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2-Methyladenosine-Substituted 2′,5′-Oligoadenylates: Conformations, 2-5A Binding and Catalytic Activities with Human Ribonuclease L

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Abstract—2-Methyladenosine-substituted analogues of 2-5A, p5′A2′p5′A2′p5′(me^{2}A), p5′(me^{2}A)2′p5′A2′p5′A, and p5′(me^{2}A)2′p5′(me^{2}A)2′p5′(me^{2}A), were prepared via a modification of a lead ion-catalyzed ligation reaction. These 5′-monophosphates were subsequently converted into the corresponding 5′-triphosphates. Both binding and activation of human recombinant RNase L by various 2-methyladenosine-substituted 2-5A analogues were examined. Among the 2-5A analogues, p5′A2′p5′A2′p5′(me^{2}A) showed the strongest binding affinity and was as effective as 2-5A itself as an activator of RNase L. The CD spectra of both p5′(me^{2}A)2′p5′A2′p5′A and p5′A2′p5′A2′p5′(me^{2}A) were superimposable on that of p5′A2′p5′A2′p5′A, indicative of an *anti* orientation about the base-glycoside bonds as in naturally occurring 2-5A.

The unique 2′,5′-oligoadenylate (2-5A) acts as a potent inhibitor of translation in vertebrate cells through the activation of a constituent latent 2-5A-dependent endoribonuclease (RNase L). This 2-5A system plays a major role in the interferon natural defense mechanism against viral infection.[1] The nucleotide bases of 2-5A are recognized by RNase L. Dramatic variation in RNase L binding and activating abilities of 2′,5′-oligoadenylates can be achieved by replacement of the adenine 8-hydrogen. For instance, complete substitution of 8-bromoadenosine (br^{8}A) for all three A's in p5′A2′p5′A2′p5′A gave a large reduction in activity; however, replacement by br^{8}A at the third adenosine of pp5′A2′p5′A2′p5′A to give pp5′A2′p5′A2′p5′(br^{8}A) provided a significant increase in RNase L activation.[2,3] When 8-methyladenosine (me^{8}A) replaced adenosine in the 2′-terminal position of the nucleotide, the resultant oligonucleotide, ppp5′A2′p5′A2′p5′(me^{8}A), was somewhat more active than parent 2-5A trimer.[4] Conformational studies of such 8-substituted 2′,5′-oligoadenylates thus have led to the hypothesis that a *syn* base-sugar orientation about the glycosidic bond of the 2′-terminal adenosine nucleotide positively influenced activating activities for RNase L.[5]

In contrast, 2-bromoadenosine (br^{2}A) introduction to the 2′-terminal position of the 2-5A molecule was of interest since it could force the nucleoside to adopt an *anti* orientation about the base-glycoside bond.[6] The analogue with br^{2}A residing in the 2′-terminal position, p5′A2′p5′A2′p5′(br^{2}A), showed the strongest binding affinity and was as effective as 2-5A itself as an activator of human recombinant RNase L.[7–9] The CD spectrum of p5′A2′p5′A2′p5′(br^{2}A) was superimposable on that of p5′A2′p5′A2′p5′A, indicative of an *anti* orientation about the base-glycoside bonds as in naturally occurring 2-5A.[9]

In this paper, we describe the syntheses of 2-methyladenosine-substituted 2-5A derivatives and their interaction with recombinant human RNase L.[7–9] This me^{2}A modification, with the same nucleotide anti-orientation as

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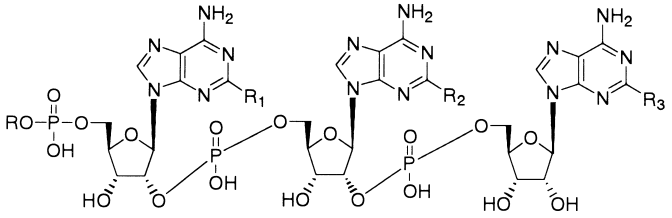


1 R = H ; $R_1 = R_2 = H$; $R_3 = CH_3$

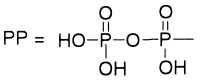
2 R = H ; $R_1 = CH_3$; $R_2 = R_3 = H$

3 R = H ; $R_1 = R_2 = R_3 = CH_3$

4 R = PP ; $R_1 = R_2 = H$; $R_3 = CH_3$

5 R = PP ; $R_1 = CH_3$; $R_2 = R_3 = H$

6 R = PP ; $R_1 = R_2 = R_3 = CH_3$



Scheme 1.

naturally occurring 2-5A, further reveals the importance of base-sugar conformation in binding to and activation of RNase L.

