Introduction of 8-methyladenosine into 2′, 5′-oligoadenylate (2-5A) 2′-terminus induces dramatic shift in binding site of RNase L

Kumi Nagaoka¹, Seiya Kito², Yoshiaki Kitamura², Yoshihito Ueno¹,² and Yukio Kitade¹,²,³

¹United Graduate School of Drug Discovery and Medical information Science, Gifu University,
²Department of Biomolecular Science, Faculty of Engineering, Gifu University and
³Center for Advanced Drug Research, Gifu University, 1-1 Yanagido, Gifu 501-1193, Japan

ABSTRACT

The 2′,5′-oligoadenylate (2-5A) system is an interferon (IFN)-regulated RNA decay pathway that provides innate immunity against viral infections. The biological action of the 2-5A system is mediated by RNase L, an endoribonuclease that becomes enzymatically active after binding to 2-5A. It has been reported that the 5′-phosphoryl group of 2-5A is required for RNase L activation. However, we have found that 5′-O-dephosphorylated 2-5A tetramer analogs with 8-methyladenosine at the 2′-terminus were more effective as an activator of RNase L than the parent 2-5A (p5′A2′p5′A2′p5′A2′p5′A2′). Introduction of 8-methyladenosine is thought to induce a dramatic shift in the binding site of RNase L.

INTRODUCTION

The 2′,5′-oligoadenylate (2-5A) system is an interferon (IFN)-regulated RNA decay pathway that provides innate immunity against viral infections. The biological action of the 2-5A system is mediated by RNase L, an endoribonuclease that becomes enzymatically active after binding to 2-5A. It has been reported that 2-5A must have at least one 5′-phosphoryl group and a minimum of three adenylyl residues in the 2′,5′-linkage.¹ We have previously reported the crystal structure of the N-terminal ankyrin repeat domain of human RNase L complexed with activator 2-5A.² We have demonstrated that the third adenine ring of 2-5A is in a syn conformation³ and that 2-5A molecules modified at the 8-position (e.g., 8-methyl) of the third adenine ring, which is thereby forced into a syn conformation, are more effective activators of RNase L than the parent 2-5A (1).³ Here, we report that the activation ability of 5′-O-dephosphorylated 2-5A analogs with an 8-methyladenosine residue at the 2′-terminus was stronger than that of the parent 2-5A (1). This increased RNase L activation ability is thought to arise from a shift in the 2-5A binding site of RNase L compared to the binding site for the parent 2-5A (1).

RESULTS

The parent 2-5A (1; p5′A2′p5′A2′p5′A2′p5′A) and 2-5A analogs (2–8) were synthesized by using phosphoramidites with a DNA/RNA synthesizer (Fig. 1). The ability of the parent 2-5A (1) and 2-5A analogs (2–8) to activate RNase L was determined by monitoring the cleavage of synthetic RNA by the activated RNase L. In this study, 5′-fluorescein-(C₃H₇)₃ (Fig. 1) was used as a substrate. Recombinant human RNase L was expressed in E. coli and purified according to the reported procedure.⁴ The reactions were analyzed by polyacrylamide gel electrophoresis. Among the 5′-O-dephosphorylated 2-5A analogs (2–8), the 2-5A analogs with 8-methyladenosine residing in the 2′-terminal position (7 and 8) were several times more effective than the parent 2-5A (1) in terms of RNase L activation ability (Fig. 2).

We next examined the stability of the 8-methylated 2-5A analog (7) against nucleolytic hydrolysis by snake venom
phosphodiesterase. Analog 7 was more resistant to nucleolytic hydrolysis than the parent 2-5A (I) (Fig. 3).

![Graph showing biological activities of RNase L complexed with 2-5A analogs.](https://example.com/graph)

**Fig. 2** Biological activities of RNase L complexed with 2-5A analogs.
Concentration of RNA substrate: 200 nM. Concentration of RNase L: 50 nM. Incubation time of 30 min. Graph shows percentage of RNA remaining.

![Graph showing stability of 2-5A analogs against snake venom phosphodiesterase.](https://example.com/graph)

**Fig. 3** Stability of 2-5A analogs against snake venom phosphodiesterase. Concentration of 2-5A analogs: 9 μM. Concentration of enzyme: 0.004 unit/mL. Reaction buffer: Tris-HCl (50 mM, pH = 8.0), MgCl₂ (10 mM) at 37 °C.

**DISCUSSION**

The analog with 8-methyladenosine in the place of adenosine at the 2’-terminus, 5’-O-dephosphorylated 2-5A tetramer (7), was significantly more effective as an activator of RNase L than the 2-5A parent. Furthermore, 7 was more stable against snake venom phosphodiesterase digestion than the parent 2-5A (I).

We also found that introduction of an 8-methyladenosine residue at the 2’-terminus of 2-5A (7 and 8) induced a shift of the 2-5A binding site of RNase L. In the parent 2-5A tetramer, the binding of three adenosine nucleotide at the 5’-terminus to RNase L is thought to induce a conformation change in the enzyme and/or result in the unmasking of an interaction domain, permitting dimerization and activation of RNase L. The 2-5A analog 2 (A2’p5’A2p5’A2’p5’A2p5’) had no RNase L activation ability under these conditions. However, the 2-5A analogs 7 (A2’p5’A2’p5’A2p5’(8MeA)) and 8 (p5’A2’p5’A2p5’A2p5’(8MeA)) were more effective than the parent 2-5A (I). Introduction of 8-methyladenosine into 2-5A at the 2’-terminus of the 2-5A analogs (7 and 8) appears to induce a dramatic shift in the binding site of RNase L. The three adenosine nucleotides from the 2’-terminus are thought to play a role in the activation of RNase L (Fig. 4).

![Diagram showing presumed model for binding pattern of 2-5A analogs 2 (A2’p5’A2’p5’A2’p5’A2) and 7 (A2’p5’A2p5’A2p5’(8MeA)).](https://example.com/diagram)

**Fig. 4** Presumed model for binding pattern of 2-5A analogs 2 (A2’p5’A2’p5’A2’p5’A2) and 7 (A2’p5’A2p5’A2p5’(8MeA)).

**REFERENCES**


*Corresponding author. E-mail: ykkitade@gifu-u.ac.jp