Structural basis for cytokinin production by LOG from Corynebacterium glutamicum

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“Lonely guy” (LOG) has been identified as a cytokinin-producing enzyme in plants and plant-interacting fungi. The gene product of Cg2612 from the soil-dwelling bacterium Corynebacterium glutamicum was annotated as an LDC. However, the facts that C. glutamicum lacks an LDC and Cg2612 has high amino acid similarity with LOG proteins suggest that Cg2612 is possibly an LOG protein. To investigate the function of Cg2612, we determined its crystal structure at a resolution of 2.3 Å. Cg2612 functions as a dimer and shows an overall structure similar to other known LOGs, such as LOGs from Arabidopsis thaliana (AtLOG), Claviceps purpurea (CpLOG), and Mycobacterium marinum (MmLOG). Cg2612 also contains a “PGGXGT XXE” motif that contributes to the formation of an active site similar to other LOGs. Moreover, biochemical studies on Cg2612 revealed that the protein has phosphoribohydrolase activity but not LDC activity. Based on these structural and biochemical studies, we propose that Cg2612 is not an LDC family enzyme, but instead belongs to the LOG family. In addition, the prenyl-binding site of Cg2612 (CgLOG) comprised residues identical to those seen in AtLOG and CpLOG, albeit dissimilar to those in MmLOG. The work provides structural and functional implications for LOG-like proteins from other microorganisms.

The term cytokinin originated from the cell division-promoting functions of these compounds1. Cytokinin phytohormones are usually N6-modified adenines such as N6-(β-isopentenyl)adenine (iP) and trans-zeatin (tZ), and they play significant roles in controlling growth and development of plants2,3. They can be conjugated with sugar moieties such as nucleotides, nucleosides, and glucosides, but these conjugated forms are biologically less active or inactive for plant cytokinin receptors2. The cytokinin biosynthetic pathway begins with dimethylallyl pyrophosphate (DMAPP), possibly originating from the mevalonate or methylerythritol phosphate pathway, being prenylated by isopentenyltransferase (IPT) (Fig. 1a). Adenylate-IPT can add DMAPP to ATP/ADP or adenylate, whereas tRNA-IPT modifies the N6-atom of adenine moiety on position 37 of tRNA4. The isopentenylated products can be converted to the typical metabolite N-(β-isopentenyl)adenosine 5′-monophosphate (iPRMP) by dephosphorylation or degradation of tRNA4. The nucleotide iPRMP might be dephosphorylated by nucleotidase and then deribosylated by nucleosidase to produce an active nucleobase5. In 2007, a one-step cytokinin activation pathway was first discovered and the novel cytokinin-activating enzyme called lonely guy (LOG) emerged6. LOG produces active cytokinins via dephosphoribosylatation, directly hydrolyzing the bond between N6-substituted bases and ribose 5′-monophosphates in cytokinin precursors such as iPRMP or trans-zeatin riboside 5′-monophosphate (tZRMP).

For many years, before the discovery of their cytokinin-producing activity, LOGs were known as possible lysine decarboxylases (LDCs) according to the Pfam database, without experimental evidence7. Recently, enzymes from several organisms, such as Oryza sativa, Arabidopsis thaliana, Claviceps purpurea, and Mycobacterium tuberculosis have been identified as LOGs by biochemical and functional studies8–10. According to morphological and metabolic analyses, the LOG-mediated one-step pathway is suggested as the major cytokinin production pathway and is pivotal for normal growth and development in Arabidopsis8. Despite a lack of evidence for the phosphoribohydrolase catalytic mechanism, the homodimeric disposition and the active site with highly conserved

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“PGGXGTXXE” motif were elucidated by structural studies on LOG proteins\(^\text{10,11}\). However, many LOG-like proteins, especially from bacteria, have remained terra incognita.

