unlike optimally compacted chromatin, and are thus more "distortable" than optimally compacted chromatin.

Registry No. BMSp, 70940-03-3; BMSpd, 93194-39-9; MSp, 86388-76-3; actinomycin D, 50-76-0; daunomycin, 20830-81-3; distamycin, 39389-47-4.

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Metal Ion Requirements and Other Aspects of the Reaction Catalyzed by M1 RNA, the RNA Subunit of Ribonuclease P from *Escherichia coli*†

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ABSTRACT: M1 RNA, the RNA subunit of ribonuclease P from *Escherichia coli*, can under certain conditions catalytically cleave precursors to tRNA in the absence of C5, the protein moiety of RNase P. M1 RNA itself is not cleaved during the reaction, nor does it form any covalent bonds with its substrate. Only magnesium and, to a lesser extent, manganese ions can function at the catalytic center of M1 RNA. Several other ions either inhibit the binding of magnesium ion at the active site or function as structural counterions. The reaction rate of cleavage of precursors to tRNAs by M1 RNA is enhanced in the presence of poly(ethylene glycol) or 2-methyl-2,4-pentanediol. Many aspects of the reaction catalyzed by M1 RNA are compatible with a mechanism in which phosphodiester bond cleavage is mediated by a metal ion.

The RNA subunit of ribonuclease P from *Escherichia coli*, M1 RNA, is a catalyst (Guerrier-Takada et al., 1983; Guerrier-Takada & Altman, 1984a). In vivo this enzyme is responsible for the maturation of the 5′ termini of tRNA molecules, cleaving P–O3′ bonds to produce 5′-phosphate and 3′-hydroxyl end groups. M1 RNA hydrolyzes phosphodiester bonds at specific sites in several different substrates, is unchanged during catalysis, and has a characteristic turnover number. The reaction of M1 with its substrates is different from the cleavage of RNA that is catalyzed by heavy-metal ions (Brown et al., 1983; Rubin & Sundaralingam, 1983) or by RNase A (Richards & Wycoff, 1971) during which P–O5′ bonds are cleaved via cyclic 2′,3′-phosphate intermediates. The self-splicing reaction of *Tetrahymena thermophila* pre-rRNA (Cech, 1983) is similar to cleavage by M1 in that P–O3′ bonds are cleaved and a divalent metal ion is required. However, M1 RNA produces a single cut in each substrate molecule,

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whereas two cleavages plus a ligation are required for excision of the *Tetrahymena* intervening sequence. We have investigated the characteristics of the reaction catalyzed by M1 RNA in an attempt to elucidate the nature of the catalytic mechanism.

Materials and Methods

Enzyme and Reagents. Acrylamide and bis(acrylamide) were obtained from Bio-Rad Corp. Ultrapure area was purchased from Schwarz-Mann. Radioactive materials were obtained from Amersham Radiochemicals Corp. PEG[1] (mol wt 6000–7500) was obtained from Matheson Coleman and Bell. All other chemicals were reagent grade.

Preparation of M1 RNA and C5 Protein. M1 RNA (Reed et al., 1982) and C5 protein (Guerrier-Takada et al., 1983) were prepared as previously described.

Preparation of Radioactive RNA. Preparation of ^{32}P-pTyr[1] (Robertson et al., 1972) and addition of ^{32}P-pCp to the 3′ terminus (Guerrier-Takada & Altman, 1984b) were carried out as described previously.

Assays for RNase P Activity. Assays for RNase P activity were carried out at 37 or 30 °C by using the buffers indicated in the figure legends. The reaction products were analyzed either on 10% nondenaturing polyacrylamide gels (Guerrier-Takada et al., 1983) or on 5% polyacrylamide gels that contained 7 M urea (Furdon et al., 1983). Complexes of enzyme and substrate were prepared as described previously (Furdon et al., 1983) from standard reaction mixtures or those containing 100 mM Mg^{2+} and 5% PEG.

When M1 RNA activity was quantitated, either by measuring Cerenkov radiation from gel slices that contained products of the reaction or by scanning X-ray films, the assays were carried out under conditions that corresponded to the linear portion of the curve showing kinetics of cleavage.

In assays of cleavage activity by M1 RNA in the presence of different metal ions, excess M1 RNA, relative to pTyr, was present in the reaction mixtures. Mixed metal ion experiments were performed as follows. The first metal ion was added to standard reaction buffer from which pTyr was absent, and the mixture, containing M1 RNA, was incubated for 2 min at 37 °C (Guerrier-Takada et al., 1983). pTyr and the second metal ion were then added, and the incubation was terminated after a further 15 min at 37 °C.

