Journal of Enzyme Inhibition and Medicinal Chemistry, April 2009; 24(2): 516–523

Inhibition of human poly(A)-specific ribonuclease (PARN) by purine nucleotides: kinetic analysis

NIKOLAOS A. A. BALATSOS[1,2], DIMITRIOS ANASTASAKIS[1], & CONSTANTINOS STATHOPOULOS[1,2]

[1]*Department of Biochemistry and Biotechnology, University of Thessaly 26 Ploutonos st., 412 21 Larissa, Greece, and* [2]*Institute of Biomedical Research & Technology (BIOMED), 51 Papanastasiou st, 412 22 Larissa, Greece*

(*Received 12 December 2007; revised 23 April 2008; accepted 15 May 2008*)

Abstract
Poly(A)-specific ribonuclease (PARN) is a cap-interacting and poly(A)-specific 3′-exoribonuclease that efficiently degrades mRNA poly(A) tails. Based on the enzyme's preference for its natural substrates, we examined the role of purine nucleotides as potent effectors of human PARN activity. We found that all purine nucleotides tested can reduce poly(A) degradation by PARN. Detailed kinetic analysis revealed that RTP nucleotides behave as non-competitive inhibitors while RDP and RMP exhibit competitive inhibition. Mg^{2+} which is a catalytically important mediator of PARN activity can release inhibition of RTP and RDP but not RMP. Although many strategies have been proposed for the regulation of PARN activity, very little is known about the modulation of PARN activity by small molecule effectors, such as nucleotides. Our data imply that PARN activity can be modulated by purine nucleotides *in vitro*, providing an additional simple regulatory mechanism.

Keywords: *PARN, mRNA, poly(A) tail, purine nucleotides, inhibition*

Introduction

Eukaryotic mRNAs are usually considered as unstable molecules and their decay is catalyzed almost exclusively by exoribonucleases [1]. Therefore, the two extreme ends of mRNAs have to be protected by such activities. Two characteristic structures, the 5′ end cap and the 3′ end poly(A) tail together with their associated proteins initially protect the mature transcript from exoribonucleases participating in mechanisms controlling the integrity of mRNA, including mRNA processing, transport, translation and stability (for reviews see [1–5]). However, and in contrast to their protective role, these post-transcriptional modifications are also involved in two of the general pathways of eukaryotic mRNA degradation. In both pathways, the first step of mRNA decay is deadenylation, i.e. the shortening of the poly(A) tail [1,2].

Several poly(A) degrading 3′ exoribonucleases have been described such as the PAN2/PAN3 nuclease the CCR4-NOT complex, and poly(A)-specific ribonuclease (PARN) (for a review, see [1]). Human PARN was originally identified as a 74 kDa enzyme [6], but a proteolytic 54 kDa fragment has also been purified [7]. PARN isolated from *Xenopus* oocytes co-purifies with two polypeptides being 74 and 62 kDa in sizes, the latter most likely being a proteolytic product that lacks the C-terminus of the former [8,9]. The important properties of PARN are its high specificity for single-stranded poly(A) and a requirement for a 3′ hydroxyl group for activity [6,10]. PARN holds another important and unique property among deadenylases, which is the ability to interact directly with both the cap structure and the poly(A) tail during deadenylation [7,11–14]. The interaction with the cap stimulates the rate of deadenylation and increases the processivity of the enzyme, however is not

Correspondence: Dr. C. Stathopoulos, Department of Biochemistry & Biotechnology, University of Thessaly, 26 Ploutonos st., 412 21 Larissa, Greece. Tel: +30-2410-565278. Fax: +30-2410-565290. E-mail: cstath@bio.uth.gr

ISSN 1475-6366 print/ISSN 1475-6374 online
DOI: 10.1080/14756360802218763

considered important for its activity [11–13]. Purine mononucleotides [10] and cap analogue [7] have been reported as inhibitors of PARN, however no systematic characterization of this inhibition is known. Sequence analysis have shown that PARN belongs to the DEDD superfamily of nucleases [15,16] and it is conserved in many eukaryotes but it is notably absent from *S. cerevisiae* and *D. melanogaster*. The crystal structure of C-terminal truncated human PARN (residues 1-430 from total 639) revealed that the enzyme forms a functional tight homodimer [17]. Each subunit is folded into two domains, a conserved R3H domain and a nuclease domain [17]. PARN also contains a RNA recognition motif (RRM), missing from the crystal structure, which harbours both poly(A) and cap binding properties [18] and stabilizes the overall structure of the enzyme [19]. The catalytic active site contains four invariant acidic amino acid residues, one glutamic acid and three aspartic acids (Asp28, Glu30, Asp292, and Asp382 in PARN) that coordinate catalytically essential divalent metal ions, such as Mg^{2+} [20,21], while His377 has been proposed to be essential for catalytic activity [17]. However, the crystal structure of the truncated PARN showed that no Mg(II) ions are harbored in the active site of the enzyme [17].

In our study we focused on molecules that might exhibit inhibitory effects on 74 kDa PARN activity, and we investigated the effect of several such nucleotides on PARN. To our knowledge, this is the first report concerning a complete kinetic study of such compounds on this enzyme. Our results suggest that all purine nucleotides tested (carrying either ribose or deoxyribose) can substantially reduce poly(A) degradation efficiency. Both GTP and ATP can significantly reduce the deadenylation activity of PARN following non-competitive kinetics, while AMP, GMP, ADP and GDP inhibit the enzyme in a competitive manner. The sugar component of the nucleotide does not seem to serve as a sign for the enzyme to discriminate between deoxy- and ribo-nucleotides. Finally, we studied the effect of Mg^{2+} in deadenylation reactions in the presence of nucleotides and we found that the inhibitory effect could be released in the case of purine tri- and diphosphate nucleotides but not for the monophosphate nucleotides.

