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Structure Report

Crystal structures and putative interface of *Saccharomyces cerevisiae* mitochondrial matrix proteins Mmf1 and Mam33

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ABSTRACT

The yeast *Saccharomyces cerevisiae* mitochondrial matrix factor Mmf1, a member in the YER057c/Yigf/Uk114 family, participates in isoleucine biosynthesis and mitochondria maintenance. Mmf1 physically interacts with another mitochondrial matrix protein Mam33, which is involved in the sorting of cytochrome b_2 to the intermembrane space as well as mitochondrial ribosomal protein synthesis. To elucidate the structural basis for their interaction, we determined the crystal structures of Mmf1 and Mam33 at 1.74 and 2.10 Å, respectively. Both Mmf1 and Mam33 adopt a trimeric structure: each subunit of Mmf1 displays a chorismate mutase fold with a six-stranded β-sheet flanked by two α-helices on one side, whereas a subunit of Mam33 consists of a twisted six-stranded β-sheet surrounded by five α-helices. Biochemical assays combined with structure-based computational simulation enable us to model a putative complex of Mmf1–Mam33, which consists of one Mam33 trimer and two tandem Mmf1 trimers in a head-to-tail manner. The two interfaces between the ring-like trimers are mainly composed of electrostatic interactions mediated by complementary negatively and positively charged patches. These results provided the structural insights into the putative function of Mmf1 during mitochondrial protein synthesis via Mam33, a protein binding to mitochondrial ribosomal proteins.

1. Introduction

Members in the YjgF/YER057c/UK114 family are highly conserved in archaea, bacteria and eukarya. They share a highly conserved primary sequence with an identity from 47% to 78%, but possess diverse biological functions (Leitner-Dagan et al., 2006; Manjasetty et al., 2004; Parsons et al., 2003; Sinha et al., 1999; Volz, 1999). The human homolog hp14.5 has been proposed as a putative translation inhibitor that can inhibit cell-free protein synthesis in the rabbit reticulocyte lysate system (Oka et al., 1995; Schmiedeknecht et al., 1996), whereas the rat homolog rp14.5 has endoribonuclease activity (Morishita et al., 1999), and the goat homolog UK114 has tumor antigen activity (Ceciliani et al., 1996). Biological functions of other homologs include the calpain activation in the bovine (Melloni et al., 1998), the purine regulation activity in *Bacillus subtilis* (Sinha et al., 1999), and photosynthesis and chromoplastogenesis in plants (Leitner-Dagan et al., 2006). A line of evidences suggested that subtle sequence changes attribute the functional divergence among the members (Thakur et al., 2010). To date, about twenty structures of this family have been determined, all of which adopt a trimeric structure with three clefts, each of which is characterized by 6–9 signature residues. These conserved clefts are able to bind diverse ligands, including L-threonine, L-serine, 2-ketobutyrate, ethylene glycol, or propionate (Kim et al., 2001; Parsons et al., 2003; Sinha et al., 1999). However, the physiological significance of these members remains unclear. The homolog from the yeast *Saccharomyces cerevisiae* was named Mmf1, for mitochondrial matrix factor. Mmf1 was proposed to be involved in isoleucine biosynthesis and intact mitochondria maintenance (Burman et al., 2007; Kim et al., 2001; Oxelmark et al., 2000).

Tandem affinity purification assays indicated that Mmf1 physically interacted with a mitochondrial acidic matrix protein Mam33 (Krogan et al., 2006), suggesting their putative functional relevance. Mam33 was found to be involved in sorting cytochrome b_2 to the mitochondrial intermembrane space via binding to its signal peptide (Seytter et al., 1998). Deletion of *MAM33* gene would lead to slower growth of yeast in glycerol medium but not in glucose medium, suggesting that Mam33 may participate in the mitochondrial oxidative phosphorylation (Muta et al., 1997; Seytter et al., 1998). Mam33 was identified as a homo-trimer or tetramer and shared a sequence identity of approximately 24–35% with the homologs from human, *Leishmania major*, *Trypanosoma brucei*,