Results

We showed in earlier experiments that M1 RNA is unchanged in size after it has been incubated with a substrate molecule in the appropriate reaction mixture (Guerrier-Takada et al., 1983). Furthermore, there is no requirement for a particular nucleotide sequence on the 5′ side of the cleavage site (Guerrier-Takada & Altman, 1984a): a 3′-terminal hydroxyl group in intact M1 RNA (see below) or a nucleotide cofactor. These characteristics distinguish the reaction catalyzed by M1 RNA from that carried out by *Tetrahymena* p-rRNA.[1] However, to determine whether M1 RNA could undergo a self-cleavage and ligation reaction during each catalytic event, transiently generating an internal free 3′-hydroxyl group that could mediate phosphodiester bond attack in the substrate molecule, we have investigated the state of

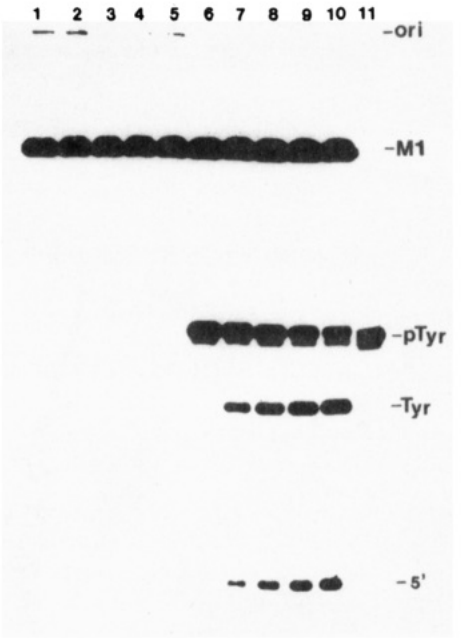

FIGURE 1: Fate of M1 RNA during cleavage of pTyr. M1 RNA (0.05 pmol labeled at the 3′ terminus with ^{32}P-pCp) was preincubated for 30 min at 30 °C in 50 mM Tris·HCl, pH 7.5, 100 mM NH_4Cl, 100 mM $MgCl_2$, and 5% PEG to ensure linear kinetics of the reaction (Guerrier-Takada et al., 1983). H_2O (lanes 1–5) or pTyr (lanes 6–10, 0.015 pmol) was added to the incubation mixture, and aliquots were removed for analysis at 0, 1, 2, 4, 8, and 20 min. The reaction mixtures were analyzed on a 5% polyacrylamide gel containing 7 M urea, 50 mM Tris–borate, and 1 mM EDTA. The aliquot taken at 20 min is not shown on the gel but gave the same result as the 8-min aliquot. The positions of pTyr, the product of the cleavage of pTyr that contains the mature tRNA sequence (Tyr), and the 5′ fragment containing extra nucleotides (5′) are marked. Some contaminating species in the pTyr preparation, which are not substrates for RNase P, are evident in the figure.

M1 RNA in a complete reaction mixture. We have examined the state of M1 when it reacts with its substrate under conditions of substrate excess and have isolated M1 RNA from the enzyme–substrate complex (Furdon et al., 1983). In these experiments M1 RNA was end-labeled with ^{32}P-pCp to enhance the sensitivity of our assays. During incubation in the standard reaction mixture or in a control mixture with no substrate (Figure 1), there is no indication that the labeled M1 RNA has yielded any fragments or formed any covalent linkages with the substrate. Only intact enzyme and substrate or the cleavage products of the substrate are apparent. Note that the end-labeled M1 RNA functions well as enzyme. We repeated a similar assay using M1 RNA isolated from the fractions that contained the enzyme–substrate complex isolated from Cs_2SO_4 density gradient analyses of reaction mixtures (Furdon et al., 1983), and we obtained the same result. If a cleaved M1 RNA intermediate (or M1 RNA covalently bound to pTyr) were present at about a 0.1% level compared to the amount of either input M1 RNA or M1 RNA in the enzyme–substrate complex, such an intermediate would have been detectable. We conclude, therefore, that it is unlikely that M1 RNA undergoes self-cleavage and ligation as has been found in the self-splicing of p-rRNA (Price et al., 1985). It is more likely that the M1 RNA mechanism is similar to the *Tetrahymena* p-rRNA self-splicing reaction in which the cyclic intervening sequence is hydrolyzed (Zaug et al., 1985). Accordingly, we have attempted to determine if cleavage may be occurring via a mechanism in which cleavage of the phosphodiester bond is mediated by a complex of a magnesium ion with water molecules (Haydock & Allen, 1985).

[1] Abbreviations: pTyr, precursor to *Escherichia coli* $tRNA^{Tyr}$; P-RNA, the RNA subunit of RNase P from *Bacillus subtilis*; C5 protein, the protein subunit of *E. coli* RNase P; PEG, poly(ethylene glycol); MPD, 2-methyl-2,4-pentanediol; Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid; CAPS, 3-(cyclohexylamino)-1-propanesulfonic acid; p-rRNA, precursor to ribosomal RNA from *Tetrahymena thermophila*.

Table I: Effect of Various Metal Ions on the Activity of M1 RNA[a]

metal ion	concentration of metal ion in buffer: 10–50 mM (alone)	10 mM plus[b] 5 mM polyamine	50 mM plus[b] 10 mM Mg^{2+}	10 mM plus[b] 60 mM Mg^{2+}
Mg^{2+}		85	70	75
Mn^{2+}	0	20	5	70
Zn^{2+}	0	0	0	0
Co^{2+}	0	0	0	0
Fe^{2+}	0	0	0	0
Ni^{2+}	0	0	0	0
Ca^{2+}	0	0	32	70
Sr^{2+}	0	0	20	65
Sm^{3+}	0	0		0

[a] Both ions added simultaneously. The polyamine can be spermine or spermidine. [b] The numbers listed are percentages of cleavage of pTyr by M1 RNA and have been obtained by scanning films of the autoradiography of acrylamide gel separations of the products of the reactions. For example, as shown in the first line of the table, at 60 mM Mg^{2+} 75% of the substrate is cleaved to make the product. The graph shown in Figure 2 illustrates the dependence of the reaction of Mg^{2+} concentration at between 10 and 50 mM. The absolute numbers shown in Figure 2 are not strictly comparable to those given in this table because the assays were not carried out under precisely identical conditions.