The soil-dwelling bacterium *Corynebacterium glutamicum* has been intensively studied for industrial applications due to its high production of amino acids, nucleotides, and vitamins\(^\text{12}\). Among these products, L-lysine has most actively drawn attention in industry\(^\text{13}\). Interestingly, *C. glutamicum* ATCC 13032 contains a gene product

**Figure 1. Overall structure of CgLOG.** (a) Biosynthetic pathway for cytokinin production. (b) Amino acid sequence alignment of LOGs. The secondary structure elements are drawn based on the structure of CgLOG. Residues involved in catalysis, AMP binding, and prenyl-group binding are indicated by red, blue, and orange-colored triangles, respectively. The PGGXGTXXE motif is indicated with a purple-colored dotted rectangle. CgLOG, MtLOG, AtLOG, OsLOG, and CpLOG are abbreviations of LOGs from *Corynebacterium glutamicum*, *Mycobacterium tuberculosis*, *Arabidopsis thaliana*, *Oryza sativa*, and *Claviceps purpurea*, respectively. (c) The monomeric structure of CgLOG presented as a cartoon diagram. The bound glycerol and phosphate molecules are shown as stick models. Secondary structure elements are labeled. (d) Dimeric structure of CgLOG. The dimeric structure of CgLOG is presented as a cartoon diagram. The bound glycerol and phosphate molecules are shown as in (c). The bottom figure is the top figure rotated horizontally by 90°.
of Cg2612 that is annotated as a possible LDC (pfam03641) and a nucleotide-binding protein. LDCs are known as pyridoxal 5′-phosphate (PLP)-dependent enzymes that convert L-lysine to cadaverine by a decarboxylation reaction. Ironically, C. glutamicum is also known to lack LDC, which results in the accumulation of L-lysine. Moreover, Cg2612 shows high amino acid similarity with LOG proteins, suggesting that Cg2612 is possibly an LOG protein. In this report, in order to elucidate the function of Cg2612, we determined its crystal structure.

Results

Overall structure of Cg2612. To investigate the function of Cg2612, we determined its crystal structure at a 2.3 Å resolution (Table 1). The asymmetric unit contained four molecules and seems to contain two distinct dimers. Molecules I, II, III, and IV of Cg2612 contain 9-191, 3-195, 3-190, and 2-195 residues visible in the electron density map, respectively. The R.M.S.D. values between these four monomeric structures are under 0.4, indicating that four monomers have quite similar structures each other. Interestingly, among four monomers in the asymmetric unit, two monomers contain a phosphate ion at each active site. We found that Lys194 from one dimer interacts with a phosphate in the active site of the other dimer (Supplementary Fig. S1). We speculate that crystal packing in I222 space group caused tetrameric arrangement as an artifact (Supplementary Fig. S1). Size-exclusion chromatography analysis suggested that Cg2612 forms a dimer (Supplementary Fig. S2). We then performed small-angle X-ray scattering (SAXS) experiment to further confirm the dimeric conformation of Cg2612 in solution, and the result indicates that Cg2612 functions as a dimer as observed in other LOGs (Supplementary Fig. S3).

The monomeric structure of Cg2612 shows an α/β fold belonging to a Rossmann fold (Fig. 1b,c). The central β-sheet which is formed by seven parallel β-strands is surrounded by eight α-helices (Fig. 1c). Dimerization of Cg2612 displays a compact domain folding. The dimerization interface is mainly composed of α5- and α6-helices, and the α4-helix partially aids in dimerization (Fig. 1d and Supplementary Fig. S4). PISA computed the buried interface area to be 1,563 Å (averaged with AB dimer and CD dimer) and the percentage of participating residues to be 24.5%. Dimerization of two polypeptides constitutes a pocket which serves as the active site, and the conserved “PGG2GTxG” motif was found in the surface of the pocket, which will be described in detail later.

