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

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Abbreviations: ITC, isothermal temperature calorimetry; K-turn, kink-turn; *Pfup*RNA, ribonuclease P RNA from *Pyrococcus furiosus*; *Phop*RNA, 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.

ABSTRACT

PhoRpp38 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 *PhoRpp38* 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 (*PhopRNA*). In order to gain insight into the *PhoRpp38* binding mode to *PhopRNA*, we determined the crystal structure of *PhoRpp38* in complex with the SL12 mutant (SL12M) at a resolution of 3.4 Å. The structure revealed that Lys35 on the β -strand (β 1) and Asn38, Glu39, and Lys42 on the α -helix (α 2) in *PhoRpp38* interact with characteristic G•A and A•G pairs in SL12M, where Ile93, Glu94, and Val95, on a loop between α 4 and β 4 in *PhoRpp38*, 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 *PhoRpp38* binds to the K-turns in SL12 and SL16 in *PhopRNA*. 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 *PhopRNA* by *PhoRpp38*.

Keywords: crystal structure; kink-turn; *Pyrococcus horikoshii*; ribonuclease P; ribosomal protein L7Ae

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 (*Phop*RNA) alone in the hyperthermophilic archaeon *Pyrococcus horikoshii* OT3 had no endoribonuclease activity, but *Phop*RNA and five archaeal homologues, *PhoPop*5, *PhoRpp*21, *PhoRpp*29, *PhoRpp*30, and *PhoRpp*38, 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 *PhoRpp*38 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 *PhoRpp*38 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 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 *P. furiosus* RNase P RNA (*Pfup*RNA), which correspond to SL12 and SL16 in *Phop*RNA [11]. However, nucleotides predicted for the K-turn in *Pfup*RNA P12 are slightly distinct from those predicted to be folded into the K-turn in

SL12, although nucleotides in *Pfup*RNA P16 are in perfect agreement with those in SL16 in *Phop*RNA [11]. That is, nucleotides at positions 182-186 in *Pfup*RNA P12 which correspond to those at positions 181-185 in *Phop*RNA 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 *Phop*RNA [9,10].

To clarify the binding mode of *Pho*Rpp38 to *Phop*RNA, 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 *Phop*RNA. 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]. *Phop*RNA 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 *PhoP*RNA [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 *PhoRpp38* were mixed at a molar ratio of 1 : 0.9 in 20 mM Tris-HCl, pH 8.0, containing 5 mM MgCl₂ 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 *PhoRpp38* in complex with SL12M was determined by molecular replacement with the program Phaser [16] using the *PhoRpp38* 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 *PhoRpp38*-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 *PhoRpp38*-SL12M complex were deposited in Protein Data Bank (ID. 5DCV).

2.5. Gel shift assay

*PhoP*RNA or its fragments was incubated with different molar ratios (0.1 ~ 10) of *PhoRpp38* or its mutants in a binding buffer (16 μ l) 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 $\times$ TBE buffer as the running buffer. The gels were stained with ethidium bromide.

2.6. Pull-down assay

*PhoP*RNA or its mutants was incubated with a mixture of *PhoRpp38* 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

3.2. Crystallization and structural determination of *PhoRpp38* in complex with *SL12M*

The structure of *PhoRpp38* in complex with *SL12M* was solved at a resolution of 3.4 Å by molecular replacement using *PhoRpp38* (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 *PhoRpp38*-*SL12M* complex, MolA (*PhoRpp38*)-MolB (*SL12M*) and MolC (*PhoRpp38*)-MolD (*SL12M*) in the asymmetric unit. The two complexes in the asymmetric unit exhibit essentially identical structures; the two *PhoRpp38* molecules (MolA and MolC) could be superimposed with an rmsd of 0.26 Å and the two *SL12M* molecules (MolB and MolD) with 0.38 Å. Although the six N-terminal residues of *PhoRpp38* are indistinct, a clear electron density is observed for residual amino acids. On the other hand, electron densities for C1-G11 and C16-C51 in two *SL12M* molecules (MolB and MolD) are clearly observed. The refinement statistics are summarized in Supplementary Table 1.

3.3. Structure of *PhoRpp38* bound to *SL12M*

The overall structure of *PhoRpp38* in complex with *SL12M* is presented in Fig. 1. Expectedly, the GAAA tetraloop in MolB interacts with the 11nt tetraloop-receptor in

Mold, and *vice versa*. *PhoRpp38* forms a globular domain comprising five α -helices and a four-stranded β -sheet. The X-ray crystal structure of the unbound *PhoRpp38* was previously solved at 2.0 Å resolution [4]. The superposition of the free and bound *PhoRpp38* structures gave the rmsd value of 0.6 Å, indicating that the structure of *PhoRpp38* is basically unaltered upon association with SL12M, except for a loop between α_4 and β_4 where hydrophobic residues are strongly involved in the interaction with a 3-nucleotide bulge in the K-turn, as described below. This loop moved by 2.5 Å compared with from the free form RNA (Supplementary Fig. S2).