Using the previously published procedure,[4,8,10] three 2′,5′-linked oligoadenylates possessing 2-methyladenosine (me^2A)[11] could be generated; namely p5′A2′p5′A2′p5′(me^2A) (**1**), p5′(me^2A)2′p5′A2′p5′A (**2**), and p5′(me^2A)2′p5′(me^2A)2′p5′(me^2A) (**3**). These structures were confirmed on the basis of NMR spectroscopy (see Table 1) and degradation methods (data not shown). Three 5′-monophosphates (**1**, **2** and **3**) were subsequently converted into the corresponding 5′-triphosphates (**4**, **5** and **6**) using methods described earlier.[2–4,9]

Earlier studies[2–4,9] have provided much evidence that effective binding to and activation of RNase L are favored by an *anti* conformation of the 5′-terminal nucleotide of 2-5A. According to the CD results (Fig. 1), p5′(me^2A)2′p5′(me^2A)2′p5′(me^2A) (**3**) does not possess a conformation remarkably different from parent p5′A2′p5′A2′p5′A. However, insofar as a decrease in RNase L binding and activation ability of p5′(me^2A)2′p5′(me^2A)2′p5′(me^2A) (**3**) was observed. It is possible that steric or electronic properties associated with 2-methyl substitution may play a role in these diminished parameters. On the other hand, the CD spectra of both p5′A2′p5′A2′p5′(me^2A) (**1**) and p5′(me^2A)2′p5′A2′p5′A (**2**) were superimposable on that of p5′A2′p5′A2′p5′A, indicative of an *anti* orientation about the base-glycoside bonds as in naturally occurring 2-5A and p5′A2′p5′A2′p5′(br^2A).[9]

Introduction of a 2-methyl substituent to the adenine ring of the 5′-terminal adenosine of 2-5A trimer, p5′(me^2A)2′p5′A2′p5′A (**2**), caused an 8-fold decrease in binding affinity to RNase L as determined by ability to compete with radiolabeled p5′A2′(p5′A2′)$_2$ p5′A3′[^{32}P]pCp in a modified assay of Knight et al.[12,13] This moderate diminution in binding also was true for the 5′-triphosphate analogue, ppp5′(me^2A)2′p5′A2′p5′A (**5**). When evaluated for their abilities to activate the RNase L, as judged by ability to stimulate the degradation of labeled poly(U) or labeled $pC_{11}U_2C_7$ by RNase L,[14] both the monophosphate (**2**) and the triphosphate (**5**) showed an approximate 5-fold decrease in activation ability (Table 2). Thus, the loss of binding ability was directly related to the loss of RNase L activation capacity.

The 2′-terminally-modified analogues, (pp)p5′A2′p5′A2′p5′(me^2A) (**1** and **4**), bound to the RNase L with at least a 2-fold increase in affinity compared with parent 2-5A (Table 2). RNase L activation by the 5′-monophosphate congener (**1**) was just as effective as by the 2-5A trimer, p5′A2′p5′A2′p5′A; however, the corresponding 5′-triphosphate (**4**) was slightly less active than 2-5A. The human RNase L has been shown to require only a 5′-monophosphate-terminated 2′,5′-oligoadenylate trimer for full activation.[8] Complete substitution of 2-5A trimer mono- (**3**) or triphosphates (**6**) with 2-methyladenines brought about one- or two-log decrease in binding affinity to the human RNase L (Table 2) and, concomitantly, a similar drop in ability to activate the nuclease L.

Table 1. Characteristic proton NMR signals of 2′,5′-oligoadenylates (500 MHz, in D_2O)

Oligomer	H-2 and H-8 (ppm)		H-1′ (ppm)	CH_3 (ppm)
pApApA	7.997	7.770	5.892 (*d*, *J* = 3.00 Hz)	
	7.932	7.752	5.759 (*d*, *J* = 3.50 Hz)	
	7.814	7.716	5.643 (*d*, *J* = 4.50 Hz)	
pApAp(me^2A) (**1**)	7.956	7.746	5.906 (*d*, *J* = 3.50 Hz)	
	7.826	7.742	5.769 (*d*, *J* = 3.50 Hz)	2.345
	7.773		5.636 (*d*, *J* = 4.00 Hz)	
p(me^2A)pApA (**2**)	8.030	7.803	5.943 (*d*, *J* = 3.20 Hz)	
	7.972	7.794	5.838 (*d*, *J* = 4.00 Hz)	2.225
	7.877		5.697 (*d*, *J* = 4.00 Hz)	
p(me^2A)p(me^2A)p(me^2A) (**3**)	7.905		5.829 (*d*, *J* = 2.50 Hz)	2.290
	7.743		5.781 (*d*, *J* = 3.50 Hz)	2.195
	7.648		5.595 (*d*, *J* = 4.00 Hz)	2.097