Table 1. Data collection and structural refinement statistics. a The numbers in parentheses are statistics from the highest resolution shell. b $R_{\text{sym}} = \Sigma |I_{\text{obs}} - I_{\text{avg}}|/I_{\text{avg}}$, where $I_{\text{obs}}$ is the observed intensity of individual reflection and $I_{\text{avg}}$ is average over symmetry equivalents. c $R_{\text{work}} = \Sigma |F_o| - |F_c|)/\Sigma |F_o|$, where $|F_o|$ and $|F_c|$ are the observed and calculated structure factor amplitudes, respectively. $R_{\text{free}}$ was calculated with 5% of the data.

![Table 1](https://www.nature.com/scientificreports/)

| Data collection | CgLOG |
|-----------------|-------|
| Space group     | I222  |
| Cell dimensions | 113.5, 130.5, 140.5 |
| $\alpha, \beta, \gamma$ (°) | 90.00, 90.00, 90.00 |
| Resolution (Å) | 50.00–2.30 (2.34–2.30) |
| $R_{\text{sym}}$ or $R_{\text{merge}}$ | 10.2 (31.9) |
| $I/O$ | 17.5 (3.5) |
| CC1/2 | 0.989 (0.63) |
| Completeness (%) | 95.2 (87) |
| Redundancy | 6.8 (3.2) |

**Refinement**

| Resolution (Å) | 50.00–2.30 |
| No. reflections | 41951 |
| $R_{\text{work}}/R_{\text{free}}$ | 18.3/23.0 |
| No. atoms | 6149 |
| Protein | 5899 |
| Glycerol/PO$_4$ | 36/10 |
| Water | 204 |
| $B$-factors | 29.69 |
| Protein | 29.76 |
| Glycerol/PO$_4$ | 38.04/20.20 |
| Water | 30.13 |
| R.m.s. deviations |
| Bond lengths (Å) | 0.015 |
| Bond angles (°) | 1.704 |
Cg2612 has LOG function. Structural comparison using the DALI server\(^\text{17}\) showed that the structure of Cg2612 is quite similar to LOG3 (AtLOG3, PDB CODE 2A33, Z-score 29.4) and LOG8 (AtLOG8, PDB CODE 1YDH, z-score 30.4) from A. thaliana. The comparison also showed that LOGs from C. purpurea (CpLOG, PDB CODE 5AJT, Z-score 26.8) and M. marinum (MmLOG, PDB CODE 3SBX, Z-score 27.7) are structural homologs of Cg2612. These structural homologs also shared amino acid identity higher than 33% with Cg2612. Because these structural homologs of Cg2612 have been identified as LOG proteins, high similarity in structure and amino acid sequence with these proteins suggests that Cg2612 functions as a LOG. To investigate the biochemical function of Cg2612, we performed lysine decarboxylase and phosphoribohydrolase activity assays on Cg2612, and compared the results with lysine decarboxylase from E. coli (EccadA). As expected, Cg2612 did not show any lysine decarboxylase activity, while EccadA showed strong activity (Fig. 2a). These results indicate that Cg2612 is not a lysine decarboxylase as inferred from previous studies on LOGs and high amino acid sequence identity of Cg2612 with LOGs. We then tested if Cg2612 has a phosphoribohydrolase activity. For this assay, we used adenosine monophosphate (AMP) as a substrate, because we could not obtain natural cytokinin precursors and it was previously reported that LOG has a phosphoribohydrolase activity against an AMP substrate\(^\text{9}\). Interestingly, we observed that Cg2612 has phosphoribohydrolase activity and this activity tends to increase upon reaction time (Fig. 2b). However, LOGs generally show higher phosphoribohydrolase activity against natural cytokinin precursors than AMP\(^\text{5,9}\), suggesting that Cg2612 might have much higher phosphoribohydrolase activity against natural cytokinin precursors than observed with AMP as a substrate. On the other hand, EccadA showed no phosphoribohydrolase activity with AMP as a substrate (Fig. 2c). These results confirm that Cg2612 belongs to the LOG family, and hereafter, we will represent Cg2612 as CgLOG.

