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# Phosphorylation Properties of Recombinant OsCPK11, a Calcium-dependent Protein Kinase from Rice

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In plants, calcium ($Ca^{2+}$)-dependent protein kinases (CDPKs) are important sensors of $Ca^{2+}$ signals. Previous research demonstrated the expression of the *OsCPK11* gene in various tissues at the transcription level, but its developmental and biochemical functions at the protein level were not determined. This study was aimed to identify biochemical characteristics of OsCPK11. GST- OsCPK11 was expressed in *E. coli* and used for an *in vitro* kinase assay. Biochemical analyses identified OsCPK11 as a CDPK. OsCPK11 autophosphorylated itself and transphosphorylated histone III-s and MBP as substrates in the presence of $Ca^{2+}$. The activity of the recombinant OsCPK11 was influenced by $Mg^{2+}$, with optimum activity detected at pH 7.0-7.5. OsCPK11 activity was not affected by $Mg^{2+}$, $Mn^{2+}$, or $Na^{+}$ in the presence of a high level of $Ca^{2+}$. Autophosphorylation of OsCPK11 decreased $Ca^{2+}$ sensitivity of OsCPK11. An anti-OsCPK11 rabbit antibody recognized 95.5 kD of GST-OsCPK11, as shown by an immunoblot analysis. These results shed light on the function of OsCPK11 in $Ca^{2+}$-mediated signaling in rice.

**Key words** : Autophosphorylation, $Ca^{2+}$-mediated signaling, CDPKs, GST-OsCPK11, transphosphorylation

## Introduction

Calcium is a ubiquitous second messenger during signal transduction in eukaryotes. In plants, calcium is regulated by many exogenous signals such as hormone, light, physical stimuli, and pathogens [11, 36, 39, 40]. The molecular decoders of calcium signals are the calcium-binding proteins, which include protein kinases regulated by calcium [42]. Calcium sensors that recognize specific calcium signals are activated and transfer the signal to downstream substrate [36, 39]. In animals, protein kinase C (PKC) and calmodulin-dependent kinases(CaMKs) are main response or to calcium signals [20]. On the other hand, calcium-dependent protein kinases (CDPKs or sometimes called as CPKs) are interspersed in plants, but they are not observed in animals except for several protozoan [5, 16]. CDPKs widely exist in different plant species and are encoded by a multi-gene family. 34 CDPK genes were identified in *Arabidopsis thaliana* [3]. And 31 CDPK genes were confirmed recently in rice [46]. The CDPK gene family comprises Ser/Thr protein kinases organized in four subgroups [7, 16]; families of varying size have been characterized in Arabidopsis, rice, wheat, maize, and poplar [5, 16]. Transcripts of CDPKs have been found in every studied plant organ. In many cases, CDPKs are strongly expressed in proliferating tissues. For example, tobacco CDPKs are expressed in rapidly growing tissues such as root tip, lateral root primordia, and vascular tissue in leaf and anther, suggesting that they might be related to cell differentiation and particular metabolic function [23, 49]. CDPKs exhibit at multiple locations including the cytosol, nucleus, plasma membrane, endoplasmic reticulum, peroxisomes, mitochondrial outer membrane and oil bodies [18].

Calcium-dependent protein kinase (CDPK) is a kind of calcium sensor which binds to calcium and phosphorylases substrate peptide shaving Ser or Thr residues [39]. CDPK has N-terminal variable domain, kinase domain, auto inhibitory domain, and calmodulin-like domain [7, 17, 20]. Calmodulin-like domain is organized in N-terminal and

