THE MODE OF ACTION OF DIVALENT CATIONS ON THE RNase CATALYZED HYDROLYSIS OF RNA AND URIDYLYL (3'-5') URIDINE. *

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Summary. Pseudo first order rate constants (k') have been measured for the RNase A catalyzed hydrolysis of uridylyl (3'-5') uridine at several ionic strengths and compositions. The k' values are independent of Mg^{2+} concentration between 0 and 10 mM. This shows that for hydrolysis of RNA, in which Mg^{2+} concentration does change k', the perturbation must be through binding of Mg^{2+} to the substrate RNA rather than to the enzyme RNase.

Introduction. It has been known for some time that the rate of some enzyme catalyzed reactions is sensitive to the concentration of cations in the reacting solution. With increasing concentrations of KCl, the activity of skin proteinase C first increases and then decreases giving a bell-shaped curve.[1] A similar curve is found for polynucleotide phosphorylase with increasing KCl concentration.[1]

The rate of hydrolysis of RNA catalyzed by pancreatic RNase A has been shown to give similar regions of activation and deactivation.[2, 3, 4] In the case of Mg^{2+}, as concentration is increased from 0.1 mM the rate of hydrolysis first increases, reaches a maximum near 5 mM and then

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decreases to a low value near 10 mM.[4] Present evidence suggests that activation and deactivation may be caused by the binding of divalent cations to the substrate RNA but binding to the RNase is also a possible explanation. In this report we present evidence which confirms that the site of action of Mg^{2+} is on the substrate RNA rather than on the enzyme.

Materials and Methods. Hydrolysis was carried out in 50 µl of a solution of 5 mM tris, pH 7.3 and other ions as desired. Reactions were stopped at the appropriate times by the addition of sodium dodecylsulfate (SDS) to 0.2%.

Uridylyl (3'-5') uridine (UpU), uridine-5'-monophosphate (5'-UMP) and cyclic (2'-3') uridine monophosphate and uridine were purchased from Sigma Co. Other chemicals were reagent grade obtained from standard sources.

Polyacrylamide gels were prepared at a concentration of 3% acrylamide by standard methods.[5] Samples were made up to 10% in sucrose and were layered on gels. Electrophoresis was carried out at 5 milliampers per gel for 1.5 hr. Absorbance profiles were recorded for gels scanned immediately after electrophoresis to prevent further diffusion which broadens bands.

Results and Conclusions. We wish to report values of the pseudo first order rate constant for the RNase A catalyzed hydrolysis of UpU. The method requires knowledge of the concentration of UpU as a function of time during hydrolysis. We have used polyacrylamide gel electrophoresis to separate UpU from its hydrolysis products which are 3'-UMP, 2'-3' cyclic UMP and uridine. Examination of the electrophoresis profiles shows that these compounds are well separated with migration distance increasing as follows: uridine < UpU < cyclic UMP < UMP (fig. 1, a, b). Since uridine is uncharged,

it remains at the top of the gel which it enters by diffusion. The profile for cyclic UMP shows two impurities one of which appears to be UMP; the other band near the top of the gel is of unknown origin (fig. 1, a).

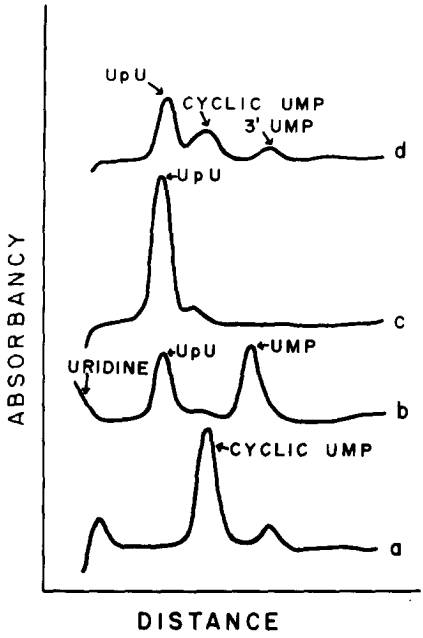

Figure 1. Electrophoresis profiles. (a) 3'-5' cyclic uridine monophosphate. (b) A mixture of uridine, uridylyl (3'-5') uridine and 5' uridine monophosphate. (c) uridylyl (3'-5') uridine, no hydrolysis (zero time control). (d) uridylyl (3'-5') uridine hydrolyzed with 20 μg/ml of RNase A for 18 min. in 5 mM $MgAc_2$, 5 mM tris pH 7.3.

