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INHIBITION OF ENDORIBONUCLEASE VI FROM *Artemia* LARVAE BY CYTIDINE 2'- PHOSPHATE

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SUMMARY. The endoribonuclease VI from *Artemia* larvae is non-competitively inhibited by cytidine 2'- phosphate with a Ki *ca* 1 μM. Neither of the cytidine monophosphates isomers with the phosphate group in the 3' or 5' position nor the cyclic 2': 3' phosphate are inhibitors at concentrations up to 100 μM. Adenosine, guanosine and uridine 2' or 3' phosphates are also ineffective in this range of concentrations. Certain polyribonucleotides are potent competitive inhibitors of the ribonuclease activity.

INTRODUCTION. *Artemia* larvae have an endoribonuclease which preferentially hydrolyzes poly (U) among a series of natural and synthetic polyribonucleotides (1). The enzyme has been purified to homogeneity and it was shown that it is very specific to hydrolyze phosphodiester bonds with a uridine residue at the 3' side of the ester bond (2). This last property distinguishes it from other eukaryotic and prokaryotic ribonucleases (3-5). The enzyme was named endoribonuclease VI (2) and its importance in the processing and sequencing of RNA molecules has been discussed (1,2).

The expression of endoribonuclease VI is controlled during the early stages of *Artemia* development by mechanisms still poorly understood (1,2,6). Recent evidence suggests that the burst in ribonuclease activity that takes place upon emergence of the nauplii is the result of the unmasking of the enzyme already present in the encysted gastrulae (7). In contrast with the detailed knowledge of the substrate specificity of this enzyme (1,2) no information is available on the existence of effectors that could modulate this ribonuclease activity. This information might be important for the understanding of the regulation of this enzymatic activity during *Artemia* development. In this communication we present evidence showing that cytidine 2'- phosphate and certain polyribonucleotides are strong inhibitors of the endoribonuclease VI from *Artemia* larvae.

MATERIALS AND METHODS

Chemicals

Baker's yeast tRNA and cytidine 2' : 3' phosphate were from Boehringer. All other mono-, di- and polynucleotides were from Sigma Chemical Co (Sr. Louis, Mo).

0006-291X/84 $1.50

Enzymes

The *Artemia* endoribonuclease VI used in this work (steps V and VI of the purification procedure) (2) was a gift of Dr. M. Quintanilla.

The enzymatic activity was determined in reaction mixtures (0.1 ml) containing: 100 mM HEPES pH 7.5, 10 mM magnesium acetate, 1 mM EDTA, 100 μg (2 A_{260} units) of poly (U), the additions indicated in each case and appropiate amounts of enzyme. After incubation at 30º, the reaction was stopped by addition of 0.1 ml of a solution containing 7.5% perchloric acid and 0.1 % uranyl acetate. After standing at 0º for 15 min, the mixtures were centrifuged and 0.1 ml of the supernatants were brought to 1 ml with distilled water and the optical density at 260 nm was measured. EDTA was included in the reaction mixtures, because the ribonuclease is inhibited by micromolar concentrations of Zn^{2+} and Cu^{2+} (data not shown).

RESULTS

We reported previously (1) that poly (U) is the best known substrate of *Artemia* endoribonuclease VI. The results in Table 1 show that the hydrolysis of poly (U) is inhibited by different polyribonucleotides. Among the homopolymers, poly (G), which is not substrate of the ribonuclease (1), is the most efficient inhibitor. The inhibition by poly (G) is counteracted by increasing the concentration of poly (U), indicating that is of competitive type. From the results in Table 1 it can be deduced that the apparent affinity of the enzyme for poly (G) is around two hundred times greater than for poly (U). Poly (A), poly (C) and yeast tRNA, which are hydrolyzed by the ribonuclease between ten to one hundred times less efficiently than poly (U) (1), are also efficient inhibitors of the hydrolysis of poly (U) at low polynucleotide / poly (U) ratio indicating that the ribonuclease has a much higher affinity for these compounds

Table 1. Effect of different polyribonucleotides on the hydrolysis of poly (U) by *Artemia* endoribonuclease. Reactions were carried out in the conditions described in Methods in the presence of different concentrations of the indicated polynucleotides. The values shown in the Table were obtained from the inhibition curves.

Compound	Amounts for fifty per cent inhibition[a] (A_{260} units/ reaction mixture)
tRNA (yeast)	0.05
Poly (A)	0.10
Poly (C)	0.10
Poly (G)	0.01
Poly (A-G-U)	0.005
Poly (A,C)	0.2
Poly (C,U)	---
Poly (A,U)	---

a/ A dash in this column indicates no detectable inhibition at a concentration of 0.2 A_{260} units / reaction mixture.