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C-terminal EF lobes and may display different $Ca^{2+}$-binding affinities [27]. CDPKs bind to calcium directly for the kinase activity, while calmodulin does not affect their kinase activity. In other words, its different from calcium/calmodulin-dependent protein kinase (CCaMK) binding to a calcium-calmodulin complex [33]. Amino acid alignment of 34 *Arabidopsis thaliana* CDPKs showed that their kinase, auto inhibitory and calmodulin-like domains have high consensus sequences except for N-terminal variable domain [14]. OsCPKs are composed of the four domains (Fig. 1) and Fig. 1C shows the structure of OsCPK11 protein. The specific function of N-terminal variable domain was not known well, but this domain is supposed to contain information of subcellular targeting [15]. Under some conditions, glycine residue of N-terminal variable region binds covalently to myristic acid and increase the interaction between protein and protein or between protein and membrane [22]. The N-terminal variable domain differs in length and amino acid sequence even within the same species. Palmitoylation also seems to be required for the stability of binding membrane and 24 AtCPKs have been shown to have potential palmitoylation sites including Cys residue [29]. The autoinhibitory domain contains a pseudo-substrate site that binds to the catalytic center in the absence of $Ca^{2+}$, resulting in an inactive state of the kinase. However, the binding of $Ca^{2+}$ to the calmodulin-like domain can induce a conformational change for the release the pseudo-substrate domain from the active site and kinase activation [15, 17, 49]. Calmodulin-like domain has EF-hand sites binding calcium. EF-hand composed of a loop of 13 amino acid residues adjacent to two α-helix and each $Ca^{2+}$ binds to each EF-hand one by one [48]. Most CDPKs in *Arabidopsis thaliana* have 4 EF-hands, but some have fewer sites. In the experiment with a deleted EF-hand sequentially, the number of EF-hand found to be important for regulation of CDPK activity by calcium [19]. In addition, study using site-directed mutagens is showed that EF-hand strongly regulated CDPK activity as it is located nearby autoinhibitory domain [50]. Therefore, it is proposed that the number and location of EF-hands determine allosteric property and active form of this enzyme. With the recent availability of crystal structures from apicomplexan CDPKs, encompassing the kinase and autoinhibitory domain, this model has been refined [44, 45]. It has been proposed that both the apo- and calcium-bound forms of the enzyme are stabilized by distinct contact sites between the kinase and regulatory domains [45]. It is tempting to speculate that, in particular, some of the amino acids involved in forming these contact sites may be subjected to post-translational modifications during the activation process [28], modulating the stability of the active or inactive form.

CDPKs phosphorylate proteins that are involved in nitrogen and carbon metabolism, defense-related processes, protein degradation, cytoskeletal organization and ABA signaling processes [31]. CDPKs in rice have an effect on its tolerance to the cold, salt, and drought stress. [38]. Transgenic rice constitutively expressing OsCPK7 and OsCPK13 showed enhanced tolerance to cold, salt and drought stress [24, 38]. And the tissue-specific expression of CDPKs which is developmentally regulated, suggests their involvement in early developmental processes such as embryogenesis, seed development and germination [1, 2]. In addition, some CDPKs mediate the accumulation of storage starch and protein in maturing seeds [4]. Activity of CDPKs is regulated not only by calcium, but also by phospholipids, 14-3-3 protein, various cations and hormones [7]. Regulation by hormone is important for growth and development and it is

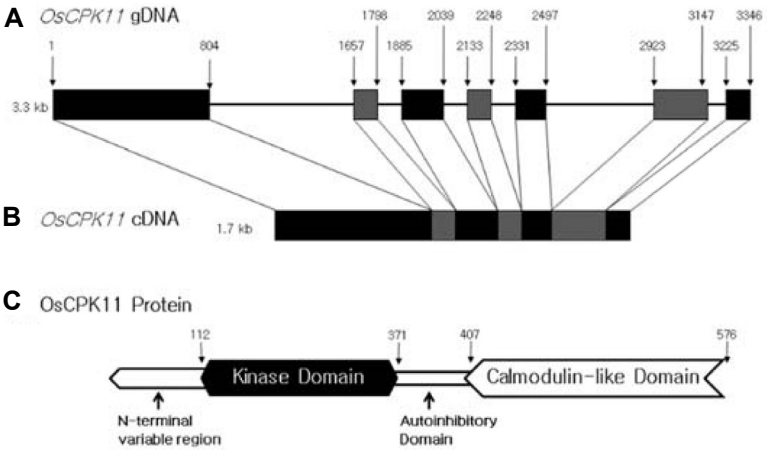


Fig. 1. Structure of OsCPK11 gene and protein. A genomic DNA (A) of OsCPK11 and its cDNA (B) were presented as well as functional domains (C) of OsCPK11 protein. Numbers in (C) indicated the sequence number of amino acids.

closely related with changing $Ca^{2+}$ concentration. Kinase activity has been determined in the absence or presence of different $Ca^{2+}$ concentrations, and the effect of further regulatory components such as lipids [10] or 14-3-3 proteins [25] was investigated. CDPKs are known to modulate calcium-dependent plant responses caused by phytohormones, mostly gibberellins (GA), cytokinins and auxins. For example, in order to compete with the natural substrates for the protein kinases, syntide-2 was microinjected into the barley aleurone protoplasts and subsequently GA-induced amylase expression, protoplast vacuolation, and amylase secretion are selectively inhibited [32].