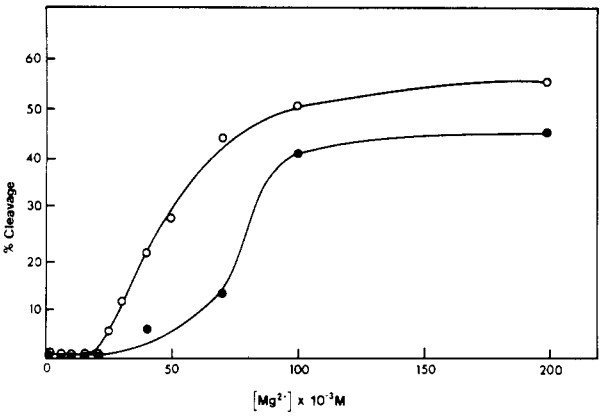

FIGURE 2: Dependence of cleavage reaction on concentration of Mg^{2+}. M1 RNA (0.25 pmol) was incubated for 15 min at 37 °C in the presence of pTyr (0.01 pmol) in 50 mM Tris·HCl, pH 7.5 (●), or in 50 mM Tris·HCl, pH 7.5, and 100 mM NH_4Cl (○) with different concentrations of $MgCl_2$. The reaction products were analyzed on a 10% polyacrylamide gel containing 89 mM Tris–borate and 2.5 mM EDTA. After autoradiography, the appropriate bands were cut out from the gel and the amount of radioactivity in each was determined by measurement of Cerenkov radiation.

Metal Ion Requirements. M1 RNA can catalyze the cleavage of tRNA precursor molecules in the presence of 5 mM spermidine or spermine (but not putrescine or cadaverine) and a divalent metal ion. We previously inferred that the polyamine acts as the structural counterion while the metal ion is necessary for the catalytic center to function (Guerrier-Takada et al., 1983). In order to explore this phenomenon further and to ascertain which metal ions could function at the catalytic center, we performed three kinds of experiments. We measured M1 RNA activity (a) in the presence of low concentrations of various metal ions with added polyamine, (b) in the presence of high concentrations of individual metal ions alone to see which ones elicit catalytic activity in the absence of added polyamine, and (c) in the presence of various concentrations and with different orders of mixing of two metal ions to determine if some inhibit the action of others.

As shown in Table I, Mg^{2+} is the only metal ion that, alone, can act as a cofactor for M1 RNA. The activity of M1 RNA increases with increasing Mg^{2+} concentration up to 100 mM (see Figure 2). Monovalent cation (NH_4^+) has a stimulatory effect on the reaction rate (Figure 2), but this effect is concentration independent between 0.1 and 2 M. In the presence of 5 mM spermine or spermidine, only Mg^{2+} and, to a much lesser extent, Mn^{2+} allow the M1 RNA reaction to proceed.

The results of the mixed metal ion experiments yielded insights into the roles of the ions as inhibitors or structural cofactors in the reaction. The behavior of M1 RNA in buffers containing Mn^{2+} and Mg^{2+} together is particularly interesting. In 10 mM Mn^{2+} and 10 mM Mg^{2+}, M1 RNA has approximately the same activity as in 20 mM Mg^{2+}. In 10 mM Mn^{2+} and 60 mM Mg^{2+}, the activity is almost equal to that in Mg^{2+} at 60 mM by itself. However, if Mn^{2+} is added to the reaction mixture before Mg^{2+}, the reaction is inhibited (Table II). With high concentration of Mn^{2+} (50 mM) plus small amounts of Mg^{2+} (10 mM), the activity is not greater than that of Mg^{2+} at 10 mM by itself. These data confirm that Mn^{2+} can substitute for, and indeed compete with, Mg^{2+} at the catalytic center.

In buffers that contained the transition metal ions Co^{2+}, Fe^{2+}, Ni^{2+}, and Zn^{2+}, M1 RNA shows no activity even with added polyamine or Mg^{2+} ions. Zn^{2+}, Co^{2+}, and Ni^{2+} at 10 mM are inhibitory in the presence of 60 mM Mg^{2+} regardless of whether they are added before or after Mg^{2+}. (In addition,

Table II: Metal Ion Competition by Order of Addition[a]

metal ion	Mg^{2+} first	Mn^{2+} or Ca^{2+} first
Mn^{2+} (10 mM)	5.5	3
Ca^{2+} (20 mM)	11	4

[a] The table lists the percentage of cleavage of substrate by M1 RNA when the indicated metal ions were in the reaction buffer, added as shown in the table and as described under Materials and Methods. The final concentration of Mg^{2+} was 10 mM.