Materials and Methods

Materials

All chemicals including purine ribonucleotides and deoxynucleotides, methylene blue and polyadenylic acid potassium salt with an average size of 300 adenosines (A_{300}) were from SigmaAldrich.

Expression and purification of recombinant PARN

The plasmid encoding full-size 74 kDa human PARN (for expression of N-terminal His_6-tagged polypeptide) was transformed into BL21(DE3) cells to express the recombinant protein as described previously [22] with some modifications. In brief, colonies were grown overnight at 37 °C in the presence of kanamycin (50 μg/mL). The cultures were then diluted (1:100) in the same medium and grown at 37 °C induced by isopropyl-1-thio-b-D-galactopyranoside (IPTG) at a final concentration of 0.1 mM. Cultures for PARN expression were allowed to grow for 3 h at 37 °C. Cells were harvested by centrifugation for 20 min at 4 °C and pellets were frozen at − 70 °C. The expressed His-tagged soluble proteins were purified following previously described protocols [22].

PARN activity assay

The enzymatic activity was determined by the methylene blue assay as described before [23]. Methylene blue buffer was prepared by dissolving 1.2 mg methylene blue into 100 mL Mops buffer (0.1M MOPS-KOH, pH 7.5, 2 mM EDTA). The standard reaction buffer contained 20 mM HEPES-KOH (pH 7.0), 1.5 mM $MgCl_2$, 100 mM KCl, 0.1 U of RNasin, 0.2 mM EDTA, 0.25 mM DTT, 10% (v/v) glycerol and 0.075–0.6 mM of poly(A). All ribonucleotides were dissolved in reaction buffer prior to use. The reactions were performed using 0.01–0.02 mM recombinant PARN. The final reaction volume was 100 μL and the reaction was performed at 30°C for 5–10 min. The reaction was terminated by mixing the reaction solution with 900 μL methylene blue buffer and the mixed solution was incubated at 30 °C for another 15 min in the dark in a water bath. The absorbance at 662 nm of 1 mL sample was measured on a Spectronic Genesys 20 spectrophotometer.

Results

Effect of purine nucleotides on PARN activity

Based on the structural similarity of adenine nucleotides with the substrate of PARN [poly(A) tail] and the deadenylation product (AMP), we investigated the effect of ATP, ADP and AMP on 74 kDa PARN activity. Figure 1A shows the effect of the adenine nucleotides on PARN activity. ATP significantly reduces the activity of the enzyme (Figures 1A, 2A and 2C) following classical Michaelis-Menten kinetics (Figure 2A). We could observe around 90% reduction of activity at 4 mM ATP after 10 minutes incubation (Figure 1A). ADP was less effective than ATP, since the activity reduction at 4 mM was approximately half of ATP. Finally AMP reduced PARN activity around 50% at higher concentrations (~14 mM). The apparent inhibition constants ($_{app}K_i$) were determined to be in the low millimolar range (1.0, 2.2 and 4.7 mM for ATP, ADP and AMP, respectively). The $_{app}K_i$ was defined

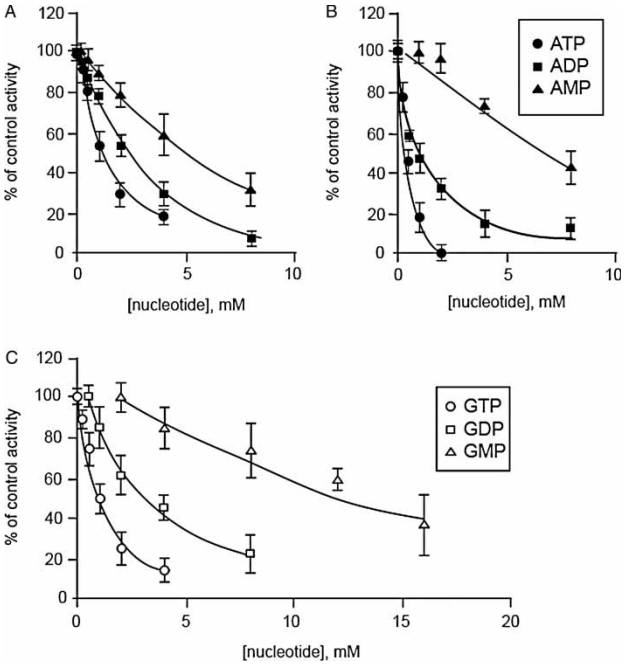

Figure 1. Effect of purine nucleotides on PARN activity. A. Relative activity (%) is presented as a function of increasing concentrations of adenosine nucleotides, 0–8 mM AMP (▲), 0–8 mM ADP (■), or 0–4 mM ATP (•). Mg^{2+} concentration is 1.5 mM. B. Same as in A, except that 0.5 mM Mg^{2+} was used and the nucleotide concentrations are 0–8 mM AMP, 0–8 mM ADP and 0–2 mM ATP. C. Same as in A, except that 0–4 mM GMP (Δ), 0–8 Mm GDP (□), or 4–16 mM GTP (○) was used. Reactions in the presence of PARN were performed as outlined in the text and Materials and Methods.

as the concentration of the nucleotide resulting in 50% inhibition of the enzymatic activity.

