5A, NW12A, BL41XU/2008G690/2009G637/2008S2-001

Crystal Structure of Arl1 in complex with Arfaptin-2 BAR domain

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1 Introduction

We have recently shown that Arfaptin-2, which was originally identified as a binding partner for the Arf and Rac1 GTPases, binds to Arl1 through its BAR domain and is recruited onto Golgi membranes [1]. There, Arfaptin-2 induces membrane tubules. Here, we report the crystal structure of the Arfaptin-2 BAR homodimer in complex with two Arl1 molecules bound symmetrically to each side, leaving the concave face open for membrane association.

2 Experiment

In this study, Arl1 and Arfaptin-2 BAR domain was overexpressed in *Escherichia coli,* purified to homogeneity, and crystallized by the sitting- drop vapor diffusion method using PEG3350 as a precipitant [2].

3 Results and Discussion

The crystals of Arl1-Gpp(NH)p-Arfaptin-2 BAR domain were screened at BL5A, BL17A, NW12A and NE3A of PF/PF-AR at KEK and the final data set was collected at the BL41XU at SPring-8 (Table 1). The diffraction data were processed using the HKL2000 program. The crystal structure was determined by the MR method using the structures of Arl1 from PDBID:1UPT and Arfaptin-2 from PDBID:1I49 as search models.

The asymmetric unit of the crystal contained an Arfaptin-2 BAR homodimer flanked by two Arl1 molecules (Figure 1). The dimeric structure of Arfaptin-2 BAR in the complex closely resembles the previously reported structure of the free form (PDBID:1I49). Each Arfaptin-2 BAR monomer unit comprises an antiparallel three-helix bundle. The two Arl1 molecules bind symmetrically to each Arfaptin-2 BAR monomer, around the kinks of the two helices.

The N-terminal amphipathic helical region of Arl1 (residues 1–13), which was removed in our con- struct for crystallization, is expected to adopt the same orientation as the concave face of the Arfaptin-2 BAR dimer. Because the amphipathic helix, along with the N-terminal attached myristoyl moiety, participates in membrane anchoring of Arl1, the crystal structure indicates that the two Arl1 molecules facilitate the direct association of the concave face of the Arfaptin-2 dimer with membranes.

This is the first crystal structure to reveal the modulation of BAR domain by Small GTPase.

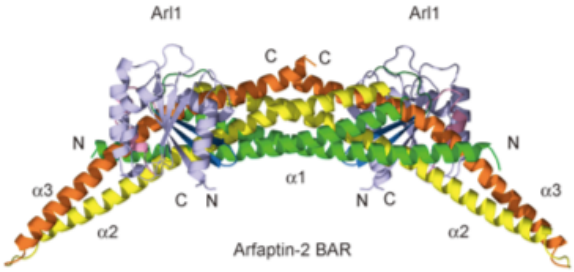

Fig. 1: The overall structure of Arl1 in complex with Arfaptin-2 BAR domain.

Table 1 Crystallographic summary

Crystal form	Arl1-Gpp(NH)p-Arfaptin-2 BAR
Crystal size (mm)	0.1x0.05x0.05
Xray source	SPring8
Beamline	BL41XU
Oscillation angle (°)	1
Exposure time	5
Wavelength (Å)	1.0000
Temperature (K)	95
Space group	P212121
a, *b*, *c* (Å)	62.7,111.1,119.8
Resolution (Å)	43.3-3.0(3.1-3.0)
R_{sym} or R_{merge}	9.4(46.2)
$I / \sigma I$	11.4(2.4)
Completeness (%)	96.5(93.4)
Redundancy	3.5(3.4)
R_{work} / R_{free}	26.2/33.3

References

[1] Z. Man, *et al*., JBC (2011) **286** 11569 –11578
[2] K. Nakamura, *et al*., JBC (2012) **287** in press
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