Both native and recombinant CDPKs found to autophosphorylate themselves [6, 16, 37]. However, it is not clear whether autophosphorylation is essential for the activities of CDPKs. For example, *in vitro* autophosphorylation activates a groundnut (*Arachis hypogea*) CDPK but inhibits one in winged bean (*Psophocarpus tetragonolobus*) [6, 37]. In other case, autophosphorylation of CDPK has no effect on the calcium-dependent activities of ground nut and soybean [6, 16]. Furthermore, activation of CDPK may be modulated by other protein kinases. For instance, tobacco (*Nicotiana tabacum*) CDPK (NtCDPK2) needs both calcium and direct phosphorylation by upstream protein kinase for its full activation [34, 35]. But it is still unknown how they work *in vivo*. With the identification of stress- or pathway-specific CDPK isoforms, the biochemical characterization is now extended to in vivo kinase activation, mediated by stimulus-induced, post-translational modification/phosphorylation of the CDPK, which may occur at all enzyme domains [28]. Dephosphorylation process is as important as phosphorylation in controlling signal pathways. A soluble phosphoserine phosphatase from winged bean shoots dephosphorylates an inactivated, autophosphorylated winged bean CDPK1 (Wb CDPK1) *in vitro* [12]. It is thought that this action releases an inhibitory effect of autophosphorylation and suggests existence of a regulatory region. Therefore, some CDPK activities are involved in regulating processes between protein kinases and phosphatases [7]. Earlier reports showed that recombinant CDPKs exhibit calcium -stimulated protein kinase activity for casein, MBP, histone III-s and syntide as substrates [21, 26, 43, 47]. But there is little information concerning the regulation of OsCPKs in response to various cations, pH, substrates and autophosphorylation. In this study, recombinant GST-linked OsCPK11 was purified from *E. coli* and its biochemical properties as kinase was determined.

## Materials and Methods

### Expression and purification of recombinant OsCPK11

Cloned pET41a(+)-OsCPK11 in *E.coli* strain BL21 was expressed and purified by GST-affinity column as describe in [8]. Transformed colony was inoculated in 5 ml of LB medium containing 50 ug/ml kanamycin and cultured overnight at 37℃ to obtain a saturated culture. 1 ml of a cultured solution was reinoculated with 1 l of LB medium containing 100 ug/ml kanamycin in a 2 l flask and incubated with shaking at 37℃ until the culture has reached the mid-log phase of growth ($A_{600}$=0.6~0.8). Isopropyl-β-D-thiogalacto-pyranoside(IPTG) was added to a final concentration of 0.2 mM to induce the expression of GST-OsCPK11. The cells were cultured for 5 hrs at 30℃ or overnight at 24℃ and they were harvested by centrifugation at 7,000× g for 5 min at 4℃. Pellet was resuspended in 10 ml of TN buffer (20 mM Tris pH 7.4, 150 mM NaCl, 1 mM EDTA, 10 uM β-mercaptoethanol, 1 mM PMSF) per 100 ml of cell culture and the suspension was frozen with liquid nitrogen and thawed in 25℃ several times to break open the cells. Lysozyme was added to the cell suspension to a final concentration of 1 mg/ml and this mixture was incubated on ice for 1 hr. The insoluble debris was removed by centrifugation at 13,000× g for 1 hr at 4℃. Supernatant was collected in a fresh tube and cell lysate was mixed with an appropriate amount of 50% slurry of glutathione-agarose resin in TN buffer. In order to bind GST-OsCPK11 protein with the resin, the mixture including both the supernatant and the resin was shaken gently overnight at 4℃. It was loaded onto the poly-prep chromatography column (Bio-Rad, USA) under gravity flow. Unbound proteins were washed away from the resin by adding 10 bed volumes of TN buffer except for PMSF to the pellet. Bound GST fusion protein was eluted from the resin using 1 bed volume of Glutathione Elution Buffer containing 10 mM reduced glutathione and 50 mM Tris-HCl, pH 8.0. All steps for the expression and purification of the OsCPK11 were followed as procedure modified from [39].

### SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

Discontinuous SDS-PAGE was performed as described in [27] using minislab gels containing 10% or 12% acrylamide. Equal amount of total proteins from each collected samples

were loaded on each lane. After electrophoresis, gel was stained with 0.1% Coomassie brilliant blue R-250 in 40% methanol and 10% acetic acid for several hours. Then, the gel was destained with 40% methanol and 10% acetic acid for 1 hr and repeated same destaining procedure twice. Molecular weight was identified by the method of [41].

