Acta Crystallographica Section F
**Structural Biology and Crystallization Communications**
ISSN 1744-3091

# Crystallization and preliminary X-ray crystallographic studies of casein kinase I-like protein from rice

**Kyoung Hun Do and Hyun Ho Park***

School of Biotechnology and Graduate School of Biochemistry, Yeungnam University, Gyeongsan, Republic of Korea

Correspondence e-mail: hyunho@ynu.ac.kr

Received 30 September 2011
Accepted 5 January 2012

Casein kinase I (CKI) is a serine/threonine protein kinase that performs various functions in the cell, such as DNA repair, cell-cycle regulation, cytokinesis, vesicular trafficking, morphogenesis and circadian-rhythm regulation. CKI proteins contain a highly conserved catalytic domain at the N-terminus and a highly diverse regulatory domain that is responsible for substrate specificity at the C-terminus. In this study, CKI from rice (riceCKI) was overexpressed in *Escherichia coli* with an engineered C-terminal His tag. RiceCKI was then purified to homogeneity and crystallized at 293 K. X-ray diffraction data were collected to a resolution of 2.0 Å from a crystal belonging to the monoclinic space group $C2$, with unit-cell parameters $a$ = 108.83, $b$ = 69.60, $c$ = 55.85 Å, $\beta$ = 109.47°. The asymmetric unit was estimated to contain one monomer.

## 1. Introduction

Casein kinases (CKs) are a family of serine/threonine protein kinases that play critical roles in cell division, cell growth, metabolism and differentiation (Tuazon & Traugh, 1991; Gross & Anderson, 1998). CKs can be divided into two subgroups, CKI and CKII, which have distinctive structures, functions and responses to effector molecules (Tuazon & Traugh, 1991).

Casein kinase I (CKI) performs various functions in both the cytoplasm and nucleus, such as DNA repair, cell-cycle regulation, cytokinesis, vesicular trafficking, morphogenesis and circadian-rhythm regulation (Gross & Anderson, 1998; Honaker & Piwnica-Worms, 2010; Rumpf *et al.*, 2010; Sugiyama *et al.*, 2010; Mehra *et al.*, 2009; Bidère *et al.*, 2009), and is highly conserved from plants to animals. CKI exclusively uses ATP as a phosphate donor to phosphorylate serine and threonine residues of target proteins. Glycogen synthase, tumour suppressor p53, the cyclic AMP-responsive element modulator CREM and type 1 protein phosphatase inhibitor 2 are well known substrates of CKI (Marin *et al.*, 1994; Milne *et al.*, 1992). CKI contains two distinct domains: a highly conserved catalytic domain at the N-terminus and a highly diverse regulatory domain that is responsible for substrate specificity at the C-terminus (Liu *et al.*, 2003; Vancura *et al.*, 1994).

Six CKI isoforms have been isolated in plants and at least 21 putative isoform sequences have been predicted in *Arabidopsis thaliana* (Klimczak *et al.*, 1995). Additional CKI genes have been isolated from a variety of plant species, including wheat, maize and rice (Dobrowolska *et al.*, 1991; Sasaki *et al.*, 2002). Although there is evidence for the involvement of plant CKIs in the regulation of hormone-related functions and plant-root development (Liu *et al.*, 2003), their physiological functions still await elucidation.

A CKI-like protein from rice (GenBank ID BAB92346; hereafter referred to as riceCKI) was identified during a sequencing project of rice chromosome I and is not well characterized (Sasaki *et al.*, 2002). Full-length riceCKI contains 472 residues with an N-terminal catalytic domain (residues 1–293) and a C-terminal regulatory domain

© 2012 International Union of Crystallography
All rights reserved

(residues 300–472). Its substrate-recognition and regulatory mechanisms are unknown.

Although several structures of the catalytic domain of CKI have been identified to date (Xu *et al.*, 1995; Longenecker *et al.*, 1996), no structures of plant CKIs are available. The structure most closely related to riceCKI is a truncated mutant of mammalian CKI (PDB entry 1cki; Longenecker *et al.*, 1996), which shares 34% sequence identity. In the present study, we overexpressed, purified and crystallized truncated riceCKI (residues 1–428), which contains part of the C-terminal regulatory domain, as a first step towards elucidating the molecular structure and catalytic mechanism of a plant CKI. Details of the atomic structure of riceCKI should enable us to understand the catalytic and regulatory mechanism of plant CKIs.