We next tested for the other purine base nucleotides (GMP, GDP and GTP) on PARN activity. All three nucleotides could reduce poly(A) degradation (Figure 1C) and the pattern of the reduction was similar to that for adenine nucleotides (compare Figures 1A and 1C). Table I summarizes the $_{app}K_i$ values (column 1.5 mM Mg^{2+}) for all six purine nucleotides. Comparisons of the values between the nucleotides with the same number of phosphate groups show that these values are similar and therefore, the enzyme does not seem to have preference for adenine or guanine.

The results presented here show that all three nucleotides could reduce PARN activity and it seems that the number of the phosphate groups play role in this reduction; the more the phosphate groups, the more the reduction of the activity.

Purine triphosphate nucleotides inhibit PARN following non-competitive kinetics

To investigate the mechanism of inhibition by ATP, we performed detailed kinetic analysis. We titrated the substrate [poly(A)] at several concentrations of ATP.

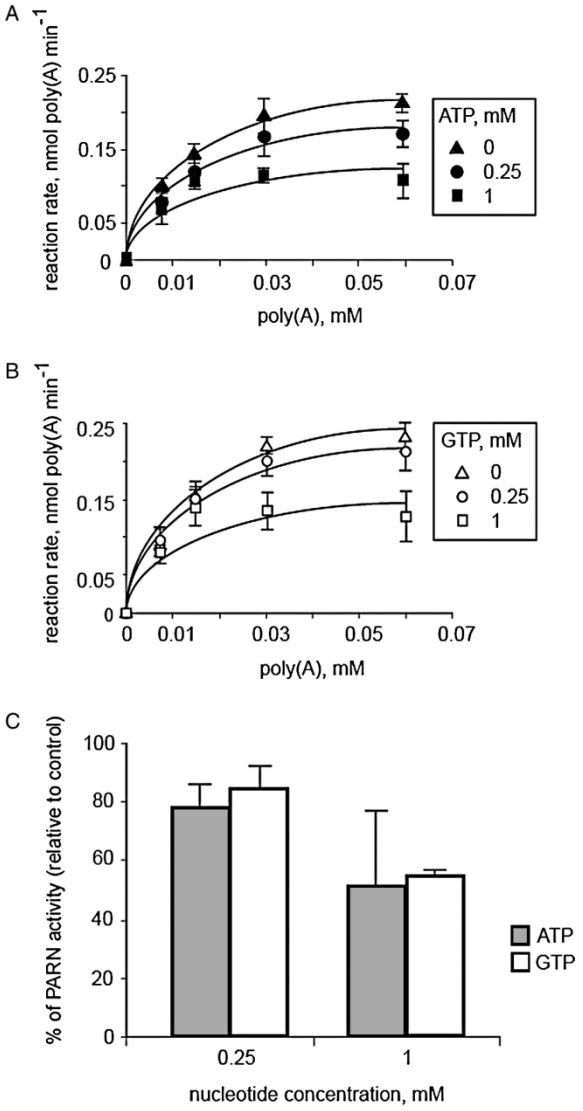

Figure 2. Purine triphosphate nucleotides inhibit PARN. A. Michaelis-Menten plot showing the effect of ATP in PARN activity. Reactions were performed as described in Materials and Methods in the presence of 0 mM (▲), 0.25 mM (•) and 1 mM (■) ATP. Representative of at least two independent experiments. B. Same as in A, except that GTP was used at 0 mM (Δ), 0.25 mM (○) and 1 mM (□). C. Comparison of ATP and GTP inhibition effect on PARN activity. Reactions were performed in the presence of the indicated ATP (shaded bars) or GTP (white bars) concentrations. The data points are averages of at least three independent experiments.

The results were plotted using Lineweaver-Burk formalism and were compatible with non-competitive inhibition (Figure 3A). In order to determine the inhibition constants (K_i) kinetically, the slopes of the lines in Figure 3A (K_M/V_{max}) were re-plotted as a function of ATP concentration (shown as inset in Figure 3A) and the K_i value was 3.2 mM (Table I, also shown as inset in Figure 3B). The same observations were made for GTP. The nucleotide behaves as non-competitive inhibitor of PARN (Figure 3B) and the calculated K_i value 3.3 mM is similar to that for ATP.

Table I. Inhibition of PARN by purine nucleotides.

Nucleotide	K_i mM	$_{app}K_i$, Mm	
		1.5 mM Mg^{2+}	0.5 mM Mg^{2+}
GTP	3.3 ± 0.5*	1.0 ± 0.1	0.5 ± 0.1
GDP	2.8 ± 0.8	3.3 ± 0.3	1.4 ± 0.5
GMP	4.5 ± 2.2	11.3 ± 3.2	4.8 ± 1.2
ATP	3.2 ± 0.8	1.0 ± 0.2	0.3 ± 0.1
ADP	0.5 ± 0.3	2.2 ± 0.7	1.0 ± 0.3
AMP	1.6 ± 0.3	4.7 ± 1.2	6.7 ± 2.2
dGTP	2.0 ± 0.1	n.d	n.d
dATP	2.8 ± 0.5	n.d	n.d

*The values listed are averages of at least three independent experiments ± experimental error.

