The *Tetrahymena* Intervening Sequence Ribonucleic Acid Enzyme Is a Phosphotransferase and an Acid Phosphatase†

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ABSTRACT: A shortened form of the *Tetrahymena* intervening sequence (IVS) RNA acts as an enzyme, catalyzing nucleotidyl transfer and hydrolysis reactions with oligo(cytidylic acid) substrates [Zaug, A. J., & Cech, T. R. (1986) *Science (Washington, D.C.) 231*, 470–475]. These reactions involve phosphodiester substrates. We now show that the same enzyme has activity toward phosphate monoesters. The 3′-phosphate of C_5p or C_6p is transferred to the 3′-terminal guanosine of the enzyme. The pH dependence of the reaction (optimum at pH 5) indicates that the enzyme has activity toward the dianion and much greater activity toward the monoanion form of the 3′-phosphate of the substrate. Phosphorylation of the enzyme is reversible by C_5-OH and other oligo(pyrimidines) such as UCU-OH. Thus, the RNA enzyme acts as a phosphotransferase, transferring the 3′-terminal phosphate of C_5p to UCU-OH with multiple turnover. At pH 4 and 5, the phosphoenzyme undergoes slow hydrolysis to yield inorganic phosphate. Thus, the enzyme has acid phosphatase activity. The RNA enzyme dephosphorylates oligonucleotide substrates with high sequence specificity, which distinguishes it from known protein enzymes.

RNA self-splicing exemplifies intramolecular catalysis in that specific cleavage–ligation reactions are accelerated many orders of magnitude beyond the basal chemical rate (Bass & Cech, 1984; Zaug et al., 1985). It has been useful to view the splice sites and cyclization sites as intramolecular substrates for an activity residing within the intervening sequence (IVS)[1] RNA (Zaug et al., 1984). This view was recently substantiated by the finding that the L – 19 IVS RNA, which is missing the 19 nucleotides that contain the cyclization sites, still retains RNA cleavage–ligation activity. When provided with oligonucleotide substrates, the L – 19 IVS RNA acts as an enzyme with nucleotidyltransferase [poly(C) polymerase] and phosphodiesterase (ribonuclease) activities (Zaug & Cech, 1986).

These two enzymatic activities of the L – 19 IVS RNA were predicted from the self-reactions of the IVS. That is, the nucleotidyltransferase activity employs the same reactions involved in IVS RNA cyclization and exon ligation, while the ribonuclease reaction is closely related to site-specific hydrolysis of the circular IVS RNA (Zaug & Cech, 1986). We now describe two additional activities of the L – 19 IVS RNA. It is a phosphotransferase with specificity for the 3′-terminal phosphate of oligo(cytidylic acid) and other oligo(pyrimidines). The phosphate is readily transferred to an oligo(pyrimidine) acceptor. At acidic pH, it is slowly transferred to water. Thus, the RNA has acid phosphatase activity.

Unlike the poly(C) polymerase and ribonuclease activities, the phosphotransferase activities were not anticipated. There are no 3′-phosphates generated during pre-rRNA self-splicing, so the requisite substrate is not even present during the biologically relevant reaction. Transphosphorylation is distinct from other L – 19 IVS RNA catalyzed reactions in that the substrate is a phosphate monoester rather than a diester. Thus, this finding extends the realm of RNA-catalyzed reactions.

Experimental Procedures

Enzyme Preparation. L – 19 IVS RNA was synthesized and purified essentially as described by Zaug and Cech (1986). In brief, RNA was transcribed from pSPTT1A3 with bacteriophage SP6 RNA polymerase in vitro and further incubated to promote self-splicing and cyclization of the IVS RNA. The RNA was subsequently incubated in $MgCl_2$ at pH 9.0 (site-specific hydrolysis conditions) to convert circular IVS RNA to L – 19 IVS RNA. The L – 19 IVS RNA was purified by polyacrylamide gel electrophoresis and Sephadex G-50 chromatography. Enzyme concentration was determined by spectrophotometry assuming a molar extinction coefficient ϵ_{260nm} of 3.26×10^6 M^{-1} cm^{-1}.

