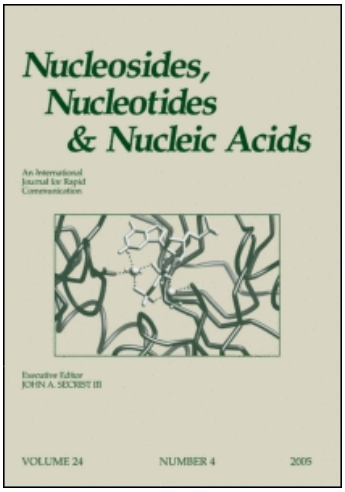

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Only One 3'-Hydroxyl Group Of ppp 5'A 2'p 5'A 2'p 5'A(2-5A) is Required for Activation of the Mouse 2-5A-Dependent Endonuclease

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ONLY ONE 3'-HYDROXYL GROUP OF ppp5'A2'p5'A2'p5'A(2-5A) IS REQUIRED FOR ACTIVATION OF THE MOUSE 2-5A-DEPENDENT ENDONUCLEASE

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Abstract. The 3'-hydroxyl groups of each of the adenosines of 2-5A trimer (ppp5'A2'p5'A2'p5'A) were sequentially replaced by hydrogen through a phosphotriester synthetic approach. Biochemical evaluation of these analogs led to the conclusion that only the 3'-hydroxy group of the second adenosine is required for activation of RNase L.

INTRODUCTION :

Interaction of the unique oligonucleotide 2-5A, produced in interferon-treated virus-infected cells, with a latent endonuclease (RNase L) can lead to RNA degradation and inhibition of translation. It appears that each individual nucleotide residue of 2-5A may assume a fundamentally different role in binding to an activation of RNase L. For instance, the purine N1 and/or 6 amino group of the first (5')nucleotide unit was vital for binding to RNase L, the purine N1 and/or 6 amino residue of the second nucleotide was of minimal importance in either binding or activation whereas the purine N1 and/or 6 amino functionality of the third nucleotide was relatively unimportant in binding but critical for RNase L activation. Herein, we demonstrate that such discrimination also occurs at the level of the ribose-phosphate backbone of 2-5A.

RESULTS AND DISCUSSION :

When the 3'-hydroxyl functionality of the 5'-terminal adenosine residue of 2-5A trimer was replaced by hydrogen, the resulting analog, ppp5'(3'dA)2'p5'A2'p5'A, was bound to RNase L just as effectively as the

parent 2-5A itself. Simultaneously, the ability of ppp5'(3'dA)2'p5'A2'p5'A to activate the 2-5A-dependent endonuclease, as judged by its ability to cause RNA degradation or to inhibit protein synthesis was diminished only by a factor of about four with respect to 2-5A trimer triphosphate. Thus the 3'-hydroxyl group of the 5'-terminal adenosine nucleotide residue of 2-5A trimer must not play a significant role in RNase L binding and only a marginal role at best in the activation of RNase L.

A wholly contrary story emerged upon examination of the analog in which the 3'-hydroxyl group of the second (from the 5'-terminus) nucleotide residue of 2-5A was substituted by hydrogen. In this instance significant decreases in binding and activation occurred. The binding efficiency of ppp5'A2'p5'(3'dA)2'p5'A decreased by a factor of approximately eight relative to 2-5A trimer. Even more dramatic, however, was the loss of ability to activate RNase L as judged by either protein synthesis inhibition (500-fold decrease in activity relative to 2-5A) or by ability to cause degradation of poly(U)(1000-fold decrease in activity relative to 2-5A). From these data, it can be concluded that the 3'-hydroxyl moiety of the second (from the 5'-terminus) residue of 2-5A makes some contribution to binding of 2-5A to RNase L but an even more vital contribution to the activation of RNase L.

When the 3'-hydroxyl substituent was converted to hydrogen in the third or 2'-terminal nucleotide residue of 2-5A, a significant increase in RNase L binding as well as activation ability occurred. According to the results of the radiobinding assay, ppp5'A2'p5'A2'p5'(3'dA) underwent an approximate 5-fold increase in binding affinity. This was accompanied by a 5-7-fold increase in ability to activate the 2-5A-dependent endonuclease as ascertained by the translational inhibition assay or by the "core-cellulose" poly(U) degradation assay.

Thus, only the 3'-hydroxy group of the second form the 5'-terminus nucleotide of 2-5A is needed for effective activation of RNase L. This finding may simplify synthetic access to derivatives of 2-5A that may be active on the intact cell.