FEBS Letters 581 (2007) 1047–1052

# Effect of magnesium ions on the thermal stability of human poly(A)-specific ribonuclease

Wei-Feng Liu[a,b], Ao Zhang[a], Yuan Cheng[b], Hai-Meng Zhou[b,*], Yong-Bin Yan[a,*]

[a] *State Key Laboratory of Biomembrane and Membrane Biotechnology, Department of Biological Sciences and Biotechnology, Tsinghua University, Beijing 100084, China*

[b] *Protein Science Laboratory of the Ministry of Education, Department of Biological Sciences and Biotechnology, Tsinghua University, Beijing 100084, China*

Received 16 January 2007; revised 2 February 2007; accepted 5 February 2007

Available online 14 February 2007

Edited by Miguel De la Rosa

**Abstract** **Poly(A)-specific ribonuclease (PARN), a member of the DEDD family, is a key enzyme involved in the deadenylation of mRNA in higher eukaryotic cells. In this research, it was found that $Mg^{2+}$ could protect PARN against thermal inactivation by increasing the midpoint of inactivation and decreasing the inactivation rate. This protective effect was unique to $Mg^{2+}$ in a concentration-dependent manner. However, the thermal unfolding and aggregation was promoted by the addition of $Mg^{2+}$ at high temperatures. These results revealed that $Mg^{2+}$ might have dual effects on PARN stability: protecting the active site but endangering the overall structural stability.**



*Keywords:* Poly(A) specific ribonuclease; Thermal stability; Divalent metal cation; Thermal aggregation; mRNA turnover

## 1. Introduction

Deadenylation of mRNAs has been demonstrated to play a critical role in both mRNA stability and translational regulation in eukaryotic cells [1,2]. As a negative regulator of gene expression, mRNA turnover is a fundamental step in several physiological processes. In this case, deadenylation is the first step of the decay of mRNA [3,4]. Moreover, the regulation of gene expression may also occur at the translation stage through the control of the poly(A) length of mRNA [1,5,6]. The circulation of mRNA has been found to contribute to the control of mRNA expression in the eukaryotic cell, and a deadenylated mRNA cannot circulate to achieve efficient translation [7]. Poly(A)-specific ribonuclease (PARN), a key enzyme involved in the deadenylation of mRNA in higher eukaryotic cells, specifically catalyzes the degradation of the mRNA poly(A) tail with a free 3′ hydroxyl group and releases 5′-AMP as the mononucleotide product [6,8–11]. Due to its crucial roles in mRNA deadenylation and further degradation, it has been found that PARN participates in many important intracellular processes such as oocyte maturation and early development [5,6,12–14].

Sequence alignment, biochemical analysis and structural studies indicated that PARN belongs to the DEDD superfamily of nucleases [14–17]. Members of the DEDD superfamily can be divided into two subgroups, DEDDh and DEDDy, based on whether the fifth conserved residue is a histidine or tyrosine [18]. The nucleases in the DEDD superfamily are proposed share a similar structure of the catalytic domain and a common catalytic mechanism [18–20]. The catalytic active site contains four invariant acidic amino acid residues, one glutamic acid and three aspartic acids, plus several other conserved residues distributed in three separate exonuclease motifs, *Exo*I, *Exo*II and *Exo*III [20]. The side-chains of these four negatively charged residues serve as the ligands for two metal ions, which are essential for the catalysis [19,21]. Similar to the other members of the DEDD family, mutational analysis [16] as well as structural studies [17] have indicated that four conserved acidic residues in the catalytic domain, Asp28, Glu30, Asp292, and Asp382, are essential for the activity of PARN. It has been proposed that PARN may utilize the two-metal ion catalytic mechanism [21–23].

Although $Mg^{2+}$ ions are the most suitable divalent metal ions for the catalytic function, the active site of PARN could also accommodate various kinds of divalent metal ions, such as $Mn^{2+}$, $Fe^{2+}$, $Zn^{2+}$, etc. [22]. Unfortunately, no metal ions are present in the crystal structure of the catalytic domain of PARN [17], which limits the understanding of the impact of the two ions on the catalysis as well as the structural stability of PARN. Magnesium, the second most abundant intracellular cation and the fourth most abundant cation in the body, has a well-established influence on intracellular biological processes [24] and the functions, stability and folding of proteins as well as RNAs (for example [25–31]). The total magnesium content in various cell types ranges from 5 to 30 mM, while the concentration of the free $Mg^{2+}$ has been determined to be in the range of 0.3–3 mM [24,32]. In this research, the effect of $Mg^{2+}$ on PARN stability was investigated by thermal inactivation and denaturation analysis. The results herein suggested that in addition to its crucial role in catalysis, $Mg^{2+}$ also played an important and unique role in PARN stability against thermal inactivation. Interestingly, $Mg^{2+}$ behaved as a destabilizer of the overall structural stability of PARN, and it could promote

*Corresponding authors. Fax: +86 10 6277 1597 (Y.-B. Yan); +86 10 6277 2245 (H.-M. Zhou).
*E-mail addresses:* zhm-dbs@tsinghua.edu.cn (H.-M. Zhou), ybyan@tsinghua.edu.cn (Y.-B. Yan).

