Biochemical and Biophysical Research Communications xxx (2018) 1–7

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Structural and biochemical characterization of the type-II LOG protein from *Streptomyces coelicolor* A3

Hogyun Seo [a, b], Kyung-Jin Kim [a, b, *]

[a] School of Life Sciences, KNU Creative BioResearch Group, Kyungpook National University, Daehak-ro 80, Buk-ku, Daegu, 41566, Republic of Korea
[b] KNU Institute for Microorganisms, Kyungpook National University, Daehak-ro 80, Buk-ku, Daegu, 41566, Republic of Korea

ARTICLE INFO

Article history:
Received 22 March 2018
Accepted 25 March 2018
Available online xxx

Keywords:
Streptomyces coelicolor
Type-II LOG
Cytokinin

ABSTRACT

Streptomyces coelicolor A3 contains *Sc5140*, a gene coding for poorly understood bacterial LOG-like protein. In this study, we determined the crystal structure of *Sc*5140 and found it resembles the overall structure of other type-II LOGs. In addition, *Sc*5140 exhibited phosphoribohydrolase activity against adenosine monophosphate (AMP), indicating that it had the same function as known type-II LOGs. Based on these results, we designated *Sc*5140 as *Sc*LOGII. We performed docking calculations of AMP into the *Sc*LOGII structure, which suggested the mode of binding for type-II LOG with their AMP substrate. The *Sc*LOGII structure uniquely exhibited a long tail-like structure at the N-terminus that was involved in hexamerization of the protein; the disordered N-terminal region (DNR). Truncation of DNR in *Sc*LOGII negatively affected both the phosphoribohydrolase activity and the oligomerization of the protein, suggesting that this region functioned in enzyme stabilization. However, results from truncation experiments using *Sc*LOGII and *Cg*LOGII, a type-II LOG homologue from *Corynebacterium glutamicum*, were quite different, leaving uncertainty regarding the general functions of DNR in type-II LOGs. Overall, the current structural work may help in understand the significance of type-II LOG protein at the molecular level.

1. Introduction

Purine-derived cytokinins are N^6-substituted adenines that are phytohormones with broad and important roles in the physiological processes of plants, such as root growth and branching [1], delay of senescence [2], chloroplast development [3], and the activation of pathogen-defense-related pathways [4]. Cytokinins are often conjugated with sugar moieties, such as nucleotides, nucleosides, and glucosides, but these forms are biologically less active or are inactive for plant cytokinin receptors [5]. Being released from their conjugates is the cytokinin-activating step, and is a key target for controlling active cytokinin levels. “Lonely guy” (LOG) was identified as an enzyme involved in this activation through direct hydrolysis of bonds between bases and phosphoribose moieties of cytokinin nucleotides in plants [6]. In addition to being plant enzymes, LOG-like proteins are also highly conserved in a variety of prokaryotes. The study of bacterial LOGs so far has been limited because they are also considered possible lysine decarboxylases [7,8].

Streptomycetes species compose the largest genus of *Actinobacteria*, and are well known for their multicellular mycelial life cycle, and antibiotic production from their complex secondary metabolism [9]. Antibiotics from *Streptomycetes* account for half of all the worlds' clinical antibiotics from natural origins [10]. In nature, *Streptomycetes* sp. inhabit soil, and are commonly saprophytic with many of them existing as endophytes and pathogens, especially of plants [11,12]. These are some of the most important groups for disclosing the ecosystems of microorganisms that exist in the ground. *Streptomyces coelicolor* A3 is a prominent model strain of *Streptomycetes*. As a soil dweller exposed to a wide variety of environmental conditions, *S. coelicolor* has many elaborate regulatory mechanism for environmental adaptation in relation to its unique existence [13]. Likewise, its genome has an abundancy of regulatory genes that respond to external stimuli and stresses [14].

Interestingly, *S. coelicolor* contains three genes that encode LOG-like proteins (*Sc5140, Sc5491,* and *Sc5651*), implying the microbial production of cytokinin and the interactions with plants. Among

* Corresponding author. Structural and molecular biology Laboratory, School of Life Sciences and Biotechnology, Kyungpook National University, 80 Daehak-ro, Buk-ku, Daegu 702-701, Republic of Korea.
E-mail address: kkim@knu.ac.kr (K.-J. Kim).

https://doi.org/10.1016/j.bbrc.2018.03.193
0006-291X/

the LOG isoforms, the gene products of *Sc5491* and *Sc5140* have the highest homology to type-I and type-II LOGs, respectively. Type-I LOGs were the first LOGs characterized in plants to be cytokinin-activating enzymes, and to be physiologically active as a dimer [6,8,15]. In comparison, type-II LOGs adopt a unique hexamer formation through an additional α-helix [7,16]. The type-II LOGs have significantly different substrate-binding sites compared to those of type-I LOGs [16]. However, heterogeneous overexpression of type-II LOG in *Escherichia coli* produced detectable amounts of isopentenyladenine, a typical cytokinin, suggesting that type-II LOG may also function as cytokinin-activating protein [16]. This is highlighted by the differences in the prenyl-group binding sites, which implies that their optimum activities for substrates are likely to vary depending on the specific type of cytokinins. In this report, we describe the crystal structure of *Sc*LOGII from *S. coelicolor*, which reveals a unique N-terminal structure. The functional implication of the N-terminal region is discussed.

