(p5'A)n, n>1 referred to as 2-5 A are prepared from ATP by 2-5 A synthetase in interferon-treated cells and are directly related to the interferon's antiviral action.[1-4] The 2-5 A binds and activates a latent 2-5 A-dependent endoribonuclease called RNase L which degrades viral mRNA resulting in an inhibition of protein synthesis.[3,4] A number of 2-5 A analogs have been prepared and their biological activities have been investigated. Some 2-5 A analogs have been reported to possess an anti-HIV activity.[5-8] Several fundamental regions of 2-5 A involved in the binding to and activation of the RNase L have been uncovered to date. The 5'-terminal polyphosophate or monophosphate, 2'-5' phosphodiester linkage, adenine base at the 5'-terminus (first) and third residues, and a chain length of more than three are necessary for the activity of the 2-5 A.[4,9-11] 2'-5' Linked α–anomeric oligoriboadenylate contains the above fundamental region except that this 2-5 A analog has α–adenosine instead of β–adenosine. Further, 3'-5' linked α-oligoribonucleotides have been reported to be useful as an antisense agent.[12] We have attempted the synthesis of 2-5 A analogs consisting of α–adenosine and the related 3'-5' linked α-oligoriboadenylates, and evaluated the effect of the difference in stereochemistry at the C-1' position to the binding of RNase L by the oligonucleotide ligand and studied biochemical and physicochemical properties of α-oligoribonucleotides.

Previously we have shown that the uranyl ion efficiently catalyzes the oligomerization of natural β-adenosine 5'-phosphorimidazolide in aqueous solution, yielding a series of 2'-5' linked β-oligoriboadenylates.[13,14] We have employed the uranyl ion-catalyzed oligomerization for the synthesis of α-oligoriboadenylates, as this method gives many kinds of oligonucleotides with different chain lengths and diferent internucleotide bonds in a one-step reaction, and is easy to perform.

α–Adenosine was prepared by a fusion reaction of 1-acetyl-2,3,5-tri-O-benzoyl-D-ribose with N^6-benzoyladenine in the presence of catalytic amount of *p*-toluene sulfonic acid at 180 °C for 15 min according to a modification of the published procedure.[12,15] After deprotection of the benzoyl group of

the products by treatment with NH_3-MeOH, the mixture was subjected to silica gel flush chromatography using 20% MeOH-CH_2Cl_2 as an eluent to give pure α–adenosine in 14 % yield. α–Adenosine was then phosphorylated with phosphoryl chloride in triethylphosphate giving α–adenosine-5'-monophosphate, [16] which was purified by column chromatography on active carbon and then on DEAE-Sephadex A-25 , in 47% yield. α–Adenosine 5'-phosphorimidazolide was obtained by the condensation reaction of α–adenosine 5'-phosphate (α–ImpA) with imidazole using triphenylphosphine and 2,2'-dipyridyl disulfide by a similar method as that of β–adenosine 5'-phosphorimidazolide (β–ImpA) synthesis.[13] The α–ImpA was oligomerized by an uranyl ion catalyst in neutral aqueous solution. Thus, oligomerization of α–ImpA (50 mM) was conducted in the presence of uranyl nitrate (2 mM) in 4.6 ml of 0.2 M N-ethylmorpholine buffer (pH 7.0) at 24 ° C for 4 d. The reaction mixture was treated with Chelex-100 to remove the uranyl ion and analyzed by HPLC on an RPC-5 column.[17] The HPLC analysis revealed the formation of α–oligoriboadenylates up to hexamer. The oligomers were separated by DEAE-Sephadex A-25 anion-exchange column chromatography with a linear gradient elution of triethylammonium hydrogen carbonate buffer and purified by preparative HPLC on ODS-silica gel, when necessary. Both 2'-5' and 3'-5' linked α–oligoriboadenylates were formed in the reaction. A cyclic dimer containing two 3'-5' bonds was also obtained along with the linear 3'-5' dimer. The oligomerization takes place as shown in the following scheme.



Scheme. Oligomerization of α-ImpA

The yields of oligomers are listed in Table 1. The ratio of the 3'-5' linkage to the 2'-5' linkage in the resulting α–oligomer increased with the increase of the chain length. Contrary to the oligomerization of

β–ImpA, in which 2'-5' linked oligoriboadenylates up to the hexadecamer were formed, no long-chained oligoribonucleotide containing fully 2'-5' linkage was obtained from α-ImpA by the uranyl ion catalyst.