### Kinase assay

#### Autophosphorylation assay

*In vitro* kinase assay was performed as [9] with some modification. For the autophosphorylation reaction, 1 ug of GST–OsCPK11 protein was mixed with reaction buffer containing 20 mM HEPES, pH 7.4, 200 nM ATP, 1 mM $Na_3VO_4$, 0.5 mM PMSF, 2 mM EDTA, 2 mM DTT and 0.5 UCi [γ-$^{32}$P] ATP (New England Nuclear) in the absence or presence of 100 uM $MgCl_2$. 0 to 1 mM $CaCl_2$ or 1 mM EGTA was added into the reaction mixture and total volume was adjusted to 10 ul. After incubation for 30 min at 30℃, reaction was terminated by adding 2.5 ul of SDS-PAGE sample buffer. All samples are boiled for 5 min at 100℃. 10 ul of each sample was loaded and separated on 10% or 12% SDS-PAGE gels. The gel was stained and destained according to previous description. It was dried with Bio-Rad Model 583 Gel Dryer (BioRad) for 30 min at 80℃ and subjected to autoradiography by exposing the gel to BioMax film (Kodak) overnight at -80℃ or for 1-5 days at room temperature. The intensity of radioactivity was calculated by Image J software (National Institutes of Health, USA).

#### Transphosphorylation assay

1 ug of substrate (histone III-s, myelin basic protein, or casein) was added to the reaction mixture prior to assay. For determining optimal pH, the buffers contained 20 mM HEPES with pH ranging from 5.0 to 10.0. To identify effect of several cations, reaction buffers were adjusted with 100 uM of NaCl, $MgCl_2$, or $MnCl_2$. And all other procedure were performed same as autophosphorylation assay. To identify effect of pre-autophosphorylation on transphosphorylation reaction, pre-autophosphorylation samples were prepared by incubation of 1 ug of GST-OsCPK11 containing 1-100 uM $CaCl_2$ in the presence/absence of 100 uM $MgCl_2$ for 10min at 30℃. And samples were incubated for 30 min at 30℃ after 1 ug of MBP was added into the mixture. Control samples were prepared by incubation of buffer only containing 1-100 uM $CaCl_2$ in the presence/absence of 100 uM $MgCl_2$ for 10 min at 30℃. And samples were incubated for 30 min at 30℃ after both 1 ug of MBP and 1 ug of GST-OsCPK11 were added into the mixture. All the reactions were terminated by adding 2.5 ul of SDS-PAGE sample buffer and following procedures were the same as described previously.

### Immunoblotting with polyclonal anti-OsCPK11 antibody

19 amino acid sequence (54-72) of PSEHSSHHSSRSTDPS TPT from OsCPK11 protein was determined. C-terminal of the peptide was added with Cys and its N-terminal was acetylated. Peptide was synthesized by the Fmoc solid-phase method by using an automated peptide synthesizer (Peptron III-R24, Peptron, Daejeon, Korea) and antibody was raised and immunoblotting was performed as described in [8].

## Results

### Autophosphorylation of recombinant OsCPK11

Calcium dependence of recombinant OsCPK11 protein was investigated using *in vitro* kinase assay. Autophosphorylation activity of GST-OsCPK11 was measured in the presence/absence of calcium. As a result, it was confirmed that GST-OsCPK11 presented calcium dependence, a major feature of CDPK (Fig. 2). These followed the prediction that EF-hands of calmodulin-like domains of CDPKs bind to calcium and that conformational change causes to release active sites. But it was not detected in the absence of calcium. Autophosphorylation activity of GST-OsCPK11 in the presence of 1 nM calcium was observed weakly and activity was

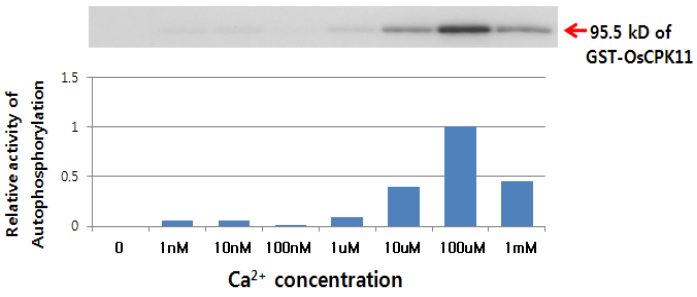


Fig. 2. $Ca^{2+}$ dependency of OsCPK11 for autophophorylation. *In vitro* kinase assay was carried out using 95.5 kD of GST-OsCPK11 for autophosphorylation with different $Ca^{2+}$ concentration. 1 μg of GST-OsCPK11 was used on each lane. It showed that OsCPK11 is calcium-dependent and the highest activity was shown in the presence of 100 uM $Ca^{2+}$.