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and *Caenorhabditis elegans* (Seytter et al., 1998). The human homolog of Mam33 was termed splicing factor 2-associated protein p32, which was proposed to be involved in the maintenance of mitochondrial oxidative phosphorylation (Muta et al., 1997). The human p32 executes diverse functions via binding to various partners (Jiang et al., 1999; Joseph et al., 1996; Matthews and Russell, 1998; Okagaki et al., 2000). Up to date, a total of 82 protein partners were identified to have physical or genetic interactions with yeast Mam33 (http://www.yeastgenome.org/). Among them, 23 partners are mitochondrial ribosomal proteins (Collins et al., 2007; Gavin et al., 2006; Krogan et al., 2006). This led us to presume that, with the assistance of Mmf1, Mam33 may take part in mitochondrial ribosomal protein synthesis.

To figure out the structural insights into the interaction pattern between Mmf1 and Mam33, we attempted to solve the crystal structure of their complex. We purified the complex of Mam33–Mmf1, but we were unable to obtain its crystal. Nevertheless, we solved the crystal structures of individual Mmf1 and Mam33 at 1.74 and 2.10 Å, respectively. In vitro biochemical assays demonstrated that Mmf1 and Mam33 can form a complex with 2:1 M ratio. Structural analysis revealed the positively charged surfaces of Mmf1 trimer are complementary to the negatively charged patches of Mam33 trimer. In addition, a docking model of Mmf1–Mam33 complex provides the structural basis for the putative binding mode of these two proteins.

2. Protein expression, purification and crystallization

The gene encoding Mmf1 and Mam33 were PCR amplified from the genomic DNA of *S. cerevisiae* S288C. The coding sequence of an additional His_6-tag was introduced at the 5′ end of the gene. The PCR product were cloned into pET28a-derived vectors and expressed at 37 °C using the transformed *Escherichia coli* BL21 (DE3) strains and 2× YT medium (OXOID LTD) supplemented with 30 μg/ml kanamycin. When the cell culture reached an OD_{600nm} of 0.6–1.0, the expression of the protein was induced with 0.2 mM isopropyl-β-D-1-thiogalactopyranoside and the cells were grown for a further 20 h at 16 °C. Cells were collected by centrifugation, resuspended in 15 ml buffer containing 100 mM NaCl, 20 mM Tris–Cl, pH 8.0. His-tagged proteins were purified using Ni^{2+} affinity columns. Eluted protein were further purified by gel filtration using a Superdex™ 200 column (GE Healthcare Bioscience) equilibrated in 100 mM NaCl, 20 mM Tris–Cl, pH 8.0 and 20 mM β-mercaptoethanol. The purity of the pooled fraction was checked by electrophoresis. Crystals of Mmf1 and Mam33 were obtained by the hanging drop diffusion method at 16 °C for 2 weeks. In each drop of crystallization, 1 μl protein sample at 10–15 mg/ml in the buffer of 50 mM NaCl, 20 mM Tris–Cl, pH 8.0 and 20 mM β-mercaptoethanol was mixed with 1 μl reservoir solution and equilibrated against 500 μl reservoir solution (25% polyethylene glycol 3550 for Mmf1 and 20% polyethylene glycol 4000 for Mam33).

3. Data collection, processing, structure determination and refinement

X-ray diffraction data were collected at 100 K in a liquid nitrogen stream using beamline 17 U with an MX225 CCD (MARresearch, Germany) at the Shanghai Synchrotron Radiation Facility (SSRF). The data were integrated and scaled with the program HKL2000 (Otwinowski and Minor, 1997).

The structures of Mmf1 and Mam33 were determined by molecular replacement method with the program MOLREP (Vagin and Teplyakov, 2010) from the CCP4 suite using the coordinates of 1JD1 and 1P32, respectively. The initial models were refined by using the maximum likelihood method implemented in REFMAC5 (Murshudov et al., 1997) as part of CCP4 program suite and rebuilt interactively in the program COOT (Emsley and Cowtan, 2004). The final models were evaluated using the programs MOLPROBITY (Davis et al., 2007) and PROCHECK (Laskowski et al., 1996). Crystallographic parameters are listed in Table 1. All structural figures were created by PyMOL (DeLano, 2002).

