BL-5A, AR-NW12A/2014G578

Crystal structure of the N-terminal domain of the human mitochondrial calcium uniporter with a novel fold

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1 Introduction

Mitochondrial Ca^{2+} homeostatis is essential for the modulation of mitochondrial functions containing fission-fusion and ATP synthesis. Excessive Ca^{2+} uptake in mitochondria induces large amount of reactive oxygen species (ROS) production and causes cell death through apoptosis and necrosis. Mitochondrial calcium uniporter (MCU) is responsible for mitochondrial calcium uptake across the IMM and is an attractive target for the regulation of cell survival and death in many pathological states. However, structural basis of Ca^{2+} channel regulation by MCU is still unknown. Here we solved the crystal structure of the MCU N-terminal domain (NTD; 75−165). The structure of MCU NTD contains a novel fold important for regulation of MCU function. We reported the structural results of MCU NTD with biochemical and functional data providing into a regulatory domain for MCU Ca^{2+} uptake activity [1,2,3].

2 Experiment

Human MCU NTD was cloned into modified pET21 vector, which includes N-terminal His_6-bacteriophge T4 lysozyme (2−161). The T4 lysozyme-MCU NTD was expressed in *E. coli* BL21-Codonplus (DE3) and purified by nickel-NTA affinity chromatography followed by size exclusion chromatography. The proteins was crystallized using the hanging-drop vapor-diffusion method at 20°C in a reservoir solution containing 25% PEG3350, 0.2 M ammonium sulfate, and 0.1 M Bis-Tris-HCl (pH 6.5). The crystal was diffracted up to 2.2 Å and X-ray diffraction data were collected using an ADSC quantum CCD detector at the beamline NW12A at the Photon Factory (Tsukuba, Japan). The space group was $P6_5$ with a unit cell parameter of a=b=98.86 Å and c=60.24 Å. The data set was indexed, processed, and scaled using the HKL2000 software package. Phases were obtained by molecular replacement using PHASER in the CCP4 program suite and structure of bacteriophage T4 lysozyme (PDB code, 2LZM) was used as a template. The MCU NTD in the initial model was manually built and refined using COOT, REFMAC5, and PHENIX.

3 Results and Discussion

The structure of MCU NTD consists of one α-helix and six β-strands that form a globular domain and two highly conserved loops (L2 and L4) which are important for regulation of MCU Ca^{2+} uptake (Fig. 1A). MCU NTD deletion or S92A mutant in the L2 loop of MCU NTD exerts a dominant negative effect [3].

MCU NTD is identified as a novel fold and we named it "MCU domain-like fold" despite the structural similarity with ubiquitin (Ub)-like and immunogloblin (Ig)-like folds (Fig. 1). MCU NTD core domain contains α-helices and β-strands in the following order: β3-β4-β6-α1-β1-β2-β3 (Fig 1A,1B). Secondary structure of MCU NTD shows different connection and directionality of β-strands in comparison with that of Ub (Fig 1A−1D,1G). It also shows different fold to β2-microglobulin, which belongs to Ig-like fold, based on different orientation and connection of β-strands such as Greek-key motif (Fig. 1E,1F,1H), suggesting that, these results provided structural basis of MCU NTD in a novel fold [3].

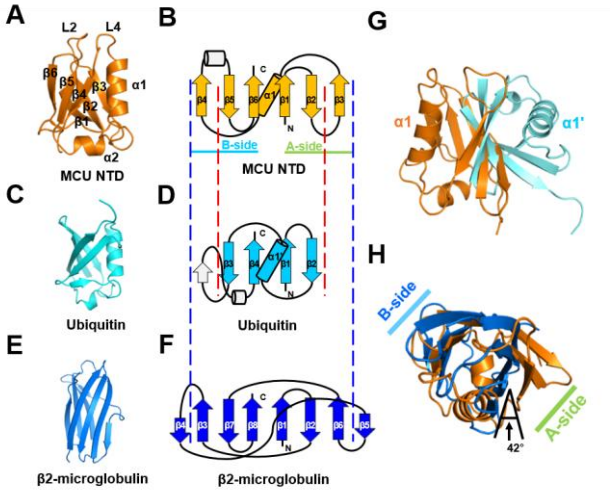

Fig. 1 (A,C,E) Overall structures of MCU NTD (A), Ubiquitin (Ub) (PDB code, 1UBQ; one of Ub-like folds) (C), and β2-microglobulin (PDB code, 1IM3; one of Ig-like folds) (E). (B,D,F) Topology of MCU NTD (B), Ub (D), and β2-microglobulin (F). α-helices and β-strands are represented by cylinders and arrows, respectively. (G) Superposition of MCU NTD (orange) and Ub (cyan). (H) Superposition of MCU NTD (orange) and β2-microglobulin (blue).

References

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