*Abbreviations:* CD, circular dichroism; DTT, dithiothreitol; IPTG, isopropyl-1-thio-β-D-galactopyranoside; PARN, poly(A) specific ribonuclease; SDS, sodium dodecyl sulfate; SDS–PAGE, SDS–polyacrylamide gel electrophoresis; $[\theta]_{MRW}$, mean residue ellipticity

0014-5793/$32.00 
doi:10.1016/j.febslet.2007.02.008

the thermal unfolding and aggregation of PARN at high temperatures.

## 2. Materials and methods

### 2.1. Bacterial strains and materials

The plasmid containing the human *parn* gene was kindly provided by Professor Anders Virtanen (Uppsala University, Sweden). The proteolytic fragment of the full-length PARN, the 54 kDa one [9], was used for the investigations in this research. *E. coli* BL21(DE3) carrying the plasmid pET33 containing the human *parn* gene was used for the expression of recombinant protein. Poly(A), methylene blue, isopropyl-1-thio-β-D-galactopyranoside (IPTG) were purchased from Sigma, dithiothreitol (DTT) was from BIOMOL, kanamycin and MOPS were from AMRESCO, and Rnasin was from Promega. All other reagents were local products of analytical grade.

### 2.2. Protein expression and purification

The expression and purification of recombinant PARN were performed according to the strategy developed by Virtanen et al. [33] with some modifications. In brief, the recombinant strains were grown at 37 °C for 12 h in LB medium containing 50 μg/ml kanamycin. The cultures were diluted (1:100) in the same medium and grown at 37 °C to reach 0.8 OD, then induced by 0.1 mM IPTG at 16 °C and finally harvested after 24 h of induction. The extracted recombinant soluble proteins were purified by metal affinity chromatography using a $Ni^{2+}$ matrix (Shenergy Biocolor BioScience & Technology), and then by gel filtration chromatography using a Superdex200 column (Amersham Biosciences). The purity of the final products was above 98% as estimated by the size exclusion chromatography and SDS–polyacrylamide gel electrophoresis. The protein concentration was determined according to the Bradford method [34] using bovine serum albumin as a standard.

### 2.3. Enzyme assay

The enzymatic activity was determined by the methylene blue assay as described before [35]. Methylene blue buffer was prepared by dissolving 1.2 mg methylene blue into 100 ml Mops buffer (0.1 mM Mops-KOH, 2 mM EDTA, pH 7.5) and the absorbance of the Mops buffer at 688 nm was adjusted to 0.6 ± 1%. The standard reaction buffer contained 1.5 mM $MgCl_2$, 100 mM KCl , 0.1 U of Rnasin, 20 mM HEPES-KOH (pH 7.0), 0.2 mM EDTA, 0.25 mM DTT, 10% (v/v) glycerol, and 0.1% BSA. The final reaction volume was 50 μl, and the reaction was performed at 30 °C for 8 min. The reaction was terminated by mixing the reaction solution with 950 μl methylene blue buffer and the mixed solution was incubated at 30 °C for another 15 min in the dark in a water bath. Then the absorbance at 662 nm of 1 ml sample was measured on an Ultrospec 4300 pro UV/visible spectrophotometer at 30 °C in the dark.

### 2.4. Thermal inactivation

The apoenzyme was pre-equilibrated with different metal ions in 20 mM Tris–HCl buffer (pH 8.0) containing 100 mM KCl, 0.5 mM DTT, 0.2 mM EDTA, and 20% (v/v) glycerol at 4 °C. Then 100 μl solution with a final enzyme concentration of 0.6 μM was incubated at different temperatures controlled by a water bath. For temperature-dependence studies, the solution was incubated for 20 min at the given temperature. For time-course studies, samples were taken at different time periods. Then the samples were cooled on ice, and the residual activity was determined using the methylene blue assay described above. The data were normalized by taking the activity of the samples incubated at 25 °C as 100%. The errors were calculated from at least three repetitions.

### 2.5. Thermal unfolding and aggregation

The thermal unfolding of PARN was monitored by recording the circular dichroism (CD) spectra on a Jasco 725 spectrophotometer using a 1 mm pathlength cell in the far ultraviolet region at different temperatures. The final concentrations of the proteins were ~1.4 μM (0.15 mg/ml). The preparation of the samples was the same as those for thermal inactivation studies. The resultant CD spectra were obtained by the subtraction of the control. The time-course thermal aggregation of PARN at 61 °C was evaluated by the turbidity at 400 nm measured on an Ultraspec 4300 pro UV/Visible spectrophotometer from Amersham Pharmacia Biotech (Uppsala, Sweden). The protein concentration for aggregation studies was ~3.7 μM (0.4 mg/ml).

