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Structural basis for recognition of a kink-turn motif by an archaeal homologue of human RNase P protein Rpp38

Kosuke Oshima [a], Yosuke Kakiuchi [a], Yoshikazu Tanaka [b, c], Toshifumi Ueda [a], Takashi Nakashima [a], Makoto Kimura [a, *], Min Yao [b]

[a] Laboratory of Biochemistry, Department of Bioscience and Biotechnology, Graduate School, Faculty of Agriculture, Kyushu University, Fukuoka 812-8581, Japan
[b] Faculty of Advanced Life Science, Hokkaido University, Sapporo 060-0810, Japan
[c] Japan Science and Technology Agency, PRESTO, Sapporo 060-0810, Japan

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ABSTRACT

*Pho*Rpp38 in the hyperthermophilic archaeon *Pyrococcus horikoshii*, a homologue of human ribonuclease P (RNase P) protein Rpp38, belongs to the ribosomal protein L7Ae family that specifically recognizes a kink-turn (K-turn) motif. A previous biochemical study showed that *Pho*Rpp38 specifically binds to two stem-loops, SL12 and SL16, containing helices P12.1/12.2 and P15/16 respectively, in *P. horikoshii* RNase P RNA (*Pho*pRNA). In order to gain insight into the *Pho*Rpp38 binding mode to *Pho*pRNA, we determined the crystal structure of *Pho*Rpp38 in complex with the SL12 mutant (SL12M) at a resolution of 3.4 Å. The structure revealed that Lys35 on the β-strand (β1) and Asn38, Glu39, and Lys42 on the α-helix (α2) in *Pho*Rpp38 interact with characteristic G•A and A•G pairs in SL12M, where Ile93, Glu94, and Val95, on a loop between α4 and β4 in *Pho*Rpp38, interact with the 3-nucleotide bulge (G-G-U) in the SL12M. The structure demonstrates the previously proposed secondary structure of SL12, including helix P12.2. Structure-based mutational analysis indicated that amino acid residues involved in the binding to SL12 are also responsible for the binding to SL16. This result suggested that each *Pho*Rpp38 binds to the K-turns in SL12 and SL16 in *Pho*pRNA. A pull-down assay further suggested the presence of a second K-turn in SL12. Based on the present results, together with available data, we discuss a structural basis for recognition of K-turn motifs in *Pho*pRNA by *Pho*Rpp38.

1. Introduction

Ribonuclease P (RNase P) is an endoribonuclease that catalyzes the processing of 5′ leader sequences from tRNA precursors (pre-tRNA) and other noncoding RNAs in all living cells [for a review see refs. 1 and 2]. We previously found *via* reconstitution experiments that RNase P RNA (*Pho*pRNA) alone in the hyperthermophilic archaeon *Pyrococcus horikoshii* OT3 had no endoribonuclease activity, but *Pho*pRNA and five archaeal homologues, *Pho*Pop5, *Pho*Rpp21, *Pho*Rpp29, *Pho*Rpp30, and *Pho*Rpp38, of the human proteins hPop5, Rpp21, Rpp29, Rpp30, and Rpp38, respectively, reconstituted RNase P activity that exhibits enzymatic properties like those of the authentic enzyme isolated from *P. horikoshii* [3,4].

The fifth protein component *Pho*Rpp38 is homologous to the *Haloarcula marismortui* ribosomal protein L7Ae [5] and human spliceosomal protein 15.5 kDa [6], which are known to bind a kink-turn (K-turn) motif. The K-turn motif is a kink in the phosphodiester backbone that causes a sharp turn in the RNA helix, and its asymmetric internal loop (3-nucleotide bulge) is flanked on its 5′ side by a section of regular base pairing (the C stem) and on its 3′ side by two consecutive irregular AG and GA pairs (the N-C stem) [7,8]. Biochemical analyses indicated that *Pho*Rpp38 binds to two peripheral stem-loops, SL12 (A116-G201) and SL16 (G229-C276), including helices P12.1/12.2 and P15/16, respectively [4]. Based on the consensus sequence, we found possible K-turn motifs at positions 141–148 and 170–174 in SL12 and at positions 241–249 and

Abbreviations: ITC, isothermal temperature calorimetry; K-turn, kink-turn; *Pfu*pRNA, ribonuclease P RNA from *Pyrococcus furiosus*; *Pho*pRNA, ribonuclease P RNA from *P. horikoshii*; pre-tRNA, precursor tRNA; rmsd, root-mean-square deviation; RNase P, ribonuclease P; SL12, stem-loop containing helices P12.1 and P12.2; SL16, stem-loop containing helices P15 and P16.