Table 1. α-Oligoriboadenylate obtained from uranyl-ion catalyzed oligomerization of α-ImpA

Oligoadenylate	Yield (% based on α-ImpA)[a]
α-pA2'pA	19
α-pA3'pA	14
α-pA2'pA2'pA	2
α-(pA)3 mixed linkage isomers	10
α-pA3'pA3'pA	4
2'-5' α-(pA)4	0.3
α-(pA)4 mixed linkage isomers[b]	13
3'-5' α-(pA)4	0.6
α-(pA)5 mixed linkage isomers[b]	8
α-(pA)6 mixed linkage isomers[b]	3

a. Yield was determined from the UV absorption at 260 nm without making correction for the hypochromicity of each oligoriboadenylate.
b. The isomers contains mainly 3'-5' linkage.

Thus, the efficiency and the selectivity of 2'-5' linked α–oligoriboadenylate formation were lower than those of the corresponding 2'-5' linked β–oligoriboadenylate formation. Steric hindrance of the adenine group at the α–position of the ribose likely suppresses the reactivity of the 2'-OH group of α–ImpA, giving a higher ratio of 3'-5' linkage in the resulting α–oligomer.

Structure of the α–oligoriboadenylates was confirmed by NMR and enzyme digestion. Figure 1 shows the NMR of 5'-dephosphorylated 2'-5' linked α–triadenylate (α–A2'p5'A2'p5'A), which was obtained by dephosphorylation of 2'-5' α-(pA)3 with alkaline phosphatase. The assignment of each signal was

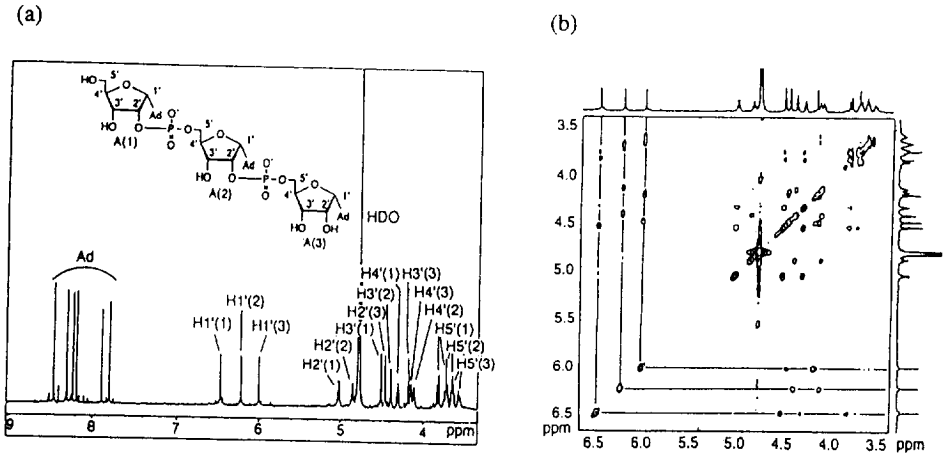


Figure 1. NMR Spectra of α-A2'pA2'pA.
(a) ^{1}H-NMR spectrum and assignment of signals. (b) HOHAHA spectrum.

done by HOHAHA spectrum. Two signals due to H2'-protons, H2'(1) and H2'(2), show down-field shift that indicates the phopshodiester groups link to the 2'-position of α–adenosine forming the 2'-5' bond. Similarly, down-field shift of the H2'-proton due to the 2'-5' internucleotide linkage was observed in other 2'-5' linked oligoriboadenylates. On the other hand, no down-field shift of the H2'-proton was observed in the corresponding 3'-5' α–oligoriboadenylates.

CD spectra of 2'-5' and 3'-5' linked α-triadenylates are shown in Figure 2 along with the corresponding β-triadenylates. α-Triadenylates showed CD spectra of reverse polarity to those of β-triadenylates in the region of 240-280 nm, reflecting the difference in stereochemistry at 1'-position of ribose. Moreover, the CD band of α-triadenylates is weaker than that of β-triadenylates. The result implies that the α-oligoribonucleotide has weak base-stacking interaction compared with the corresponding β-oligoribonucleotide.

