Poly(A) polymerase from *Escherichia coli* adenylylates the 3'-hydroxyl residue of nucleosides, nucleoside 5'-phosphates and nucleoside(5')oligophospho(5')nucleosides (Np_nN)

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The capacity of *Escherichia coli* poly(A) polymerase to adenylylate the 3'-OH residue of a variety of nucleosides, nucleoside 5'-phosphates and dinucleotides of the type nucleoside(5')oligophospho(5')nucleoside is described here for the first time. Using micromolar concentrations of [α -³²P]ATP, the following nucleosides/nucleotides were found to be substrates of the reaction: guanosine, AMP, CMP, GMP, IMP, GDP, CTP, dGTP, GTP, XTP, adenosine(5')diphospho(5')adenosine (Ap₂A), adenosine(5')triphospho(5')adenosine (Ap₃A), adenosine(5')tetraphospho(5')adenosine (Ap₄A), adenosine(5')pentaphospho(5')adenosine (Ap₅A), guanosine(5')diphospho(5')guanosine (Gp₂G), guanosine(5')triphospho(5')guanosine

(Gp₃G), guanosine(5')tetraphospho(5')guanosine (Gp₄G), and guanosine(5')pentaphospho(5')guanosine (Gp₅G). The synthesized products were analysed by TLC or HPLC and characterized by their UV spectra, and by treatment with alkaline phosphatase and snake venom phosphodiesterase. The presence of 1 mM GMP inhibited competitively the polyadenylation of tRNA. We hypothesize that the type of methods used to measure polyadenylation of RNA is the reason why this novel property of *E. coli* poly(A) polymerase has not been observed previously.

Keywords: Ap₄A; dinucleoside polyphosphates; nucleoside 5'-phosphates; nucleosides; poly(A) polymerase.

Diverse effects have been described for dinucleoside polyphosphates (Np_nN) in metabolic regulation and cell signalling [1–4]. Specific and nonspecific degradative enzymes towards Np_nN have been described [5,6] and more recently our laboratory has investigated the mechanism of synthesis of dinucleotides by ligases [7]. These enzymes catalyse the synthesis of Np_nN by different mechanisms involving formation of: (a) an E–X–AMP complex, where X represents an acidic residue (firefly luciferase [8] and aminoacyl tRNA synthetases [9]); (b) an E–AMP complex without any need for an acid residue (T4 DNA ligase [10] and T4 RNA ligase [11]); or (c) both types

of complexes (acetyl-CoA synthetase [12] and acyl-CoA synthetase [13]). Common to all of these mechanisms are the liberation of PP_i and the activation of the synthesis Np_nN by pyrophosphatase [7].

As described recently by us, T4 RNA ligase also recognizes some dinucleotides as RNA acceptors and, in the presence of an RNA donor (pCp), cytidylylates their 3'-OH ends through reactions (1) and (2) [14]

Where AppCp is 5'-adenylylated cytidine-5',3'-bisphosphate and Gp₄G is guanosine(5')tetraphospho(5')guanosine. Based on the above, we wondered whether enzymes other than ligases that liberate PP_i could also catalyse the synthesis and/or modification of dinucleoside polyphosphates [7]. In this context, we tested *Escherichia coli* poly(A) polymerase (ATP:RNA adenylyltransferase, EC 2.7.7.19) and found, unexpectedly, that this enzyme adenylylates the 3'-OH end of a variety of nucleosides, nucleoside 5'-phosphates and dinucleoside polyphosphates. In our view this is a novel property of this enzyme whose assigned role is to add a tail of poly(A) to the 3'-OH terminus of mRNA.