* Corresponding author. Laboratory of Biochemistry, Department of Bioscience and Biotechnology, Faculty of Agriculture, Graduate School, Kyushu University, 6-10-1 Hakozaki, Higashi-ku, Fukuoka 812-8581, Japan.
E-mail address: mkimura@agr.kyushu-u.ac.jp (M. Kimura).

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259–264 in SL16, where they contain asymmetric internal loops with G-C rich pairs [9,10]. Recently, hydroxyl radical-mediated footprinting carried out by Lai *et al*. predicted the presence of two K-turn motifs in stem-loop structures containing helices P12 and P16 in *Pyrococcus furiosus* RNase P RNA (*Pfu*pRNA), which correspond to SL12 and SL16 in *Pho*pRNA [11]. However, nucleotides predicted for the K-turn in *Pfu*pRNA P12 are slightly distinct from those predicted to be folded into the K-turn in SL12, although nucleotides in *Pfu*pRNA P16 are in perfect agreement with those in SL16 in *Pho*pRNA [11]. That is, nucleotides at positions 182–186 in *Pfu*pRNA P12 which correspond to those at positions 181–185 in *Pho*pRNA were highly susceptible to hydroxyl radical cleavage [11], while nucleotides at positions 141–148 and 170–174 were predicted to be fold into the K-turn in *Pho*pRNA [9,10].

To clarify the binding mode of *Pho*Rpp38 to *Pho*pRNA, we have determined the crystal structure of *Pho*Rpp38 in complex with the SL12 fragment (SL12M) at a resolution of 3.4 Å. Moreover, structure-based mutational analysis indicated that amino acids involved in the interaction with the K-turn in SL12 were also responsible for the binding to the K-turn in SL16, suggesting the presence of two *Pho*Rpp38 copies on *Pho*pRNA. Subsequently, a pull-down assay using His-tagged *Pho*Rpp38 further suggested the presence of at least two K-turns in SL12.

2. Materials and methods

2.1. Materials

The Prime STAR Mutagenesis Basal kit was obtained from Takara Bio (Shiga, Japan). Oligonucleotides were purchased from Sigma–Aldrich (St. Louis, USA). Phusion High-Fidelity DNA polymerase was purchased from NEW ENGLAND Biolabs (Ipswich, Massachusetts, USA). All other chemicals were of analytical grade for biochemical use.

2.2. Preparation of proteins and RNAs

*Pho*Rpp38 and its mutants were prepared, as described previously [4]. *Pho*pRNA and its fragments were prepared by *in vitro* transcription with T7 RNA polymerase using corresponding double stranded DNA as a template [12]. The purified RNAs were annealed by heating up to 90 °C for 5 min and cooling down to 4 °C over 5 min, and then used for further experiments. A 51nt-SL12 mutant designated SL12M (133U-151G/168C-180G) was designed based on the deduced secondary structures of P12.1 and P12.2 in *Pho*pRNA [9], in which the GAAA tetra-loop and its receptor sequence (11 nucleotide tetra-loop receptor of the group I intron domain 4–6) were introduced between G151 and C168, and the 5'- and 3'-terminus, respectively [13,14]. Furthermore, GG and its complementary CC sequences were added to enhance the transcription efficiency, at the 5'- and 3'- terminus, respectively (Supplementary Fig. S1).