These results show that PARN does not discriminate the kind of purine, but adenine and guanine can equally and effectively reduce enzymatic activity. Furthermore, purine triphosphate nucleotides are non-competitive inhibitors of PARN.

Purine mono- and diphosphate nucleotides inhibit PARN following competitive kinetics

Subsequently, we followed the same approach as before and performed kinetic analysis of the mono- and diphosphate nucleotides. We used a range of nucleotide concentrations around the calculated $_{app}K_i$ from Table I. Interestingly, we found that the nucleotides inhibited PARN following competitive kinetics (Figures 3C and 3D). Table I summarizes all the inhibitory constants for purine nucleotides.

These data point towards a direct competition of AMP, ADP and the substrate for the same binding-site on PARN.

DeoxyATP and deoxyGTP reduce PARN activity non-competitively

Following the previous observations, we asked whether the sugar component of the nucleotide had any effect on PARN activity. Towards this direction, we performed deadenylation reactions as described above, including dATP or dGTP in the reaction mixture. Figure 4 shows the Lineweaver-Burk plot using the data from deadenylation reactions in the presence of dATP or dGTP. In both cases, deoxy-nucleotides reduced enzymatic activity non-competitively. K_i values were 2.8 and 2.0 mM for dATP and dGTP, respectively (Figure 4A and 4B and insets therein, and Table I). These results show that the sugar's nature does not alter the inhibition pattern. The fact that K_i values for ATP and dATP are very close is supportive of our measurements.

These results show that PARN does not discriminate between ribose and deoxy-ribose in the nucleotide

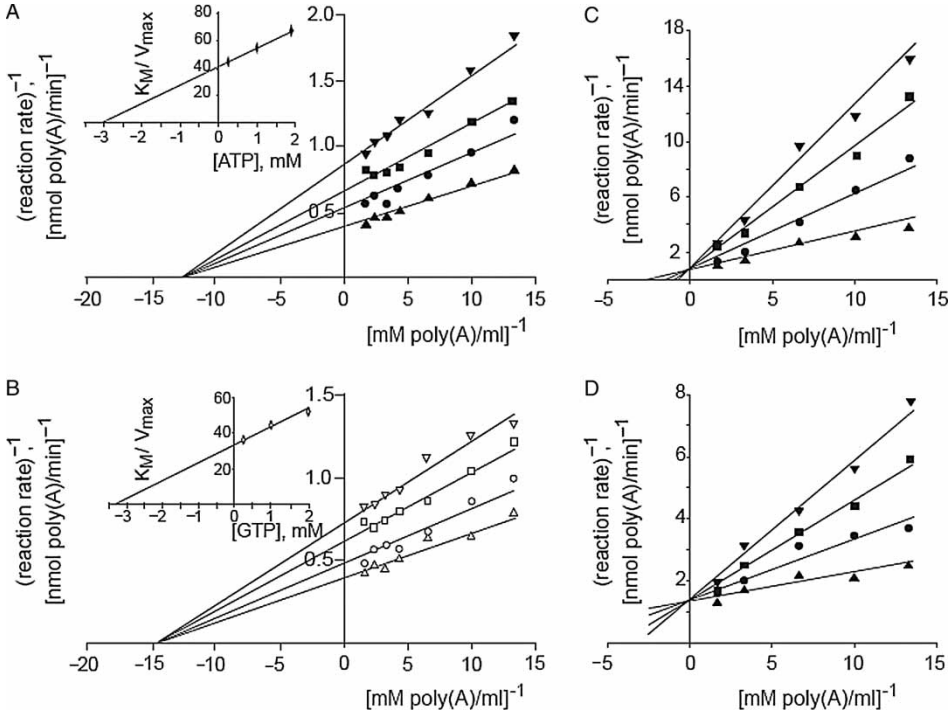

Figure 3. Kinetic analysis of purine phosphate nucleotides inhibition of PARN: ATP (A) and GTP (B) are noncompetitive inhibitors; AMP (C) and ADP (D) are competitive inhibitors. Reactions were performed as described in Materials and Methods. Double reciprocal plots 1/v versus 1/[substrate] for PARN activity in the presence of GTP or ATP are shown. The ATP concentrations (filled markers in A) and GTP (empty markers in B) were 0 mM (triangle), 0.25 mM (circle), 1 mM (square) and 2 mM (inverted triangle). AMP concentrations were 0 mM (▲), 2 mM (•), 4 mM (■) and 6 mM (▼). ADP concentrations were 0 mM (▲), 1 mM (•), 2 mM (■) and 3 mM (▼). Representative of at least three independent experiments. Insets in A and B: The slopes (K_M/V_{max}) of the double reciprocal lines versus the nucleotide concentrations.