Active site of CgLOG. In order to elucidate an active site and a substrate binding mode of CgLOG, we tried to determine the structure in complex with AMP or cytokinin. However, neither co-crystallization nor soaking of AMP or cytokinin into the CgLOG crystal was successful. We then superposed our structure with MmLOG in complex with AMP\(^\text{18}\). The active site of CgLOG is located near the “PGG\(_3\)GT\(_{\text{xy}}\)E” motif. The phosphate moiety was hydrogen bonded with main chains of Gly116, Ala117, and Gly118, and side chains of Thr119 and Ser19 (Fig. 3a). The ribose moiety is mainly stabilized by hydrogen bond interactions between Arg99 and two hydroxyl groups of the ribose moieties (Fig. 3a). To stabilize the adenine ring, a mixture of hydrophobic and hydrophilic
residues, Met96, Lys100, and Glu121, form an adenine binding site (Fig. 3a). Two proposed catalytic residues, Arg99 and Glu122, are located in the vicinity of the bond to be hydrolyzed; a covalent bond between adenine-N9 and ribose-C1 (Fig. 3a). Among the residues involved in AMP binding and enzyme catalysis, Gly116, Ala117, Gly118, Thr119, Glu121, and Glu122 are located in the “PGGXGTXXE” motif, indicating that the motif serves as a nucleotide binding site as suggested by other LOG structures. In our current structure, one phosphate and one glycerol molecule are bound at the AMP binding site and these molecules mimic the stabilization of the phosphate moiety and the ribose ring, respectively (Fig. 3a and Supplementary Fig. S5). In order to confirm the involvement of these residues in AMP binding and enzyme catalysis, we performed site-directed mutagenesis experiments. As expected, substituting these crucial residues with alanines resulted in almost complete loss of phosphoribohydrolase activity (Fig. 3b). One exception is S19A mutant which shows higher activity than the wild-type. Most of the residues that involved in AMP stabilization are conserved in all LOGs, and the active site

Figure 3. Active site of CgLOG. (a) Stereo view of the CgLOG AMP binding site. The CgLOG structure is superposed with LOG from M. marinum (MmLOG) in complex with AMP. The bound AMP molecule in MmLOG is shown with the CgLOG structure. Secondary structure elements are labeled. CgLOG is shown as a cartoon diagram. Residues involved in AMP binding are shown as line models. The bound AMP molecule was prepared as in Fig. 1b. The bound glycerol and phosphate molecules are labeled and shown as stick models. The hydrogen bonds involved in stabilization of the glycerol and phosphate molecules are shown with red dotted lines. The PGGXGTXXE motif is depicted with a green color. The covalent bond (a bond between adenine-N9 and ribose-C1) hydrolyzed by the enzyme is indicated by a star symbol. (b) Site-directed mutagenesis experiments. The AMP and adenine standard are indicated. (c) Prenyl-group binding site. The CgLOG structure is superposed with AtLOG3. Two monomers of CgLOG are distinguished with colors of grey and salmon and those of AtLOG3 are with colors of cyan and light-blue. The bound AMP molecule is shown as a stick model in magenta. Two catalytic residues (R39 and E122 in CgLOG) and residues involved in the constitution of the prenyl-group binding site are shown as line models. The putative prenyl-group binding site is labeled and shown with a cyan circle.
conformation observed in CgLOG further supports the classification of this protein as a LOG family enzyme (Fig. 1b).

Stabilization of the prenyl-group, the N\(^{6}\)-modifying moiety of cytokinin precursors, still remains unclear due to the absence of a LOG structure in complex with a natural substrate or cytokinin. However, the binding site of the N\(^{6}\)-prenyl group could be inferred from configuration of the adenine moiety and positioning of N\(^{6}\) atom of AMP bound in MmLOG. Superposition of the CgLOG structure with MmLOG in complex with AMP also leads us to speculate the prenyl-group binding site of CgLOG. In CgLOG, Met96, His97, Lys100, Glu125, and Trp129 seem to form a prenyl-group binding site (Fig. 3c). The prenyl-group binding locates at the dimer interface and especially Glu125 and Trp129 residues are provided from a neighboring molecule (Fig. 3c). Importantly, these residues are identical to those found in AtLOG3, OsLOG, and CgLOG (Figs 1b and 3c). Based on these observations, we propose that CgLOG utilizes cytokinin precursors as substrates that are similarly used by other LOGs from plants or plant-interacting fungi.