## 3. Results and discussion

### 3.1. Effect of $Mg^{2+}$ on PARN thermal inactivation

As mentioned above, the concentration of the free $Mg^{2+}$ in various cell types ranges from 0.3 to 3 mM [24,32]. To investigate the effect of the coordination of $Mg^{2+}$ in the active site on the stability of PARN, thermal inactivation was performed by incubating the enzyme at given temperatures varying from 25 °C to 65 °C for 20 min followed by the measurement of the residual activity. As shown in Fig. 1, in the absence of $Mg^{2+}$, the activity of PARN decreased continuously with the increasing of the temperature, and the enzyme was totally inactivated at temperatures above 49 °C. With the addition of 3 mM $Mg^{2+}$, PARN could retain most of its activity (above 90%) at moderate temperatures, followed by an abrupt decrease of its activity from 45 °C to 60 °C. By fitting the data in Fig. 1 into a two-state model, it was found that the midpoints of the temperature, where the enzyme lost 50% of its activity were about 53 °C and 42 °C for the samples with and without 3 mM $Mg^{2+}$, respectively. These results clearly indicated that the addition of 3 mM $Mg^{2+}$ could effectively enhance the thermal stability of PARN. Similar protective effects of $Mg^{2+}$ was also observed in other enzymes (for example [27,30,31]).

To further characterize the effect of $Mg^{2+}$ concentration on the stabilization of PARN, the enzyme was incubated with different concentrations of $Mg^{2+}$, and the time-course inactivation by heating the enzyme at 49 °C was measured (Fig. 2). The inactivation of PARN at high temperatures could be well-fitted to a first-order process. As the concentration of $Mg^{2+}$ was increased from 0 to 3 mM, the inactivation rate gradually slowed down, and meanwhile the residual activity increased significantly. However, only minor differences could be observed when the $Mg^{2+}$ concentration was increased from 3 to 6 mM, which suggested that a physiological concentration of $Mg^{2+}$ was efficient for the stabilization of PARN. The temperature dependence of the inactivation rate (Fig. 3) followed a first-order process. In the absence of $Mg^{2+}$, a considerable loss of the activity occurred at temperatures around body temperature. In contrast, in the presence of 3 mM $Mg^{2+}$, the inactivation rate was remarkably slower, particularly at high temperatures (Fig. 3C).

### 3.2. Effect of divalent metal ions on PARN thermal inactivation

The effects of the coordination of different metal ions on PARN activity were determined by the activity assay. As shown in Fig. 4A, of the four divalent ions, $Mg^{2+}$ was the best for PARN activity. In the presence of 3 mM $Mn^{2+}$, $Ca^{2+}$, and $Co^{2+}$, the activity gradually decreased about 5-, 15-, and 78-fold, respectively. Considering that the different substrates were used in the activity assay, our results were consistent with those in the literature [22], and suggested that although the active site of PARN could accommodate various kinds of divalent metal ions, $Mg^{2+}$ is the most suitable one for the

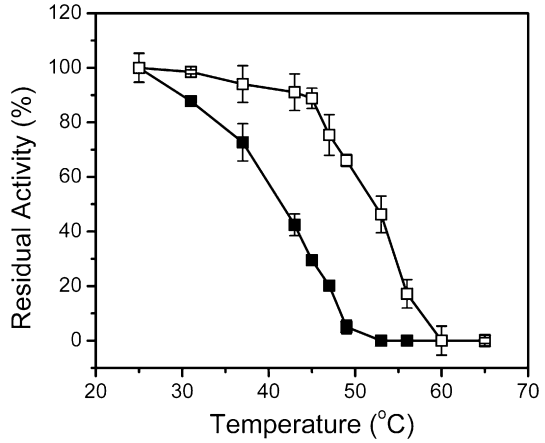


Fig. 1. Effect of $Mg^{2+}$ on the thermal inactivation of PARN. The enzyme was dissolved in 20 mM Tris–HCl buffer containing 100 mM KCl and 20% (v/v) glycerol, pH 8.0, in the presence (open squares) or absence (closed squares) of 3 mM $Mg^{2+}$. The solutions were incubated at the given temperatures for 20 min, cooled on ice, and then the activity was measured at 37 °C. The final enzyme concentration was 0.6 μM. The data were normalized by taking the activity of the samples incubated at 25 °C as 100%. The errors were calculated from at least three repetitions.