SL12M folds into a standard K-turn structure, juxtaposing the two helices (C stem and N-C stem) (Supplementary Fig. S3). As is the case for other K-turn structures [22], two residues, G19 and G20, in the 3-nucleotide bulge participate in stacking interactions with the C and N-C stems, respectively, and U21 is directed away from the K-turn into solution. Furthermore, a coaxial base stack consisting of G20, A34, A23, and A24 constitutes a central hydrophobic core for organizing the K-turn structure. When the degree of bending defined by Moore *et al.* was measured, the bending angle for the K-turn in SL12M is 55.7°, which is in accordance with the average calculated for other K-turn structures [22,23].

3.4. Interaction between *PhoRpp38* and SL12M

PhoRpp38, like other L7Ae family proteins, primarily interacts with nucleotides in the N-C stem and at the 3-nucleotide bulge. In the N-C stem interaction, the side-chain of Lys35 at a loop between β_1 and α_2 forms hydrogen bonding with G20 and also with a backbone phosphate of A34. The side-chain of Lys42 at α_2 forms hydrogen bonds with backbone phosphates of C31 and G33 (Fig. 2). In addition, the side-chains of Asn38 and Glu39 at α_2 bind to G33. As for the 3-nucleotide bulge structure, Ile93 at a loop between α_4 and β_4 forms a hydrophobic interaction with G20, and Glu94 and

Val95 at the loop hydrogen bond G19 and a phosphate backbone between G19 and G20, respectively (Fig. 2).

Crystal structures of several L7Ae family proteins in complex with their cognate RNAs have been reported [22]. When the structure of *PhoRpp38* in complex with SL12M was superimposed on known homologous proteins in complex with K-turn structures [6,23], the two-helices containing the 3-nucleotide bulge were found to be highly similar, giving rmsd values of around 0.4 Å (Supplementary Fig. S4). This observation indicates a canonical interaction between *PhoRpp38* and the K-turn.

3.5. Characterization of *PhoRpp38* mutants

Thermodynamic analysis indicated that *PhoRpp38* interacts with *PhopRNA* with a stoichiometry of 1 : 1, suggesting that *PhoRpp38* simultaneously binds both K-turn motifs in SL12 and SL16 with two RNA-binding sites [12]. To test this assumption, mutants N38/E39/K42A and I93/E94/V95A, in which Asn38, Glu34, and Lys42 on $\alpha 2$ and Ile93, Glu94, and Val95 on the loop between $\alpha 4$ and $\beta 4$ in *PhoRpp38* were simultaneously replaced by Ala, respectively, were prepared and characterized in terms of their binding potency to three *PhopRNA* fragments using gel shift assay. In this assay, two *PhopRNA* mutants Δ SL12 and Δ SL16, in which SL12 and SL16 were individually deleted from *PhopRNA*, were prepared, as described previously [12]. In addition, mutant Δ SL12/ Δ SL16, in which SL12 and SL16 were simultaneously deleted from *PhopRNA*, was prepared, and the three mutant RNAs were characterized with respect to affinity to the mutant proteins. As shown at left gels in Fig. 3, the wild-type *PhoRpp38* retained binding affinity to Δ SL12 and Δ SL16, in agreement with the result obtained by ITC [12]. As for the mutant proteins, N38/E39/K42A retained considerable binding affinity to Δ SL12, while it had little affinity to Δ SL16 and Δ SL12/ Δ SL16 (Fig. 3, middle gels). In contrast, I93/E94/V95A had little binding affinity to the three mutant

*Phop*RNAs (Fig. 3, right lanes). This result indicated that amino acid residues at the loop between $\alpha 4$ and $\beta 4$ are involved in the interaction with the K-turn present in SL16, as well as in SL12, a finding that is inconsistent with the assumption that *PhoRpp38* binds the two stem-loops simultaneously with two RNA binding sites, but suggests that each *PhoRpp38* binds K-turns in SL12 and SL16 in *Phop*RNA individually.