Structural comparison of CgLOG with other LOGs. To compare CgLOG with other LOGs, we superposed the CgLOG structure with other LOG proteins such as AtLOG3, CpLOG, and MmLOG. Although the overall folds of all four LOG structures are quite similar to each other, CgLOG exhibited somewhat unique structural features. Compared with the three other LOGs, CgLOG has an extra helix in the C-terminal region and contains extended connecting loops of \(\alpha 3\)-\(\beta 4\) and \(\alpha 4\)-\(\beta 5\) (Fig. 4a). Notably, the extended connecting loop of \(\alpha 3\)-\(\beta 4\) is located near the AMP binding site (Fig. 4a). In AtLOG3 this region is distorted, in CgLOG this region showed high a b-factor. However, in MmLOG, this region contains the Glu80 residue that forms direct a hydrogen bond with the hydroxyl group of the ribose ring (Fig. 4b). These observations indicate that this region is quite diverse in various LOGs and stabilization of the ribose ring seems to occur somewhat differently in each protein. Except for structural differences in this region, LOGs have similar AMP binding modes (Fig. 4b). The conserved residues in "PGGXGTXXE" motifs along with other conserved residues contribute to AMP stabilization (Figs 1b and 4b and Supplementary Fig. S6). One exception is found in MmLOG; Ala19 and Asp120 are involved in AMP stabilization while serine and glutamate residues are located at the corresponding positions in other LOGs (Fig. 4b). Moreover, all four LOGs have two catalytic residues, and Arg99 and Glu122 in CgLOG are located at the same positions, indicating that these LOGs catalyze this reaction via the same catalytic mechanism. The comparison of the prenyl-group binding sites provides insights into LOG substrate specificity. As observed in CgLOG, AtLOG3 and CpLOG contain the residues Met96, His97, Lys100, Glu125, and Trp129 at the prenyl-group binding site (Fig. 4c). However, MmLOG has a glaring discrepancy in the prenyl-group binding site compared with CgLOG, AtLOG3, and CpLOG. At the prenyl-group binding site in MmLOG, Asp124, Glu128, and Trp96 residues are located at the positions corresponding to glutamate, tryptophan, and histidine residues, respectively, in the other three LOGs (Fig. 4c). These comparisons suggest that CgLOG might produce cytokinins similar to those produced by LOGs from plants and plant-interacting fungi. However, MmLOG might produce different types of cytokinins than CgLOG, AtLOG3 and CpLOG, which drives us to speculate that mammalian-interacting bacteria like those in the Mycobacterium genus seem to utilize different types of cytokinins.

Discussion

Our structural and biochemical studies on Cg2612, a protein previously annotated as a LDC, imply that this protein functions as LOG. Because LOGs are enzymes that catalyze the production of cytokinins, here we can raise the question if C. glutamicum truly synthesizes cytokinins. As we mentioned above, the first reaction for cytokinin production is the prenylation of ATP/ADP/AMP or tRNA by IPTs, and these enzymes can be divided into two categories depending on whether they utilize nucleotides or tRNA as a substrate. Adenylate-IPT is usually found in higher plants or phytopathogens (known as Fas4) as a main regulator for cytokinin levels, while tRNA-IPT (MiaA) is ubiquitous to improve the efficiency and fidelity of the codon-anticodon interaction during translation. It was reported that M. tuberculosis H37Rv produces cytokinins such as iP and tZ. Interestingly, genome analysis of M. tuberculosis H37Rv revealed that this strain contains a gene coding for tRNA-IPT (MiaA), but not for adenylate-IPT. This analysis intimated that the tRNA-mediated cytokinin activation is a major pathway for cytokinin production in bacteria, although it was considered to be a minor pathway in Arabidopsis. Like M. tuberculosis, the genome of C. glutamicum ATCC 13032 only contains a gene coding for tRNA-IPT (Cg2130, MiaA). In addition, Cg2130 exhibits 56.9% amino acid sequence identity with Rv2727c. These results indicate that C. glutamicum seems to produce cytokinins by a tRNA-mediated activation mechanism similar to the mechanism in M. tuberculosis. Thus, studies on cytokinin detection and biosynthesis in C. glutamicum are needed.

