SUPPORTING INFORMATION

Hierarchical and Helical Self-assembly of ADP-ribosyl Cyclase into Large-scale Protein Microtubes

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EXPERIMENTAL METHODS

Protein Sample Preparation

Expression and purification of ADP-ribosyl cyclase proteins were performed with procedures as previously described (S1). The Pichia expression vector pPICZαA (Invitrogen, Carlsbad, CA) was used to express the ADP-ribosyl cyclase protein. The expressed protein was secreted as a soluble component directed by the yeast mating α-factor signal sequence framed in the vector. The soluble component protein was purified by phenylsepharose and cation-exchange SP5-PW column chromatography (Waters, Milford, MA). The elusion peak was characterized as active ADP-ribosyl cyclase by monitoring its enzymatic production of cyclic GDP-ribose (S2). Purified protein was concentrated to 8 mg/mL using Centriprep 10 filters (Amicon, Beverly, MA) and frozen in liquid nitrogen until use.

Fabrication of crystalline microtubules

The hexagonal tubular crystalline structures were obtained by a hanging-drop vapor diffusion method (S3) at room temperature. The drops were formed by mixing 1μL 5 mg/mL cyclase protein with 1μL precipitates containing 100 mM imidazole, pH 7.5, 11-12% PEG 4000. Addition of 0.02% sodium azide into the drop helped prevent the growth of microbes. The concentration of PEG 4000 was important for

the formation of tubular structures. Higher or lower PEG 4000 produced either no crystalline microtubules or solid thin crystalline plates.

Scanning electron microscopy

SEM measurements were performed with a Hitachi S4500 Scanning Electron Microscope at the Cornell Integrated Microscopy Center (CIMC). Freshly prepared microtube samples were not used directly for SEM characterization because of abundant water hosted in samples. Also, a dehydration process was avoided because it can totally destroy microtubular structures. To maintain their architecture, the samples were fixed by incubating them with 5% glutaraldehyde for 10 min. After several cycles washing with distilled water, the fixed samples were negatively stained with 0.2% uranyl acetate for about 5 min. Before mounting for SEM imaging, the dehydrated samples were carbon coated to increase their conductivity.

X-ray crystallography

X-ray diffraction data were collected at the Cornell High-Energy Synchrotron Source (CHESS) A1 station under the protection of liquid nitrogen cryo-stream at 100 K. The crystal to detector distance was optimized and set to 450 mm to separate spots due to the long unit cell dimension along b-axis. A total of 360 frames with an oscillation angle of 1° were collected from a single microtube using a Quantum Q-210 CCD detector. To obtain a scaled and merged data set, all diffraction frames were processed by using the program Denzo/Scalepack suite at 4.5 Å resolution (S4). The crystal lattice is not perfect due to a large mosaic spread ranging from 2.0 to 2.9. Therefore, we cut off the data set to 4.5 Å resolution to reduce the noise caused from the mosaic spread.

The crystalline tube belongs to space group $P2_1$. The solvent content estimation shows that there are three dimers of ADP-ribosyl cyclase molecules in the crystallographic asymmetric unit. The tube structure was solved by a molecular replacement method with the program Phaser (S5). The dimeric model of published ADP-ribosyl cyclase (PDB ID: 1LBE) was used as a search model for its initial

assembly within the unit cell. The initial structural assembly was further refined against the diffraction data at 4.5 Å resolution. Due to the low resolution data, tight non-crystallographic symmetry (NCS) and chemical restraints were applied during refinements. The refined model explains the data quite well with an R factor of 29.98% and a free R factor of 30.79%. All refinements were done in REFMAC (S6). A summary of structural refinement and model statistics are listed in Table S1.

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Table S1. Data collection and refinement statistics.

Data collection	
Cell dimensions	55.66, 356.81, 55.66
a, b, c (Å)	90.0, 120.1, 90.0
α, β, γ (º)	
Space group	$P2_1$
Crystal to detector distance (mm)	450
Range of mosaic spread	2.0-2.9
Resolution (Å)	100-4.5 (4.66-4.5)
Unique reflections	10307
Redundancy	6.2 (5.4)
I/σ (I)	22.9 (15.4)
R_{merge} (%)	7.8 (12.4)
Completeness (%)	94.2 (93.1)
Refinement	
Resolution (Å)	100-4.5
R factor (%)	29.98
R_{free} factor (%)	30.79
Protein atoms	12042
Number of molecules in the unit cell	12
Overall B ($Å^2$)	62.46
R.M.S. deviations	
Bond lengths (Å)	0.006
Bond angles (º)	0.894

Values in parentheses are from the highest resolution shell.

$R_{merge} = \Sigma|I - \langle I \rangle|/\Sigma I$, where I is the integrated intensity of a given reflection.

$R = \Sigma||Fobs| - |Fcalc||/\Sigma|Fobs|$. R_{free} was calculated using 5% of data excluded from refinement.