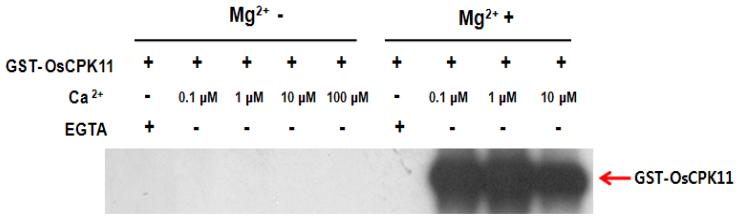


Fig. 3. Effect of $Mg^{2+}$ on $Ca^{2+}$ dependence of autophosphorylation. It was determined that GST-OsCPK11 does not show any autophosphorylation in the absence of $Mg^{2+}$ by *in vitro* kinase assay. Strong signals were shown in the presence of both $Ca^{2+}$ and $Mg^{2+}$ together.

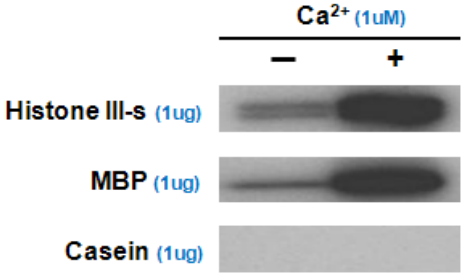


Fig. 4. Searching for the substrates of OsCPK11. 1 ug of GST-OsCPK11 and each 1 ug of Histone III-s, MBP, and casein as a substrate were used for the assay and all buffers contained 100 uM $Mg^{2+}$. Histone III-s and MBP found to be good substrates for the phosphorylation reaction in the $Ca^{2+}$ dependent manner.

strongest with 100 uM calcium. Though calcium-dependency results were shown in the presence of $Mg^{2+}$, it was not detected with 100 uM calcium in the absence of $Mg^{2+}$ (Fig. 3). GST-OsCPK11 did not show a kinase activity for itself with $Mg^{2+}$ only, but its activity was shown when 100 nM $Ca^{2+}$ is present with $Mg^{2+}$.

### Transphosphorylational property of recombinant OsCPK11

In order to determine the transphosphorylational property of GST-OsCPK11, 1 μg of histone III-S, MBP, and casein as a substrate. Histone III-S and MBP were clearly phosphorylated with $Ca^{2+}$ dependent manner. But casein showed no signal with or without $Ca^{2+}$ (Fig. 4). So histone III-S and MBP could be used for later assays. *In vitro* kinase assay was performed with varying pH from 5 to 10 using GST-OsCPK11 (Fig. 5). Activity of GST-OsCPK11 was optimal at pH 7.0-7.5. Calcium-dependency for each substrate was identified by the same procedure as autophosphorylation method with or without $Mg^{2+}$ (Fig. 6 and Fig. 7). GST-OsCPK11 in the absence of $Mg^{2+}$ was activated only in the presence of 100 uM $Ca^{2+}$, but GST-OsCPK11 with $Mg^{2+}$ also showed an strong signal in the presence of 1 uM $Ca^{2+}$. In the presence of 100 uM $Ca^{2+}$, activity of GST-OsCPK11 was confirmed regardless of any cation present (Fig. 8). Activity of GST-OsCPK11 in the absence of $Ca^{2+}$ seemed to be weaker compared with one in the presence of 100 uM $Ca^{2+}$. It was determined whether autophosphorylation of OsCPK11 affected its transphosphorylation (Fig. 9).

Pre-autophosphorylation was carried out by incubating with MBP for 30 min after incubation for 10 min with GST-OsCPK11. Control samples were prepared by incubating with MBP and GST-OsCPK11 for 30 min after incubation for 10 min with buffer only. $Mg^{2+}$ enhanced the phosphorylation activity in the control sample and it was $Ca^{2+}$-dependent. $Mg^{2+}$ also enhanced the phosphorylation activity in the pre-autophosphorylation sample but it did not seem to be $Ca^{2+}$-dependent. As a result, pre-autophosphorylation showed that signal intensity was not increased when $Ca^{2+}$ concentration increased from 1 uM to 100 uM.