Some soil bacteria communicate with plants via cytokinins. For instance, Rhodococcus fascians produces cytokinins to infect to a wide range of plants and causes diseases in plant hosts such as a leafy gall syndrome. The virulence of C. glutamicum in plant species has not yet been reported. However, because C. glutamicum is a soil-dwelling bacterium in nature, investigating C. glutamicum–plant communication through cytokinins is of interest for agricultural applications. Furthermore, recent studies on cytokinin production in M. tuberculosis, a mammalian pathogen, suggest that cytokinin production by microbes is not limited to communication with plants, but rather can be applied to wide cross-kingdom communications.

Methods

Protein preparations. The gene coding for LOG from Corynebacterium glutamicum ATCC 13032 (CgLOG) was amplified from chromosome of C. glutamicum by polymerase chain reaction (PCR) with primers: forward, 5-GGCCCATATGACTTCGCTTTTTGGACGCCGGC-3, and reverse, 5-GGCCCTCGAGCATTTTTGGTGTGGTGGAGTTC-3. The PCR product was then subcloned into pET30a (Novagen) with 6xHis at the C-terminus. The resulting expression vectors pET30a: CgLOG was transformed into...
E. coli BL21 (DE3) strain and was grown on LB medium containing 100 mg l\(^{-1}\) kanamycin at 37 °C to OD600 of 0.6. After induction with 1.0 mM 1-thio-\(\beta\)-D-galactopyranoside (IPTG) for a further 20 h at 18 °C, the culture was harvested by centrifugation at 5,000 \(\times\) 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 lysate was bound to Ni-NTA agarose column (Qiagen). After washing with buffer A containing 20 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 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. Site-directed mutagenesis experiments were performed using the QuikChange site-directed mutagenesis kit (Stratagene). The production and purification of the CgLOG mutants were carried out by the same procedures as described for the wild-type protein. CadA from E. coli (EcCadA) was prepared by the procedure similar to CgLOG.

Crystallization, data collection and structure determination. Crystallization of the purified proteins were initially performed by the hanging-drop vapor-diffusion method 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 CgLOG 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 2 day using a reservoir solution consisting of 0.2 M DL-malic acid, pH 7.0 and 24% PEG 3350 and reached maximal 3.10 Å³ Da⁻¹ was 0.1 nm. LOG 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 2 day using a reservoir solution consisting of 0.2 M DL-malic acid, pH 7.0 and 24% PEG 3350 and reached maximal dimensions of approximately 0.3 × 0.3 × 0.1 mm. For the cryo-protection the crystals, glycerol of 30% glycerol in reservoir solution was used. 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 CgLOG crystal diffracted to resolution of 2.3 Å. The data was then indexed, integrated, and scaled using the HKL2000 program²³. Crystals of CgLOG belonged to the I-centered orthorhombic space group I222 with unit cell dimensions of a = 113.51 Å b = 130.50 Å c = 140.51 Å. With four CgLOG molecules per asymmetric unit, the crystal volume per unit of protein mass was approximately 3.10 Å³ Da⁻¹, which corresponds to a solvent content of approximately 60.31%²⁴. The structure of CgLOG was solved by molecular replacement method using MOLREP²⁵ with LOG from A. thaliana (AtLOG, PDB CODE 2A33) as a search model. The model building was performed using the program WinCoot²⁶ and the refinement was performed with REFMAC5²⁷. The data statistics are summarized in Table 1. The refined models of CgLOG was deposited in the Protein Data Bank (PDB CODE 5ITS).