Preparation of Substrates. C_5p^*Cp and A_6p^*Cp were prepared from C_5-OH and A_6-OH, respectively, with T_4 RNA ligase (New England Nuclear), p*Cp, and ATP. Products were purified by 20% polyacrylamide–7 M urea gel electrophoresis and Sephadex G-25 chromatography. C_5p^* was prepared from C_5p^*Cp by treatment with calf intestinal phosphatase and β-elimination (Winter & Brownlee, 1978). Unlabeled C_5p was prepared in a similar manner with unlabeled pCp as donor in the ligase reaction. Concentration was determined by spectrophotometry using a molar extinction coefficient ϵ_{270nm} of 30×10^3 M^{-1} cm^{-1}.

Preparation of E-p.* Unlabeled L – 19 IVS RNA (16 pmol) was incubated with 5.2 pmol of C_5p^* in 50 mM NaOAc, pH 5.0, and 20 mM $MgCl_2$ at 42 °C for 10 min. The reaction was stopped by the addition of EDTA to 40 mM. The E-p* was purified from unreacted C_5p^* by column chromatography on Sephadex G-100–120, which was equilibrated in 0.01 M Tris-HCl, pH 7.5, 0.25 M NaCl, and 0.001 M EDTA. The

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[1] Abbreviations: IVS, intervening sequence; L – 19 IVS RNA (read "L minus 19"), a 395-nucleotide linear RNA missing the first 19 nucleotides of the IVS; CHES, 2-(cyclohexylamino)ethanesulfonic acid; EDTA, ethylenediaminetetraacetic acid; MES, 2-(*N*-morpholino)-ethanesulfonic acid; Tris, tris(hydroxymethyl)aminomethane; p*, ^{32}p within an oligonucleotide (for example, C_5p^*Cp is CpCpCpCpC[^{32}P]-pCp).

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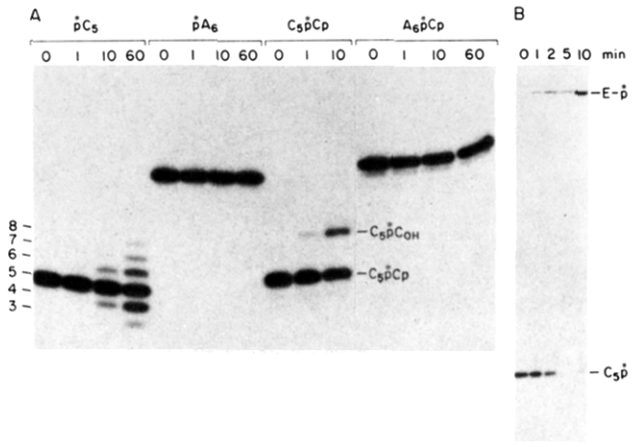

FIGURE 1: The L – 19 IVS RNA catalyzes the dephosphorylation of oligo(cytidylic acid) 3′-phosphate. (A) L – 19 IVS RNA (0.16 μM) was incubated with p^*C_5 (10 μM), p^*A_6 (10 μM), C_5p^*Cp (~2 nM), and A_6p^*Cp (~3 nM) in 20 mM $MgCl_2$ and 50 mM Tris-HCl, pH 7.5, at 42 °C for the times indicated. Reaction products were separated by electrophoresis in a 20% polyacrylamide–7 M urea sequencing gel, an autoradiogram of which is shown. (B) L – 19 IVS RNA (0.2 μM) was incubated with C_5p^* (~2 nM) as above. The phosphoenzyme E-p* is the L – 19 IVS RNA with a 3′-terminal phosphate monoester. Gel electrophoresis and autoradiography as in (A). Only a portion of the 5-min sample was loaded on the gel.

fractions that contained the E-p* complex were pooled and precipitated with 3 volumes of ethanol. The dried precipitate was then dissolved in H_2O.

RESULTS

The L – 19 IVS RNA Dephosphorylates C_6p. When oligo(cytidylic acid) with a 3′-terminal hydroxyl group is incubated with the L – 19 IVS RNA enzyme in 20 mM $MgCl_2$, it is converted to oligo(C) with both larger and smaller chain lengths (Figure 1A) as described previously (Zaug & Cech, 1986). Both cleavage and rejoining occur in substrate excess, as shown in Figure 1A, while only cleavage occurs in enzyme excess (Zaug & Cech, 1986). The reaction is specific for oligo(C); for example, pA_6-OH is unreactive under the same conditions (Figure 1A).