2.3. Crystallization of PhoRpp38 in complex with SL12M

Purified SL12M and *Pho*Rpp38 were mixed at a molar ratio of 1: 0.9 in 20 mM Tris–HCl, pH 8.0, containing 5 mM $MgCl_2$ and 200 mM KCl, and then concentrated to 4 mg/ml using Amicon Ultra Centrifugal Filters (Merck, Darmstadt, Germany). The mixture was crystallized after incubation at 50 °C for 10 min. Crystals suitable for X-ray analysis were obtained by sitting drop vapor diffusion at 20 °C from a crystallization buffer, 50 mM sodium cacodylate (pH 6.5) containing 2% isopropanol and 200 mM calcium acetate, in which 2 μL of the sample was mixed with the same amount of the crystallization buffer. X-ray diffraction experiments were performed at SPring-8 (Harima, Japan) and Photon Factory (Tsukuba, Japan) under the proposal numbers 2014A1193/2014B1295/2014B1292/2015A6524/2015A1117/2015A1114 (SPring-8), and 2013R-26/2014G022/2014G105/2014G080/2015G067 (Photon Factory). Diffraction data were indexed, integrated, scaled, and merged with the program XDS [15]. The data statistics are shown in Supplementary Table 1.

2.4. Structure determination

The structure of *Pho*Rpp38 in complex with SL12M was determined by molecular replacement with the program Phaser [16] using the *Pho*Rpp38 structure [4] (PDB ID 2CZW) and the K-turn in the L30e-mRNA complex [17] (PDB ID 1T0K) as search probes. Phaser determined two molecules of the *Pho*Rpp38-SL12M complex in an asymmetric unit. Jelly body refinement with the program REFMAC5 [18] and rigid body refinement with the program phenix.refine [19] were carried out for two datasets, and then their electron density map was averaged using the program phenix.multi_crystal_average [20]. The atomic coordinate was built for the averaged electron density map manually with the program Coot [21]. Individual atomic coordinate refinement and individual ADP refinement were performed using the program phenix.refine. The refinement statistics are summarized in Supplementary Table 1. The coordinates of the *Pho*Rpp38-SL12M complex were deposited in Protein Data Bank (ID. 5DCV).

2.5. Gel shift assay

*Pho*pRNA or its fragments was incubated with different molar ratios (0.1–10) of *Pho*Rpp38 or its mutants in a binding buffer (16 μl) containing 20 mM Tris–HCl (pH 8.0), 200 mM NH_4Cl, 10 mM $MgCl_2$, 2 mM DTT, 2 mM EDTA, and 20% glycerol at 75 °C for 10 min. Samples of each set were loaded onto a 6% polyacrylamide gel separately. Electrophoresis was performed for 80 min at 150 V after pre-running the gel for 30 min, with 1 × TBE buffer as the running buffer. The gels were stained with ethidium bromide.

2.6. Pull-down assay

*Pho*pRNA or its mutants was incubated with a mixture of *Pho*Rpp38 with and without His-tag in a buffer 50 mM Tris–HCl, pH 8.0, containing 5 mM $MgCl_2$ and 200 mM NaCl at 50 °C for 10 min, and the complexes were applied onto a HisAccept column (Nacalai Tesque, Kyoto, Japan). After incubation for 30 min at room temperature and washing extensively, the complexes bound to the column were eluted by a buffer containing 0.5 M imidazole, and the effluent was subjected to SDS-PAGE analysis.

3. Results

3.1. Crystallization and structural determination of PhoRpp38 in complex with SL12M

The structure of *Pho*Rpp38 in complex with SL12M was solved at a resolution of 3.4 Å by molecular replacement using *Pho*Rpp38 (PDB ID 2CZW) [4] and the K-turn motif of L30e-mRNA complex (PDB ID 1T0K) [17] as search models. The structure has been refined to an R_{free} of 31.13% (R_{work} of 26.28%), having geometries close to ideal with an rmsd of 0.006 Å and 1.382° from ideal values for bond lengths and angles, respectively. The final model contains 2 copies of the *Pho*Rpp38-SL12M complex, MolA (*Pho*Rpp38)-MolB (SL12M) and MolC (*Pho*Rpp38)-MolD (SL12M) in the asymmetric unit. The two complexes in the asymmetric unit exhibit essentially identical structures; the two *Pho*Rpp38 molecules (MolA and MolC) could be