BL-1A, BL-5A, BL-17A, NW12A and NE3A/ 2012G009

Preliminary crystallographic studies on the large-conductance mechanosensitive channel in different conformational states

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1 Introduction

The capability of sensing mechanical force (mechanosensation) is a pivotal physiological function fundamental to both prokaryotic and eukaryotic organisms, including archaea, bacteria, plants and animals. It is crucial for their survival in the constantly changing environments. Underlying the mechanosensation processes are mechanosensitive (MS) channels that are capable of detecting and responding to the mechanical stimuli by opening their pores to conduct substances across the membrane. In prokaryotes, the MS channels are involved in the control of cell volume. By responding to the increased membrane tension induced by acute environmental osmolarity decrease, these channels will open their central pores to release the intracellular turgor pressure and thus help bacteria to survive under hypoosmotic downshock [1].

Among the three different types of bacterial MS channels (MscL, MscS and MscM) identified so far, MscL (mechanosensitive channel of large-conductance) gates at the highest pressure threshold near the rupture limit of baterial membrane and has the largest single-channel conductance at ~3.5 nS [2]. Previously, it was discovered that MscL undergoes drastic conformational changes leading to the formation of a large open pore with diameter up to 30 Å during the transition from closed to open states [3]. To gain detailed insights into the gating mechanism of MscL, it is indispensable to solve the structures of MscL at different conformational states. Structural studies on MscL have proven to be highly challenging not only because it is an integral membrane protein but also because of the inherent flexible nature of the channel which may cause the conformational heterogeneity in the purified protein sample or within the crystal sample. After years of persistent and pioneering work, Rees lab at Caltech have obtained two crystal structures of MscL homologs, namely a pentameric channel at the closed state from *Mycobacterium tuberculosis* [4] and a tetrameric channel at the pre-expanded intermediate state from *Staphylococcus aureus* [5]. For the next stage, the structures of MscL at the open state and/or the subconducting state are highly anticipated in the field. Our current aim is to solve and characterize the structures of MscL at various conformations, ideally of the same homolog with identical oligomeric state. The ultimate goal is to provide high-resolution views on the dynamic gating process of MscL and promote the understanding on the molecular basis of mechanical force sensing.

2 Experiment

Constructs of various MscL homologs from archaea and bacteria were expressed in *E. coli*. and purified through nickel affinity chromatography and size exclusion chromatography. The MscL protein at the open-state conformation was obtained by introducing charged residues into the constriction site of the channel through site-directed mutagenesis. As a general procedure for membrane protein purification, various kinds of detergents (such as alkyl-glucosides or alkyl-maltosides) and mixture of two/three different species of detergents have been attempted for preparing proteins samples in micelles with different curvature stresses imposed on the channel. The initial crystallization conditions have been identified by using commercial screening kits such as the MemGold I and MemGold II (Molecular dimensions). Extensive optimization strategies, including pH screening and detergent/additive screening, have been carried out. The MscL crystals were grown by the hanging or sitting drop vapour diffusion method at 16 °C. To stabilize the crystals, crosslinking with glutaraldehyde through vapour diffusion has been tried. For cryoprotection, crystals were soaked in a stabilizing solution containing 15% glycerol and then flash-frozen by immersing into liquid nitrogen.

To identify the best crystals suitable for structural studies, over 300 samples have been screened at the BL-5A, BL-17A, NW12A, NE3A and BL-1A beamlines in Photon Factory. Two useful datasets of medium resolution have been collected on BL17A and BL1A, respectively. The data were processed using HKL2000 or iMosflm. Analysis of non-crystallographic symmetry through self-rotation function was performed by using Molrep in CCP4.

3 Results and Discussion

Due to the highly fragile nature of the MscL crystals, numerous cracks occurred within the large MscL crystals (large rods measuring 0.3-0.4 mm at the longest dimension) during cryoprotection procedure. During the crystal screening experiments at different beamlines of PF, we have found that the BL1A micro-focus beam with a size of 10 μm × 25 μm proved to be extremely helpful in avoiding the cracks on the crystals. A high-quality single crystal diffraction dataset has been collected on this beamline. To minimize radiation damage, the helix mode for data collection has been applied by choosing two to three spots on the same crystals and then the data were merged to yield a complete set after integration and scaling.