When oligo(cytidylic acid) with a 3′-terminal phosphate is incubated with excess L – 19 IVS RNA in 20 mM $MgCl_2$, the substrate is converted to a product with reduced electrophoretic mobility (Figure 1A). This abrupt reduction in electrophoretic mobility, equivalent to an increase of approximately three nucleotides on a sequencing ladder, is exactly that obtained by treating the substrate with alkaline phosphatase (not shown in Figure 1; an example is shown in Figure 2). Thus, it appeared that the product was C_6-OH. When the substrate is internally labeled (C_5p^*Cp), the labeled phosphate is retained in the product (Figure 1A). On the other hand, when the substrate is terminally labeled (C_5p^*), the oligonucleotide product is unlabeled and the L – 19 IVS RNA becomes labeled (Figure 1B). These findings confirmed that the reaction involves removal of the 3′-terminal phosphate of the substrate.

Dephosphorylation is specific for the 3′-phosphate of oligo(cytidylic acid). The 5′-phosphate of pC_5 is not reactive (Figure 1A), and neither phosphate is removed from pCp (data not shown).[2] Neither A_6Cp (Figure 1A) nor pA_6p (not shown) is a substrate.[2] On the basis of this limited sample, it appears that there is both a minimum length requirement

[2] We estimate that 0.1% reaction would have been detected.

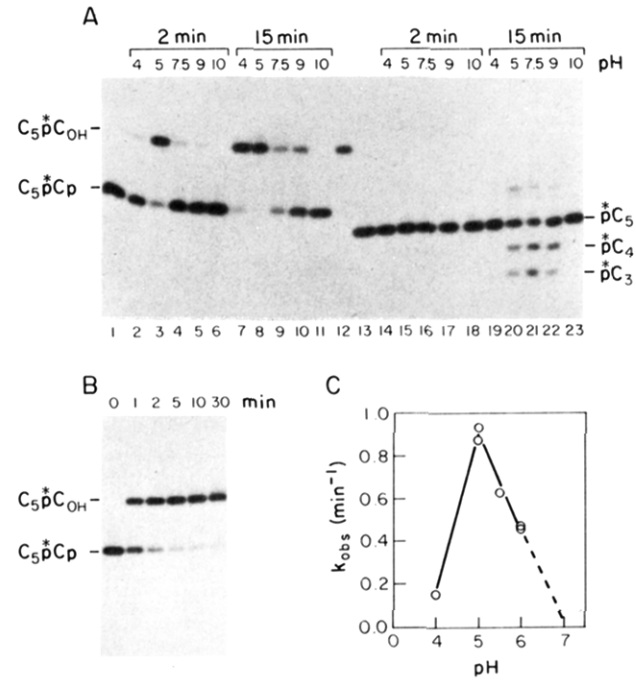

FIGURE 2: Effect of pH on the phospho transfer and nucleotidyl transfer reactions. (A) Lane 1, untreated C_5p^*Cp; lanes 2–11, C_5p^*Cp (15 nM) incubated with excess L – 19 IVS RNA (500 nM) in 20 mM $MgCl_2$ and 50 mM buffer (NaOAc for pH 4.0 and 5.0, Tris-HCl for pH 7.5, CHES for pH 9.0 and 10.0); lane 12, C_5p^*Cp treated with calf intestinal phosphatase to provide a marker for C_5p^*C-OH; lane 13, untreated p^*C_5; lanes 14–23, p^*C_5 (15 nM) incubated with excess L – 19 IVS RNA (500 nM) as in lanes 2–11. Reactions proceeded at 42 °C for the indicated times, after which they were stopped by the addition of an equal volume of urea sample buffer containing 50 mM EDTA. (B) C_5p^*Cp (~2 nM) was incubated with L – 19 IVS RNA (0.2 μM) at pH 5.0 for the times indicated. (C) Data similar to those shown in (B) except with 15 nM C_5p^*Cp were quantitated by liquid scintillation counting of the sliced gel. Semilogarithmic plots, which were linear for the first three or four time points, were used to determine $t_{1/2}$. The observed first-order rate constant (k_{obsd}) was calculated as $(\ln 2)/t_{1/2}$. NaOAc buffer was used for pH 4.0 and 5.0, MES for pH 5.5 and 6.0, and Tris-HCl for pH 7 (estimate based on a single point that showed ~50% reaction).

and a sequence requirement for a substrate and that the requirements are similar to those of the nucleotidyl transfer activity of the L – 19 IVS RNA (Zaug & Cech, 1986).