MATERIALS AND METHODS

Materials

Poly(A) polymerase from *E. coli* was from Amersham Pharmacia Biotech (Code E2180Y, lot numbers: K101-7; K-101-11; K-101-12). One unit of enzyme is the amount

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Abbreviations: AppCp, 5'-adenylylated cytidine-5',3'-bisphosphate;

Ap₂A, adenosine(5')diphospho(5')adenosine or diadenosine

diphosphate; Ap₃A, adenosine(5')triphospho(5')adenosine; Ap₄A,

adenosine(5')tetraphospho(5')adenosine; Ap₅A,

adenosine(5')pentaphospho(5')adenosine; Gp₂G,

guanosine(5')diphospho(5')guanosine; Gp₃G,

guanosine(5')triphospho(5')guanosine; Gp₄G,

guanosine(5')tetraphospho(5')guanosine; Gp₅G,

guanosine(5')pentaphospho(5')guanosine; Np_nN,

nucleoside(5')oligophospho(5')nucleosides.

Enzymes: alkaline phosphatase (EC 3.1.3.1); inorganic

pyrophosphatase (EC 3.6.1.1); phosphodiesterase from

Crotalus durissus (EC 3.1.4.1); poly(A) polymerase from

Escherichia coli (EC 2.7.7.19).

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that incorporates 1 nmol AMP into tRNA in 10 min at 37 °C and pH 7.9, with ATP as substrate. Those preparations contained 7744 U·mg⁻¹ protein. When required, the enzyme was diluted in a buffer containing: 25 mM Tris/HCl pH 7.9, 0.5 M NaCl, 0.1 mM dithiothreitol and 10% glycerol. Alkaline phosphatase (grade I; EC 3.1.3.1), phosphodiesterase from *Crotalus durissus* (EC 3.1.4.1), yeast inorganic pyrophosphatase (EC 3.6.1.1) and tRNA from brewer's yeast were from Boehringer Mannheim. [α -³²P]ATP (3000 Ci·mmol⁻¹) was from Dupont NEN. Guanylyl (3'→5') adenosine (GpA) and adenylyl (3'→5') adenosine (ApA) were from Sigma. TLC silica-gel fluorescent plates were from Merck. X-ray films were from Konica Corporation. Radioactively labelled nucleotides were quantified by the use of an InstantImager (Packard Instrument Co.). HPLC was carried out in a Hewlett Packard chromatograph (model 1090), with a diode array detector, commanded by an HPLC ChemStation. The Hypersil ODS column (4.6 × 100 mm) was from Hewlett Packard.

Adenylylation of nucleosides, nucleotides and dinucleoside polyphosphates catalysed by *E. coli* poly(A) polymerase

Unless indicated otherwise the reaction mixtures contained 50 mM Tris/HCl pH 7.9, 10 mM MgCl₂, 2.5 mM MnCl₂, 250 mM NaCl, 1 mM dithiothreitol, 0.05% mg·mL⁻¹ BSA, 1 U·mL⁻¹ desalted pyrophosphatase, ATP, plus either nucleosides, nucleotides or dinucleoside polyphosphates, and enzyme as indicated. After incubation at 37 °C the reaction mixtures were analysed by TLC or HPLC. When indicated, the reaction mixtures were either treated with 50 µg·mL⁻¹ alkaline phosphatase for 1 h at 37 °C or, after inactivation of the phosphatase, treated further with phosphodiesterase.

TLC. The reaction mixtures (usually 0.01 mL) contained (0.002–0.1 mM) [α -³²P]ATP (10–60 µCi·mL⁻¹). Aliquots (1.5 µL) of the reactions were taken, spotted on silica gel plates, along with standards, and developed in dioxane/ammonium hydroxide/water 6 : 1 : 5 (v/v/v) or 6 : 1 : 6 (v/v/v). Nucleotide spots were localized with a 253-nm wavelength light and the radioactivity measured by

autoradiography and/or with an InstantImager. The experimental values given below are representative of the data obtained from two to five determinations with deviations of less than 20%.

HPLC. Aliquots (0.01 mL) of the reaction mixtures (usually in a volume of 0.05–0.1 mL) were transferred into 0.14 mL water and kept at 95 °C for 1.5 min. After chilling, the mixtures were filtered and a 0.05-mL aliquot injected into a Hypersil ODS column (4.6 × 100 mm), as indicated. Elution was performed at a flow rate of 0.5 mL·min⁻¹ with a 20-min linear gradient (5–30 mM) of sodium phosphate (pH 7.5), in 20 mM tetrabutylammonium bromide/20% methanol (buffer A) followed by a 10-min linear gradient (30–100 mM) of sodium phosphate (pH 7.5) in buffer A.