Zn^{2+} has been found to have the same inhibitory effect in the presence of C5 regardless of the order of addition.) Pb^{2+} and Cu^{2+} also fail to function as divalent cation cofactors in the cleavage by M1 RNA, but we have not investigated the action of these ions as extensively as those listed in Table I. In 10 mM Mg^{2+} plus 50 mM Ca^{2+} (or Sr^{2+}) M1 RNA has about 50% activity, but neither of these cations promotes activity by itself even with added polyamine. In fact, when added before Mg^{2+}, Ca^{2+} inhibits the reaction (Table II). We attempted to replace Mg^{2+} by Sm^{3+} at several concentrations since Teeter et al. (1980) showed that Sm^{3+} was able to substitute for Mg^{2+} in the four principal binding sites of the cation in the crystal structure of tRNA. No activity was observed at any of these concentrations.

The experiments with two metal ions indicate that the binding site at the catalytic center of M1 RNA must be one from which a bound metal cannot easily escape. The first metal ion added in the competition experiments is present for 2 min prior to addition of substrate and Mg^{2+} and then for a further 15 min, during which time clear effects on the enzymatic activity of M1 RNA in the presence of Mg^{2+} can be observed. Conversely, when Mg^{2+} is added first, the binding is so tight and the rate of dissociation so slow that no ion added subsequently interferes with the rate of cleavage.

These data together show that Mg^{2+}, Ca^{2+}, Sr^{2+}, and, to a lesser extent, Mn^{2+} can perform the electrostatic shielding function and preserve the structural properties of the two RNA molecules necessary to keep the substrate and enzyme in appropriate conformations. Only Mg^{2+} can function efficiently at the catalytic center.

Solutes That Enhance the Reaction Rate of M1 RNA. Poly(ethylene glycol) (PEG) [poly(ethylene oxide)] is known to enhance the rate of various biochemical reactions (Zimmerman & Pheiffer, 1983) and the ordered structure of DNA

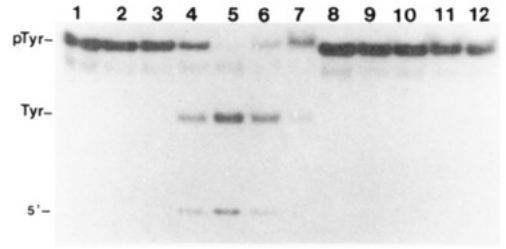

FIGURE 3: Cleavage reaction in buffers containing PEG. M1 RNA (0.25 pmol) was incubated for 10 min at 37 °C in the presence of pTyr (0.01 pmol) in buffer X (50 mM Tris·HCl, pH 7.5, 100 mM NH_4Cl, 10 mM $MgCl_2$) with different concentrations of PEG. Lane 1, buffer X without M1 RNA; lane 2, buffer X; lane 3, buffer X + 5% PEG; lane 4, buffer X + 10% PEG; lane 5, buffer X + 15% PEG; lane 6, buffer X + 20% PEG; lane 7, buffer X + 28% PEG; lane 8, buffer X lacking $MgCl_2$ plus 10 mM EDTA; lane 9, as lane 8 plus 10% PEG; lane 10, as lane 8 plus 15% PEG; lane 11, as lane 8 plus 20% PEG; lane 12, as lane 11 with *no* EDTA.

in solution (Lerman, 1971). These effects are achieved through the creation of high local concentrations of macromolecules, possibly because of localized effects of excluded volume. Some organic solvents can also enhance catalytic rates and simultaneously alter the specificity of biochemical reactions of nucleic acids (Schimmel & Soll, 1979). The way in which these organic solvents affect enzymatic reactions must be different from the mode of action of PEG. For example, changes in conformation of enzyme or substrate that depend on hydrophobic interactions will occur more easily in solutions containing organic solvents.

We have found that, in the presence of PEG, no polyamine or protein cofactor is necessary for the activity of M1 RNA (Figure 3). In fact, in solutions containing 10 mM Mg^{2+} and 15% PEG, M1 RNA activity is stimulated about 5-fold compared to the reaction carried out in 10 mM Mg^{2+} and 5 mM spermidine. However, as we observed before, Mg^{2+} is absolutely required. No activity is seen if Mg^{2+} is omitted from the reaction mixture (Figure 3, lanes 8–12); the experiments shown were done with excess M1 RNA to give maximum sensitivity of the assay. (Quantitation was performed with approximately equimolar amounts of enzyme and substrate.) None of the other ions listed in Table I, including Mn^{2+}, can substitute for Mg^{2+} in the presence of 10% PEG. If 10 mM Ca^{2+} or Sr^{2+} is added to solutions containing PEG and 10 mM Mg^{2+}, the activity of M1 RNA is enhanced 2–3-fold, probably because these ions act as structural counterions and optimize the conformation of M1 RNA in the PEG solution. In solutions containing 100 mM Mg^{2+}, optimal M1 RNA activity is seen in the presence of 5% PEG (data not shown). The maximum stimulation by PEG is 10-fold compared to the rate seen with M1 RNA in 100 mM Mg^{2+} alone and about 2-fold compared to the rate of the holoenzyme complex (M1 RNA plus excess C5 protein) in 10 mM Mg^{2+}. The turnover number of M1 RNA under these optimal conditions, about 40 mol of product (mol of enzyme)$^{-1}$ min^{-1}, approximates that which could be expected of the intracellular turnover number for RNase P, assuming about 100 molecules of enzyme per cell and a 20-min generation time. PEG has a very slight stimulatory effect on the action of the holoenzyme complex in 10 mM Mg^{2+}. Increases in the concentration of monovalent cations up to 0.5 M in the presence of PEG or 2 M in the absence of PEG have little effect on the turnover number of M1 RNA.

