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Atomic resolution structure of the *E. coli* YajR transporter YAM domain

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ABSTRACT

YajR is an *Escherichia coli* transporter that belongs to the major facilitator superfamily. Unlike most MFS transporters, YajR contains a carboxyl terminal, cytosolic domain of 67 amino acid residues termed YAM domain. Although it is speculated that the function of this small soluble domain is to regulate the conformational change of the 12-helix transmembrane domain, its precise regulatory role remains unclear. Here, we report the crystal structure of the YAM domain at 1.07-Å resolution, along with its structure determined using nuclear magnetic resonance. Detailed analysis of the high resolution structure revealed a symmetrical dimer in which a belt of well-ordered poly-pentagonal water molecules is embedded. A mutagenesis experiment and a thermal stability assay were used to analyze the putative role of this dimerization in response to changes in halogen concentration.

1. Introduction

The *Escherichia coli* transporter YajR (ecYajR) is a member of the major facilitator superfamily (MFS) of secondary active transporters [1]. Despite initial efforts to elucidate its physiological function, the precise nature of its substrate remains a mystery. Recently, we have reported the crystal structure of the full-length ecYajR, and used it to illustrate the functional roles of its signature motif (motif A) found in most MFS transporters [2]. Apart from the canonical 12-helix transmembrane (TM) domain of the MFS transporter, ecYajR contains a 67-residue, carboxyl-terminal, cytosolic, soluble YAM domain. The naming of YAM reflects the fact that the folding topology and/or sequence homology of this small soluble domain of YajR is similar to that in arabinose efflux permeases (AraEP) as well as in the metal binding domain (MBD) of P-type ATPase transporters. While its functional roles during the transport process remain unknown, the fact that the YAM domain is highly conserved in all known YajR transporters of a variety of Gram-negative bacterial species suggest that its role is essential for the proper functioning of the transporter (Fig. 1A). Importantly, ecYajR exhibits high levels of thermal stability, which is independent of the TM core. Also, the *in vitro* thermal stability of the purified YAM domain increases in response to both increases in pH, and halogen ion concentration (see Fig. S6 in Ref. [2]).

During the determination of the crystal structural of the full-length membrane protein ecYajR which had a medium resolution (3.15 Å), we determined the atomic-resolution crystal structure of the isolated YAM domain at 1.07 Å as well as its nuclear magnetic resonance (NMR) structure. We demonstrate that the YAM domain forms a homodimer in the crystal and possibly in solution as well. The mutation L38A introduced at the dimer interface significantly reduced the thermal stability of the YAM domain, especially at higher concentration of NaCl. These observations suggest that homo-dimerization of the YAM domain may serve as a regulatory mechanism for the full length YajR transporter.

2. Methods

2.1. Protein expression, purification and crystallization

The YAM domain (*i.e.* amino acid residues 388–454 of ecYajR) protein fused with His_6-tag was expressed in the *E. coli* BL21 (DE3). The fusion protein was purified by Ni–NTA affinity chromatography. After removing His6-tag, the protein was further purified by size-exclusion chromatography. The protein samples for NMR were concentrated to ~1.5 mM in 50 mM phosphate buffer (pH 6.3) containing 150 mM NaCl, 1 mM DTT, and 1 mM EDTA.

Crystals of YAM domain were obtained by the hanging drop vapor diffusion method at 16 °C. To get high quality crystals, 1 μl

Abbreviations: MFS, major facilitator superfamily; YAM, YajR/AraEP/MBD domain.