RESULTS

GTP, GDP, GMP and guanosine as acceptors of AMP

As stated in the introduction, the synthesis of Ap₄N catalysed by both T4 DNA ligase [10] and T4 RNA ligase [11] implies the formation of an E–AMP complex and transfer of the AMP moiety to NTP to form Ap_nN. Before testing the ability of poly(A) polymerase to synthesize Ap₄N we tried, with no success, to detect the formation of the E–AMP complex by incubating the enzyme with [α -³²P]ATP followed by SDS/PAGE. Nevertheless, synthesis of Ap₄G by poly(A) polymerase was approached in the presence of [α -³²P]ATP and GTP. The reaction mixtures were incubated for 0, 60, 120 and 240 min in the presence of pyrophosphatase and analysed by TLC. The synthesis of a new compound migrating between ATP and ADP was observed (Fig. 1, left panel). This compound was sensitive to alkaline phosphatase giving rise to a faster migrating new spot (Fig. 1, left panel). When the reaction mixture was incubated further in the presence of snake venom phosphodiesterase (after previous inactivation of the alkaline phosphatase by heating), a major spot migrating in the same position as AMP was obtained (Fig. 1, right panel). Altogether, these experiments can be viewed as if poly(A) polymerase adds a residue of AMP (from [α -³²P]ATP) to

Fig. 1. Synthesis of pppGpA catalysed by *E. coli* poly(A) polymerase. A reaction mixture (0.03 mL) was set up as described, containing 0.002 mM [α -³²P]ATP (0.45 µCi), 1 mM GTP and 0.9 U poly(A) polymerase. At the indicated times of incubation, aliquots were taken and subjected to TLC before and after treatment by alkaline phosphatase (left panel). A reaction mixture containing GpA (a) was further treated with snake venom phosphodiesterase (b) and subjected to TLC (right panel). (+) Indicate the reaction mixtures treated with alkaline phosphatase (AP) or phosphodiesterase (PDE). Silica gel plates were developed in dioxane/ammonium hydroxide/water 6 : 1 : 6 (v/v/v).

Fig. 2. Synthesis of pppGpA catalysed by *E. coli* poly(A) polymerase: analysis by HPLC. A reaction mixture (0.125 mL) containing 0.2 mM ATP, 0.8 mM GTP and 3.75 U poly(A) polymerase was incubated for 16 h at 37 °C. (A) Analysis by HPLC of an aliquot of the reaction mixture. (B) Analysis of the reaction mixture after treatment with alkaline phosphatase. (C) Same mixture as in (B) but supplemented with commercial GpA. (D) UV spectra of a standard of GpA and of compound c.

GTP generating pppGp*A: the treatment with alkaline phosphatase renders Gp*A, and further incubation in the presence of phosphodiesterase generates unlabelled guanosine and labelled AMP(p*A). The presumptive labelled Gp*A obtained in that way, after 2 h incubation, contained all of the radioactivity originally present as [α - 32 P]ATP and comigrated with a GpA standard.

Fig. 3. Synthesis of pppNpA and pNpA catalysed by *E. coli* poly(A) polymerase. The reaction mixtures (0.01 mL) contained 0.02 mM [α - 32 P]ATP (0.3 μ Ci), 0.3 U poly(A) polymerase supplemented with 1 mM GTP, dGTP, CTP, UTP or XTP in (A), or 1 mM GMP, AMP, CMP, XMP or IMP in (B). After 1.5 and 3 h incubation aliquots were taken, treated with alkaline phosphatase and subjected to TLC. Silica gel plates were developed in dioxane/ammonium hydroxide/water 6 : 1 : 6 (v/v/v).