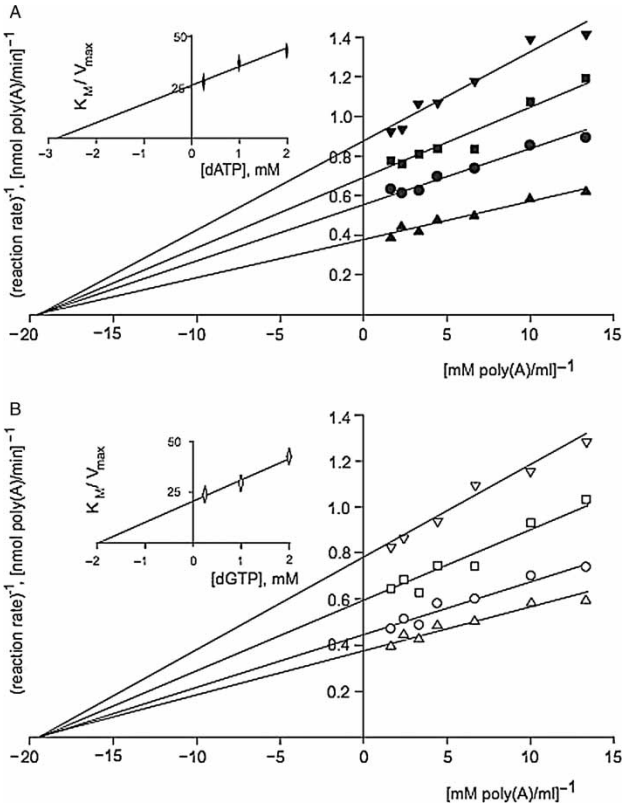

Figure 4. Purine triphosphate deoxynucleotides inhibit PARN: dATP (A) and dGTP (B) are noncompetitive inhibitors. Double reciprocal plots 1/v versus 1/[substrate] for PARN activity in the presence of dATP or dGTP are shown. The dATP concentrations (filled markers in A) and dGTP (empty markers in A) were 0 mM (triangle), 0.25 mM (circle), 1 mM (square) and 2 mM (inverted triangle). Representative of at least three independent experiments. Insets: The slopes (K_M/V_{max}) of the double reciprocal lines versus the nucleotide concentrations.

composition. The experiments described above clearly indicate that it is the number of the phosphate groups that regulate the effectiveness of PARN activity, and not the purine base or the sugar.

Mg^{2+} releases the inhibition by RDPs and RTPs, but not RMPs

Purine nucleotides (in particular adenine nucleotides) serve as substrates in the form of metal ion complexes in enzymatic reactions taking part in central metabolic processes. To investigate whether the observed inhibition of PARN by purine nucleotides was due to Mg^{2+} deprivation, we performed deadenylation reactions in the presence of Mg^{2+} concentrations ranging from 0.5 to 15 mM, which are below and above the concentrations of the nucleotides in the reaction and their K_i values (Table I). Figure 5A shows that when Mg^{2+} is increased, the inhibition of PARN is released. At 15 mM Mg^{2+} (3–5 fold of K_i value) in the presence of 1 mM ATP or GTP, the enzymatic activity is totally restored compared to the control. When Mg^{2+} concentration was lower than the concentration

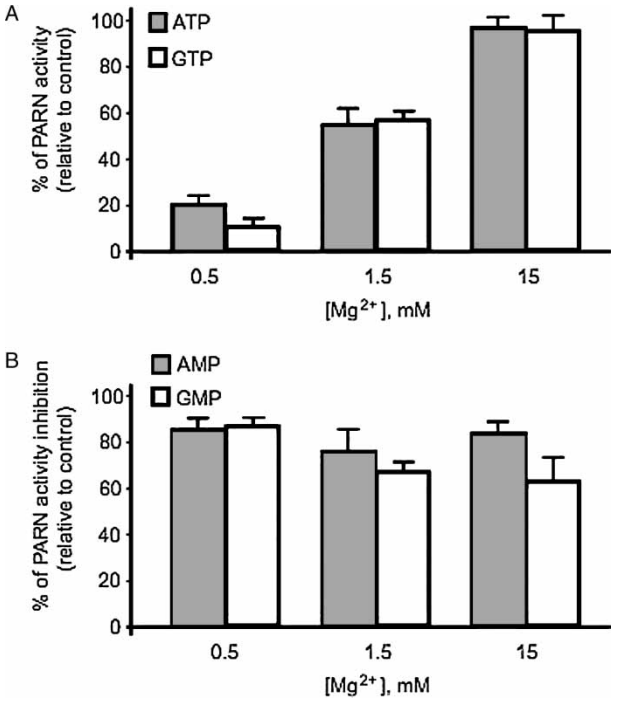

Figure 5. Effect of Mg^{2+} in the inhibition of PARN by purine nucleotides. A. Relative activity inhibition by 1.5 mM ATP (grey bars) or 1.5 mM GTP (white bars) in the presence of 0, 1.5 and 15 mM Mg^{2+}. B. Release of PARN activity inhibition by 1.5 mM AMP (grey bars) or 1.5 mM GMP (white bars) in the presence of 0, 1.5 and 15 mM Mg^{2+}. The results are mean values from at least three independent experiments.

of nucleotides, PARN activity could not be restored, compared to the standard conditions (compare Figures 1A and 1B). This observation is reflected to the calculated $_{app}K_i$ values for 0.5 and 1.5 mM Mg^{2+} (Table I). Upon increase of Mg^{2+} concentration well above AMP or GMP the release of inhibition was not significant, compared to the almost full restoration of activity in the case of RTPs (compare Figures 5A and 5B).

These observations suggest that inhibition of PARN by RTPs can be attributed to the sequestration of Mg(II) ions from the enzyme, rather than a direct interaction of the nucleotides with PARN. Further, Mg(II) ions alter purine di- and triphosphate nucleotides inhibitory effect on PARN activity, but does not interfere with the strong inhibitory effect of AMP or GMP.