When UpU is hydrolyzed by pancreatic RNase A the electrophoresis profiles show that the UpU band diminishes as a function of time of hydrolysis and bands due to UMP and cyclic UMP increase (fig. 1, c, d). The separation obtained by electrophoresis therefore allows peak height (h) or the band area to be measured at various times of hydrolysis for the remaining nonhydrolyzed UpU. For pseudo first order kinetics we expect a straight line on a plot of ln h_o/h

versus time (fig. 2). The slope of this line then gives the pseudo first order rate constant (k').

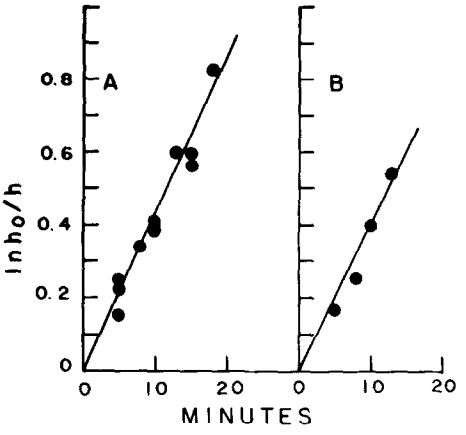

Figure 2. Graphical determination of the pseudo first order rate constant for the hydrolysis of uridylyl (3'-5') uridine by RNase A. (A) hydrolysis in 5 mM $MgAc_2$, 5 mM tris pH 7.3. (B) hydrolysis in 5 mM tris pH 7.3, no magnesium ions.

Values of k' were determined for UpU in the following solutions: (1) 5 mM tris no Mg^{2+}, (2) 5 mM tris, 5 mM $MgAc_2$, (3) 5 mM tris, 10 mM $MgAc_2$, (4) 5 mM tris, 1 mM $MgAc_2$, 0.1 M NH_4Cl, (5) 5 mM tris, 1 mM $MgAc_2$, 0.1 M NH_4Cl, 4 mM EDTA (Table 1). The pH of all solutions was 7.3. The observed values of k' for hydrolysis in these solutions are the same within experimental error.

The k' value for the hydrolysis of ribosomal RNA (rRNA) and ribosomes goes from a low value (2×10^{-4} sec^{-1}) at 0.1 mM $MgAc_2$ to a high value (12×10^{-2} min^{-1}) at 5 mM $MgAc_2$ and back to a low value at 10 mM $MgAc_2$.[4] This shows that the concentration of Mg^{+2} ions has a major effect on the structure of either the rRNA or RNase. The fact that there is little or no change in

k' for the hydrolysis of UpU over the same concentration range (Table 1) shows that the rRNA must be the species which changes structure as the concentration of magnesium ions is changed and that the RNase structure is not significantly changed.

Table 1: The Pseudo First Order Rate Constants for the Hydrolysis of UpU and rRNA.

Ionic Conditions*	UpU (20 μg/ml, RNase)	rRNA(0.01 μg/ml)
no Mg^{2+}	4.0×10^{-2}	
1 mM Mg^{2+}		$(1 \text{ to } 2) \times 10^{-2}$
5 mM Mg^{2+}	4.3×10^{-2}	$(6 \text{ to } 12) \times 10^{-2}$
10 mM Mg^{2+}	5.4×10^{-2}	$(1 \text{ to } 2) \times 10^{-2}$
0.1M NH_4Cl, 1 mM Mg^{2+}	5.6×10^{-2}	
0.1M NH_4Cl, 1 mM Mg^{2+}, 4 mM EDTA	5.0×10^{-2}	

* All solutions contain 5 mM tris, pH 7.3.

It should also be noted that the rate constant for hydrolysis of RNA is much faster than the k' for UpU (note RNase concentrations in Table 1). This shows that the secondary and tertiary structures of the rRNA produce a few regions in the RNA chain which are in highly strained configurations which lower the energy barrier for hydrolysis. We expect UpU to be in a low energy state since it is unlikely that a tertiary structure for UpU exists in solution.

The near constancy of k' for UpU shows that strained configurations are not present to any significant extent.

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