The effect of PEG on the reaction catalyzed by M1 RNA can be explained if an association of M1 RNA molecules is required for catalysis. PEG could enhance the formation of dimers or other oligomers of M1 RNA through local excluded volume effects. Accordingly, we measured the dependence of the reaction rate on M1 RNA concentration. The data, shown in Figure 4, indicate a second-order dependency of the catalytic activity on M1 RNA concentration. One interpretation of these results is that dimers of M1 RNA carry out the RNase P reaction. While the *rate* of catalysis is increased in solutions containing PEG as we might expect, the nature of the dependence of the catalytic activity on M1 RNA concentration is unchanged.

When the activity of M1 RNA is assayed in solutions that contain 100 mM Mg^{2+} and 10% 2-methyl-2,4-pentanediol (MPD),[1] a stimulation of 10-fold in rate is observed. However, in solutions containing 10 mM Mg^{2+}, MPD does not enhance the rate of cleavage by M1 RNA, with or without added C5 protein. Ethanol does not stimulate the M1 RNA reaction as well as does MPD. In solutions containing 100 mM Mg^{2+}, a peak of stimulation of about 2-fold is seen in 10% ethanol. Thirty percent ethanol inhibits the reaction. The specificity of the M1 RNA reaction is unchanged by addition of any of the solutes we discuss above. The tolerance to these solutes of the reaction catalyzed by M1 RNA may help our attempts to crystallize the M1 RNA.

pH Dependence of the RNase P Reaction. The pH dependence of the reactions catalyzed by both M1 RNA and RNase P holoenzyme is similar. In each case, little activity is seen below pH 5.5. As the pH is raised, a plateau is reached

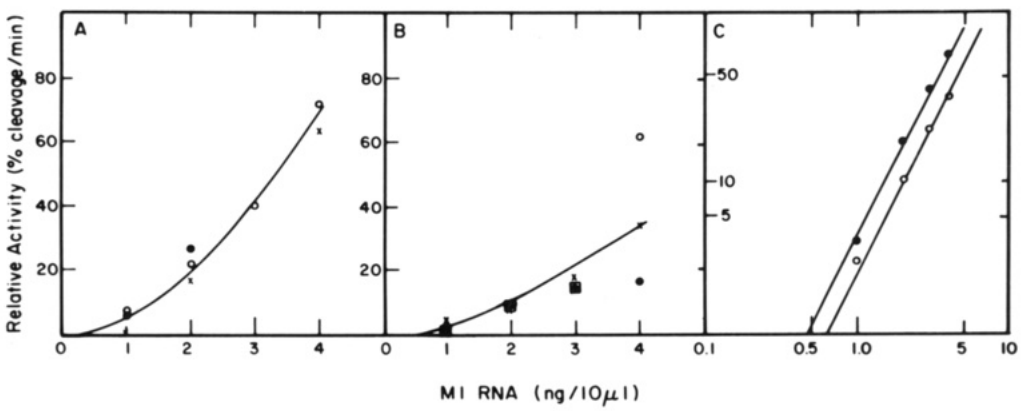

FIGURE 4: Dependence of M1 RNA activity on M1 RNA concentration. Initial reaction rates were measured in reaction mixtures containing 100 mM Mg^{2+}, and the amount of M1 RNA is shown (see Materials and Methods). Each data point represents the result of a reaction rate determination at the particular concentration of M1 RNA indicated. Absolute values of cleavage rates have been normalized for small variations in experimental conditions to facilitate comparison of several experiments on the same scale. The rates of cleavage shown in panels A and B are, therefore, not directly comparably with each other on an absolute scale. A particular symbol in each panel denotes data from one series of experiments. (A) No PEG added; (B) 5% PEG added; (C) data points taken from panels A (○) and B (●) and replotted on a log–log scale.

