BL-5A/BL-17A/2013G018

Crystal structure of mitochondrial calcium uptake 1

Yuequan Shen*
State Key Laboratory of Medicinal Chemical Biology, Nankai University, 94 Weijin Road, Tianjin 300071, China

1 Introduction

Mitochondrial calcium uptake is a critical event in various cellular activities. Two recently identified proteins, the mitochondrial Ca^{2+} uniporter (MCU), which is the pore-forming subunit of a Ca^{2+} channel, and mitochondrial calcium uptake 1 (MICU1), which is the regulator of MCU, are essential in this event. However, the molecular mechanism by which MICU1 regulates MCU remains elusive. In this study, we report the crystal structures of Ca^{2+}-free and Ca^{2+}-bound human MICU1. Our studies reveal that Ca^{2+}-free MICU1 forms a hexamer that binds and inhibits MCU. Upon Ca^{2+} binding, MICU1 undergoes large conformational changes, resulting in the formation of multiple oligomers to activate MCU. Furthermore, we demonstrate that the affinity of MICU1 for Ca^{2+} is approximately 15-20 μM. Collectively, our results provide valuable details to decipher the molecular mechanism of MICU1 regulation of mitochondrial calcium uptake [1].

2 Experiment

The MICU1-xtal protein was crystallized using the hanging drop vapor diffusion method mixed 1:1 with a reservoir solution of 8% PEG 3350 and 0.075 M ammonium citrate tribasic pH 7.0. After microseeding, the crystals grew much bigger. Crystals were grown for one week at 20 °C and frozen in a cryoprotectant consisting of the reservoir solution supplemented with 30% ethylene glycol. The Se-Met crystals were produced in the same conditions used for the wild-type protein, with the exception that 1.5 mg/ml Se-Met protein was used for crystallization.

The MICU1-xtal-deltaC protein was crystallized using the sitting drop vapor diffusion method equilibrated against a 43% reservoir solution composed of (w/v) 2-methyl-2,4-pentanediol and 10 mM $CaCl_2$. Crystals were grown for 3 weeks at 4 °C, and the reservoir solution itself was used as a cryoprotectant.

Wild-type data and single anomalous data were collected at the element Se peak wavelength on the BL17U1 station of the Shanghai Synchrotron Radiation Facility (SSRF) and then were processed using the HKL2000 software [2]. The Ca^{2+}-free MICU1-xtal structure was determined using single-wavelength anomalous dispersion (SAD) at the resolution of 3.2 Å. The Ca^{2+}-bound MICU1-xtal-deltaC structure was determined using molecular replacement at a resolution of 2.7 Å.

3 Results and Discussion

The Ca^{2+}-free MICU1-xtal structure includes four regions (Figure 1A and 1B): the N-domain, the N-lobe, the C-lobe and the C-helix. The N-domain (residues 103-177) consists of three α-helices and three antiparallel -strands. The N-lobe (residues 183-318) and the C-lobe (residues 319-445) are composed of six and seven α-helices, respectively. The C-helix (residues 446-476) is one long helix that does not specifically interact with the primary Ca^{2+}-free MICU1-xtal core.

The topology of the Ca^{2+}-bound form is similar to the topology of the Ca^{2+}-free form (Figure 1B and 1C). Two calcium ions are present in the Ca^{2+}-bound MICU1-xtal-deltaC structure. One calcium ion is bound to the EF hand (referred to as the canonical EF1) that consists of the NH3 and NH4 helices of the N-lobe. The second calcium ion is bound to the EF hand (also known as the canonical EF4) that is composed of the CH6 and CH7 helices of the C-lobe. Both calcium ions bound to sites of the conventional EF hand, as predicted by previous studies. Furthermore, other two helix-loop-helix structural units (referred to as pseudo EF2 and pseudo EF3) were found in the MICU1 structure. These units are composed of the NH5 and NH6 helices for pseudo EF2 as well as the CH4 and CH5 helices for pseudo EF3. Apparently, these two sites were unable to bind calcium ions, although they contain helix-loop-helix structural units (Figure 1C). Therefore, each N- and C-lobe contains two helix-loop-helix structural units that were capable of binding only one calcium ion.

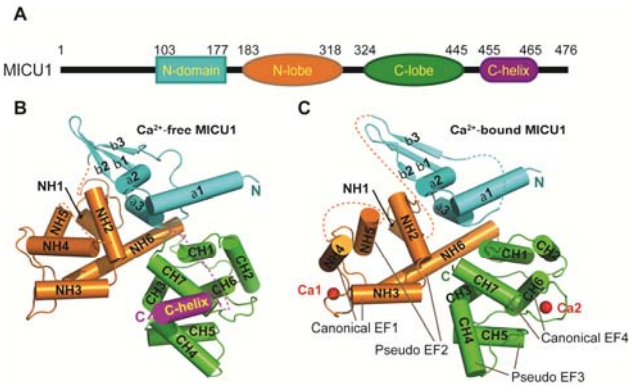

Figure 1 Crystal structures of Ca^{2+}-free and Ca^{2+}-bound human MICU1. (**A**) A schematic drawing of human MICU1. (**B,C**) Cartoon representation of the overall structure of MICU1 in the Ca^{2+}-free and the Ca^{2+}-bound state. The N-domain, N-lobe, C-lobe, C-helix and Ca^{2+} are colored cyan, orange, green, purple and red, respectively.

Acknowledgement

We are grateful to the staff at the beamline BL-5A and BL-17A at Photon Factory (Tsukuba, Japan) for excellent technical assistance during data collection.

References

[1] Wang L, Yang X, Wang Z, Liu Y, Feng J, Zhu Y, Shen Y, *EMBO J* 33(6), 594-604 (2014).

[2] Otwinowski Z, Minor W, *Method Enzymol* **276**, 307-326 (1997).

* yshen@nankai.edu.cn