Formation and Stability of E-p. We next investigated the fate of the phosphate that is removed from C_5p in the presence of L – 19 IVS RNA. At neutral pH no inorganic phosphate is formed during the reaction, as judged by thin-layer chromatography of the reaction products (data not shown). When the reaction is conducted in the presence of C_5p^*, it becomes clear that the phosphate is transferred to the L – 19 IVS RNA (Figure 1B). Treatment of the phosphorylated L – 19 IVS RNA (hereafter called E-p) with alkaline phosphatase leads to quantitative release of the radioactivity in the form of inorganic phosphate (data not shown). Thus, the dephosphorylation of the substrate is accomplished by transphosphorylation. The structure of E-p has been determined; the phosphate is esterified through the 3′-O of the 3′-terminal guanosine residue (G414) of the RNA (Zaug and Cech, unpublished results).

The rate of conversion of C_5p to C_5-OH + E-p is pH-dependent with an optimum around pH 5.0. A sample of the data is shown in Figure 2. The phospho transfer reaction is essentially pH-independent in the range pH 7.5–9 and proceeds at a rate similar to that of the nucleotidyl transfer reaction (Figure 2A). At pH 5, the phospho transfer reaction is accelerated more than 20-fold, while the nucleotidyl transfer

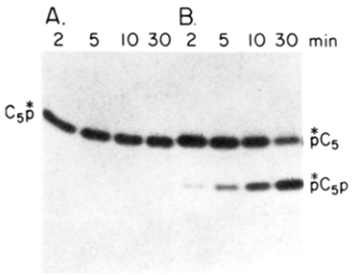

FIGURE 3: Phosphorylation of the enzyme is reversible. L – 19 IVS RNA (0.2 μM) was phosphorylated by incubation with 2.5 μM unlabeled C_5p for 5 min at 42 °C at pH 5.0. A trace amount of (A) C_5p^* (1.2 nM) or (B) p^*C_5 (20 nM) was then added to the unlabeled E-p, and incubation was continued for the times shown.

reaction is unaffected. At pH 4, the enzyme still has substantial phospho transfer activity while its nucleotidyl transfer activity is greatly diminished. Since the enzyme is probably starting to denature below pH 5 (Zaug et al., 1985), the phospho transfer activity profile in this range presumably reflects the inactivation of the enzyme rather than the pH optimum for the transfer step. Data such as those shown in Figure 2B were quantified, and the resulting rate constants are summarized in Figure 2C.

The covalent intermediate formed during the reaction of pC_5-OH with the L – 19 IVS RNA (E-pC) is alkali-labile; at pH 9.0 it undergoes hydrolysis, releasing the nucleotide pC and regenerating the free enzyme (Zaug & Cech, 1986). We therefore investigated the stability of the phosphate monoester in the phosphoenzyme E-p. There is no detectable hydrolysis of the phosphate monoester at pH 9.0, conditions in which E-pC treated in parallel gave release of pC, or at any other pH in the range 7.0–9.0 (data not shown).

At acidic pH, on the other hand, the terminal phosphate monoester of E-p underwent slow hydrolysis. When E-p was incubated at pH 5.0, the phosphate was released as P_i at a rate of approximately 10%/h (data not shown). The rate was slower at pH 4.0 and at pH 6.0 than at pH 5.0. Release of P_i was also observed during the reaction of C_5p^* with L – 19 IVS RNA at pH 5.0 at reaction times of 2 and 4 h. Thus, the L – 19 IVS RNA has acid phosphatase activity. This hydrolytic reaction is so slow, however, that we have not attempted to demonstrate multiple turnover.

Phosphorylation of the Enzyme Is Reversible. When unlabeled E-p is incubated with a trace amount of C_5p^*, very little reaction takes place (Figure 3A). In contrast, when the same E-p is incubated with a trace amount of labeled pC_5-OH, labeled pC_5p is progressively formed (Figure 3B). The products normally formed by incubation of pC_5-OH with excess L – 19 IVS RNA, pC_4-OH and pC_3-OH (Zaug & Cech, 1986), are not observed. Thus, E-p is inactive as a nucleotidyltransferase but is readily subject to a reverse phosphorylation reaction.

The reversibility of phosphorylation of the L – 19 IVS RNA was confirmed by reacting the enzyme with C_5p^* to form E-p*, purifying the E-p*, and incubating it with unlabeled C_5-OH. A labeled product corresponding to C_5p^* was produced (data not shown). This approach allowed to rapid screening of a series of nucleotides and oligonucleotides for their ability to reverse the phosphorylation reaction. As shown in Table I, of the oligonucleotides tested only UCU and C_4U had activity comparable to that of C_5. It remains possible that some of the other oligonucleotides have a high K_m and would have detectable activity at a higher concentration.