Discussion

This is the first report where the inhibitory mechanism of purine nucleotides on PARN activity is studied in detail. We show that all purine nucleotides can reduce PARN activity. Interestingly, the kinetic analysis revealed that the number of the phosphate groups of the mononucleotide alters the type of inhibition: competitive for mono- and diphosphate nucleotides,

non-competitive for triphosphate ones. Finally, Mg(II) ions release inhibition of the tri- and diphosphate nucleotides, but had no effect on the inhibitory action of monophosphate nucleotides.

Although several strategies have been reported to modulate PARN activity *in vitro*, they mainly focus on interactions of PARN with auxiliary protein factors and the cap [7,12,24–31]. However little is known about possible modulation mechanisms of the enzyme that are facilitated by small molecule effectors, with the exception of detailed kinetic analysis of aminoglycosides' inhibition [32].

Part of the work presented here refers to the effect of ATP and GTP on full-size PARN activity. Both nucleotides can inhibit the enzyme non-competitively (Figures 1–3) with K_i values 3.3 and 3.2 mM, respectively. Considering that the intracellular nucleotide concentration varies markedly among tissues of the same species and in mammalian cytosol (3–10 mM for ATP) [33], the calculated kinetic parameters that derived from our study are compatible with the known intracellular conditions. Moreover, our data suggest an allosteric effect on the enzyme, where the tri-phosphate nucleotides act as non-competitive inhibitors of PARN and presumably compete with cap for binding in the RRM domain. Our data imply that increased GTP and/or ATP concentration around the translational machinery would minimize PARN preference for cap and consequent poly(A) degradation. Furthermore, during translation the poly(A) tail of the mRNA is continually shortened. This process continues until a critical minimum length has been reached which seem to be approximately 10–12 adenylate residues in lower eukaryotes and twice this size in metazoans [34]. In any case the tail does not seem to be removed completely by the responsible deadenylase. Concerning PARN, our data could provide information towards a possible explanation for this phenomenon. A possible explanation could be that the high local concentration of the produced 5′-AMP during deadenylation inhibits competitively PARN and releases the poly(A)-shortened mRNA substrate from the enzyme. This mechanism is independent from the presence of ATP/GTP or the essential divalent ions such as Mg^{2+} (see also below) in the translation microenvironment.

In a second step we performed kinetic analysis of the inhibition by 5′-AMP and 5′-GMP. Interestingly, we observed that these nucleotides inhibited PARN too, but the type of inhibition is competitive (Figure 3), suggesting a simple product inhibition mechanism of regulating PARN activity during deadenylation. This indicates that the product of deadenylation can inhibit the enzyme and could compete for substrate binding. ADP and GDP inhibited PARN competitively, too. The inhibition constants in Table I (K_i and $_{app}K_i$) show that purine diphosphate nucleotides interact stronger than the monophosphate ones with the active site of the enzyme. These results imply that the enzyme is able to discriminate among mono-, di- and tri-phosphate groups. Moreover, the mono- and diphosphate nucleotides interact with PARN in the active site, while the tri-phosphate nucleotides with a different domain of the enzyme. The RRM domain could facilitate such an interaction, since it is known that the cap binds this domain and ATP/GTP are structurally similar to the cap.

Finally, the involvement of Mg(II) ions during deadenylation was examined because the binding of Mg^{2+} with the phosphate groups of purine nucleotides (in particular with ATP) is an important aspect of enzyme-catalysed reactions. It has been proposed that Mg^{2+} binds only the β-phosphate of ATP at pH 7.0 [35], that Mg-ATP is a β,γ-bidentate at pH 8.0 [36], a mixture of α,β-, β,γ- and α,γ- bidentates [37], or that interacts with α- and β-phosphates of ADP and all α-, β-, γ- phosphates of ATP [38].

The role of Mg(II) on PARN activity is, so far, elusive. Biochemical studies clearly show that it is important for activity and it has been proposed that the active site of PARN harbours two divalent ions resembling the 3′exonuclease active site of *E. coli* DNA pol I [21]. Furthermore, it has been reported that neomycin B (and other aminoglycosides) inhibit PARN and the inhibition could be released by Mg^{2+}. As an explanation of this effect, it has been proposed that aminoglycosides bind in the active site of the enzyme and displace catalytically important divalent metal ions [32]. However, and in contrast to the available biochemical data, the crystal structure of a truncated but active 54 kDa PARN showed no Mg(II) ions in the active site [17].

In this work, we show that tri- or diphosphate nucleotides do not inhibit PARN when Mg^{2+} is present in excess. Therefore, at least an equimolar amount of the ion should bind the nucleotides and inactivate their effect on PARN, while at the same time Mg^{2+} interacts with the active site as well. The competitive inhibition of PARN by AMP or GMP is almost independent of Mg^{2+} (Figure 5, compare Figures 1A and 1B). In contrast, upon increase of [Mg^{2+}] the inhibition of PARN by ATP or GTP is released suggesting that Mg-RTP complexes do not inhibit the enzyme. RDPs inhibit the enzyme competitively, therefore bind to the enzyme and also deplete PARN from the available Mg^{2+}. Based on the previous observations we can reasonably assume that the combination of the local concentration of Mg^{2+} and ATP/GTP or ADP/GDP affects PARN activity; when the deadenylation reaction is active (and 5′-AMP is released) no metal ion involvement occurs.

A similar example has been reported for the modulation of succinic semialdehyde dehydrogenase (SSADH) activity from *A. thaliana*. SSADH is inhibited by AMP, ADP, and ATP ($K_i = 2.5–8$ mM).