Further evidence for the synthesis of GpA derivatives was obtained incubating poly(A) polymerase in the presence of unlabelled ATP and GTP and analysing the mixture by HPLC. As shown in Fig. 2, two clear peaks (a and b) with retention times (R_t) of 30.5 and 32.2 min were obtained. These peaks presented UV spectra compatible with the presence of one adenosine and one guanosine residue in the same molecule. Treatment with alkaline phosphatase rendered a unique peak c with a R_t of 7.6 min and an area equivalent to the sum of the areas of peaks a and b. Compound c coeluted with a GpA standard and both (as well as peaks a and b) presented identical UV spectra (Fig. 2D). This result confirms that poly(A) polymerase catalyses, in the presence of GTP and ATP, the synthesis of a compound(s) that after phosphatase treatment yields GpA. The two GpA derivatives formed in the presence of GTP (Fig. 2, peaks a and b) probably correspond to the expected pppGpA (peak b) and to ppGpA (peak a), the latter due to the presence of some GDP in the GTP sample.

The specificity of the enzyme towards others guanine derivatives was tested in reaction mixtures containing 0.02 mM [α - 32 P]ATP and 1 mM guanosine, GMP, GDP or GTP. The mixtures were incubated for 60 min and analysed by TLC before and after phosphatase treatment. Radioactive spots that should correspond to GpA, pGpA, ppGpA and pppGpA were observed. Treatment with alkaline phosphatase transformed those spots into a single spot corresponding to GpA. From the radioactivity present in that spot, the calculated relative efficiencies of the guanine

Fig. 4. Synthesis of pGpA and pCpA catalysed by poly(A) polymerase: characterization by HPLC. Reactions mixtures (0.05 mL) containing 1 mM ATP, 0.5 mM GMP or 0.5 mM CMP as acceptors, 1.5 U poly(A) polymerase and other components as indicated in Materials and methods, were incubated for 48 h at 37 °C and subjected to HPLC. The chromatograms obtained before (A and D) and after (B and E) treatment of the reaction mixtures with alkaline phosphatase (AP) and, after further treatment with phosphodiesterase (PDE) (C and F), are shown.

derivatives as acceptors of AMP were: Guo (100); GMP (78); GDP (33) and GTP (13), where 100% represents the incorporation of 102 nmol [α - 32 P]AMP·min⁻¹·mg·protein⁻¹ (data not shown).

Specificity of nucleoside 5'-triphosphates (NTP) and nucleoside 5'-monophosphates (NMP) as acceptors of AMP

The capacity of poly(A) polymerase to add a residue of AMP to a variety of NTPs was analysed by reacting [α - 32 P]ATP with GTP, dGTP, CTP, UTP, and XTP. The reaction mixtures were incubated for 1.5 and 3 h and, after alkaline phosphatase treatment, analysed by TLC. From the radioactivity present under the Np*A patches, the following relative efficiencies were calculated: GTP (100); CTP (69); dGTP (26); XTP (10) and UTP (< 2) (Fig. 3A); 100% represents the incorporation of 13 nmol [α - 32 P]AMP·min⁻¹·mg·protein⁻¹.

Similarly, the specificity of the enzyme for different NMPs as acceptors of AMP in the presence of [α - 32 P]ATP was also approached. From the corresponding NpA spots (Fig. 3B) the following relative efficiencies were calculated: GMP (100); CMP (82); IMP (59); AMP (32) and XMP (< 2), where 100% represents the incorporation of 79 nmol [α - 32 P]AMP·min⁻¹·mg·protein⁻¹. The synthesis and characterization of the GMP and CMP derivatives (pGpA and pCpA) were also analysed in reaction mixtures containing unlabelled ATP and (GMP or CMP). Peaks corresponding to pGpA and pCpA were clearly detected by HPLC (Fig. 4, panels A and D). Treatment with alkaline phosphatase promoted formation of GpA and CpA,

respectively (Fig. 4, panels B and E). This treatment also unveiled a peak corresponding to ApA, most probably due to the degradation of pppApA (synthesized from ATP) by alkaline phosphatase (Fig. 4 panels B and E). Additional treatment with phosphodiesterase, after inactivation of alkaline phosphatase, rendered AMP plus the expected nucleosides (guanosine, or cytidine, and adenosine) (Fig. 4, panels C and F). Further characterization of pGpA, pCpA, GpA, CpA, and ApA was obtained from their UV spectra (not shown).