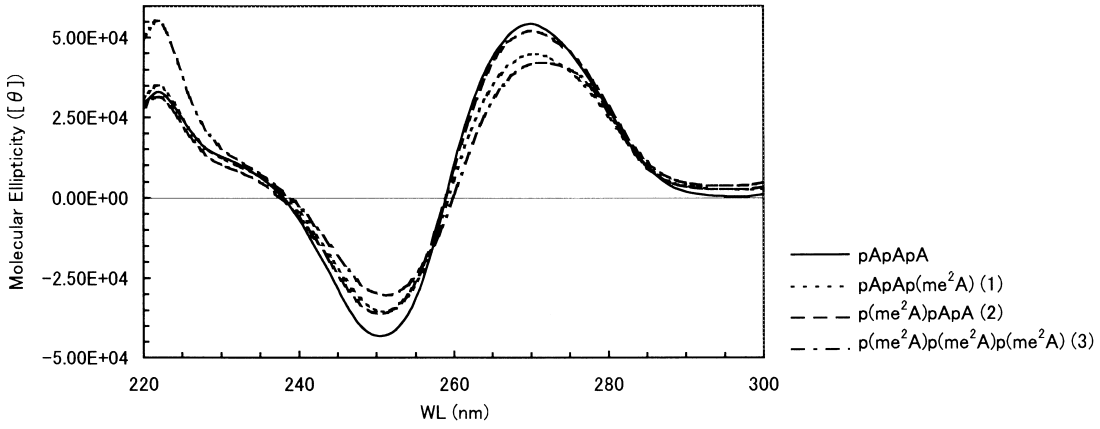


Figure 1. CD spectra of 2′,5′-oligoadenylates.

Table 2. Biological activities of the purified human-recombinant RNase L by 2′,5′-oligoadenylates

Oligomer	Binding[a] (IC_{50} nM)	C_{rel}[c]	Activation[b] (EC_{50} nM)	C_{rel}[c]	R_n[d]
pApApA	7.3	1	0.15 (0.2)	1 (1)	1
pApAp(me^{2}A) (**1**)	2.6	0.4	0.13 (0.2)	0.9 (1)	0.4
p(me^{2}A)pApA (**2**)	57	8	0.84 (0.7)	6 (2.7)	1.4
p(me^{2}A)p(me^{2}A)p(me^{2}A) (**3**)	1060	146	16 (30)	107 (120)	1.4
pppApAp(me^{2}A) (**4**)	0.72	0.1	0.24 (0.3)	2 (1.5)	0.06
ppp(me^{2}A)pApA (**5**)	24	3	0.68 (0.8)	5 (4)	0.7
ppp(me^{2}A)p(me^{2}A)p(me^{2}A) (**6**)	105	15	4.6 (4)	31 (20)	0.5

[a]Binding ability of 2′,5′-oligoadenylates to recombinant human RNase L as measured by displacement of the probe $p(A2'p)_3A3'[^{32}P]p5'Cp$.
[b]Activation of purified recombinant human RNase L as measured by the degradation of poly (U) $3'[^{32}P]p5'C3'p$ and by degradation of $[^{32}P]pC_{11}U_2C_7$ (in parentheses).
[c]C_{rel} was defined as the relative concentration of analogue required to displace 50% of the probe or to cause 50% degradation of substance.
[d]R_n was calculated from the quotient (binding IC_{50})/(activation EC_{50} for the poly(U) assay). The quotient for pApApA was then set equal to 1, and all other quotients were normalized to that value.

These results show that substitution of 2-methyladenosine for adenosine in the 2′-terminal nucleotide position of 5′-monophosphorylated 2-5A trimer supported nuclease activation to the same extent as parent 2-5A. This finding suggests that the RNase L enzyme may be able to adapt to either *syn* or *anti* conformation of the 2′-terminal nucleotide, perhaps through modulation of the nucleotide conformation itself.

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