The L – 19 IVS RNA Is a Phosphotransferase. The phospho transfer reactions described thus far were all done

Table I: Relative Activity of Different Acceptors in the Transphosphorylation Reaction[a]

acceptor	activity	acceptor	activity
UTP	–	UUU	–
CTP	–	UCU	++
UC	–	CCU	+/–
CC	–	AU_3	–
AA	–	GU_3	–
GU	–	C_4U	++
UU	–	C_5	++
CU	–	U_6	+/–
AUU	–	dC_5	–

[a] E-p* was incubated with 10 μM oligonucleotide (or mono- or dinucleotide) in 20 mM $MgCl_2$ and 50 mM Tris-HCl, pH 7.5, at 42 °C for 30 min. Transfer of the phosphate to the oligonucleotide was assayed by sequencing gel electrophoresis and autoradiography. ++, approximately the same activity as C_5; +/–, barely detectable activity, estimated as ~1% that of C_5; –, no activity detectable under these conditions.

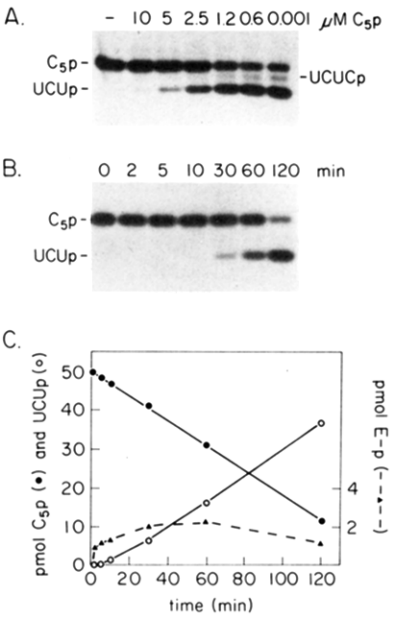

FIGURE 4: The L – 19 IVS RNA acts catalytically as a phosphotransferase. (A) Decreasing concentrations of C_5p^* were incubated for 30 min with 0.32 μM L – 19 IVS RNA and 100 μM unlabeled UCU-OH. The specific radioactivity of C_5p^* was adjusted by addition of unlabeled C_5p to keep the amount of radioactivity constant among samples. The small amount of intermediate band seen in some reactions is presumed to be UCUCp formed by attack of UCU on an E-pCp covalent intermediate. (– lane) C_5p^* prior to incubation. (B) C_5p^* (2.5 μM) incubated with 0.16 μM L – 19 IVS RNA and 200 μM unlabeled UCU-OH. (C) Quantitation of data shown in (B), including labeled E-p, which ran near the top of the gel. In all cases, incubation was in 20 mM $MgCl_2$ and 50 mM MES, pH 6.0, at 42 °C.

in enzyme excess. To prove that the L – 19 IVS RNA could act catalytically, it was necessary to show that each enzyme molecule could mediate the dephosphorylation of multiple substrate molecules. This was accomplished by incubation of L – 19 IVS RNA with a molar excess of C_5p^* and an even greater molar excess of unlabeled UCU, to act as a phosphate acceptor. As shown in Figure 4A, L – 19 IVS RNA was capable of transferring the 3′-terminal phosphate from C_5p to UCU. Treatment of the product with RNase T2 and thin-layer chromatography confirmed that the phosphate had been transferred from a C to a U residue (data not shown). The time course in Figure 4B was done under conditions of lower enzyme concentration (0.16 μM) and higher acceptor concentration (200 μM) than those used in the experiment of Figure 4A. Quantitation of the data (Figure 4C) showed that

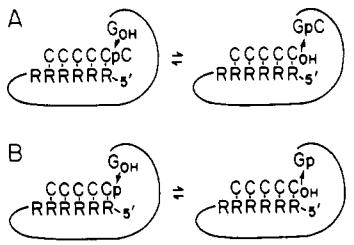

FIGURE 5: Single active site model for the activity of the L – 19 IVS RNA on phosphate diester and phosphate monoester substrates. (A) Reversible nucleotidylation of the L – 19 IVS RNA, proposed to be the key step in the poly(C) polymerase reaction (Zaug & Cech, 1986). (B) Reversible phosphorylation of the L – 19 IVS RNA, which allows the enzyme to act as a phosphotransferase. In both cases, the oligo(C) substrate base pairs to the oligo(pyrimidine) binding site (six R's) to form a noncovalent complex. The binding site is nucleotides 22–27 of the IVS RNA and has the sequence GGAGGG (M. D. Been and T. R. Cech, submitted for publication). Nucleophilic attack by the 3′-hydroxyl of the active site guanosine, G414, leads to formation of E-pC (A) or E-p (B) and the release of C_5. The complex folded core structure of the IVS is depicted as a simple curved line.