Effect of pH value and metals

The effect of pH value on the rate of synthesis of pGpA was determined in reaction mixtures containing 0.02 mM [α - 32 P]ATP (0.2 μ Ci), 1 mM GMP, 0.3 U poly(A) polymerase and 100 mM Tris/HCl buffer at pH values of 7.0, 7.4, 7.9, 8.5 and 8.8. Aliquots were taken after 60 min incubation, treated with alkaline phosphatase, subjected to TLC and the radioactivity under GpA counted. The reaction rate increased steadily from a pH value of 7.0 up to a maximum at pH 7.9. Beyond this point the enzyme activity decreased to around 30% of the maximum at pH 8.8 (data not shown).

E. coli poly(A) polymerase requires Mg²⁺ or Mn²⁺ to incorporate AMP into tRNA and the activity is highest when the ions are present at 10 mM and 2.5 mM, respectively [15]. The effect of the Mn²⁺ concentration in the incorporation of AMP into GTP was assayed using standard reaction mixtures containing 0.02 mM [α - 32 P]ATP, 1 mM GTP 10 mM MgCl₂, and variable concentrations of MnCl₂. The activity was very low in the absence of Mn²⁺

Fig. 5. Diguanosine polyphosphates as acceptors of AMP. The reaction mixtures (0.01 mL) set up as described in Materials and methods, contained 0.02 mM [α - 32 P]ATP (0.6 μ Ci), 0.3 U poly(A) polymerase, 1 mM GTP (as a control), or 1 mM each of the indicated Gp_nG . After incubation for 30 and 60 min, aliquots were taken, treated with alkaline phosphatase and subjected to TLC. Encircled areas represent the UV absorbing spots corresponding to the dinucleotide indicated by the arrow. Lanes: 1, no addition of nucleotide; 2, GTP; 3, Gp_2G ; 4, Gp_3G ; 5, Gp_4G ; 6, Gp_5G . Silica gel plates were developed in dioxane/ammonium hydroxide/water 6 : 1 : 6 (v/v/v).

(< 1%), and relative rates of 13.3, 41.6, 95.9 and 100% were obtained at 0.5, 1, 2 and 4 mM Mn^{2+} concentration, respectively (data not shown).

Apparent K_m value for ATP

The apparent K_m value for ATP as substrate for the incorporation of AMP into tRNA is ≈ 0.05 mM [15,16]. A K_m value of 0.025 mM was obtained for ATP, in the presence of 1 mM GMP and variable (0.01–0.08 mM) concentrations of [α - 32 P]ATP. A similar K_m value for ATP (0.026 ± 0.003 mM) was obtained in the presence of 1 mM GTP as adenylyl acceptor (data not shown). The V_{max} values determined in the above experiments with GMP or GTP as cosubstrates, were ≈ 92 and ≈ 21 nmol $\cdot$ min $^{-1}\cdot$ mg $^{-1}$, respectively. In the same assay conditions and in the presence of 0.1 mM ATP and increasing concentrations of tRNA, the V_{max} value determined for the adenylylation of tRNA was 406 nmol $\cdot$ min $^{-1}\cdot$ mg $^{-1}$.