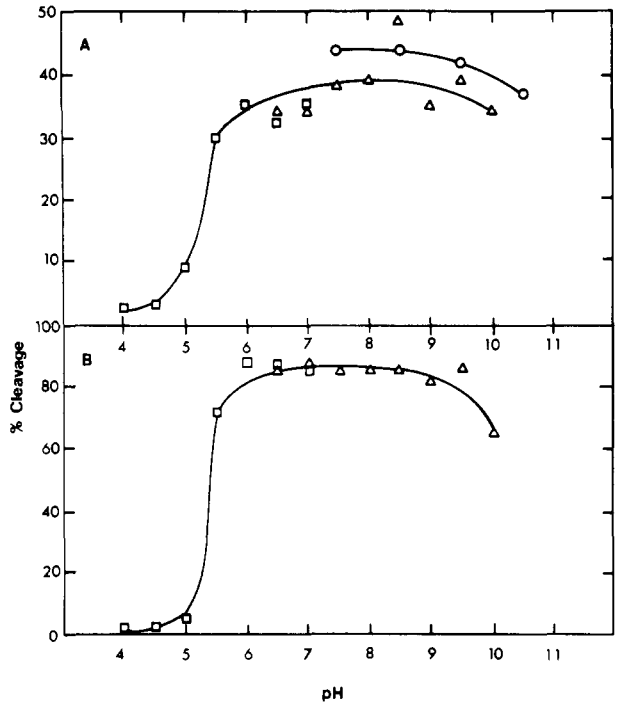

FIGURE 5: pH dependence of cleavage reaction. M1 RNA (0.015 pmol) was incubated for 10 min at 37 °C in the presence of pTyr (0.01 pmol) in buffer containing 60 mM $MgCl_2$ and 100 mM NH_4Cl and 50 mM of either Na(OAc) (□), Tris·HCl (△), or CAPS (○). (A) M1 RNA alone as the source of enzymatic activity; (B) M1 RNA plus C5 protein complex as the source of enzymatic activity.

that is maintained between pH 5.5 and 9.5 (Figure 5). Above pH 9.5 and below pH 5.5 the rate of reaction decreases. This drop in activity could possibly be related to ionization of the bases.

Conformational States of M1 RNA. The dependence on the concentration of Mg^{2+} ions of the reaction catalyzed by M1 RNA exhibits a sigmoidal character (Figure 2) characteristic of a cooperative transition. This dependence is observed whether or not a monovalent cation is present. The need for a high concentration of Mg^{2+} to potentiate the activity of M1 RNA can be alleviated by the addition of C5 protein, 5 mM polyamine (though the latter is not as efficient a cofactor), or PEG. We have shown that both C5 protein and Mg^{2+} can induce a structural change in M1 RNA which, we have suggested, primes the active state (Guerrier-Takada & Altman, 1984b). Evidence for this structural change in M1 RNA in solution has been obtained from analyses of partial digests with RNase T_1 of M1 RNA in solution (Guerrier-Takada & Altman, 1984b).

Discussion

Nucleolytic reactions catalyzed by RNA, in which 5′-phosphate and 3′-hydroxyl groups are generated in the products, are mediated by a mechanism that is not yet fully understood. Hypotheses have been advanced to explain the self-splicing reaction of the *T. thermophila* rRNA precursor. In this case the nature of the specific nucleotide sequences involved appears to be important to the reaction mechanism [see Cech (1983) for review and Price et al. (1985)]. The cleavage of tRNA precursors by M1 RNA cannot be identical in all respects with the rRNA splicing reaction since a 3′-hydroxyl end group of M1 RNA is not important in its reaction.

Gardiner et al. (1985) have described the ion and solvent dependence of the reaction governed by the RNA subunit (P-RNA) of RNase P from *B. subtilis*, using a synthetic tRNA precursor as substrate. Our findings are in agreement with their general conclusions. Although we [see Guerrier-Takada et al. (1983) and unpublished data] have determined that P-RNA behaves very similarly to M1 RNA on the natural substrate pTyr, Gardiner et al. (1985) found significantly different characteristics of the reaction with the synthetic substrate. The nature of the substrate used may be very important in the determination of the relevant physiological features of reactions catalyzed by RNA. Using pTyr as substrate, we have attempted to elucidate the characteristics of the metal ion dependence of catalysis by M1 RNA and to determine if an hypothesis advanced to explain the mechanism of self-cleavage by *Tetrahymena* p-rRNA (Haydock & Allen, 1985) is also compatible with the observed aspects of the reaction catalyzed by M1 RNA.

Metal Ion Effects. The minimal assumption necessary to systemize the data of Tables I and II is that there be two separate types of metal binding sites: catalytic and structural. This assumption is supported by the metal binding properties of protein enzymes such as thermolysin (Feder et al., 1971) and liver alcohol dehydrogenase (Eklund et al., 1976). In addition, a comparison of ionic radii and charge polarizability, which has been useful in the analysis of the binding of metal ions to tRNA (Teeter et al., 1980) and nucleic acids (Saenger, 1984), is important because the catalytic site is probably more limited in size and steric properties than the structural sites. Thus we note that the ionic radius of Ca^{2+} is one-third greater (0.86 vs 1.14 Å) than that of Mg^{2+}. Therefore, Ca^{2+} cannot substitute for Mg^{2+} to promote the catalytic activity of M1 RNA. There is no reaction when Ca^{2+} (or Sr^{2+}) is present alone, and these ions inactivate catalytic sites when added to reaction mixtures before Mg^{2+}. On the other hand, the high-charge density of Ca^{2+} and Sr^{2+} permits them to substitute for Mg^{2+} at the structural sites, thereby explaining the almost full reactivity realized with 10 mM Mg^{2+} and 50 mM Ca^{2+} (and the approximately 50% level realized with 50 mM Sr^{2+}).