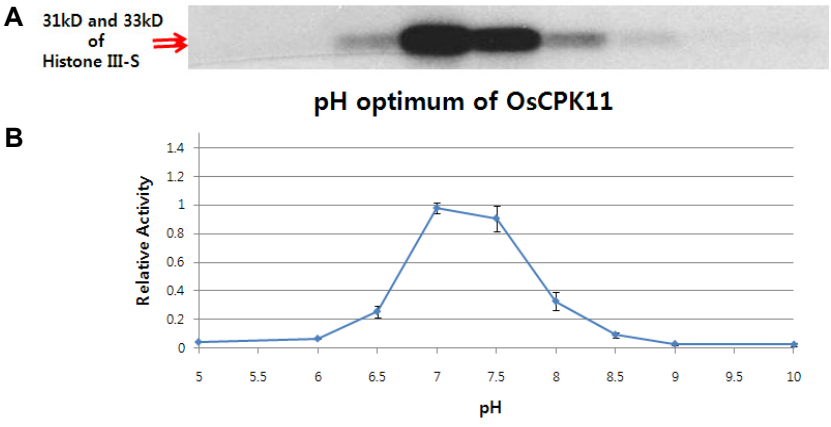


Fig. 5. Optimal pH for the transphosphorylation of OsCPK11. 1 ug of GST-OsCPK11 and 1 μg of Histone III-s were included in the buffer with different pH ranging from 5.0 to 10.0. All buffers contained 1 uM $Ca^{2+}$ and 100 uM $Mg^{2+}$. Optimum pH for the transphosphorylation of OsCPK11 seemed to be pH 7.0 to 7.5.

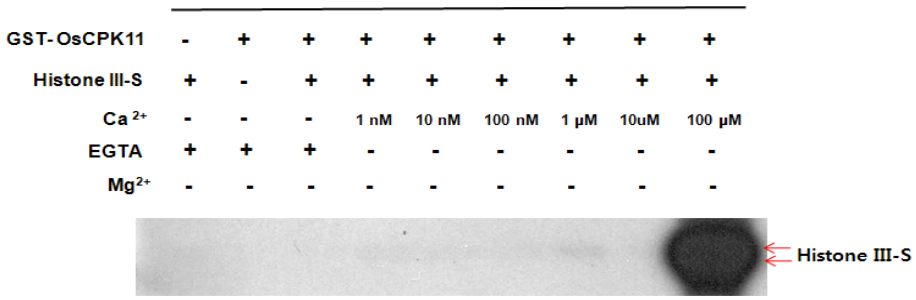


Fig. 6. $Ca^{2+}$ dependence of transphosphorylation in the absence of $Mg^{2+}$. 1 ug of Histone III-s was used as a transphosphorylation substrate for the 1 ug of GST-OsCPK11. In the absence of $Mg^{2+}$, Histone III-s was barely phosphorylated up to 10 uM $Ca^{2+}$, but was strongly phosphorylated in the presence of 100 uM $Ca^{2+}$.

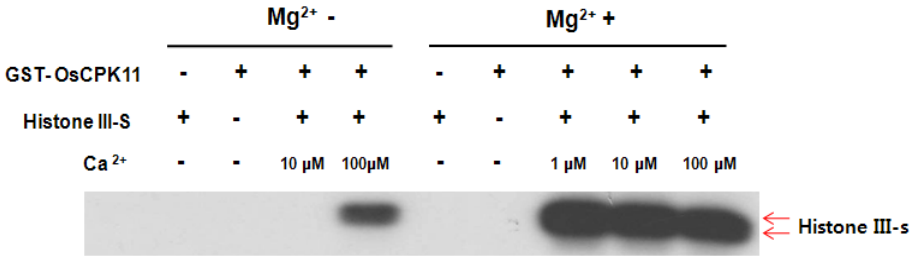


Fig. 7. Effect of $Mg^{2+}$ on $Ca^{2+}$ dependence of transphosphorylation. 1 ug of GST-OsCPK11 and 1 ug of Histone III-s as substrate were used for the assay and buffers contained either no $Mg^{2+}$ (left panel) or with 100 uM $Mg^{2+}$ (right panel). In the absence of $Mg^{2+}$, results were the same as Fig. 6. In the presence of $Mg^{2+}$, GST-OsCPK11 phosphorylated Histone III-s in the presence of $Ca^{2+}$ as low as 1 uM.

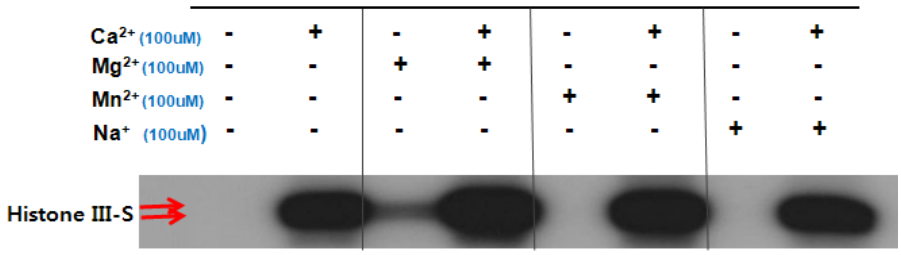


Fig. 8. Effect of several cations on $Ca^{2+}$ dependence of transphosphorylation. GST-OsCPK11 was incubated with Histone III-s as a substrate. In the presence of 100 uM $Ca^{2+}$, strong phosphorylation activity of OsCPK11 was shown regardless of any cation present. In the absence of $Ca^{2+}$, Histone III-s was a little bit phosphorylated with 100 uM $Mg^{2+}$.