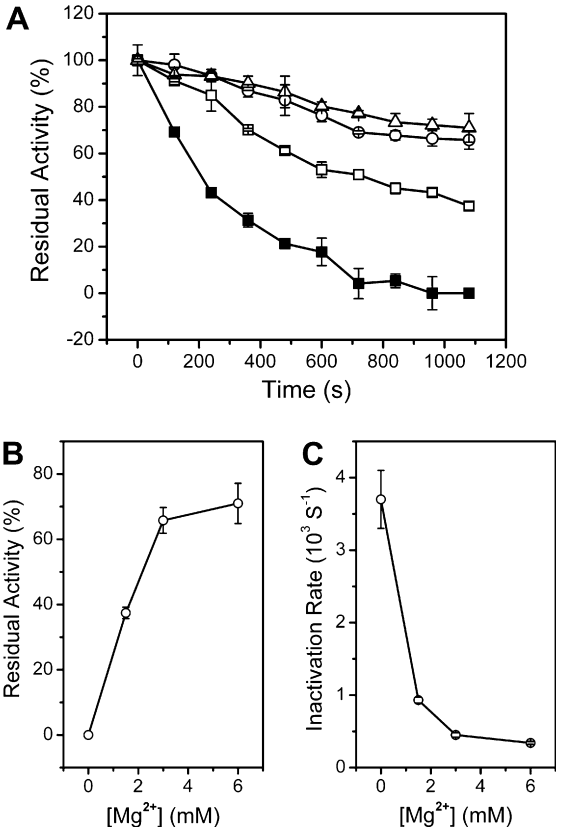


Fig. 2. Concentration-dependent effect of $Mg^{2+}$ on the thermal stability of PARN. (A) Time-course study of PARN inactivation in the presence of 0 (closed squares), 1.5 (open squares), 3 (open circles) or 6 mM (open triangles) $Mg^{2+}$ at 49 °C. (B) Dependence of the residual activity of PARN incubated at 49 °C for 20 min on $Mg^{2+}$ concentration. (C) Dependence of the inactivation rate at 49 °C on $Mg^{2+}$ concentration. The experimental details are the same as those described in Fig. 1.

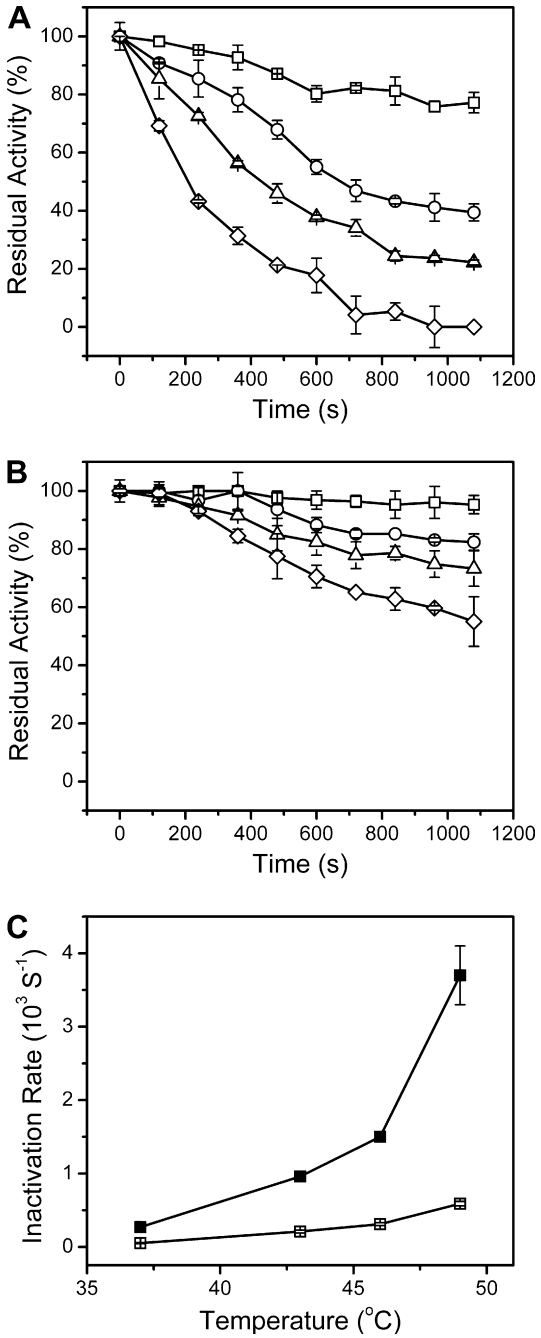


Fig. 3. Time-course study of the thermal inactivation of PARN in the presence (B) or absence (A) of 3 mM $Mg^{2+}$ at 37 °C (squares), 43 °C (circles), 46 °C (triangles) or 49 °C (diamonds). The dependence of inactivation rate on temperature is summarized in panel C.

catalytic function. To investigate whether the other metal ions had a stabilizing effect similar to that of $Mg^{2+}$ on the structural stability of PARN, thermal inactivation was performed by incubating the enzyme in the presence or absence of 3 mM $Mg^{2+}$, $Mn^{2+}$, $Ca^{2+}$, or $Co^{2+}$ at 49 °C. In the presence of 3 mM $Mn^{2+}$, $Ca^{2+}$, or $Co^{2+}$, the time-course inactivation curve was similar to the apo-enzyme, and the enzyme was totally inactivated after 20 min incubation at 49 °C. The inactivation rate (Fig. 4C) of the sample with the addition of 3 mM $Ca^{2+}$ or $Co^{2+}$ was almost the same as that of the apo-enzyme, while

3 mM $Mn^{2+}$ could moderately decrease the inactivation rate. A comparison of Fig. 4A and C indicated that the stabilizing effects of the metal ions were closely related to their impact on the catalytic activity of PARN. Among the four divalent ions studied here, only $Mg^{2+}$ had a significant protective effect on the thermal stability of PARN, which suggested that this stabilizing effect was unique to $Mg^{2+}$. These results also suggested that the effect of $Mg^{2+}$ was not due to the minor difference in the solution conditions induced by the addition of 3 mM metal ions, but due to the binding of $Mg^{2+}$ to the protein.