3.6. Stoichiometry of the interaction of *PhoRpp38* with *Phop*RNA in vitro

The present finding suggested that there are two *PhoRpp38* copies in *P. horikoshii* RNase P; one molecule binds to SL12 and the other to SL16. To test this assumption, *Phop*RNA was incubated with a mixture of *PhoRpp38* with and without His-tag, and the complexes were purified using a nickel affinity column. In this assay, it could be expected that if the assumption is correct, two protein bands corresponding to *PhoRpp38* and His-tagged *PhoRpp38* would be detected. Indeed, SDS-PAGE analysis of the bound fractions clearly showed two bands corresponding to *PhoRpp38* and His-tagged *PhoRpp38* (Fig. 4, lane 2 of the upper gel). When *Phop*RNA was incubated with *PhoRpp38* and His-tagged *PhoRpp38* with a molar ratio of 3 : 1, the complex containing 3 molar excesses of *PhoRpp38* over His-tagged *PhoRpp38* gave a more distinct protein band corresponding to *PhoRpp38* than that containing an equal molar ratio of the proteins (Fig. 4, lane 3 of the upper gel). In contrast, incubation of *PhoRpp38* with *Phop*RNA gave no protein band (Fig. 4, lane 4 of the upper gel). Furthermore, incubation of His-tagged *PhoRpp38* with *Phop*RNA gave a single band corresponding to *PhoRpp38* with His-tag (Fig. 4, lane 1 in the upper gel), and that of His-tagged *PhoRpp38* with *PhoRpp38* in the absence of *Phop*RNA gave no band corresponding to *PhoRpp38* (Fig. 4, lane 5 of the upper gel)). This strongly suggested that two *PhoRpp38* molecules bind the K-turns present in SL12 and SL16 in *Phop*RNA individually.

We further tested the stoichiometry of the interaction between *PhoRpp38* and *PhopRNA* using the *PhopRNA* mutants, Δ SL12, Δ SL16, and Δ SL12/ Δ SL16, in the expectation that incubation of His-tagged *PhoRpp38* and *PhoRpp38* with Δ SL12 or Δ SL16 might give a single band corresponding to His-tagged *PhoRpp38*. The result showed a faint band corresponding to *PhoRpp38* as well as a clear band corresponding to His-tagged *PhoRpp38* in the mixture containing His-tagged *PhoRpp38*, *PhoRpp38*, and Δ SL16, suggesting that at least two *PhoRpp38* molecules are able to bind to K-turns in SL12 (Fig. 4, lane 3 of the lower gel). In contrast, incubation of the two proteins with Δ SL12 gave a single band corresponding to His-tagged *PhoRpp38* (Fig. 4, lane 2 of the lower gel). This result suggested the presence of an additional K-turn motif in SL12. Then, we searched for a second K-turn consensus sequence in SL12 and found that nucleotides at positions 129-133 and 180-187 can be folded into a K-turn motif (Supplementary Fig. S5).

4. Discussion

Although the *PhoRpp38*-SL12 complex was crystallized previously, the crystals thus obtained diffracted at a low resolution of 7.0 Å [12]. Recently, various RNA-engineering strategies for crystallization have been developed to promote crystal contact formation, exploiting well-characterized RNA motifs such as kissing loops, tetraloops and tetraloop receptors [for a review see ref. 13]. Hence, a series of constructs, having these RNA motifs inserted into the SL12 structure, were generated and crystallized in the presence of *PhoRpp38*. As a result of crystallization trials, the SL12 mutant designated SL12M (Supplementary Fig. S1), where the loop (GUGA) connecting helix P12.2 was replaced by a GAAA tetraloop, and the GAAA tetraloop receptor was introduced in helix P12.1, yielded crystal forms that allowed the structure to be solved at 3.4 Å resolution. The crystal structure of *PhoRpp38* in complex with SL12M determined in this study supported the secondary structure of the proposed

K-turn in SL12 [9,10].

Biochemical and structural studies of *PhoRpp38* suggested that *PhoRpp38* simultaneously binds to K-turns in SL12 and SL16 with two RNA binding sites. This assumption was, however, incorrect, because the present structure-based mutational analysis of *PhoRpp38* indicated that amino acids involved in the binding to the K-turn in SL12 are also responsible for the binding to the K-turn present in SL16. A subsequent pull-down assay further suggested the presence of an additional *PhoRpp38* copy bound to SL12 in *PhopRNA*. A search for a second K-turn consensus sequence predicted nucleotides at positions 129-133 and 180-186 to be folded into the K-turn in *PhopRNA* SL12. This prediction is in agreement with the recent results derived from hydroxyl radical-mediated footprinting, showing that nucleotides at positions 180-187 in *PfupRNA* P12 (corresponding to 179-186 in *PhopRNA*) were highly susceptible to hydroxyl radical cleavage [11]. It is thus likely that three *PhopRNA* copies bind to the individual K-turns present in SL12 and SL16; two *PhoRpp38* copies bind to SL12 and one copy to SL16 in *PhopRNA*.