## 2. Materials and methods

### 2.1. Expression and purification

To express C-terminally His-tagged enzyme, the coding region including the regulatory domain of riceCKI (corresponding to residues 1–428) was cloned into pET26b. The plasmid was transformed into *Escherichia coli* BL21 (DE3) competent cells and its expression was induced in LB medium when the $OD_{600}$ reached 0.75 by treating the bacteria with 0.5 m*M* isopropyl $\beta$-D-1-thiogalactopyranoside (IPTG) overnight at 293 K. Cells expressing riceCKI were pelleted by centrifugation, resuspended and lysed by sonication in 25 ml lysis buffer (20 m*M* Tris pH 7.9, 500 m*M* NaCl, 25 m*M* imidazole). The lysate was centrifuged at 16 000 rev min$^{-1}$ for 1 h at 277 K, after which the supernatant fractions were applied onto a gravity-flow column (Bio-Rad) packed with Ni–NTA affinity resin (Qiagen). Unbound bacterial proteins were removed from the column using wash buffer (20 m*M* Tris pH 7.9, 500 m*M* NaCl, 60 m*M* imidazole). The C-terminally His-tagged riceCKI was eluted from the column using elution buffer (20 m*M* Tris buffer pH 7.9, 500 m*M* NaCl, 250 m*M* imidazole). The elution fractions were collected on a 500 µl scale to 3 ml. Fractions containing more than 80% homogeneous riceCKI, as shown by SDS–PAGE, were selected, combined and concentrated to 10–15 mg ml$^{-1}$ using a concentration kit (Millipore). The concentrated protein was applied onto a Superose 6 gel-filtration column (GE Healthcare) that had been pre-equilibrated with a solution consisting of 20 m*M* Tris pH 8.0, 150 m*M* NaCl. RiceCKI (molecular weight 51 000 Da) eluted at around 16.5 ml and was collected and concentrated to 6–7 mg ml$^{-1}$. The peak was confirmed to contain riceCKI by SDS–PAGE. Purified riceCKI contained the additional residues AAALEHHHHHH at the C-terminus. The hexa-His tag at the C-terminus was not removed.

### 2.2. Crystallization

Crystallization conditions were initially screened at 293 K by the hanging-drop vapour-diffusion method using screening kits from Hampton Research (Crystal Screen, Crystal Screen 2, Natrix, MembFac, Crystal Screen Lite, Crystal Screen Cryo and Index HT) and Emerald BioStructures (Wizard I, II, III and IV). Initial crystals were grown on plates by equilibrating a mixture consisting of 1 µl protein solution (6–7 mg ml$^{-1}$ protein in 20 m*M* Tris pH 8.0, 150 m*M* NaCl) and 1 µl condition No. 41 from Crystal Screen Lite [0.1 *M* Na HEPES pH 7.5, 5%(*v*/*v*) 2-propanol, 10%(*w*/*v*) polyethylene glycol 4000] against 0.4 ml reservoir solution. Crystallization was further optimized using a range of concentrations of protein, polyethylene glycol 4000 and 2-propanol and a range of pH values. Crystals appeared within two months and grew to maximum dimensions of 0.3 × 0.3 × 0.1 mm (Fig. 1) in the presence of 0.1 *M* Na HEPES pH 7.6, 5%(*v*/*v*) 2-propanol, 12%(*w*/*v*) polyethylene glycol 4000. The crystals were triangle-shaped and diffracted to a resolution of 2.0 Å.

### 2.3. Crystallographic data collection

For data collection, crystals were transiently soaked in a solution corresponding to the reservoir solution supplemented with 40%(*v*/*v*) glycerol. The soaked crystals were then cooled in liquid nitrogen. A native diffraction data set was collected from a single crystal to 2.0 Å resolution using a MAR CCD detector (crystal-to-detector distance of 250 mm, 1° oscillation per image, total rotation angle of 180°) on beamline BL-4A at Pohang Accelerator Laboratory (PAL), Republic of Korea. The data sets were indexed and processed using *HKL*-2000 (Otwinowski & Minor, 1997).