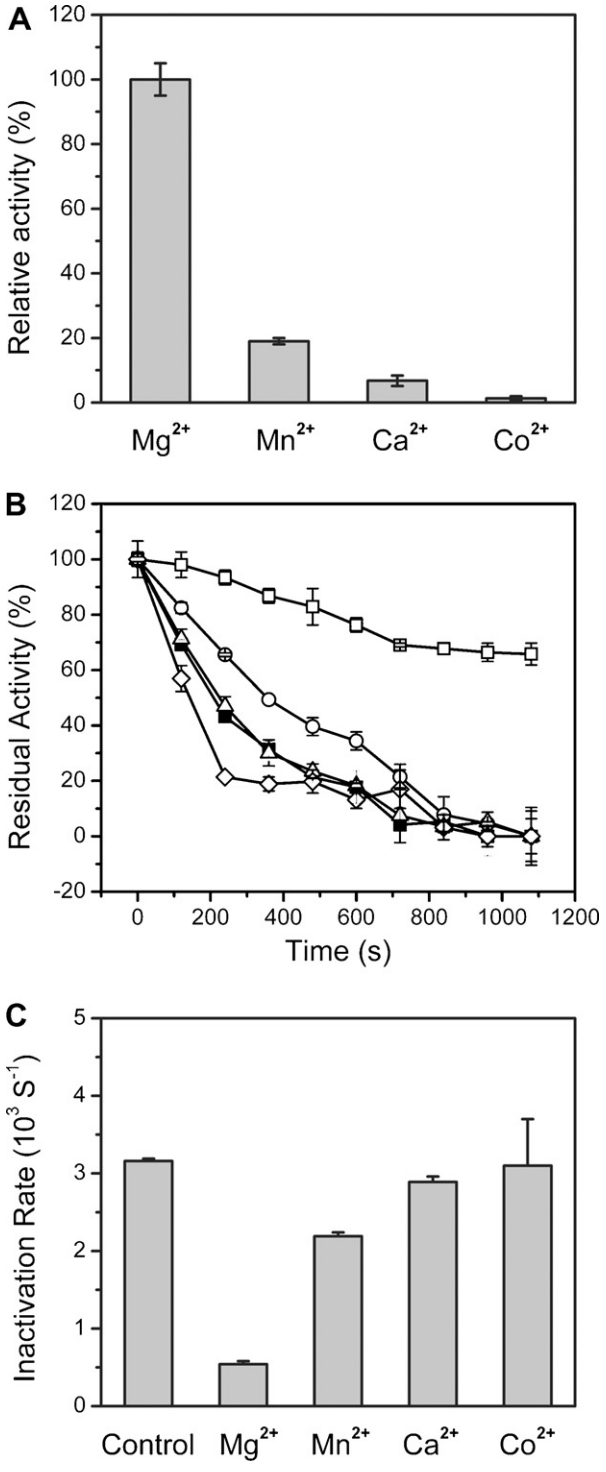


Fig. 4. Effect of divalent metal ions on the activity (A), time-course thermal inactivation (B) and inactivation rate at 49 °C (C). The residual activity in panel B was measured after incubating the enzyme solutions in the presence (open squares) or absence (closed squares) of 3 mM $Mg^{2+}$, $Mn^{2+}$ (circles), $Ca^{2+}$ (triangles), or $Co^{2+}$ (diamonds) at 49 °C.