under these conditions 11 substrate molecules were dephosphorylated per enzyme molecule after 120 min. Phosphorylation of the enzyme precedes substantial product formation, consistent with E-p being an obligatory intermediate in the reaction. At steady state 63% of the enzyme is present as E-p.

Discussion

We have shown that the L – 19 IVS RNA has transphosphorylation activity toward 3′-phosphorylated oligo(C) substrates. The properties of the transphosphorylation reaction indicate that it is taking place in the same active site as the poly(C) polymerase and ribonuclease reactions (Figure 5). The properties include the specificity of the reactions for oligo(C) substrates, the production of oligo(C) products with 3′-hydroxyl termini, and the formation of similar covalent enzyme–substrate complexes. The presumptive intermediate is a phosphoenzyme, E-p, in the case of the phosphotransferase reaction and a nucleotidyl enzyme, E-pC or E-$(pC)_n$, in the case of the poly(C) polymerase and ribonuclease reactions (Zaug & Cech, 1986). In both cases the presumptive covalent intermediate involves a phosphate ester linkage through the 3′-O of G414 of the L – 19 IVS RNA (Zaug and Cech, unpublished results).

The transphosphorylation reaction is readily reversible. The phosphate can be transferred from the enzyme to an acceptor with a 3′-hydroxyl group, such as C_5 or UCU. With C_5p and UCU as cosubstrates, the L – 19 IVS RNA can catalyze the reaction C_5p + UCU-OH → C_5-OH + UCUp. The proposed pathway is

$$E + C_5p \rightleftharpoons E{\cdot}C_5p \overset{C_5\text{-OH}}{\rightleftharpoons} E\text{-}p \overset{UCU\text{-}OH}{\rightleftharpoons} E\text{-}p{\cdot}UCU\text{-}OH \rightleftharpoons UCUp + E$$

Thus, the L – 19 IVS RNA has transphosphorylation activity resembling that of *Escherichia coli* alkaline phosphatase (Reid & Wilson, 1971; Coleman & Gettins, 1983), acid phosphatase, and a variety of other phosphotransferases that form covalent enzyme–substrate intermediates (Knowles, 1980). In addition, the L – 19 IVS RNA phosphoenzyme can transfer its phosphate to water at pH 4 and 5, indicating that it has acid phosphatase activity.

As the pH is lowered from 7.5 to 5.0, the rate of the transphosphorylation reaction increases substantially. In this same pH range, the 3′-phosphate of C_5p is converted to a monoanion [$pK_a \simeq 6.0$, based on the value for cytidine 3′-phosphate from Ts'o (1974)]. Protonation of a phosphate monoester makes it possible for it to react like a diester (Benkovic & Schray, 1973). Thus, it seems reasonable that an enzyme known to react with diesters could use the same mechanism to react with monoester monoanions. The acidic pH requirement for hydrolysis of the phosphoenzyme can be similarly explained if the reaction occurs by attack of water on the phosphate monoester monoanion. The pH independence of transphosphorylation between pH 7.5 and pH 9.0 strongly suggests that the monoester dianion is also reactive, albeit at a rate <5% that of the monoester monoanion. The reactivity of the monoester dianion is surprising and perhaps indicates that the enzyme provides electrophilic assistance to the departure of the leaving group with a proton or metal ion.

At alkaline pH the phosphodiester bond following G414 is labile in the circular IVS RNA (Zaug et al., 1984), in the pre-rRNA (Inoue et al., 1986), and in E-pC (Zaug & Cech, 1986), whereas the phosphomonoester bond following G414 is stable in E-p. Specific hydrolysis of the phosphodiester bonds involves attack of hydroxide ion (Zaug et al., 1985). It is not surprising that attack of hydroxide ion on the phosphate monoester dianion of E-p might be prohibited due to electrostatic repulsion (Kirby & Younas, 1970).