## 3. Results and discussion

His-tag affinity chromatography followed by gel-filtration chromatography produced 90% pure riceCKI and no contaminating bands were observed upon SDS–PAGE analysis (Fig. 1). The calculated monomeric molecular weight of riceCKI including the C-terminal His tag was 51 000 Da and its size-exclusion chromatography elution

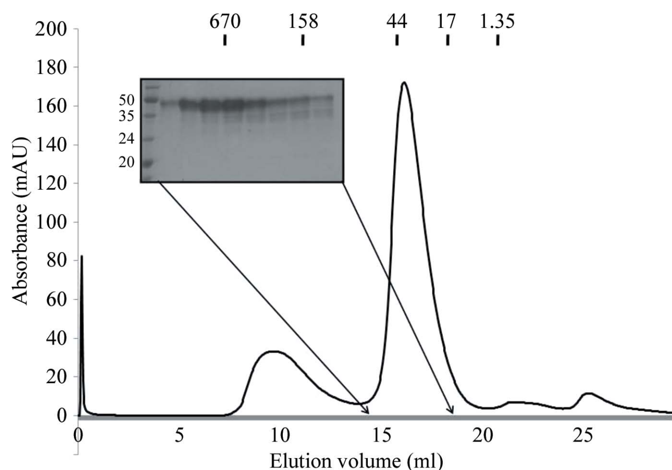


**Figure 1**
Gel-filtration chromatography and SDS–PAGE of riceCKI. Molecular weights are labelled in kDa.

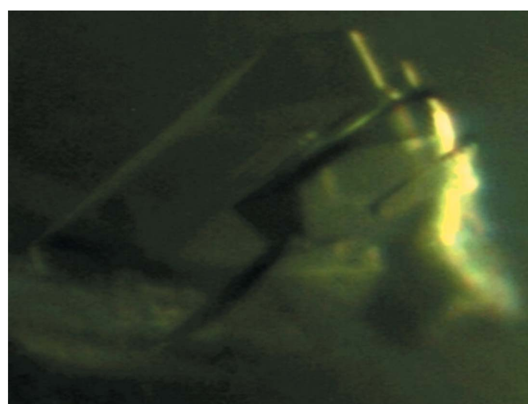

**Figure 2**
Crystals of casein kinase I-like protein from rice (riceCKI). Crystals grew in two months in the presence of 0.1 *M* Na HEPES pH 7.6, 5%(*v*/*v*) 2-propanol, 12%(*w*/*v*) polyethylene glycol 4000. The approximate dimensions of the crystals were 0.3 × 0.3 × 0.1 mm.

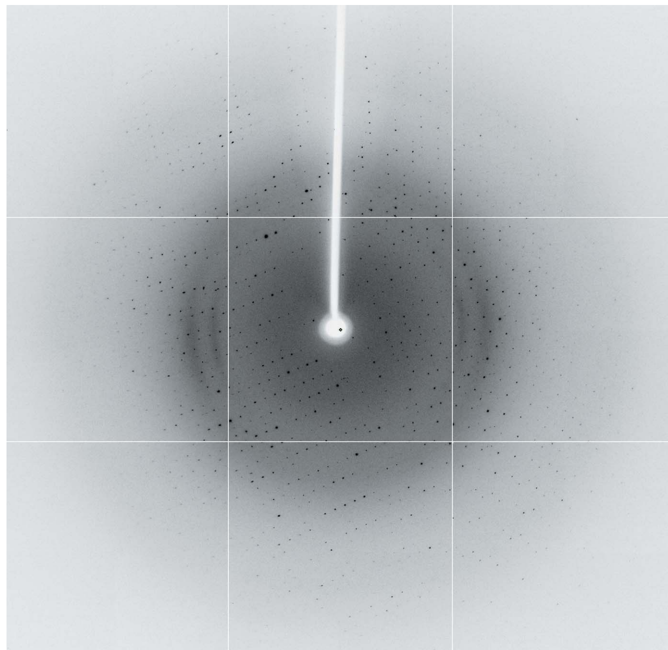

**Figure 3**
A diffraction image (1° oscillation) of the riceCKI crystal with 2.0 Å resolution limit.

peak suggested that it exists as a monomer in solution (Fig. 1). Gel-filtration standards (Bio-Rad) consisting of a mixture of molecular-weight markers (thyroglobulin, 670 000 Da; globulin, 158 000 Da; ovalbumin, 44 000 Da; myoglobulin, 17 000 Da; vitamin $B_{12}$, 1350 Da) were used for size calibration.

An initial needle-shaped crystal that diffracted poorly was obtained from Crystal Screen Lite condition No. 41 (Hampton Research). Optimization of the crystallization conditions using a range of concentrations of protein, polyethylene glycol 4000 and 2-propanol and a range of pH values led to diffraction-quality crystals with a triangular shape (Fig. 2). The optimized crystals grew to dimensions of 0.3 × 0.3 × 0.1 mm in two months and diffracted to 2.0 Å resolution (Fig. 3). The crystals belonged to space group *C*2, with unit-cell parameters $a = 108.83$, $b = 69.60$, $c = 55.85$ Å, $\beta = 109.47°$. Diffraction data statistics are given in Table 1.

