The roles of cosolutes on the hammerhead ribozyme activity

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ABSTRACT

The hammerhead ribozyme is often used for gene regulations in a cell. One of the major differences between in vitro and in cell conditions is the molecular crowding. However, the influence of crowding conditions on the ribozyme activity is still unclear. Here, we investigated the activity of the hammerhead ribozyme under molecular crowding condition by ethylene glycol (EG), poly (ethylene glycol) (PEG), dextran, and Ficoll. These cosolutes enhanced the hammerhead ribozyme activity by 2–7-times, and larger-sized PEGs exhibited the greater activity. More importantly, the rate constant at 1 mM Mg2+ and 37°C increased about 1000-times and 500-times upon the addition of 20 wt% PEG8000 and PEG2000, respectively. Additionally, the ribozyme still retained the cleavage activity even at 50°C in the presence of PEG8000. Our results suggest that the ribozyme activity can be enhanced by the addition of the crowding reagent even at a low magnesium ion concentration and even at a high temperature.

INTRODUCTION

The hammerhead ribozyme sequence is one of the small catalytic RNA motifs found in plant RNA viruses, satellite RNA, viroids, and repetitive satellite DNAs.1 The ribozyme catalyzes the cleavage and ligation of the RNA during rolling circle replication. The minimal conserved motif of hammerhead ribozyme sequence derived from natural RNA sequences consists of three helices that intercalate at the highly conserved catalytic core.2 The presence of this motif is sufficient to support the RNA cleavage in the presence of high Mg2+ concentrations in vitro. Although the free Mg2+ concentration is not more than 1 mM at the intracellular condition, the ribozyme activity remains high.3,4 One of the major differences between in vitro and in vivo conditions is the macromolecule concentration because cells generally contain macromolecules occupying 20–40% of the total volume (called the molecular crowding).3,6 In vitro solutions containing high concentrations of water-soluble cosolutes such as EG, PEG, dextran, and Ficoll are often used as a model system of the molecular crowding.7,8 We have previously found that molecular crowding is one of the most important environmental factors for the nucleotide conformation and its stability.10–13 However, the influence of the crowding conditions on the ribozyme activity is still unclear. Here, we investigated the activity of a hammerhead ribozyme (Figure 1) under the molecular crowding condition induced by cosolute.

MATERIALS AND METHODS

The ribozyme RNA was enzymatically prepared, and the FAM (6-carboxy 1 fluorescein)–labeled substrate RNA was chemically prepared. The RNAs were purified by 20% PAGE (polyacrylamide gel electrophoresis) containing 7 M urea, followed by the ethanol precipitation. The cosolutes were purchased from Wako, Sigma, Amersham Bioscience, or TCI and used without further purification. The substrate strand was annealed with the ribozyme strand in 50 mM NaCl, 50 mM Tris-HCl (pH 7.0), and 0.1 mM Na2EDTA with or without the cosolutes by heating to 60°C for 2 min and then cooling to 37°C. To assure the single-turnover condition, the ribozyme RNA was used in 20-times excess over the substrate RNA. The cleavage reaction was initiated by the addition of MgCl2. An aliquot of the reaction solution was removed and quenched in 80% formamide, 10 mM Na2EDTA, and 0.2% blue dextran, and immediately frozen on ice. The reaction products were quantified by 20% PAGE containing 7 M urea using FUJIFILM FLA-5100. The observed rate constant was obtained from the plot of a fraction of the substrate cleaved versus time by fitting to the equation, \( F = F_0 \left(1 - e^{-kt}\right) \) using KaleidaGraph.
4.0, where \( F \) is the fraction of the substrate cleaved, \( F_{\text{max}} \) is the maximum fraction cleaved, \( t \) is time, and \( k \) is the observed rate constant for cleavage.

RESULTS AND DISCUSSION

The ribozyme activity to cleave the substrate RNA (Figure 1) was measured in the absence and presence of 20 wt% cosolute of EG, PEG with an average molecular weight (MW) of 200 or 8000 (PEG200 or PEG8000, respectively), dextran10 (MW = 1 \( \times \) 10^6), dextran70 (MW = 7 \( \times \) 10^6), or Ficoll70 (MW = 7 \( \times \) 10^6). Figures 2 shows the cleavage yield for the ribozyme reaction performed at 10 mM MgCl\(_2\) and 37°C for 35 s. The amount of the cleaved DNA increased when the cosolutes were added to the solution. The rate constant \( (k) \) for the substrate RNA cleavage indicated that PEG8000 and PEG200 increased the reaction rate by 6.6- and 3.6-times, respectively, and EG, dextran10, dextran70, and Ficoll70 showed 2.0-2.8-times greater rate constant than that obtained in a solution without the cosolutes, where the reaction was monophasic and the product yield approached ~80% both in the absence and presence of the cosolutes. Although lower ribozyme activity was observed at 1 mM MgCl\(_2\) in the absence of cosolutes, the rate constant at 1 mM Mg\(^{2+}\) increased about 1000-times and 500-times upon the addition of 20 wt% PEG8000 and PEG200, respectively. In the presence of PEG8000, the dissociation constant for Mg\(^{2+}\) was smaller than that in the absence of PEG8000. This observation suggests that the cosolutes reduces the requirement of Mg\(^{2+}\) concentration for the ribozyme reaction. Moreover, although the ribozyme activity decreased generally at higher temperatures due to denaturation of the active form, the cosolutes appeared to protect from the thermal denaturation. The cosolutes except EG accelerated the reaction by >7-times at 50°C, and the ribozyme activity remained efficient even at 50°C when PEG8000 was added. The results suggest that the molecular crowding enhances the stability of the active structure of the ribozyme structure.

CONCLUSION

The ribozyme activity was enhanced by addition of the cosolutes. We also found the decrement of the Mg\(^{2+}\) concentration required for an effective ribozyme activity and the protection of the ribozyme from the thermal denaturation when the cosolutes were presented. Therefore, the cosolutes can play the role to proceed the ribozyme reaction at a low magnesium ion concentration and even at a high temperature.

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REFERENCES