Solution SAXS measurements. Small-angle X-ray scattering (SAXS) measurements were carried out using the 4C SAXS II beamline of the Pohang Accelerator Laboratory (Pohang, Korea). A sample-to-detector distance (SDD) of 4.00 m and 1.00 m for SAXS were used. The magnitude of scattering vector, q = (4π/λ) sin θ, was 0.1 nm⁻¹ < q < 6.50 nm⁻¹, where 2θ is the scattering angle and λ is the wavelength of the X-ray beam source. All scattering measurements were carried out at 4 °C by using a FP50–HL refrigerated circulator (JULABO, Germany). The SAXS data were collected in six successive frames of 0.1 min each to monitor radiation damage. Measurements of CgLOG were carried out over a small concentration range 0.5 ~ 4.5 mg/ml. Each 2D SAXS pattern was radial averaged from the beam center and normalized to the transmitted X-ray beam intensity, which was monitored with a scintillation counter placed behind the sample. The Rg (radius of gyration) values were estimated from the scattering data using Guinier analysis²⁸. The molecular mass (MM) was calculated from the scattering curve based on the Qθ method²⁹. The pair distance distribution p(r) function was obtained through the indirect Fourier transform method using the program GNOM³⁰.

Lysine decarboxylase activity assay. The activity of LDC was determined by measuring residual concentration of α-l-lysine using lysine oxidase and peroxidase. After LDC reaction, lysine oxidase converts remaining lysine into 6-amino-2-oxohexanoate, NH₃, and H₂O₂ and then the hydrogen peroxide is reduced by peroxidase with 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS). The oxidized ABTS is detected by spectrophotometry method in absorbance at 412 nm. The assay was performed at 30 °C in a total volume 200 μl containing 100 mM potassium phosphate, pH 6.0, 0.1 M α-l-lysine, 0.2 mM pyridoxal 5-phosphate, and 25 μg of purified enzymes. The reaction was stopped by heating the reaction mixture at 100 °C for 5 min. After centrifugation at 13,500 × g for 1 min, 2X reaction solution that contains 0.1 unit ml⁻¹ lysine oxidase and 1 unit ml⁻¹ peroxidase in potassium phosphate buffer is added to the reaction mixture.

Phosphoribohydrolase activity assay. The phosphoribohydrolase activity was determined by detecting adenosine ring compounds separated by thin layer chromatography (TLC) method. Enzyme reactions were carried out in the mixture of 20 mM AMP, 36 mM Tris-HCl, pH 8.0, and 23 μM purified enzymes at 30 °C and then the reactions were stopped by heating the mixture at 95 °C for 1.5 min. The reaction mixtures were then dotted on PEI-cellulose-F plastic TLC sheet (Merck Millipore). The mobile phase was 1 M sodium chloride. After development in the TLC chamber, the sheet was dried completely. Adenine ring-including compounds were detected by UV lamp (290 nm).

Size-exclusion chromatographic analysis. To investigate the oligomerization of CgLOG, analytical size-exclusion chromatography was performed using a Superdex 200 10/300 column (GE Healthcare) at NaCl concentrations of 150 mM. 300 μl of protein samples with concentration of 3 mg/ml were analyzed. The molecular weights of the eluted samples were calculated based on the calibration curve of standard samples.

References
1. Skoog, E. & Armstrong, D. J. Cytokinins. Annual review of plant physiology 21, 359–384 (1970).
2. Sakakibara, H. Cytokinins: activity, biosynthesis, and translocation. Annu Rev Plant Biol 57, 431–49 (2006).
3. Mok, D. W. & Mok, M. C. Cytokinin Metabolism and Action. Annu Rev Plant Physiol Plant Mol Biol 52, 89–118 (2001).
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