Assuming the presence of one monomer in the crystallographic asymmetric unit, the Matthews coefficient ($V_M$) was calculated to be 2.17 $Å^3$ $Da^{-1}$, which corresponds to a solvent content of 43.26% (Matthews, 1968). The molecular-replacement phasing method was conducted with *Phaser* (McCoy *et al.*, 2007), using a truncated mutant of mammalian CKI (PDB entry 1cki), which shares 34% sequence identity, as a search model. A clear solution with rotation-function and translation-function *Z* scores of 26.2 and 24.8, respectively, was initially obtained. Initial refinement with *REFMAC*5 (Vagin & Teplyakov, 2010) using the initial *Phaser* model gave an $R_{work}$ of 35.8% and an $R_{free}$ of 40.7%. Further structural refinement is in progress.

**Table 1**
Diffraction data statistics for riceCKI crystals.

Values in parentheses are for the highest resolution shell.

| | |
|---|---|
| X-ray source | BL-4A, PAL |
| Wavelength (Å) | 0.9999 |
| Space group | *C*2 |
| Unit-cell parameters (Å, °) | $a$ = 108.83, $b$ = 69.60, $c$ = 55.85, $\beta$ = 109.47 |
| Resolution limits (Å) | 50–2.0 |
| No. of observations | 98488 |
| No. of unique reflections | 26484 |
| Mean $I/\sigma(I)$ | 33.7 (6.4) |
| Completeness (%) | 99.9 (100) |
| $R_{merge}$ | 10.5 (31.8) |

We are grateful to Dr Yeon Gil Kim of BL-4A at Pohang Accelerator Laboratory. This study was supported by a grant from the Korea Healthcare Technology R&D Project, Ministry of Health and Welfare, Republic of Korea (A110057).

## References


Bidère, N. *et al.* (2009). *Nature (London)*, **458**, 92–96.
Dobrowolska, G., Muszyńska, G. & Shugar, D. (1991). *Biochim. Biophys. Acta*, **1080**, 221–226.
Gross, S. D. & Anderson, R. A. (1998). *Cell. Signal.* **10**, 699–711.
Honaker, Y. & Piwnica-Worms, H. (2010). *Oncogene*, **29**, 3324–3334.
Klimczak, L. J., Farini, D., Lin, C., Ponti, D., Cashmore, A. R. & Giuliano, G. (1995). *Plant Physiol.* **109**, 687–696.
Liu, W., Xu, Z.-H., Luo, D. & Xue, H.-W. (2003). *Plant J.* **36**, 189–202.
Longenecker, K. L., Roach, P. J. & Hurley, T. D. (1996). *J. Mol. Biol.* **257**, 618–631.
Marin, O., Meggio, F., Sarno, S., Andretta, M. & Pinna, L. A. (1994). *Eur. J. Biochem.* **223**, 647–653.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
McCoy, A. J., Grosse-Kunstleve, R. W., Adams, P. D., Winn, M. D., Storoni, L. C. & Read, R. J. (2007). *J. Appl. Cryst.* **40**, 658–674.
Mehra, A., Shi, M., Baker, C. L., Colot, H. V., Loros, J. J. & Dunlap, J. C. (2009). *Cell*, **137**, 749–760.
Milne, D. M., Palmer, R. H., Campbell, D. G. & Meek, D. W. (1992). *Oncogene*, **7**, 1361–1369.
Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.
Rumpf, C., Cipak, L., Dudas, A., Benko, Z., Pozgajova, M., Riedel, C. G., Ammerer, G., Mechtler, K. & Gregan, J. (2010). *Cell Cycle*, **9**, 2657–2662.
Sasaki, T. *et al.* (2002). *Nature (London)*, **420**, 312–316.
Sugiyama, Y., Hatano, N., Sueyoshi, N., Suetake, I., Tajima, S., Kinoshita, E., Kinoshita-Kikuta, E., Koike, T. & Kameshita, I. (2010). *Biochem. J.* **427**, 489–497.
Tuazon, P. T. & Traugh, J. A. (1991). *Adv. Second Messenger Phosphoprotein Res.* **23**, 123–164.
Vagin, A. & Teplyakov, A. (2010). *Acta Cryst.* D**66**, 22–25.
Vancura, A., Sessler, A., Leichus, B. & Kuret, J. (1994). *J. Biol. Chem.* **269**, 19271–19278.
Xu, R.-M., Carmel, G., Sweet, R. M., Kuret, J. & Cheng, X. (1995). *EMBO J.* **14**, 1015–1023.