2. Materials and methods

2.1. Protein preparations

The *Sc*LOGII gene was amplified by polymerase chain reaction (PCR) using synthesized gene with codon optimization for expression in *Escherichia coli* cells as a template. The PCR product was then subcloned into pET30a (Novagen), and the resulting expression vector pET30a:*Sc*LOGII was transformed into the *E. coli* BL21 (DE3) strain, which was grown on LB medium containing 100 mg l^{-1} kanamycin at 37 °C to OD600 of 0.6. After induction by addition of 1.0 mM isopropyl β-D-1-thiogalactopyranoside (IPTG), the culture was further incubated for 20 h at 18 °C. The cells were then harvested by centrifugation at 5000 × g for 15 min at 4 °C. The cell pellet was resuspended in ice-cold buffer A (40 mM Tris–HCl, pH 8.0) and disrupted by ultrasonication. The cell debris was removed by centrifugation at 11,000 × g for 1 h, and the supernatant was applied to a Ni-NTA agarose column (Qiagen). After washing with buffer A containing 15 mM imidazole, the bound proteins were eluted with 300 mM imidazole in buffer A. Further purification was carried out by applying the HiTrap Q ion exchange chromatography and size exclusion chromatography. The degree of protein purify was confirmed by sodium dodecyl sulfate polyacrylamide gel electrophoresis. All purification steps were performed at 4 °C. The purified proteins were concentrated to 30 mg ml^{-1} in 40 mM Tris–HCl, pH 8.0, and stored at −80 °C for crystallization trials. The production and purification of the *Sc*LOGII$_{\Delta DNR}$ was carried out by the same procedures as described for the wild-type protein.

2.2. Crystallization of ScLOGII

Crystallization of the purified proteins were initially performed by the hanging-drop vapor-diffusion technique at 20 °C using commercially available sparse-matrix screens from Hampton Research and Emerald BioSystems. Each experiment consisted of mixing 1.0 μl protein solution with 1.0 μl reservoir solution and then equilibrating it against 0.5 ml of the reservoir solution. The *Sc*LOGII crystals were observed from several crystallization screening conditions. After several optimization steps using the hanging-drop vapor-diffusion method, the best-quality crystals appeared in 4 day using a reservoir solution consisting of 0.1 M citrate (pH 5.5) and 20% PEG 3350 and reached maximal dimensions of approximately 0.2 × 0.2 × 0.2 mm. The crystals were transferred to a cryoprotectant solution containing 0.1 M citrate (pH 5.5), 30% PEG 3350, and 30% (v/v) glycerol, extracted with a loop larger than the crystals, and flash-frozen by immersion in liquid nitrogen.

2.3. Data collection and structure determination of ScLOGII

Data were collected at the 7A beamline at the Pohang Accelerator Laboratory using a QUANTUM 270 CCD detector (San Diego, CA, USA) at the wavelength of 0.97934 Å. The *Sc*LOGII crystal diffracted to resolution of 2.5 Å. The data was then indexed, integrated, and scaled using the HKL2000 software suite [17]. The *Sc*LOGII crystals belonged to the I-centered cubic space group *I*23 with unit cell dimensions of $a = b = c = 206.77$ Å. With four *Sc*LOGII molecules per asymmetric unit, the crystal volume per unit of protein mass was approximately 3.68 $Å^3$ Da^{-1}, which corresponds to a solvent content of approximately 66.29% [18]. The structure of *Sc*LOGII was determined by molecular replacement method with the CCP4 version of *MOLREP* [19] using the structure of type-II LOG from *C. glutamicum* (*Cg*LOGII, PDB code 5WQ3, 65% sequence identity) as a search model. The model building was performed using the program *WinCoot* [20] and the refinement was performed with *REFMAC5* [21]. The data statistics are summarized in Table 1. The refined model of *Sc*LOGII was deposited in the Protein Data Bank (PDB code 5ZI9).

2.4. Size-exclusion chromatographic analysis

To investigate the oligomerization of *Sc*LOGII and *Sc*LOGII$_{\Delta DNR}$, analytical size-exclusion chromatography was performed using a Superdex 200 10/300 column (GE Healthcare) at NaCl concentrations of 150 mM. Protein samples of 1 ml with concentration of 4 mg ml^{-1} were analyzed. The molecular weights of the eluted samples were calculated based on the calibration curve of standard samples.

Table 1
| Data collection and structural refinement statistics.

	*Sc*LOGII
PDB code	5ZI9
Data collection	
Wavelength (Å)	0.97934
Unit cell (*a*, *b*, *c*; γ) (Å; °)	206.77; 90.0
Space group	*I23*
Solvent content (%)	66.29
Protein chains in AU	4
Resolution range (Å)	50.00–2.50
Highest resolution shell (Å)	2.54–2.50
Unique reflections	49440
Redundancy	8.7 (4.5)
Completeness (%)	98.0 (96.8)
R_{merge} (%)	10.1 (41.3)
Average I/σ(I)	16.7 (3.8)
CC1/2	0.374
Refinement	
R (%)	18.5
R_{free} (%)	23.7
Mean B value ($Å^2$)[a]	32.0
B from Wilson plot ($Å^2$)	27.3
RMS deviation bond lengths (Å)	0.015
RMS deviation bond angles (°)	1.762
Number of amino acid residues	900
Number of water molecules	69
Ramachandran plot	
Most favored regions (%)	98.0
Additional allowed Regions (%)	2.0

[a] Mean B value is for both protein atoms and the solvent molecules.

Please cite this article in press as: H. Seo, K.-J. Kim, Structural and biochemical characterization of the type-II LOG protein from *Streptomyces coelicolor* A3, Biochemical and Biophysical Research Communications (2018), https://doi.org/10.1016/j.bbrc.2018.03.193