As shown in Table 1, two complete dataset have been collected using crystal form A and B, respectively. The A-form crystal diffracted to 3.5 Å at BL-1A and belongs to the $P2_12_12_1$ space group with unit cell dimensions of *a*

=84.69 Å, *b* =130.44 Å and *c* =185.13 Å. In comparison, the B-form crystal shows a different space group ($P2_12_12$) and the unit cell dimensions are: *a* =99.17 Å, *b* =147.36 Å and *c* =149.25 Å. Although the B-form crystal only diffracted to about 4.2 Å resolution, it adopts a completely different crystal packing pattern and may represent a conformation different from the molecules in the A-form crystal.

To detect the non-crystallographic symmetry within the crystal, we have run self-rotation function through Molrep program using the data of A-form crystal. It appears that the self-rotation peak at 72° Chi angle is fairly weak (Fig. 1), suggesting that the five-fold non- crystallographic symmetry might not be strictly maintained in the homo-oligomeric channel assembly within the crystal.

Currently, we are making every effort to further improve the quality of both crystal forms to overcome the anisotropic problem and achieve higher resolution. In parallel, functional analyses through single-channel electrophysiology methods are performed to test the activity of the MscL protein reconstituted on the giant unilamella vesicles.

Table 1: Data-collection statistics

Crystal form	A	B
Beamline	BL-1A	BL-17A
Wavelength (Å)	1.1	1.0
Resolution (Å)	50-3.5	41-4.2
Space group	$P2_12_12_1$	$P2_12_12$
Unit cell parameters(Å)		
A	84.69	99.17
B	130.44	147.36
C	185.13	149.25
No. of reflections		
Observed	155836	148162
Unique	25441	16510
Completeness (%)	97.5 (98.7)#	99.7 (100.0)#
$I/\sigma(I)$	13.2 (1.6)#	9.3 (2.8)#
R_{merge} (%)	11.7 (88.5)#	10.2 (78.4)#

Numbers in parentheses refer to the statistical data for the highest resolution shell at 3.63-3.5 Å (A-form) and 4.43-4.2 Å (B-form).

Acknowledgement

We thank the PF staff members for their excellent technical support during crystal screening and data collection, Xiuying Liu for the assistance with crystal loading and handling, Mei Li for the help with the B-form dataset collection. This research project is financially supported by the "National Thousand Young Talents Program" from the Office of Global Experts Recruitment in China, the "135" Project of Chinese Academy of Sciences (CAS), the "Startup Funding for the Awardees of the Outstanding Ph.D. Thesis Fellowship" from CAS, and the "Innovative Research Group Funding" from Chinese Natural Science Foundation.

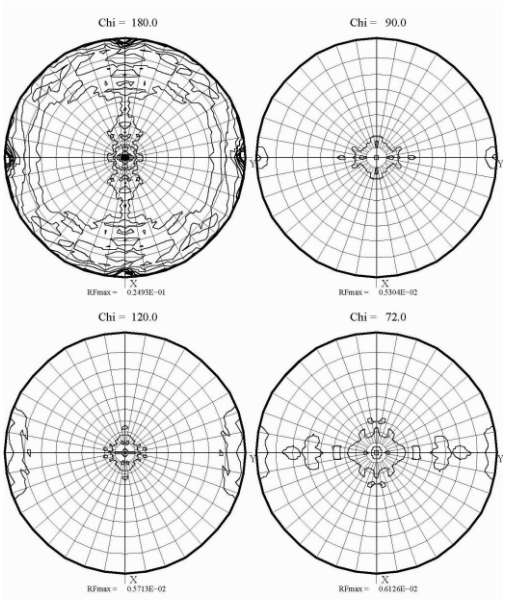

Fig. 1: The self rotation function maps of the A-form MscL crystal computed by Molrep program at Chi angles of 180°, 120°, 90° and 72°.

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