The mechanism of inhibition was competitive for AMP, noncompetitive for ATP, and mixed competitive for ADP. However, Mg(II) ions had no effect on the inhibitory effect of the nucleotides [39].

Our data, together with all the above mentioned studies, are supportive of a simple mechanism by which PARN-mediated deadenylation is regulated via simple interactions of the enzyme with purine nucleotides in combination with the Mg^{2+} concentration. These interactions consist of either direct competition to the active site or allosteric effects. Within this context, a high concentration of ATP/GTP in the translation machinery microenvironment could ensure that PARN would be less active on a capped and polyadenylated mRNA, since the enzyme would bind the nucleotide instead of the cap. Furthermore, eIF4E and PABPC would limit the access of PARN to mRNA. In such a microenvironment, PARN would remain "silent" through the protection of its mRNA accession structures, i.e. the poly(A) and the cap, but also through allosteric inhibition by small and abundant molecules, such as ATP or GTP. Hence, a purine nucleotide can act as an allosteric effector molecule at the same time. When mRNA degradation proceeds, deadenylation occurs as the first step until the tail is shortened down to a critical length where the deadenylase is not active; it could be the produced 5′-AMP that finally inhibits the enzyme, without any dependence on Mg^{2+}, and then other exonucleases (such as the exosome complex) take over and fully degrade the mRNA. However, further experimentation is needed to test these scenarios. Furthermore, our observation that purine deoxynucleotides inhibit PARN at high concentrations could reflect the fact that in nuclear microenvironments the enzyme remains silent.

PARN is considered an important mediator of mRNA turnover in human. On the other hand, purine nucleotide analogues are among the first chemotherapeutic agents to be introduced for the medical treatment of cancer. They act as antimetabolites, competing with physiological nucleosides and interacting with intracellular targets to induce cytotoxicity [40]. The elucidation of the biochemical mechanism of anticancer action, together with the identification of novel putative molecular targets, is considered essential for potentiating their antitumor effects. Our study could provide an additional mechanism of action of such compounds on a potentially important molecular target of human cells.

Acknowledgements

We thank Professor Anders Virtanen, Uppsala University (Uppsala, Sweden) for kindly providing us the plasmid for PARN expression and for valuable comments. We also thank Dimitra Levendi, Stamatina Giannouli and Athanasios Kyritsis for support and technical assistance. This work was supported in part by a Research Grant from the University of Thessaly Research Committee.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

[1] Meyer S, Temme C, Wahle E. Messenger RNA turnover in eukaryotes: Pathways and enzymes. Crit Rev Biochem Mol Biol 2004;39:197–216.
[2] Parker R, Song H. The enzymes and control of eukaryotic mRNA turnover. Nat Struct Mol Biol 2004;11:121–127.
[3] Sachs AB, Varani G. Eukaryotic translation initiation: There are (at least) two sides to every story. Nat Struct Biol 2000; 7:356–361.
[4] Shatkin AJ, Manley JL. The ends of the affair: Capping and polyadenylation. Nat Struct Biol 2000;7:838–842.
[5] Wilusz CJ, Wormington M, Peltz SW. The cap-to-tail guide to mRNA turnover. Nat Rev Mol Cell Biol 2001;2:237–246.
[6] Korner CG, Wahle E. Poly (A) tail shortening by a mammalian poly(A)-specific 3′-exoribonuclease. J Biol Chem 1997;272: 10448–10456.
[7] Martinez J, Ren YG, Thuresson AC, Hellman U, Astrom J, Virtanen A. A 54-kDa fragment of the Poly(A)-specific ribonuclease is an oligomeric, processive, and cap-interacting Poly(A)-specific 3′ exonuclease. J Biol Chem 2000;275: 24222–24230.
[8] Copeland PR, Wormington M. The mechanism and regulation of deadenylation: Identification and characterization of Xenopus PARN. Rna 2001;7:875–886.
[9] Varnum SM, Hurney CA, Wormington WM. Maturation-specific deadenylation in Xenopus oocytes requires nuclear and cytoplasmic factors. Dev Biol 1992;153:283–290.
[10] Astrom J, Astrom A, Virtanen A. Properties of a HeLa cell 3′ exonuclease specific for degrading poly(A) tails of mammalian mRNA. J Biol Chem 1992;267:18154–18159.
[11] Dehlin E, Wormington M, Korner CG, Wahle E. Cap-dependent deadenylation of mRNA. Embo J 2000;19: 1079–1086.
[12] Gao M, Fritz DT, Ford LP, Wilusz J. Interaction between a poly(A)-specific ribonuclease and the 5′ cap influences mRNA deadenylation rates in vitro. Mol Cell 2000;5:479–488.
[13] Martinez J, Ren YG, Nilsson P, Ehrenberg M, Virtanen A. The mRNA cap structure stimulates rate of poly(A) removal and amplifies processivity of degradation. J Biol Chem 2001; 276:27923–27929.
[14] Opyrchal M, Anderson JR, Sokoloski KJ, Wilusz CJ, Wilusz J. A cell-free mRNA stability assay reveals conservation of the enzymes and mechanisms of mRNA decay between mosquito and mammalian cell lines. Insect Biochem Mol Biol 2005;35: 1321–1334.
[15] Korner CG, Wormington M, Muckenthaler M, Schneider S, Dehlin E, Wahle E. The deadenylating nuclease (DAN) is involved in poly(A) tail removal during the meiotic maturation of Xenopus oocytes. Embo J 1998;17:5427–5437.
[16] Moser MJ, Holley WR, Chatterjee A, Mian IS. The proofreading domain of Escherichia coli DNA polymerase I and other DNA and/or RNA exonuclease domains. Nucleic Acids Res 1997;25:5110–5118.
[17] Wu M, Reuter M, Lilie H, Liu Y, Wahle E, Song H. Structural insight into poly(A) binding and catalytic mechanism of human PARN. Embo J 2005;24:4082–4093.
[18] Nilsson P, Henriksson N, Niedzwiecka A, Balatsos NA, Kokkoris K, Eriksson J, Virtanen A. A multifunctional RNA

recognition motif (RRM) in poly(A)-specific ribonuclease (PARN) with cap and poly(A) binding properties. J Biol Chem 2007.