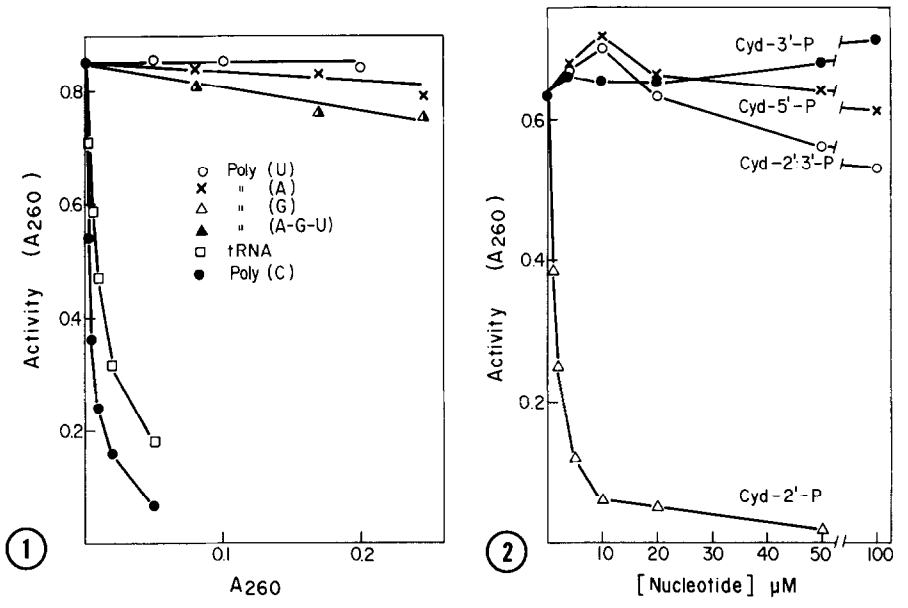

Fig 1.- Differential effect of the alkaline digest of different polynucleotides on the ribonuclease activity. Solutions of the different polynucleotides were brought to 0.25 M KOH and they were incubated at 30º for 15 h and then neutralized with HCl. Ribonuclease activity was determined as stated in Methods in the presence of the indicated amounts of the hydrolyzates.

Fig 2.- Differential effect of cytidine monophosphates on the ribonuclease. Activity was determined in reaction mixtures (see Methods) containing the indicated concentrations of nucleotides.

(about 10 fold) than for poly (U). Among the heteropolymers tested, poly (A-G-U), which is not substrate, is the most effective inhibitor.

In order to confirm that the observed inhibitions were due to the polyribonucleotides, we subjected these compounds to alkaline hydrolysis and looked for the effect of the alkaline digests on the enzymatic hydrolysis of poly (U). The results in Fig 1 show that poly (A), poly (G) and poly (A-G-U) lose their inhibitory effect after alkaline hydrolysis, indicating that the observed inhibitions were in fact due to these polyribonucleotides. Surprisingly, the alkaline hydrolyzates of poly (C), tRNA (Fig 1) and poly (A,C) (not shown) were much more efficient as inhibitors than the intact polymers. All these results taken together strongly indicated that the inhibition by the alkaline hydrolyzates was due to cytidine nucleotides. Consistent with this view was the fact that the alkaline hydrolyzate of poly (C) is 3 to 4 times more efficient as inhibitor than the tRNA digest (Fig 1) in agreement with the cytosine abundance in the tRNAs (8). From the results in Figure 2 it can be concluded that in fact cytidine 2'- phosphate strongly and specifically inhibits the *Artemia* endoribonuclease. Fifty per cent inhibition is attained at a concentration 0.5-1 µM. None of the cytidine monophosphate isomers in the 3' or 5' positions nor the cyclic 2': 3' phosphate are inhibitors at concentrations

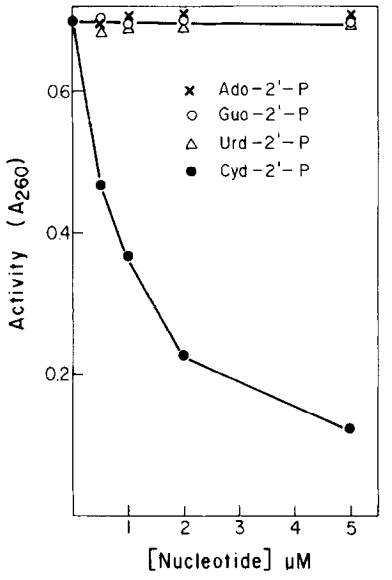

Fig 3.- Nucleotide base specificity for the inhibition of the ribonuclease. Assay conditions as stated in Methods in the presence of the indicated concentrations of the nucleotides.

up to 100 μM (Fig 2). Contrary to that found in the case of the polynucleotides (see above), the inhibition by cytidine 2'- phosphate is not counteracted by increasing the concentration of poly (U). The results in Figure 3 show the nucleotide base specificity required to produce this inhibition. As could be expected from the results in Figure 1, the 2'- phosphates of adenosine, guanosine and uridine are ineffective. We have also tested as inhibitors, dinucleoside monophosphates that are not (or very poorly) hydrolyzed by the ribonuclease (2). The compounds tested were: ApA, ApU, CpC, CpU, GpG, GpU, UpG, UpC, UpA and UpUpUp. None of these compounds produce detectable inhibition of the hydrolysis of poly (U) at concentrations up to 100 μM.

The results shown in this communication demonstrate important differences between the *Artemia* endoribonuclease VI and other ribonucleases sensitive to inhibition by nucleotides, such as ribonucleases T_2 from *Aspergillus oryzae* (9) and M from *Aspergillus saitoi* (10). The inhibition of these ribonucleases by nucleoside monophosphates has a broader specificity with respect to the nature of the base and the position of the phosphate group in the ribose moiety. Moreover, the apparent affinity of the *Artemia* ribonuclease for cytidine 2'- phosphate is 10^2 and 10^3 times greater than those found in the cases of ribonucleases M and T_2 respectively (9,10). The implication of our findings in the regulation of the endoribonuclease VI activity during *Artemia* early development is now under investigation.

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