4. In vitro assays of the complex between Mmf1 and Mam33

The cells expressing Mmf1 and Mam33, respectively, are mixed and lysed by sonication after three cycles of freezing/thawing. The mixed proteins were purified using Ni^{2+} affinity columns. Eluted protein were further purified by gel filtration using a HiLoad 16/60 Superdex 200 column (GE Healthcare) equilibrated in 100 mM NaCl, 20 mM β-mercaptoethanol and 20 mM Tris–Cl, pH 8.0, and with a flow rate of 1.0 ml/min. The purity and the components of the pooled fraction were checked by electrophoresis. The buffer was filtered with a 0.22-mm membrane and thoroughly degassed.

Isothermal titration calorimetry assays were carried out with Microcal iT-200 isothermal titration calorimeter (GE Healthcare, Germany). According to manufacturer's instructions, about 0.2 ml Mmf1 protein (150 μM) in the Tris–Cl buffer, pH 8.0 was loaded into the cell. The titration syringe was loaded with Mam33 at a concentration of about 400 μM. Titrations were carried out at 25 °C with 20 injections at an interval of 2 min. A protein-free buffer was set as control. Results of the titration curves were analyzed using Origin 7.5 software (Northampton, MA).

Sedimentation velocity assays were performed on a Proteomelab XL-A/XL-I analytical ultracentrifuge (Beckman Coulter Instruments) with an An-60 Ti rotor, one cell assembled by sapphire windows, a double-sector 12 mm length charcoal-filled Epon centerpiece and UV/vis absorbance optical system. All assays were conducted at 60,000 rpm and 20 °C. Four hundred microliters of Mmf1–Mam33 (C = 1.3 mg/ml) and Mmf1 (C = 4.0 mg/ml) were loaded for measurement with 420 μl of buffer solution as the ref-

Table 1
Data collection and refinement statistics.

Data processing	Mmf1	Mam33
Space group	*H3*	*I2$_1$3*
Unit cell (Å)	77.62, 77.62, 43.91	118.76, 118.76, 118.76
Resolution range (Å)	50.00–1.74 (1.80–1.74)[a]	50.00–2.10 (2.18–2.10)
Unique reflections	9,969 (929)	16,404 (1,605)
Completeness (%)	99.3 (94.5)	99.9 (100)
$\langle I/\sigma(I)\rangle$	14.8 (7.2)	37.7 (10.3)
R_{merge}[b] (%)	10.4 (25.0)	8.1(39.3)
Average redundancy	11.0 (7.8)	22.0 (21.3)
Refinement statistics		
Resolution range (Å)	38.81–1.75	41.99–2.10
R-factor[c]/*R*-free[d] (%)	18.1/22.2	22.7/25.2
Number of protein atoms	957	1,442
Number of water atoms	77	87
RMSD[e] bond lengths (Å)	0.011	0.010
RMSD bond angles (°)	1.139	1.040
Mean B factors (Å^2)	22.8	18.4
Ramachandran plot[f] (residues, %)		
Most favored (%)	98.4	100
Additional allowed (%)	1.6	0
PDB entry	3QUW	3QV0

[a] The values in parentheses refer to statistics in the highest bin.
[b] $R_{merge} = \sum_{hkl}\sum_i|I_i(hkl) - \langle I(hkl)\rangle|/\sum_{hkl}\sum_i I_i(hkl)$, where $I_i(hkl)$ is the intensity of an observation and $\langle I(hkl)\rangle$ is the mean value for its unique reflection; Summations are over all reflections.
[c] R-factor = $\sum_h|Fo(h) - Fc(h)|/\sum_h Fo(h)$, where Fo and Fc are the observed and calculated structure-factor amplitudes, respectively.
[d] R-free was calculated with 5% of the data excluded from the refinement.
[e] Root-mean-square deviation from ideal values.
[f] Categories were defined by Molprobity.