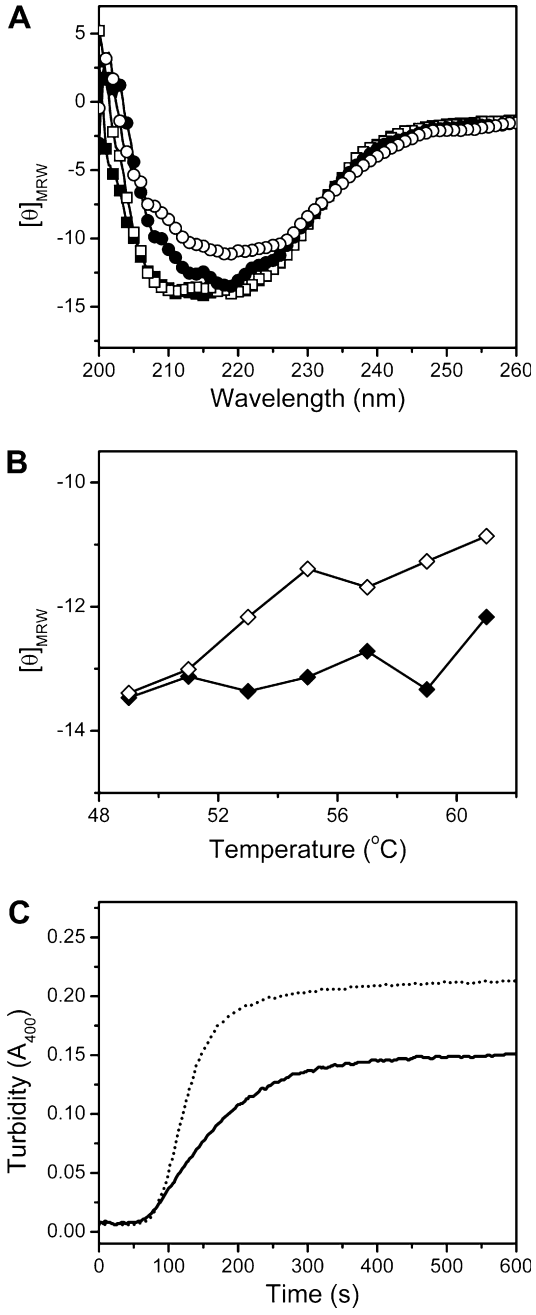


Fig. 5. Effects of 3 mM $Mg^{2+}$ on PARN thermal unfolding (A,B) and aggregation (C). (A) The CD spectra of PARN at 49 °C (squares) and 61 °C (circles) in the presence (open symbols) and absence (filled symbols) of 3 mM $Mg^{2+}$. The final concentration of PARN was 0.15 mg/ml. The CD data are presented as the mean residue ellipticity ($[\theta]_{MRW}$) expressed in [$10^3$ deg cm$^2$ dmol$^{-1}$]. (B) The transition curve of PARN thermal unfolding in the presence (open symbols) or absence (filled symbols) of $Mg^{2+}$ monitored by $[\theta]_{MRW}$ at 222 nm. (C) The time-course aggregation of PARN in the presence (dotted line) or absence (solid line) of $Mg^{2+}$ monitored by the absorbance at 400 nm. The final concentration of PARN was 0.4 mg/ml.

### 3.3. Effect of $Mg^{2+}$ on PARN thermal unfolding and aggregation

The above results clearly indicated that the existence of $Mg^{2+}$ could protect PARN against thermal inactivation. CD spectroscopy, which is a sensitive tool to evaluate secondary structure compositions and has been widely used in protein folding studies, was carried out to probe the effect of $Mg^{2+}$ on the overall structural stability of PARN. No significant difference was observed between the CD spectra of the apo- and the holo-enzyme recorded at temperatures below 51 °C (Fig. 5A). As seen in Fig. 1, the apo-enzyme was totally inactivated at about 50 °C, while the holo-enzyme could remain most of its activity at temperatures below 50 °C. The CD data further suggested that the unfolding of the flexible active site proceeded the unfolding of the overall structure, and this conclusion is quite consistent with those found in other enzymes [36,37]. The shape of the CD spectra of both samples revealed two negative peaks at around 208 and 222 nm, which is consistent with the fact that the protein contains considerable amounts of helical structures [17]. At higher temperatures, the ellipticity at 222 nm of both samples decreased (Fig. 5B), which suggested that the native secondary structures of PARN began to unfold. It is worth noting that the change of the sample in the presence of 3 mM $Mg^{2+}$ was much greater than that of the sample without $Mg^{2+}$. This suggested that the existence of $Mg^{2+}$ could promote the thermal unfolding of PARN, which is quite different from the thermal inactivation results. The CD spectra recorded at 61 °C showed a dominant negative peak at about 218 nm, which indicated an increase of the β-sheet structures when the samples were incubated at this temperature. This phenomenon suggested that aggregates might form at high temperatures, as has been observed in many enzymes (for example [38]). Thus the time-course thermal aggregation of PARN at 61 °C was further studied by the turbidity monitored by the absorbance at 400 nm (Fig. 5C). The lag time of both samples was similar (about 70 s). However, the aggregation rate of the sample in the presence of 3 mM $Mg^{2+}$ was much faster than the apo-enzyme, and the highest absorbance at 400 nm for the sample in the presence of $Mg^{2+}$ was much higher than the apo-enzyme. This observation was consistent with the CD results, and suggested that the existence of $Mg^{2+}$ could promote the formation of aggregates by increasing the aggregation rate. One possible explanation of this destabilizing effect of $Mg^{2+}$ at high temperatures is that the binding of $Mg^{2+}$ to the protein might minimize the negative charge repulsion during the formation of intermolecular interactions in aggregates. Such dissimilar effects of $Mg^{2+}$ on enzymatic and structural stability were also observed during the reactivation and refolding of creatine kinase [28].

