Symposium date: 8th September 2008
Nucleic Acids Symposium Series No. 52 519–520
doi:10.1093/nass/nrn263

# Molecular crowding effect on metal ion binding properties of the hammerhead ribozyme

**Shu-ichi Nakano[1], Yuichi Kitagawa[2], Hisae Tateishi Karimata[2,#] and Naoki Sugimoto[1,2,*]**

[1]Frontier Institute for Biomolecular Engineering Research (FIBER), and [2]Department of Chemistry, Faculty of Science and Engineering, Konan University, 8–9–1 Okamoto, Higashinada–ku, Kobe 658–8501, Japan

## ABSTRACT

**Although metal ion is essential for the DNA and RNA folding, there are a limited number of reports describing the influence of molecular environments on the metal ion binding. Here, we investigated the binding properties of $Mg^{2+}$ and $Na^{+}$ toward the hammerhead ribozyme in the presence of PEG [poly(ethylene glycol)] as a cosolute that changes the solvent property of the reaction. PEG8000 (PEG with the average molecular weight of 8000) increased the reaction rate at lower $Mg^{2+}$ concentrations but not at higher concentrations. We also found that PEG8000 unchanged the number of $Mg^{2+}$ bound while the binding cooperativity of $Na^{+}$ in the reaction without divalent metal ion was altered. The change of $Na^{+}$ condensation by cosolute has also been reported for diffusely bound $Na^{+}$ to Watson-Crick base pairs. It is thus supposed that diffusely bound $Na^{+}$ stabilizes the ribozyme active form in the absence of divalent metal ion. This study provides insights into the RNA-metal ion interaction and the hammerhead ribozyme activity under the molecular crowding condition occurred in a cell and on biosensor devices.**

## INTRODUCTION

Natural ribozymes catalyze the site-specific RNA cleavage, and their folding into an active form and the catalysis are intimately related. The ribozyme activity is facilitated by metal ion that stabilizes the catalytic active form and may also play a direct role in the catalysis (1). The hammerhead ribozyme is one of the smallest ribozymes, and its truncated ribozyme motif, identified as a minimum catalytic active sequence, cleaves the substrate RNA at a high and moderate $Mg^{2+}$ concentrations (2). The crystal structure and the molecular simulation studies for the hammerhead ribozyme (3.4) suggest the general acid-base catalysis by the nucleotide bases of G12 and G8 and the $Mg^{2+}$ binding near the catalytic site as a structural role rather than the catalytic role (Fig. 1). The model is consistent with the observations that the ribozyme activity emerges even without divalent metal ion when there exists a high concentration of monovalent ion (5-7).

In this study, we investigated the binding properties of $Mg^{2+}$ and $Na^{+}$ to the hammerhead ribozyme under the molecular crowding of PEG that changes the solvent property. In a living cell, the molecular environment is distinct from an aqueous solution, for example, the number of free water molecules is greatly reduced (8) and there is significant steric crowding arise from large-sized biomolecules, called the molecular crowding. The experimental system using a PEG-containing solution allows quantitative investigations of biomolecules under the molecular crowding (9). Our previous study showed that PEG increased the reaction rate of the truncated hammerhead ribozyme (10), while Watson-Crick base pairs of an oligomer duplex were destabilized by PEG (11-13). This study reveals the binding properties of $Mg^{2+}$ and $Na^{+}$ to the hammerhead ribozyme in the presence of PEG, and provides insights into the metal ion binding to an RNA under the molecular crowding condition.

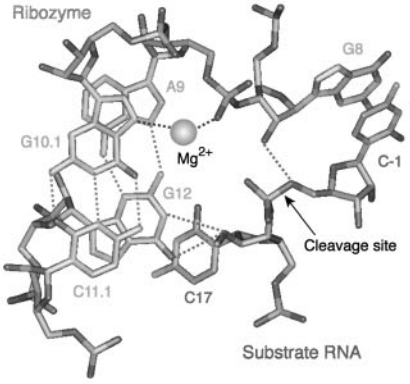


**Fig. 1** Interactions near the active site of the hammerhead ribozyme suggested from the crystal structure (3).

## MATERIALS AND METHODS

A *trans*-acting ribozyme of the truncated hammerhead ribozyme motif derived from *Schistosoma mansoni* were prepared by the run-off transcription using the T7 RNA polymerase and the template DNA duplex containing the T7 RNA polymerase promoter sequence. The substrate RNA labeled with FAM (6-carboxyl fluorescein) at the 5' end was synthesized chemically. The reaction was carried out at pH 7.0 and 37 ˚C under the single turnover condition. After the reaction, aliquots of the reaction were loaded on a 20% polyacrylamide gel containing 7 M urea.

The observed rate constant ($k_{obs}$) of the reaction was determined by the fit of the plots of the fraction of a cleaved fragment at a desired site versus time to a single-exponential equation.