### Immunoblotting analysis

Serum obtained from a immunized rabbit with OVA-conjugated 19 amino acids (PSEHSSHHSSRSTDPSTPT) deduced from OsCPK11 gene was assayed to determine an antibody activity. Immune serum was specific to the peptide deduced from OsCPK11 gene. Immunoblotting results showed that GST-OsCPK11 was detected by immune serum while pre-immune serum did not show any clear band (Fig. 10).

## Discussion

Calcium is an important factor in preventing physiological disorders in plant tissues during growth and development [30]. Calcium ions are the most important regulator of CDPKs activity and CDPKs are different in their affinity for $Ca^{2+}$. For example, soy bean CDPK isoforms α and γ display $Ca^{2+}$ activation thresholds that differ by more than ten-fold (isoform α shows a very low threshold around 60 nM calcium). Thus, low level $Ca^{2+}$ may selectively activate CDPK α and much higher level of $Ca^{2+}$ would activate both isoforms [26]. This study showed that both $Ca^{2+}$ and $Mg^{2+}$ are important for the autophosphorylation of OsCPK11. In almost all studied cases, autophosphorylation was observed in CDPKs and it was $Ca^{2+}$-dependent except for WbCDPK

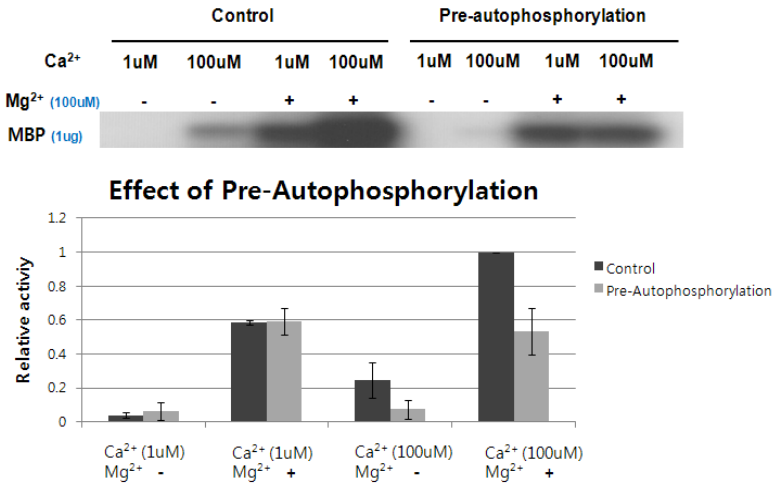


Fig. 9. Effect of pre-autophosphorylation of OsCPK11 on its transphosphorylation. Pre-autophosphorylation samples were prepared by incubating with MBP for 30 min after incubation with GST-OsCPK11 for 10 min. Control samples were prepared by incubating with MBP and GST-OsCPK11 for 30 min after incubation for 10 min with buffer only. $Mg^{2+}$ enhanced the phosphorylation activity in the control sample and could be calcium-dependent. $Mg^{2+}$ also enhanced the phosphorylation activity in the pre-autophosphorylation sample, but it did not seem to be calcium-dependent.

[23]. Unlike the substrate phosphorylation reaction, autophosphorylation of WbCDPK is $Ca^{2+}$-independent and is not inhibited by the calmodulin antagonist [37]. It was identified that autophosphorylation of OsCPK11 showed $Ca^{2+}$-dependent and that $Mg^{2+}$ was essential for the reaction. Recombinant OsCPK11 exhibited $Ca^{2+}$ requirement for the substrate phosphorylation activity. In the presence of $Ca^{2+}$ OsCPK11 phosphorylated Histone III-s and MBP but not casein. In the presence of 100 uM $Ca^{2+}$, $Mg^{2+}$ was required for autophosphorylation, but $Mg^{2+}$ was not always essential for the transphosphorylation. It was expected that activity condition

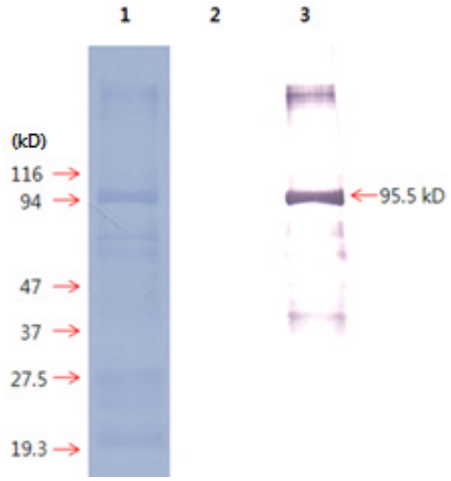


Fig. 10. Immunoblotting analysis of GST-OsCPK11 using immune serum. GST-OsCPK11 samples were subjected to SDS-PAGE on 10% polyacrylamide gel, transferred onto nitrocellulose membrane and processed to immunoblotting. Lane 1, stained with Coomassie Brilliant blue (numbers indicated the molecular weights of the protein markers); lane 2, treated with pre-immune serum; lane 3, treated with immune serum.