### 3.4. Conclusions

As a member of the DEDD exonuclease family, PARN contains two divalent metal ions in its active site, and $Mg^{2+}$ is the most suitable divalent metal ion for the catalytic function of degrading mRNA poly(A) tails. In this research, it was found that in the presence of $Mg^{2+}$, the midpoint of PARN thermal inactivation increased about 10 °C compared to the apo-enzyme (Fig. 1), and the inactivation rate was gradually slowed down (Figs. 2,3). The protective effects of $Mg^{2+}$ against PARN thermal inactivation was unique to $Mg^{2+}$ in an a concentration-dependent manner, and the PARN residual activity after heat treatment reached a platform at $Mg^{2+}$ concentrations above 3 mM. However, the thermal unfolding and aggregation was significantly promoted by the addition of $Mg^{2+}$. These results revealed that $Mg^{2+}$ might have dual effects on PARN stability: protecting the active site against denaturation, but destabilizing the overall structural stability of the protein. Considering that the intracellular concentration of free $Mg^{2+}$ can be affected by the binding of some factors to the specific cell receptors [32,39], the dual functions of $Mg^{2+}$ suggested that $Mg^{2+}$ might be one of the regulators in the activity and stability of PARN in the cells. The present research provided a starting point for further research in understanding the unique effects of $Mg^{2+}$ on the catalysis and regulation of PARN as well as other members in the DEDD family.

*Acknowledgements:* The authors thank Professor Anders Virtanen (Department of Cell and Molecular Biology, Uppsala University, Sweden) for kindly providing the plasmid of pE33 PARN. This investigation was supported by Grants 30270318 (to H.-M. Zhou) and 30500084 (to Y.-B. Yan) from the National Natural Science Foundation of China, and grant JCqn2005040 (to Y.-B. Yan) from the Tsinghua Basic Research Foundation.

## References

[1] Mitchell, P. and Tollervey, D. (2000) mRNA stability in eukaryotes. Curr. Opin. Genet. Develop. 10, 193–198.
[2] Meyer, S., Temme, C. and Wahle, E. (2004) Messenger RNA turnover in eukaryotes: pathways and enzymes. Crit. Rev. Biochem. Mol. Biol. 39, 197–216.
[3] Wilusz, C.J., Wormington, M. and Peltz, S.W. (2001) The cap-to-tail guide to mRNA turnover. Nat. Rev. Mol. Cell Biol. 2, 237–246.
[4] Caponigro, G. and Parker, R. (1996) Mechanisms and control of mRNA turnover in *Saccharomyces cerevisiae*. Microbiol. Rev. 60, 233–249.
[5] Reverdatto, S.V., Dutko, J.A., Chekanova, J.A., Hamilton, D.A. and Belostotsky, D.A. (2004) MRNA deadenylation by PARN is essential for embryogenesis in higher plants. RNA 10, 1200–1214.
[6] Copeland, P.R. and Wormington, M. (2001) The mechanism and regulation of deadenylation: identification and characterization of *Xenopus* PARN. RNA 7, 875–886.
[7] Wells, S.E., Hillner, P.E., Vale, R.D. and Sachs, A.B. (1998) Circularization of mRNA by eukaryotic translation initiation factors. Mol. Cell 2, 135–140.
[8] Åström, J., Åström, A. and Virtanen, A. (1992) Properties of a HeLa cell 3′ exonuclease specific for degrading poly(A) tails of mammalian mRNA. J. Biol. Chem. 267, 18154–18159.
[9] Martìnez, J., Ren, Y.G., Thuresson, A.C., Hellma, U., Åström, J. and Virtanen, A. (2000) 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. 275, 24222–24230.
[10] Virtanen, A. and Åström, J. (1997) Function and characterization of poly(A)-specific 3′ exoribonuclease. Prog. Mol. Subcell. Biol. 18, 199–220.
[11] Körner, C.G. and Wahle, E. (1997) Poly(A) tail shortening by a mammalian poly(A)-specific 3′-exoribonulease. J. Biol. Chem. 272, 10448–10456.
[12] Dehlin, E., Wormington, M., Körner, C.G. and Wahle, E. (2000) Cap-dependent deadenylation of mRNA. EMBO J. 19, 1079–1086.
[13] Seal, R., Temperley, R., Wilusz, J., Lightowlers, R.N. and Chrzanowska-Lightowlers, Z.M.A. (2005) Serum-deprivation stimulates cap-binding by PARN at the expense of eIF4E, consistent with the observed decrease in mRNA stability. Nucl. Acids Res. 33, 376–387.
[14] Körner, C.G., Wormington, M., Muckenthaler, M., Schneider, S., Dehlin, E. and Wahle, E. (1998) The deadenylating nuclease (DAN) is involved in poly(A) tail removal during the meiotic maturation of *Xenopus* oocytes. EMBO J. 17, 5427–5437.