At pH 5 the phosphoenzyme undergoes very slow hydrolysis but readily transfers its phospho group to C_5-OH. The rate of the hydrolysis reaction is 2–3 orders of magnitude slower than that of the phospho transfer reaction, even though H_2O is present at 55 M and the oligonucleotide at <1 μM. Thus, C_5-OH is a better acceptor than H_2O by a factor exceeding 10^{10}. [Such a large factor is not unusual for phosphotransferases; for example, Ray et al. (1976) report that phosphoglucomutase transfers a phosphate to the C-6 hydroxyl of glucose 1-phosphate at a rate 3×10^{10} times greater than that of transfer to H_2O.] The difference in rate is much too large to be explained by the greater nucleophilicity of the 3′-hydroxyl of C_5-OH than H_2O, which could perhaps account for a factor of 10 (Lohrmann & Orgel, 1978; Kirby & Varvoglis, 1967). Most of the difference in rate probably reflects the ability of the enzyme to utilize the binding energy from its interaction with nonreacting portions of C_5-OH to accelerate the reaction (Jencks, 1975). For example, specific binding interactions could precisely position the 3′-hydroxyl of C_5-OH for displacement of the phosphate from the enzyme, but would not be available to facilitate the addition of water. Furthermore, the catalytic apparatus may not be fully assembled until the acceptor oligonucleotide is in place and water is absent (Koshland, 1963; Knowles, 1980).

We are only beginning to understand how the L – 19 IVS RNA catalyzes phospho transfer. The overall transfer reaction is undoubtedly facilitated by the formation of a covalent bond between the enzyme and the phosphate of the substrate. Such covalent catalysis is common in enzyme-catalyzed group transfer reactions (Jencks, 1969; Walsh, 1979). Binding sites within the IVS RNA for the oligo(pyrimidine) substrate (Zaug & Cech, 1986) and for the nucleophilic G residue at its own 3′ end (N. K. Tanner and T. R. Cech, unpublished results) contribute to the catalysis of the transfer reactions. These binding interactions presumably place the 3′-hydroxyl group of G414 in an optimal orientation for nucleophilic attack on the terminal phosphate of C_5p (Figure 5B) or on an internal phosphate of C_5-OH (Figure 5A). We suspect that catalysis might also involve a specific role for Mg^{2+} [Steffens et al., 1973, 1975; Anderson et al., 1977; see also Zaug et al. (1985) and Guerrier-Takada et al. (1986)] and general acid–base catalysis [see Cech and Bass (1986)], but we have no direct evidence for such mechanisms.

One unanswered question concerns the extremely low extent of nucleotidyl transfer with the C_5p substrate at neutral pH. Since C_5-OH is readily attacked at the phosphate following the fourth C to produce E-pC, why is C_5p not attacked at the equivalent phosphate to produce E-pCp? Perhaps the terminal phosphate of C_5p is coordinated to Mg(II) or serves as a hydrogen bond acceptor, resulting in a preferred mode of binding different from that of C_5-OH.

Finding an enzyme that has both phosphodiesterase and phosphomonoesterase activity is unusual but not unprecedented. Exonuclease III (Richardson & Kornberg, 1964), P1 nuclease, and mung bean nuclease (Shishido & Ando, 1982) all have 3′-phosphatase activity.

The L − 19 IVS RNA is unique among known enzymes in its ability to remove 3′-phosphates from RNA with high substrate specificity. *E. coli* and mammalian alkaline phosphatases are nonspecific. These enzymes remove 5′-, 3′-, and 2′-phosphates from RNA with little regard for the base sequence of the molecule (Garen & Levinthal, 1960; Harkness, 1968). Polynucleotide kinase has 3′-phosphatase, 2′-phosphatase, and cyclic 2′,3′-phosphatase activity (Cameron & Uhlenbeck, 1977; Weber, 1985). Substrates as diverse as U_5p, A_6Cp, and pCp are readily dephosphorylated, and where careful kinetic measurements have been made, the rates of dephosphorylation of different RNA substrates seldom vary by more than a factor of 2 (Weber, 1985). P1 nuclease and mung bean nuclease have limited preference for certain nucleoside 3′-monophosphates (Shishido & Ando, 1982). The L − 19 IVS RNA, on the other hand, transfers the 3′-phosphate of RNA to a donor molecule with high substrate specificity; C_5p and C_6p are substrates, whereas pCp and A_6pCp are not. This length and sequence specificity is explained by the requirement that the substrate must bind to the enzyme by Watson–Crick base pairing to the guanosine-rich active site (Figure 5). If this model is correct, it should be possible to alter the sequence specificity of the phosphotransferase by site-specific mutagenesis of the active site.