of autophosphorylation is different from one of transphosphorylation *in vivo*. Soybean CDPKs showed broad pH optimum in a range of pH 6.0-10.0 [26]. Unlikely, the pH optimum of OsCPK11 were found to be in a range between pH 7.0 and pH 7.5 and it is similar to the green alga, *Dunaliella salina* CDPK that showed a peak activity at pH 7.5 [13]. Cations such as $Mg^{2+}$, $Mn^{2+}$, or $Na^{+}$ did not seem to affect the activity of OsCPK11 in the presence of 100 uM $Ca^{2+}$. However, *in vitro* kinase assay also showed that pre-autophosphorylation of GST-OsCPK11 arrested the sensitivity to $Ca^{2+}$. This result was similar to one of [12] that autophosphorylation inhibits kinase activity of WbCDPK1 in winged bean. Various stimuli could change intracellular ionic condition including increase of $Ca^{2+}$. OsCPK11 might be autoinhibited with a low $Ca^{2+}$ concentration and activated by high $Ca^{2+}$ concentration. However, if substrates were not sufficient with high $Ca^{2+}$ concentration, autophosphorylation could occur more frequently than transphosphorylation. And OsCPK11 might become insensitive to increasing amount of substrates. Anti-OsCPK11 rabbit antibody was produced against a specific N-terminal variable region of OsCPK11. GST-OsCPK11 was detected with immune serum. It can be used as a tool to determine expression and cellular localization of OsCPK11 at the protein level. These results in conjunction with one described in [27] should help better understanding and navigating the function of OsCPK11 in $Ca^{2+}$-mediated signaling in rice. It will be necessary to determine the proteomic data of various OsCPKs for further study.

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## 초록 : 벼의 칼슘-의존적 단백질 카이네즈인 재조합 OsCPK11의 인산화 특성

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식물에서, 칼슘-의존적 단백질 카이네즈(CDPKs)는 $Ca^{2+}$ 신호전달에서 중요한 $Ca^{2+}$ 수용체이다. 벼(*Oryza sativa* L.)의 CDPKs인 3개의 OsCPKs는 생물정보에 대한 분석이 이루어졌으나, OsCPK11 유전자는 연구가 완전히 수행되지 않았다. 다양한 조직에서 OsCPK11 유전자가 전사수준에서 발현한다는 것은 알려져 있으나, 단백질 수준에서 발현과 생화학적인 특징은 잘 알려져 있지 않다. 이 연구는 OsCPK11의 몇 가지 생화학적 특징을 알아보기 위해 이루어졌다. 먼저 *in vitro*에서 *E. coli*를 이용하여 GST-OsCPK11를 발현시키고, 카이네즈 활성 측정과 칼슘-의존적 단백질 카이네즈로서 OsCPK11의 생화학적 분석도 수행하였다. OsCPK11은 스스로 자가인산화하며, $Ca^{2+}$의 존재 하에서 기질로서 histone III-s와 MBP로 인산기 전달 작용을 수행한다. 재조합 OsCPK11의 활성은 $Mg^{2+}$에 의해 영향을 받으며, pH 7.0-7.5에서 최적의 활성을 보인다. 또한 OsCPK11의 활성은 높은 수준의 $Ca^{2+}$가 존재하는 조건에서는 $Mg^{2+}$, $Mn^{2+}$, $Na^{+}$의 영향을 받지 않는다. 또한 OsCPK11의 자가인산화는 OsCPK11의 $Ca^{2+}$ 민감도를 감소시키는 것으로 밝혀졌다. 마지막으로, OsCPK11의 N-말단 다양화 지역으로 토끼 항체를 만들었고, immunoblot을 기초로 polyclonal antibody는 95.5 kD의 GST-OsCPK11를 인식하는 것으로 나타났다. 이 결과는 벼의 $Ca^{2+}$ 매개 신호전달에서 OsCPK11의 기능을 더 잘 이해하는데 도움을 줄 것이며, 심화 연구를 위해 다양한 OsCPKs의 단백질 정보를 결정하는 것이 필요할 것이다.