In the X-ray structure (Teeter et al., 1980) of tRNA, Sm^{3+} was found to occupy the same sites as Mg^{2+}, but its larger radius and higher charge make it inappropriate as a replacement for Mg^{2+} in the catalytic function of M1 RNA; therefore, its lack of activity with Sm^{3+} is not surprising. Mn^{2+} is only slightly larger and more polarizable than Mg^{2+} and is also expected to have a spherically symmetric charge distribution (Huheey, 1978). Thus, it might be expected that when polyamines or Mg^{2+} have satisfied the structural requirements for M1 RNA, Mn^{2+} can replace Mg^{2+} at catalytic sites. However, because of its tendency to bind to nucleotide bases, Mn^{2+} will have an effect on the structure of M1 RNA different from that of Mg^{2+} and can inhibit the reaction when added before Mg^{2+}, as we have observed. (If the order is reversed, Mg^{2+} binding prevents Mn^{2+} from disrupting the structure.) Co^{2+}, Fe^{2+}, Ni^{2+}, and Zn^{2+} are all considerably more polarizable acids than Mg^{2+}, and they can bind to nucleotide lone pairs (Saenger, 1984), thus distorting the structural sites in M1 RNA. None of these four metals shows activity at any concentration. Zn^{2+}, Co^{2+}, and Ni^{2+} inhibit the reaction.

Although fewer data on metal ion effects are available for the two other systems in which RNA acts catalytically, namely, the reaction of RNase P from *B. subtilis* (Gardiner et al., 1985) and the self-splicing of rRNA from *Tetrahymena* (Zaug et al., 1983), it is important to make comparisons. A consistent hypothesis is that all three share a common metal ion complex as catalyst but that RNA–RNA recognition and

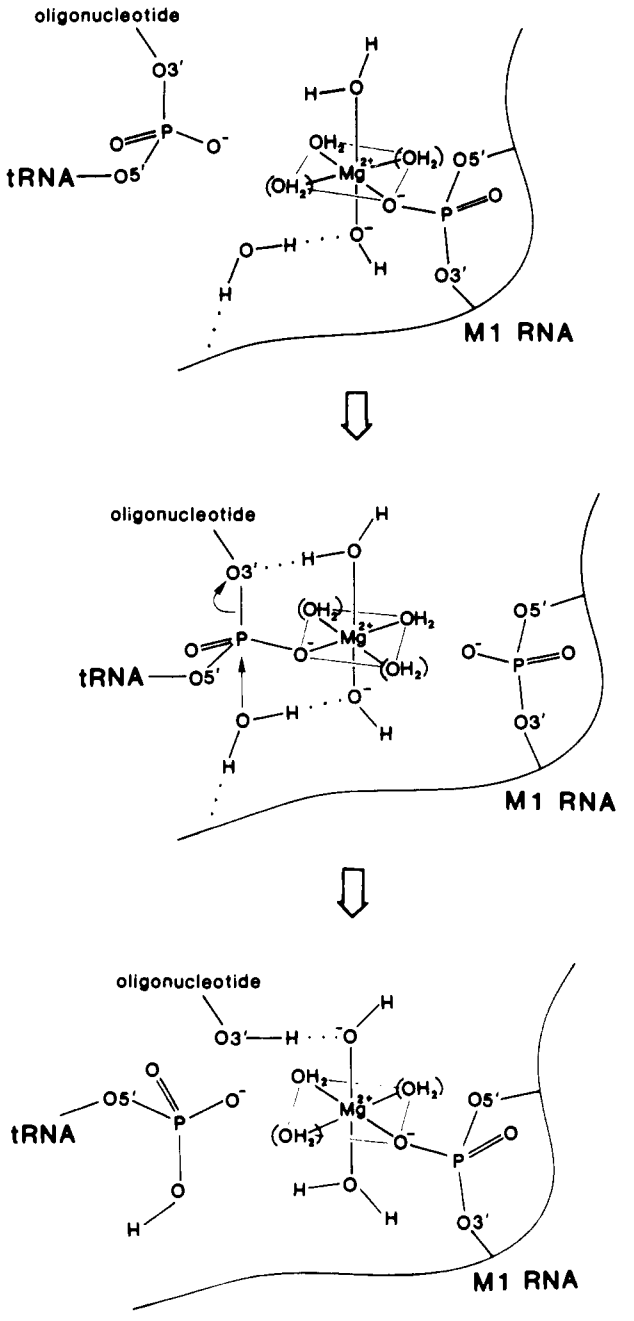

FIGURE 6: Hypothetical electronic mechanism of tRNA precursor hydrolysis by M1 RNA of RNase P. The reaction is catalyzed by a Mg–H_2O complex that is initially bound to a phosphate of M1 RNA. Mg^{2+} is formally shown as hexacoordinated, but may well be tetracoordinated as indicated by the parentheses around the two equatorial water ligands. In the top panel a water molecule from the solvent that will participate in hydrolysis is positioned by a hydrogen bond to an O or N atom in M1 RNA. In the middle and bottom panels the tRNA precursor substrate is bound by the water molecule attached to M1 RNA and passes through a transition state prior to cleavage of the "extra" oligonucleotide and the addition of OH to its O5′ terminal phosphate. After the reaction steps shown here, a solvent water chain between the axial ligands of Mg^{2+} recocks the enzyme for the next cycle.

structural features differ considerably. The concentration of metal ion required in each case for the catalysis itself appears to be small (5–10 mM).

As in the case of the M1 RNA reaction, Mn^{2+} is the only ion that can substituted for Mg^{2+} under certain conditions and still produce enzymatic activity comparable to the native system for both *B. subtilis* P-RNA and *Tetrahymena* rRNA precursor.