Despite the presence of three K-turn motifs in *PhopRNA*, *PhoRpp38* bindings to the K-turns in SL12 and SL16 do not appear to be equivalent after considering the available biochemical data described below. That is, the finding that N38/E39/K42A, in which Asn38, Glu39, and Lys42 in *PhoRpp38* were simultaneously replaced by Ala, retained binding affinity to SL16 in Δ SL12, whereas it had little activity with SL12 in Δ SL16 (Fig. 3), suggests that the K-turn in SL16 folds into a conformation distinct from that in SL12. Taking the crystal structure of *PhoRpp38* in complex with SL12M into account, amino acids Ile93, Glu94, and Val95 in N38/E39/K42A may be responsible for recognition of the 3-nucleotide bulge structure of SL16 formed in Δ SL12. It can be thus assumed that the 3-nucleotide bulge structure of SL16 adopts a conformation distinct from that of SL12. It is known that the binding of L7Ae family proteins to their cognate RNAs forms additional protein binding sites on RNAs, thereby enabling subsequent assembly of RNPs [23,24]. Since P16 in SL16, to which the *PhoPop5-PhoRpp30*

complex binds, is in close proximity to the K-turn in SL16 [25], it is likely that the *PhoRpp38* binding to the K-turn in SL16 leads to a specific conformation of the 3-nucleotide bulge structure, the K-turn thus optimizing a double-stranded structure (P16) in SL16 for the *PhoRop5-PhoRpp30* complex binding. Structural studies of *PhopRNA* in complex with *PhoRpp38* will provide more insight into the structural basis for the recognition of the K-turns in *PhopRNA* by *PhoRpp38*.

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Figure legends

Figure 1. Structures of *PhoRpp38* bound SL12M

A, The crystal structure of *PhoRpp38* in complex with SL12M observed in the asymmetric units. *PhoRpp38* is represented by its ribbon diagram with MolA and MolC colored cyan and green, while SL12M is drawn in stick form with MolB and MolD colored red and blue. B, The crystal structure of *PhoRpp38* in complex with SL12M. The protein is represented by its ribbon diagram, while SL12M is drawn in stick form. (For interpretation of the references to colors in this figure legend, the reader is referred to the web version of this paper.)

Figure 2. Interaction of *PhoRpp38* with SL12M

In the interaction between *PhoRpp38* and SL12M, Lys35 on $\beta 1$ and Asn38, Glu39, and Lys42 on $\alpha 2$ form a hydrogen bond network with nucleotide residues in the N-C helix, and Ile93, Glu94, and Val95 on the loop between $\alpha 4$ and $\beta 4$ bind the three-nucleotide bulge in SL12M. *PhoRpp38* is drawn in ribbon form, while SL12M in skeletal form.

Figure 3. Gel shift assay of the interaction of *PhoRpp38* or its mutants with *PhoP*RNA mutants

Δ SL12 (upper lanes), Δ SL16 (middle lanes), or Δ SL12/ Δ SL16 (lower lanes) was incubated with *PhoRpp38* or its mutants, N38/E39/K42A and I93/V95/E94A, in a binding buffer (16 μ l) containing 20 mM Tris-HCl, pH 8.0, 5 mM MgCl₂, and 200 mM NaCl at different molar ratios (0.1 ~ 10) as indicated on gels. Electrophoresis was performed, as described in Materials and methods. The protein-RNA complexes were detected using ethidium bromide-stained 6% polyacrylamide gels.

Figure 4. Pull-down assay of the interaction of *PhoRpp38* and His-tagged

PhoRpp38 with *PhopRNA*

PhopRNA (upper gel) or its mutants (lower gel) was incubated with mixtures of *PhoRpp38* and His-tagged *PhoRpp38* with different molar ratios as indicated, and the complexes were purified by a nickel affinity column. The eluted fractions were subjected to SDS-PAGE. + indicates the mixtures containing an equimolar ratio of *PhoRpp38* and His-tagged *PhoRpp38*, while 3+ indicates the mixtures containing 3 molar excesses of *PhoRpp38* over His-tagged *PhoRpp38*. Protein markers (M) include trypsin inhibitor (20.0 kD) and lysozyme (14.3 kD).

Figure

Figure 1

Figure 2

Figure 3

Figure 4

A structural model of *PhopRNA* in complex with *PhoRpp38*, *PhoPop5*, and *PhoRpp30*

In this docking model, three *PhoRpp38* were readily able to locate on K-turns in SL12 and SL16 without steric hindrance of the *PhoPop5*-*PhoRpp30* complex which bridges P3 and P16 helices in *PhopRNA*.