A series of sequence-specific 3′-phosphate dephosphorylating enzymes would provide a useful tool for RNA biochemistry and recombinant RNA manipulations. However, the RNA enzyme described here has cleavage–ligation activity as well as the dephosphorylation activity. Unless these activities can be separated, the usefulness of the enzyme as a reagent for dephosphorylation of RNA is limited.

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REFERENCES

Anderson, B., Milburn, R. M., Harrowfield, J. M., Robertson, G. B., & Sargeson, A. M. (1977) *J. Am. Chem. Soc. 99*, 2652–2661.

Bass, B. L., & Cech, T. R. (1984) *Nature (London) 308*, 820–826.

Benkovic, S. J., & Schray, K. J. (1973) *Enzymes (3rd Ed.) 8*, 235–236.

Cameron, V., & Uhlenbeck, O. C. (1977) *Biochemistry 16*, 5120–5126.

Cech, T. R., & Bass, B. L. (1986) *Annu. Rev. Biochem. 55*, 599–629.

Coleman, J. E., & Gettins, P. (1983) *Adv. Enzymol. Relat. Areas Mol. Biol. 55*, 381–452.

Garen, A., & Levinthal, C. (1960) *Biochim. Biophys. Acta 38*, 470–483.

Guerrier-Takada, C., Haydock, K., Allen, L., & Altman, S. (1986) *Biochemistry 25*, 1509–1515.

Harkness, D. R. (1968) *Arch. Biochem. Biophys. 126*, 513–523.

Inoue, T., Sullivan, F. X., & Cech, T. R. (1986) *J. Mol. Biol. 189*, 143–165.

Jencks, W. P. (1969) *Catalysis in Chemistry and Enzymology*, McGraw-Hill, New York.

Jencks, W. P. (1975) *Adv. Enzymol. Relat. Areas Mol. Biol. 43*, 219–410.

Kirby, A. J., & Varvoglis, A. G. (1967) *J. Am. Chem. Soc. 89*, 415–423.

Kirby, A. J., & Younas, M. (1970) *J. Chem. Soc. B*, 1165–1172.

Knowles, J. R. (1980) *Annu. Rev. Biochem. 49*, 877–919.

Koshland, D. R., Jr. (1963) *Cold Spring Harbor Symp. Quant. Biol. 28*, 473–480.

Lohrmann, R., & Orgel, L. E. (1978) *Tetrahedron 34*, 853–855.

Ray, W. J., Jr., Long, J. W., & Owens, J. D. (1976) *Biochemistry 15*, 4006–4017.

Reid, T. W., & Wilson, I. B. (1971) *Enzymes (3rd Ed.) 4*, 373–415.

Richardson, C. C., & Kornberg, A. (1964) *J. Biol. Chem. 239*, 242–250.

Shishido, K., & Ando, T. (1982) in *Nucleases* (Linn, S. M., & Roberts, R. J., Eds.) pp 155–185, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Steffens, J. J., Sampson, E. J., Siewers, I. J., & Benkovic, S. J. (1973) *J. Am. Chem. Soc. 95*, 936–938.

Steffens, J. J., Siewers, I. J., & Benkovic, S. J. (1975) *Biochemistry 14*, 2431–2440.

Ts'o, P. O. P. (1974) in *Basic Principles in Nucleic Acid Chemistry*, Vol. I, pp 462–463, Academic, New York.

Walsh, C. (1979) *Enzymatic Reaction Mechanisms*, W. H. Freeman, San Francisco.

Weber, T. R. (1985) Ph.D. Thesis, University of Illinois.

Winter, G., & Brownlee, G. G. (1978) *Nucleic Acids Res 5*, 3129–3139.

Zaug, A. J., & Cech, T. R. (1986) *Science (Washington, D.C.) 231*, 470–475.

Zaug, A. J., Grabowski, P. J., & Cech, T. R. (1983) *Nature (London) 301*, 578–583.

Zaug, A. J., Kent, J. R., & Cech, T. R. (1984) *Science (Washington, D.C.) 224*, 574–578.

Zaug, A. J., Kent, J. R., & Cech, T. R. (1985) *Biochemistry 24*, 6211–6218.