Mechanism and pH Dependence of the Reaction Catalyzed by M1 RNA. The reaction catalyzed by M1 RNA has three aspects that, taken together, distinguish it from other reactions that cleave RNA: production of 5′-phosphate and 3′-hydroxyl groups in the products, absence of transient cleavage products or covalent bonds which link substrate to enzyme, and a strict requirement for Mg^{2+} for optimal activity. The simple reaction mechanism proposed by Haydock and Allen (1985) can account for these observations (Figure 6). It is an application of the S_N2 in-line displacement mechanism familiar from the chemistry of the phosphodiester bond (Cooperman, 1976; Knowles, 1980). However, the details of the binding of a Mg–H_2O complex to M1 RNA in this model, or the active site pocket in which the substrate interacts with both M1 RNA and the metal ion complex, remain to be elucidated.

M1 RNA has an activity vs. pH profile that is constant from pH 5.5 to pH 9.3 and falls off on either end (Figure 5). At first glance, this may not be what one would expect for a hydrolysis reaction that involves the Mg^{2+} ion–water complex proposed by Haydock and Allen, since in aqueous solution the pK_a of $Mg(OH_2)_6$ is 11.42 (Huheey, 1978). However, large pK_a perturbations (compared to those seen for ion complexes in free solution) are a frequent occurrence in protein enzymes (Fersht, 1977; Cotton et al., 1979). Another possible explanation of the pH profile of the cleavage by M1 RNA is that the pK_a is controlled by moieties on a nucleoside base or sugar of M1 RNA.

Structural Considerations. The discussion has been concerned only with the role of metal ions at the catalytic center. The catalysis itself, as indicated by the cooperative nature of the dependence of the reaction on the concentration of Mg^{2+} (Figure 2) and its stimulation by C5 protein, may involve a rearrangement of the conformation of M1 RNA (Guerrier-Takada & Altman, 1984b). However, elucidation of such changes in a molecule the size of M1 RNA (377 nucleotides) will require extensive chemical and enzymological studies or, preferably, determination of a crystal structure of the enzyme.

ADDED IN PROOF

The shape of the pH dependence of the reaction catalyzed by M1 RNA in the presence of Mn^{2+} is the same as that for Mg^{2+}, but the whole curve is shifted down by about 0.5 pH unit. Since the pK for hydrated Mn^{2+} is 10.70 and that for Mg^{2+} is 11.42, the *shift* in the curve is compatible with the general mechanism illustrated in Figure 6.

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Cell-Free Synthesis of Tumor-Type Poly(A) Polymerase†

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ABSTRACT: Previous studies in this laboratory suggested that in adult liver, either the gene for the tumor-type poly(A) polymerase is poorly transcribed or the mRNA for this enzyme is largely not expressed. To test these possibilities, total RNA from rat liver and Morris hepatoma 3924A RNA were isolated by using a guanidine thiocyanate method; poly(A+) RNA and poly(A−) RNA were separated by oligo(dT)–cellulose chromatography and used for translation in a rabbit reticulocyte lysate system. After in vitro translation, the products were immunoprecipitated with either purified anti-tumor poly(A) polymerase antibodies or control immunoglobulins. When the polypeptides translated from poly(A+) or poly(A−) hepatoma RNA were precipitated with immune sera, a unique [^{35}S]methionine-labeled 35-kilodalton (kDa) protein was observed. This band was not apparent when control serum was used for the immunoprecipitation. The radiolabeled 35-kDa polypeptide was not evident when the products were incubated with highly purified tumor nuclear poly(A) polymerase prior to immunoprecipitation. Prior incubation of the translation products with bovine serum albumin instead of poly(A) polymerase had no effect on the immunoprecipitation. This 35-kDa protein was not apparent when liver poly(A+) RNA was used to direct translation. These data demonstrate that (a) the tumor enzyme is not synthesized as a precursor, (b) tumor mRNA, but not normal liver mRNA, contains detectable sequences coding for tumor-type poly(A) polymerase, and (c) poly(A) polymerase mRNA also exists as a poly(A−) population.

Eukaryotic poly(A) polymerase (EC 2.7.7.19) is a ubiquitous enzyme present in a great variety of cells [for reviews, see Jacob & Rose (1983) and Edmonds (1982)]. The major function of the enzyme appears to be the posttranscriptional addition of a poly(A) tract to the 3′ termini of mRNAs. Although poly(A) polymerase is largely localized in the nucleus, it is also present in the mitochondria (Jacob et al., 1972), microsomes (Wilkie & Smellie, 1968), ribosomes (Milchev et al., 1980), and cytosol (Tsiapalis et al., 1975) fractions. Over the years, our laboratory has been studying the structure, function, and posttranslational modifications of nuclear poly(A) polymerase (Rose et al., 1977, 1978; Rose & Jacob, 1979, 1980).

Recent studies have shown that the major nuclear poly(A) polymerase from normal liver is structurally and immunologically distinct from the corresponding enzyme of transplanted (Stetler & Jacob, 1984) and of azo dye induced primary hepatomas (Stetler et al., 1984). Polyclonal antibodies raised against hepatoma nuclear poly(A) polymerase reacted specifically with the tumor enzyme, but not with the liver enzyme (Stetler & Jacob, 1984). These studies have shown that the difference in the molecular weights of the tumor and liver nuclear poly(A) polymerase is not due to proteolytic

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