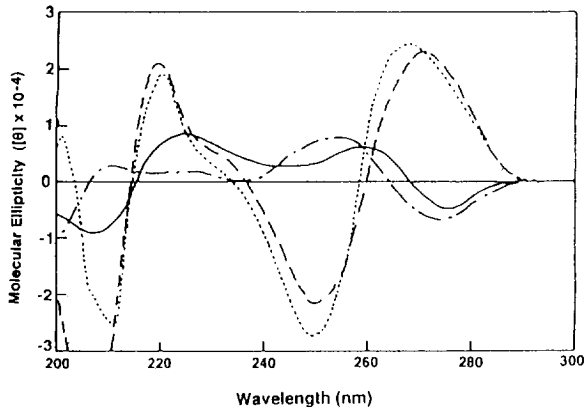


Figure 2. CD spectra of linkage isomers of α- and β-ApApA
β-A2'pA2'pA, — — —; β-A3'pA3'pA; ····· ;
α-A2'pA2'pA, ——— ; α-A3'pA3'pA —·—

Stability of 2'-5' and 3'-5' linked α–ApA against three nucleases was studied along with the corresponding β–ApA, to assess the nuclease resistance of α–oligoribonucleotide. The enzymatic hydrolysis was monitored by HPLC and half-lives of the oligomers are listed in Table 2. Snake venom phosphodiesterase degraded both β– and α–oligomers containing 2'-5' or 3'-5' linkage, but α–oligomers showed substantial resistance compared with the corresponding β–oligomers. Under the conditions where natural β–A3'p5'A was degraded with RNase T2, other dimers, β–A2'p5'A, α–A3'p5'A and α–A2'p5'A, remained intact. Nuclease P1 degraded α–A3'p5'A, although its half-life was longer than that of β-A3'p5'A. On the other hand, α–A2'p5'A or β–A2'p5'A was completely resistant against nuclease P1. The improved resistance of 3'-5' linked α–oligoriboadenylate against some nucleases is consistent with the results observed with 3'-5' linked α–oligoribouridylates[18] and α–oligodeoxynucleotides.[19]

Table 2. Stability of linkage isomers of α- and β-ApA against nucleases

Oligoadenylate	Half life against nuclease (h)		
	SVPDE [a]	Nuclease P1[b]	RNaseT2 [c]
β-A2'pA	0.06	not cleaved[d]	not cleaved[d]
β-A3'pA	0.06	0.13	0.21
α-A2'pA	0.30	not cleaved[d]	not cleaved[d]
α-A3'pA	0.53	2.00	not cleaved[d]

a. ApA (8.4 nmol, 0.21-0.25 ODU_{260}) was incubated with snake venom phsphodiesterase (0.2 units) in 30 μl of 0.02 M Tris-HCl (pH 8.0) in the presence of 0.01 M $MgCl_2$ at 37 °C.

b. ApA (8.4 nmol) was incubated with nuclease P1 (0.03 μg) in 50 μl of 0.01 M ammonium acetate buffer (pH 5.3) at 37°C.

c. ApA (8.4 nmol) was incubated with RNase T2 (0.005 μg) in 50 μl of 0.2 M sodium acetate buffer (pH 4.5) in the presence of 2 mM EDTA at 37 °C.

d. No cleavage with the nuclease was observed after 48 h.

Binding of 2'-5' or 3'-5' linked α–triadenylates [α–(pA)3] with RNase L from rabbit reticulocyte lysate and mouse L-cell extract was evaluated by radiobinding assay using ^{125}I -labeled 2-5 A as a probe.[20] The concentrations of α–triadenylate which displaces 50 % of the bound ^{125}I 2-5 A probe to RNase L are shown in Table 3, as a measure of the binding ability. The α–2-5 A, α-pA2'pA2'pA, was 1000 times less effective in the binding with RNase L from mouse L cell extract compared with the natural β–2-5A, β-pA2'pA2'pA. 3'-5' α–Triadenylate showed essentially no binding ability as natural 3'-5' β–(pA)3.

Table 3. Binding ability of 2-5 A analog to RNase L

Oligoadenylate	IC_{50} (M)[a]	
	Rabbit reticulocyte lysate	Mouse L cell extract
β-pA2'pA2'pA	1×10^{-6}	5×10^{-8}
α-pA2'pA2'pA	$>5 \times 10^{-5}$	4×10^{-5}
α-pA3'pA3'pA	$>5 \times 10^{-5}$	$>5 \times 10^{-5}$

a. Concentration of oligoadenylate which displaces 50 % of ^{125}I-labeled 2-5 A probe bound to RNase L from rabbit reticulocyte lysate or mouse L cell extract.

In conclusion, short-chained α–oligoriboadenylates with 2'-5' or 3'-5' linkage were formed by uranyl-ion catalyzed oligomerization of α–ImpA. The α–oligoriboadenylates showed strong nuclease resistance, but weak biological activity as an analog of 2-5 A. The results imply that stereochemistry at the 1'-position of ribose is crucial for the recognition of 2-5 A analogs by RNase L.

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