[19] Zhang A, Liu WF, Yan YB. Role of the RRM domain in the activity, structure and stability of poly(A)-specific ribonuclease. Arch Biochem Biophys 2007;461:255–262.

[20] Ren YG, Kirsebom LA, Virtanen A. Coordination of divalent metal ions in the active site of poly(A)-specific ribonuclease. J Biol Chem 2004;279:48702–48706.

[21] Ren YG, Martinez J, Virtanen A. Identification of the active site of poly(A)-specific ribonuclease by site-directed mutagenesis and Fe(2 +)-mediated cleavage. J Biol Chem 2002;277: 5982–5987.

[22] Nilsson P, Virtanen A. Expression and purification of recombinant poly(A)-specific ribonuclease (PARN). Int J Biol Macromol 2006;39:95–99.

[23] Cheng Y, Liu WF, Yan YB, Zhou HM. A nonradioactive assay for poly(a)-specific ribonuclease activity by methylene blue colorimetry. Protein Pept Lett 2006;13:125–128.

[24] Balatsos NA, Nilsson P, Mazza C, Cusack S, Virtanen A. Inhibition of mRNA deadenylation by the nuclear cap binding complex (CBC). J Biol Chem 2006;281:4517–4522.

[25] Gao M, Wilusz CJ, Peltz SW, Wilusz J. A novel mRNA-decapping activity in HeLa cytoplasmic extracts is regulated by AU-rich elements. Embo J 2001;20:1134–1143.

[26] Gherzi R, Lee KY, Briata P, Wegmuller D, Moroni C, Karin M, Chen CY. A KH domain RNA binding protein, KSRP, promotes ARE-directed mRNA turnover by recruiting the degradation machinery. Mol Cell 2004;14:571–583.

[27] Lai WS, Kennington EA, Blackshear PJ. Tristetraprolin and its family members can promote the cell-free deadenylation of AU-rich element-containing mRNAs by poly(A) ribonuclease. Mol Cell Biol 2003;23:3798–3812.

[28] Lin WJ, Duffy A, Chen CY. Localization of AU-rich element-containing mRNA in cytoplasmic granules containing exosome subunits. J Biol Chem 2007;282:19958–19968.

[29] Moraes KC, Wilusz CJ, Wilusz J. CUG-BP binds to RNA substrates and recruits PARN deadenylase. Rna 2006;12: 1084–1091.

[30] Seal R, Temperley R, Wilusz J, Lightowlers RN, Chrzanowska-Lightowlers ZM. Serum-deprivation stimulates cap-binding by PARN at the expense of eIF4E, consistent with the observed decrease in mRNA stability. Nucleic Acids Res 2005;33:376–387.

[31] Tran H, Schilling M, Wirbelauer C, Hess D, Nagamine Y. Facilitation of mRNA deadenylation and decay by the exosome-bound, DExH protein RHAU. Mol Cell 2004;13: 101–111.

[32] Ren YG, Martinez J, Kirsebom LA, Virtanen A. Inhibition of Klenow DNA polymerase and poly(A)-specific ribonuclease by aminoglycosides. Rna 2002;8:1393–1400.

[33] Schwiebert EM, Zsembery A. Extracellular ATP as a signaling molecule for epithelial cells. Biochim Biophys Acta 2003;1615: 7–32.

[34] Garneau NL, Wilusz J, Wilusz CJ. The highways and byways of mRNA decay. Nat Rev Mol Cell Biol 2007;8:113–126.

[35] Son TD, Roux M, Ellenberger M. Interaction of Mg2 + ions with nucleoside triphosphates by phosphorus magnetic resonance spectroscopy. Nucleic Acids Res 1975;2: 1101–1110.

[36] Cohn M, Hughes TR, Jr. Nuclear magnetic resonance spectra of adenosine di-and triphosphatetriphosphate. II. Effect of complexing with divalent metal ions. J Biol Chem 1962;237: 176–181.

[37] Ramirez F, Marecek JF. Coordination of magnesium with adenosine 5′-diphosphate and triphosphate. Biochim Biophys Acta 1980;589:21–29.

[38] Huang SL, Tsai MD. Does the magnesium(II) ion interact with the alpha-phosphate of adenosine triphosphate? An investigation by oxygen-17 nuclear magnetic resonance. Biochemistry 1982;21:951–959.

[39] Busch KB, Fromm H. Plant succinic semialdehyde dehydrogenase. Cloning, purification, localization in mitochondria, and regulation by adenine nucleotides. Plant Physiol 1999; 121:589–597.

[40] Galmarini CM, Mackey JR, Dumontet C. Nucleoside analogues and nucleobases in cancer treatment. Lancet Oncol 2002;3:415–424.