## RESULTS AND DISCUSSION

In our previous study, we found accelerations of the hammerhead ribozyme-catalyzed RNA hydrolysis rate carried out at 10 mM $MgCl_2$ in the cosolute-containing solutions such as PEG8000 (PEG with the average molecular weight of 8000) (10). Here, we obtained the $MgCl_2$ and NaCl concentration profiles in the presence and absence of 20 wt% PEG8000. The reaction rate was increased by adding PEG8000 in the reaction buffers containing a lower $Mg^{2+}$ concentration, and an efficient ribozyme activity was observed even with a relatively low $Mg^{2+}$ concentration (<1 mM). However, the rate acceleration by PEG8000 was not observed with higher $Mg^{2+}$ concentrations. The log $k_{obs}$-log [$Mg^{2+}$] plot showed that both solutions gave the same plateau of $k_{obs}$=3.4 $min^{-1}$ at 37 °C and the slope close to 1 in a low $Mg^{2+}$ concentration range. It was thus proposed that PEG8000 unchanged the maximum reaction rate and the number of $Mg^{2+}$ required for the reaction.

We also investigated the reaction using high and moderate concentrations of NaCl without $MgCl_2$. As observed for the reaction with $Mg^{2+}$, the monophasic kinetic traces were obtained and PEG8000 increased the reaction rate, leading to an efficient ribozyme activity even at moderate $Na^+$ concentrations. The log $k_{obs}$-log [$Na^+$] plot for the reactions with and without PEG8000 showed the linearity over the examined NaCl concentration range but their slopes were obviously different. The data suggests a reduction of the $Na^+$ condensation around the RNAs when adopting the catalytic active conformation. It is mentioned that reduction of the sodium ion condensation by PEG8000 has also been reported for diffusely bound $Na^+$ to Watson-Crick base pairs (12,13). Therefore, it is supposed that the ribozyme active form is stabilized by diffusely bound $Na^+$, and the molecular crowding study using PEG can be used for investigations of the metal ion binding to an RNA.

## CONCLUSION

It has been regarded that the truncated hammerhead ribozyme exhibits the activity only at a moderate or high $Mg^{2+}$ concentration or a very high monovalent ion concentration. However, we observed the catalytic activity even near the physiological salt concentrations under the molecular crowding by PEG. We also found that diffusely bound $Na^+$ could stabilize the ribozyme active structure. Because the ribozyme exhibits the catalytic activity by folding into a defined tertiary structure, cosolute effects on the RNA folding were also elucidated from our results.

## ACKNOWLEDGMENT

This work was supported in part by Grants-in-Aid for Scientific Research and the "Academic Frontier" Project (2004-2009) from the Ministry of Education, Culture, Sports, Science and Technology, Japan, and the Hirao Taro Foundation of the Konan University Association for Academic Research.

## REFERENCES

1. Bevilacqua, P. C., Brown, T. S., Nakano, S. and Yajima, R. (2004) *Biopolymers*, **73**, 90-109.
2. Uhlenbeck, O. C. (1987) *Nature*, **328**, 596-600.
3. Martick, M. and Scott, W. G. (2006) *Cell*, **126**, 309-320.
4. Lee, T. S., Lopez, C .S., Giambasu, G. M., Martick, M., Scott, W. G. and York, D. M. (2008) *J. Am. Chem. Soc.*, **130**, 3053-3064.
5. Murray, J. B., Seyhan, A. A., Walter, N. G., Burke, J. M. and Scott, W. G. (1998) *Chem. Biol.*, **5**, 587-595.
6. Curtis, E. A. and Bartel, D. P. (2001) *RNA*, **7**, 546-552.
7. Inoue, A., Takagi, Y. and Taira, K. (2004) *Nucleic Acids Res.*, **32**, 4217-4223.
8. Luby-Phelps, K. (2000) *Int. Rev. Cytol.*, **192**, 189-221.
9. Zimmerman, S. B. and Minton, A. P. (1993) *Annu. Rev. Biophys. Biomol. Struct.*, **22**, 27-65.
10. Karimata, H., Nakano, S. and Sugimoto, N. (2006) *Nucleic Acids Symp. Ser.*, **50**, 81-82.
11. Nakano, S., Karimata, H., Ohmichi, T., Kawakami, J. and Sugimoto, N. (2004) *J. Am. Chem. Soc.*, **126**, 14330-14331.
12. Karimata, H., Nakano, S. and Sugimoto, N. (2007) *Bull. Chem. Soc. Jpn.*, **80**, 1987-1994.
13. Nakano, S., Wu, L., Oka, H., Karimata, H. T., Kirihata, T., Sato, Y., Fujii, S., Sakai, H., Kuwahara, M., Sawai, H. and Sugimoto, N. (2008) *Mol. BioSys.*, **4**,579-588.

* Corresponding author. E-mail: sugimoto@konan-u.ac.jp

# Current Address: Department of Chemistry, University of Illinois at Urbana-Champaign, USA.