Dinucleotides as acceptors of AMP in the reaction catalysed by poly(A) polymerase

The similarity in their 3'-OH ends among nucleoside 5'-phosphates, RNA and a dinucleotide [nucleoside(5')oligophospho(5')nucleoside] directed us to test whether these dinucleotides could be also acceptors of AMP in the presence of ATP and poly(A)polymerase. Reaction mixtures containing [α - 32 P]ATP and Gp_4G or GTP (as a control) were incubated in the presence of

Fig. 6. Influence of GMP on the adenylation of tRNA catalysed by poly(A) polymerase. Reaction mixtures (0.01 mL) were set up containing 0.1 mM [α - 32 P]ATP (0.6 μ Ci), 0.375 U poly(A) polymerase and variable concentrations of tRNA, in the absence and presence of 1 mM GMP (as indicated in the figure). After 1 h incubation aliquots were taken, treated with alkaline phosphatase and analysed by TLC. The radioactivity under each lane of the TLC plate surrounded by the dotted line was assumed to correspond to the adenylylated tRNA synthesized in the course of the reaction. A standard of tRNA migrates in that position as visualized with an UV lamp. Silica gel plates were developed in dioxane/ammonium hydroxide/water 6 : 1 : 6 (v/v/v).

enzyme, treated with alkaline phosphatase and analysed by TLC. The mixtures containing GTP or Gp_4G revealed the presence of radioactive spots corresponding, respectively, to the expected GpA and to a new compound that migrated slightly ahead of a standard of Gp_4G . Both compounds were recovered as [α - 32 P]AMP after inactivation of the phosphatase and additional treatment with phosphodiesterase (data not shown). These experiments conveyed the idea that poly(A) polymerase catalyses the synthesis of Gp_4GpA from ATP and Gp_4G .

The adenylylation of Gp_nG as acceptors of AMP in the poly(A) polymerase catalysed reaction was also studied, and the following relative efficiencies were calculated: Gp_2G (100), Gp_3G (39), Gp_4G (24) and Gp_5G (10) (Fig. 5). In parallel, the relative efficiencies for diadenosine polyphosphates (Ap_nA) were also determined: Ap_2A (13); Ap_3A (9); Ap_4A (7) and Ap_5A (6) (data not shown). In these experiments, 100% represents the incorporation of 130 nmol [α - 32 P]AMP $\cdot$ min $^{-1}\cdot$ mg $^{-1}$ protein.

Comparative adenylylation of several nucleotides, catalysed by poly(A) polymerase

As shown by Sippel in 1973 [15], ApA and other 3'→5' dinucleotides (NpN) inhibited substantially the incorporation of AMP into primer tRNA. Moreover, when ApA was used as a primer, at a final concentration of 5 mM, the incorporation of AMP into acid precipitable material was only 3.9% of the radioactivity incorporated in the presence of 11.5 μ M primer tRNA. Prompted by these results, we compared the adenylylation of 3'→5' dinucleotides (ApA

and GpC) with that of GMP and the influence of GMP on the adenylylation of both ApA and GpC, all at 1 mM. The reaction mixtures were either directly, or after alkaline phosphatase treatment, analysed by TLC (data not shown). Phosphatase treatment did not change the mobility of the adenylyl derivatives of ApA or GpC (presumptive ApAp*A and GpCp*A) in contrast with the GMP derivative (pGp*A). From this experiment the following relative efficiencies, as acceptors of AMP, were calculated: GpC (100), GMP (36), ApA (16). In the presence of GMP and either ApA or GpC, the adenylylation of GMP and that of ApA or GpC decreased.

It was of interest to study the adenylylation of GMP in the presence of tRNA. The reaction mixtures contained increasing concentrations of tRNA (1.9–15.4 μM) in the absence or presence of a fixed (1 mM) concentration of GMP. Samples of the reaction mixtures were taken after 60 (Fig. 6) and 120 min incubation (data not shown), treated with alkaline phosphatase and subjected to TLC. With increasing tRNA concentrations, the incorporation of [α - ^{32}P]AMP increased in the corresponding tracks. In the presence of GMP, formation of GpA (i.e. pGpA) was observed, together with a clear inhibition of tRNA adenylylation, particularly evident at the lower (1.9–3.8 μM) tRNA concentrations (Fig. 6). Representing the radioactivity incorporated along the tRNA migration lane (as indicated in Fig. 6) vs. the concentration of tRNA in the absence or presence of GMP in a Lineweaver–Burk plot, the following information was obtained: GMP behaved as a competitive inhibitor of tRNA adenylylation with a K_i value $\approx$ 1.2 mM; the apparent K_m values for tRNA in the absence or presence of GMP were 0.01 and 0.02 mM, respectively. The influence of the concentration of tRNA on the adenylylation of GMP was quantified following the synthesis of pGpA by HPLC. In the presence of 0.2 mM ATP, 1 mM GMP and increasing concentrations of tRNA (0.8, 3.8 and 15.4 μM) the synthesis of pGpA was inhibited 1, 7 and 44%, respectively (data not shown).