[15] Moser, M.J., Holley, W.R., Chatterjee, A. and Mian, I.S. (1997) The proof-reading domain ofEscherichia coli DNA polymerase I and other DNA and/or RNA exonuclease domains. Nucl. Acids Res. 25, 5110–5118.
[16] Ren, Y.-G., Martínez, J. and Virtanen, A. (2002) Identification of the active site of poly(A)-specific ribonuclease by site-directed mutagenesis and $Fe^{2+}$-mediated cleavage. J. Biol. Chem. 277, 5982–5987.
[17] Wu, M.S., Reuter, M., Lilie, H., Liu, Y.Y., Wahle, E. and Song, H.W. (2005) Structural insight into poly(A) binding and catalytic mechanism of human PARN. EMBO J. 24, 4082–4093.
[18] Zuo, Y. and Deutscher, M.P. (2001) Exoribonuclease superfamilies: structural analysis and phylogenetic distribution. Nucl. Acids Res. 29, 1017–1026.
[19] Mian, I.S. (1997) Comparative sequence analysis of ribonucleases HII, III, II PH and D. Nucl. Acids Res. 25, 3187–3195.
[20] Bernad, A., Blanco, L., Lazaro, J., Martin, G. and Salas, M. (1989) A conserved 3′–5′ exonuclease active site in prokaryotic and eukaryotic DNA polymerases. Cell 59, 219–228.
[21] Steitz, T.A. and Steitz, J.A. (1993) A general two-metal-ion mechanism for catalytic RNA. Proc. Natl. Acad. Sci. USA 90, 6498–6502.
[22] Ren, Y.-G., Kirsebom, L.A. and Virtanen, A. (2004) Coordination of divalent metal ions in the active site of poly(A)-specific ribonuclease. J. Biol. Chem. 279, 48702–48706.
[23] Beese, L.S. and Steitz, T.A. (1991) Structural basis for the 3′–5′ exonuclease activity of *Escherichia coli* DNA polymerase I: a two metal ion mechanism. EMBO J. 10, 25–33.
[24] Hartwig, A. (2001) Role of magnesium in genomic stability. Mutat. Res./Fundamen. Mol. Mech. Mutagen. 475, 113–121.
[25] Zhang, Y.X., Zhu, Y., Xi, H.W., Liu, Y.L. and Zhou, H.M. (2002) Refolding and reactivation of calf intestinal alkaline phosphatase with excess magnesium ions. Int. J. Biochem. Cell Biol. 34, 1241–1247.
[26] Cate, J.H., Hanna, R.L. and Doudna, J.A. (1997) A magnesium ion core at the heart of a ribozyme domain. Nat. Struct. Mol. Biol. 4, 553–558.
[27] Menendez, M., Rivas, G., Diaz, J.F. and Andreu, J.M. (1998) Control of the structural stability of the tubulin dimer by one high affinity bound magnesium ion at nucleotide N-site. J. Biol. Chem. 273, 167–176.
[28] Park, Y.D. and Zhou, H.M. (2000) Effect of $Mg^{2+}$ during reactivation and refolding of guanidine hydrochloride-denatured creatine kinase. J. Protein Chem. 19, 193–198.
[29] Cao, Z.F., Luo, W. and Zhou, H.M. (1999) Effect of $Mg^{2+}$ on the thermal inactivation and unfolding of creatine kinase. Int. J. Biochem. Cell Biol. 31, 1307–1313.
[30] Kanaya, S., Oobatake, M. and Liu, Y.Y. (1996) Thermal stability of *Escherichia coli* ribonuclease HI and its active site mutants in the presence and absence of the $Mg^{2+}$ ion – proposal of a novel catalytic role for $Glu^{48}$. J. Biol. Chem. 271, 32729–32736.
[31] Janeway, C.M.L., Xu, X., Murphy, J.E., Chaidaroglou, A. and Kantrowitz, E.R. (1993) Magnesium in the active site of *Escherichia coli* alkaline phosphatase is important for both structural stabilization and catalysis. Biochemistry 32, 1601–1609.
[32] Altura, B.M., Durlach, J. and Seelig, M.S. (1987) Magnesium in Cellular Processes and Medicine, Karger AG, Basel, Switzerland.
[33] Virtanen, A., Martinez, J. and Ren, Y.G. (2001) Purification of poly(A)-specific ribonuclease. Method. Enzymol. 324, 303–309.
[34] Bradford, M.M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–252.
[35] Cheng, Y., Liu, W.-F., Yan, Y.-B. and Zhou, H.-M. (2006) A nonradioactive assay for poly(A)-specific ribonuclease activity by methylene blue colorimetry. Protein Pept. Lett. 13, 125–128.
[36] Zhou, H.-M. and Tsou, C.-L. (1986) Comparison of activity and conformation changes during refolding of urea-denatured creatine kinase. Biochim. Biophys. Acta 869, 69–74.
[37] Tsou, C.-L. (1998) Active site flexibility in enzyme catalysis. Ann. N.Y. Acad. Sci. 864, 1–8.
[38] He, H.-W., Zhang, J., Zhou, H.-M. and Yan, Y.-B. (2005) Conformational change in the C-terminal domain is responsible for the initiation of creatine kinase thermal aggregation. Biophys. J. 89, 2650–2658.
[39] Sun, G. and Budde, R.J.A. (1997) Requirement for an additional divalent metal cation to activate protein tyrosine kinases. Biochemistry 36, 2139–2146.