GUANYLYL 2'-5' GUANOSINE AS AN INHIBITOR OF RIBONUCLEASE T_1

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SUMMARY

Guanylyl 2'-5' nucleosides were tested for their ability to inhibit the depolymerization activity of ribonuclease T_1. It was found that the guanylyl 2'-5' purines had a significant inhibitory effect. Guanylyl 2'-5' guanosine was the strongest inhibitor. However, guanylyl 2'-5' pyrimidine nucleosides did not inhibit at all.

INTRODUCTION

2'-5' Diribonucleoside monophosphates are synthetic oligonucleotides which differ from the natural 3'-5' diribonucleoside monophosphates only in the nature of the diester bond. The 2'-5' oligonucleotides are not hydrolyzed by ribonucleases, but being analogous to the substrates, we would expect them to be inhibitors to RNases. Podder (1) showed that RNase T_1 is capable of synthesizing guanylyl 2'-5' guanosine, but this oligomer is resistant to enzymatic hydrolysis. From Podder's results it seems that guanylyl 2'-5' guanosine can form a complex with the enzyme and, therefore, can serve as a potential inhibitor.

In this publication the inhibition of ribonuclease T_1 by different guanylyl 2'-5' nucleosides is reported.

MATERIALS AND METHODS

RNase T_1 (E.C.2.7.7.26) was purchased from Calbiochem, B. grade, 100000 enzyme units per milligram. [^{14}C]U was from Amersham. Uridine 2'-3' cyclic phosphate was prepared according to Shugar's method (2).

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Guanylyl 2'-5' nucleosides were prepared according to Lapidot and Barzilay (3). The concentrations of all oligonucleotides were determined by spectrophotometric measurements at pH = 1.0 at the appropriate λ max.

G3'-5'[^{14}C]U was prepared by enzymatic synthesis with RNase from *Aspergillus Clavatus* according to Bauer *et al.* (4), except that the uridine was a mixture of radioactive and non-radioactive material.

The reaction mixture for the determination of the hydrolysis rate of G3'-5'[^{14}C]U contained in a total volume of 40 µl the following: Tris-HCl buffer pH 7.5 (0.1 M), RNase T_1 (0.004 units), G3'-5'[^{14}C]U (0.238 mM, 32,000 cpm) and 1.19 mM of the appropriate G2'-5'N. Reactions were carried out in duplicates at 30°C. 5, 10 and 20 minutes after the introduction of the substrate, samples of 5 µl were put on Whatman 3 MM paper, immediately dried, and subjected to high voltage electrophoresis at pH 3.5 (0.02 M acetate buffer, 45 volts/cm) for 1 hour. It was then delivered through a radio-chromatogram scanner (Packard Model 7201), and the radioactive areas were cut out and read accurately in a scintillation counter (Tri Carb Model 3380, Packard).

The experimental data (initial velocities and Dixon plot) were evaluated to the appropriate equation by the least squre method with the aid of a desk computer.

RESULTS AND DISCUSSION

Our method of measuring the hydrolytic activity of RNase T_1 by measuring the hydrolysis of a dinucleoside monophosphate is similar to that used by Whitfeld and Witzel (5). The main difference between the two methods is the use of a radioactive substrate which made the measurements much easier and more accurate. All the above measurements were within the linear range of the hydrolysis of G3'-5'[^{14}C]U.

Fig. 1 shows the rate of hydrolysis of G3'-5'[^{14}C] in the presence and absence of G2'-5'G. It is clear from Fig. 1 that G2'-5'G serves as an inhibitor of RNase T_1.

It is known that the interaction of RNase T_1 with oligonucleotides does not depend only on the guanosine moiety. This is evident from the differences in the Km and the Vm between G3'-5'A, G3'-5'C, G3'-5'G, and G3'-5'U (5). The above conclusion can also be drawn from Table I which shows the effect of G2'-5'A, G2'-5'C, G2'-5'G, and G2'-5'U on the hydrolysis of G3'-5'[^{14}C]U by RNase T_1.

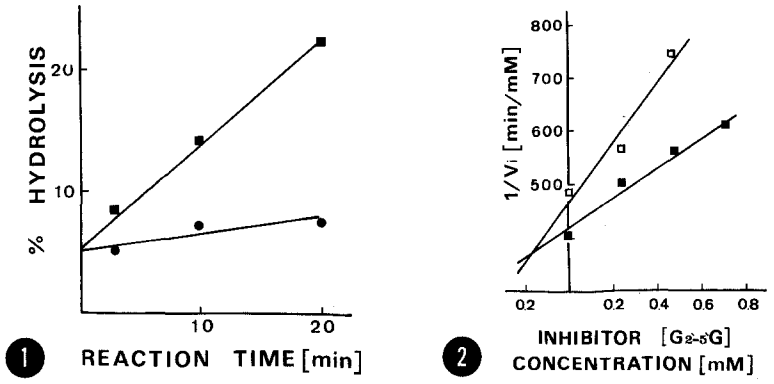

Fig. 1: *Kinetics of G3'-5'[^{14}C]U hydrolysis.* For details see Materials and Methods. ■—■ without inhibitor; ●—● with 1.19 mM G2'-5'G.

Fig. 2: *Dixon plot for G2'-5'G.* For details see Materials and Methods. The concentrations of the substrate (G3'-5'[^{14}C]U) were 0.476 mM (□—□) and 0.952 mM (■—■).

Table I: *Inhibitions of RNase T_1 by guanylyl 2'-5' nucleosides*

Compound	Percent inhibition
G2'-5'C	0
G2'-5'U	0
G2'-5'A	27
G2'-5'G	82

For details see Materials and Methods.

It is clear from Table I that the dinucleoside monophosphates which have a pyrimidine at the 3' OH end, do not have an inhibitory effect on RNase T_1. On the other hand, the dinucleoside monophosphates containing a purine base at the 3' OH end are effective inhibitors.

As G2'-5'G was found to be the strongest inhibitor, we measured its Ki with the aid of a Dixon plot (6) (Fig. 2). From the junction of the

lines in Fig. 2, the Ki of G2'-5'G was found to be 0.165 mM, and is in the region of the Ki of guanosine 3'-monophosphate which is known to be a strong inhibitor to RNase T_1 (7). The Km for the hydrolysis of G3'-5'-[^{14}C]U, calculated from the intercept with the abcissa was 0.115 mM, which is in good agreement with the previous value of 0.238 mM (8).

As it is known that dinucleoside monophosphates can penetrate into cells (9), it seems that G2'-5'G can be used as a RNase inhibitor in biological systems.

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