The apparent K_m values for GMP, GTP, ApA and Gp₂G, as substrates acceptors for the adenylyl residue, were determined in the presence of 0.02 mM [α - ^{32}P]ATP and variable concentrations of the indicated compounds. The values obtained (in parentheses) were: GMP (2 mM), GTP (1 mM), ApA (0.8 mM) and Gp₂G (0.4 mM). The corresponding V_{max} values were 160, 19, 75 and 162 nmol·min⁻¹·mg⁻¹, respectively.

DISCUSSION

In the early 1960s, an enzyme able to add poly(A) tails to RNA was purified from *E. coli* ribonuclein particles [17]. This enzyme was discovered before a physiological function could be assigned to it. Interest in this activity was rekindled when poly(A) tails were described in most eukaryotic mRNAs [18,19]. A physiological role for this enzyme was envisaged after short fragments of poly(A) flanking the 3'-OH end of mRNA were also described in *E. coli* [20]. Recently, two different poly(A) polymerases have been reported in *E. coli* extracts: poly(A) polymerase I encoded by the *pcnB* locus of *E. coli* [21] and poly(A) polymerase II [22]. The experiments described here were performed with commercial poly(A) polymerase (type I) from *E. coli*. Although the presumed role of

poly(A) polymerase from *E. coli* is to add poly(A) tails to RNA, we show here what we view as a novel property of this enzyme, i.e. its capacity to adenylylate the 3'-OH ends of nucleoside, nucleotides and dinucleotides of the type nucleosides(5')oligophospho(5') nucleosides.

In the experimental conditions described in this paper (i.e. a ratio ATP : NpnN of 0.02), the adenylylation of dinucleotides takes place, most probably, at only one of their 3'-OH ends. This view is based on experiments performed in the presence of 1 mM Gp₄G and 0.02 mM ATP as substrates, and analysing the products of the reaction by HPLC. Under these conditions, a new chromatographic peak was observed with a spectrum compatible with the occurrence of two guanines and one adenine.

The fact that this activity had not been described previously might be related to the methods of assay and the substrates used to determine poly(A) polymerase activity (reviewed in [23]). The RNA-(A)_n products synthesized in the assay mixtures usually exhibit a labelled tag in the RNA or in the poly(A) part of the molecule. Since the first description of poly(A) polymerase activity [24], the products of the reaction are usually determined by acid precipitation [15,25,26] or phenol extraction and ethanol precipitation [27,28]. The labelled precipitate is either directly counted for radioactivity or counted after gel electrophoresis and autoradiography [29,30]. The products of the reaction may also be retained in oligo(dT)-cellulose filters or in poly(U)-Sephadex columns [23]. In all of these procedures, adenylylated nucleotides of the kind reported here may pass unnoticed as they are soluble in acid and ethanol and are probably not retained by oligo(dT) or poly(U) residues.

According to our findings, formation of adenylylated mono-, di- and triphosphate nucleosides could also take place in parallel with the synthesis of poly(A) tails and may explain the inhibition by nucleosides 5'-phosphates observed with poly(A) polymerases from several sources [23,31–34]. In this way, the catalytic property of the enzyme to adenylylate nucleoside 5'-phosphates could influence the relative rate of polyadenylation of *E. coli* mRNA *in vivo*. Apart from its value as a tool to modify nucleotides, this property of the enzyme could have physiological significance as millimolar concentrations of nucleosides/nucleotides may compete with the adenylylation of RNA *in vivo*.

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