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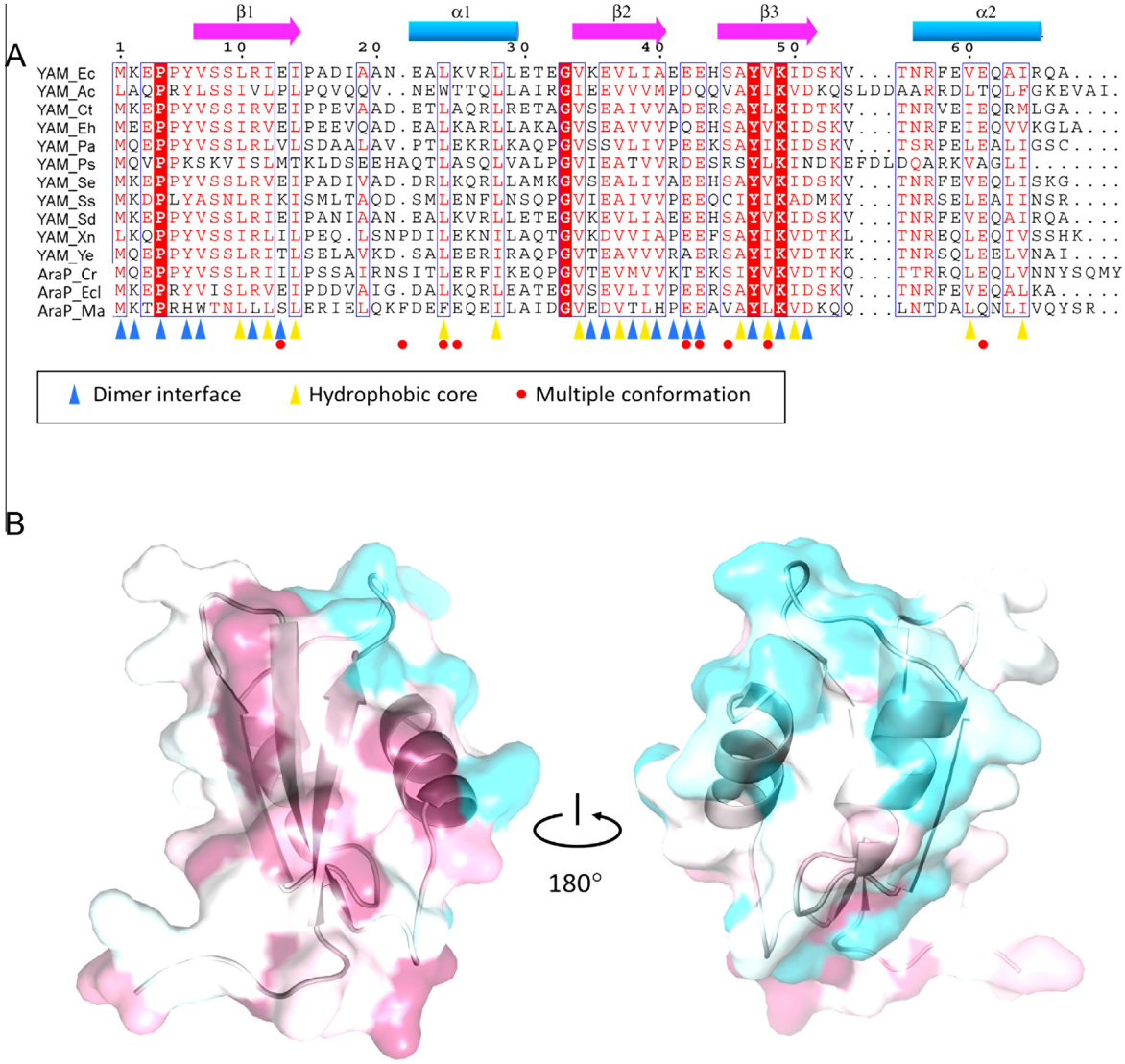

Fig. 1. Conservation of amino acid sequence of the YAM domain. (A) Alignment of amino acid sequences of YajRs from a variety of Gram-negative bacteria. Ec: *E. coli* YajR; Ac: *Acinetobacter calcoaceticus* YajR; Ct: *Cronobac terturicensis* YajR; Eh: *Escherichia hermannii* YajR; Pa: *Pantoea anantis* YajR; Ps: *Pseudoalteromonas* YajR; Se: *Salmonella enteria* YajR; Ss: *Serratia symbiotica* YajR; Sd: *Shigella dysen*teriae YajR; Xn: *Xenorhabdus nematophila* YajR; Ye: *Yersinia enterocolitica* YajR; Cr: *Candidatus Regiellainsecticola* AraP; Ecl: *Enterobacter cloacae* AraP; and Ma: *Methylomicrobium album* AraP. Identical residues are colored in white on red background, similar ones in red on white background. Secondary structural elements of YAM domain from ecYajR are indicated above the sequence alignment. Positions involved in the hydrophobic core are marked with orange triangles at the bottom, those involved in the dimer interface as blue triangles, and those of multiple conformations as red dots. Sequences were aligned with the program *ClustalX* and formatted with *ESPript*. (B) 3D distribution of conserved residues in the YAM domain. The most conserved regions among YajR transporters are in magenta, and the least conserved region in cyan. The 'β-sheet' surface (left) and the hydrophobic core are more conserved than the 'α-helix' surface (right). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

concentrated protein of 10 mg/ml was mixed with 1 μl crystallization solution containing 0.1 M HEPES (pH 7.5), 50 mM $CdSO_4$, and 1.0 M sodium acetate. Crystals were cryo-protected with mother liquor supplemented with 20% (v/v) glycerol and flash-cooled in liquid nitrogen. For iodine-derivative, native crystals were soaked in the mother liquor supplemented with 300 mM NaI or KI for 1–2 min.

2.2. Data collection and determination

Diffraction data for the 'native' YAM crystal were collected up to 1.2 Å resolution at the 17U beamline of the Shanghai Synchrotron Radiation Facility (SSRF). The highest resolution data set (up to 1.0 Å) was collected at the 17A beamline of the Photon Factory synchrotron facility (KEK, Japan). Iodine-derivative anomalous data were collected at the 41XU beamline of SPring-8 synchrotron facility (Japan). All data were processed with the program HKL2000 [3].

Initial phases were calculated with the program *Phenix.autosol* [4] using cadmium anomalous signals of the 1.2-Å data. A nearly complete model (Val7–Ala67) was automatically built with *Phenix.autobuild*. After several cycles of geometry-restrained positional and B-factor refinement with *Phenix.refine*, R_{work} dropped to 24.9% (R_{free} 27.1%). Then refinement was carried out using the 1.07 Å resolution data, and R_{work} further dropped to 18.3% (R_{free} 19.7%) after refinement. Model validation was carried out using the *Molprobity* utility [5].

All NMR spectra were acquired at 303 K on an Agilent DD2 600 spectrometer. Backbone and nonaromatic, nonexchangeable side chain resonance assignments of YAM domain were achieved by standard heteronuclear correlation experiments using $^{15}N/^{13}C$-labeled samples and confirmed by a 3D ^{15}N-seperated NOESY