Biochemical Analysis of Protein Kinase Activities in the Dry Seeds of Rice

Motoki Kanekatsu and Reiko Takai

(Faculty of Agriculture, Tokyo University of Agriculture and Technology, 3-5-8 Saiwai-cho, Fuchu, Tokyo 183-8509, Japan)

Key words: Protein kinase, Rice, Seed germination.

In rice production, the knowledge of the mechanism of seed germination serves as a base for improving irregular germination and decline in germination rate under low-temperature conditions in direct seeding cultivation. Expression of many genes, synthesis of plant hormones and activation of various metabolism systems take place in the initial process of germination (Bewley, 1997; Koornneef et al., 2002). In cereal seeds, Gibberellins (GAs) are synthesized in the embryo, diffuse to the aleurone layers, and induce expression of GAMyb and then α-amylase after water absorption in dry seeds (Lovegrove and Hooley, 2000). Since the activators of various gene expression and metabolism, and mediators of GA signaling, need to function immediately after absorbing water in seeds, they must already exist as active forms in the dry seeds before water uptake. However, these factors have not yet been identified in the dry seeds. It is generally accepted that protein kinases, which catalyze the phosphorylation of substrate proteins, have roles in signal transduction, control of gene expression and activation of various metabolisms (Edelman et al., 1987). Therefore, protein kinase might be one of the key factors that play important roles in the initial process of seed germination. Although protein kinases expressed in the seeds after water absorption or after the treatment with plant hormones have been reported in oat (Hutty and Phillips, 1995) and wheat (Anderberg and Walker-Simmons, 1992), their enzyme activities in the dry seeds before water uptake are not clear. In this research, therefore, we analyzed biochemically the activities of the protein kinases in dry seeds of rice.

Materials and Methods

1. Preparation of crude seed proteins

Crude seed proteins were prepared at 4°C. Rice (Oryza sativa L. cv. Nipponbare) dry seeds (about 2 g fresh weight), stored at 4°C in the dark for two years after harvest, were frozen in liquid nitrogen to stop cellular reactions. The frozen seeds were homogenized with a Polytron (Kinematika, Switzerland) in 10 ml of Buffer A [50 mM Tris-HCl (pH 7.4), 1 mM EDTA, 10 mM 2-mercaptoethanol (2-ME), 0.1 mM MgCl2, 0.1 mM phenylmethylsulfonyl fluoride and 10% glycerol] containing 50 mM KCl and centrifuged at 22,000 × g for 20 min. The resulting supernatant was used as a crude protein extract in the present study.

2. Assay of protein kinase activity

The reaction mixture (50 µl) contained 40 mM Tris-HCl (pH 8.0), 2 mM dithiothreitol (DTT), 3 mM MnCl2, 10 µg of phosphate acceptor such as casein (Sigma Chemicals Corp., USA), whole histone (type IIA, Sigma Chemicals Corp., USA) or myelin basic protein (Sigma Chemicals Corp., USA), 2 µCi [γ-32P]ATP and the crude protein extract. After incubation (60 min at 30°C), protein phosphorylation was stopped by addition of 50 µl of SDS-PAGE sample buffer containing 2% SDS, 10% 2-ME and bromophenol blue, then the mixture was boiled for 3 min. The [32P]-labelled proteins were detected by SDS-PAGE followed by autoradiography (Kanekatsu et al. 1993a).

Results and Discussion

A crude protein extract from rice dry seeds was incubated with three phosphate acceptors, casein, whole histone and myelin basic protein (MBP), in the presence of [γ-32P]ATP to detect protein kinase activities. Under this condition, the phosphorylation of whole histone and MBP was detected at a low level (Fig.1). On the other hand, marked activity of protein kinase was detected in the extract when casein was used as a phosphate acceptor. When the crude protein extract was boiled for 3 min and then incubated with casein in the presence of [γ-32P]ATP, no detectable activity was found. Moreover, the phosphorylated protein was not detected in the absence of phosphate acceptors. These results suggest that at least one protein kinase, which can phosphorylate casein, is present as an active form in dry seeds of rice.

There are many reports on protein kinases that can...
Plant Production Science Vol.8, 2005

Phosphorylate casein in plant cells. Casein kinase II (CK-II) is one of these casein phosphorylating type kinases and is well-characterized biochemically. CK-II is a ubiquitous protein kinase and may have an important role in the regulation of cellular functions, such as cell division and growth, gene expression, and DNA replication (Pinna, 1990; Kanekatsu et al., 1993a, 1995). There is a possibility that CK-II is involved in regulation of seed germination. This kinase (i) utilizes GTP besides ATP as phosphate donors in vitro; and (ii) is sensitive to heparin and stimulated by poly-Lys (Kanekatsu and Ohtsuki, 1993b). To examine the existence of CK-II in the crude protein extract from rice dry seeds, we assayed protein kinase activity using $[^{32}\text{P}]\text{GTP}$ as a phosphate donor instead of $[^{32}\text{P}]\text{ATP}$ and detected phosphorylation of casein by the kinase(s) in the extract (Fig.2). In addition, this kinase activity was stimulated by poly-Lys (10 µg/ml), and reduced by heparin (1 µg/ml). These results strongly suggest that CK-II is one of the protein kinases expressed in the dry seeds of rice.

In higher plants, calcium-dependent protein kinases (CDPK) are the most abundant serine/threonine kinase and are key elements in signaling processes mediated by $\text{Ca}^{2+}$ (Sheen, 1996). Since a CDPK of Arabidopsis can phosphorylate casein (Urao et al., 1994), we examined the effect of $\text{Ca}^{2+}$ on the activities of the protein kinases in the crude extract from rice.
We found that the phosphorylation of casein was stimulated significantly by Ca\(^{2+}\) (between 0.1 mM and 1 mM) in the reaction mixture (Fig. 3). This result indicates that one of the protein kinases in dry seeds of rice is a calcium-dependent type enzyme. Gibberellins (GAs), which are important promoters of seed germination in many plants, increase the level of Ca\(^{2+}\) in aleurone cells of wheat (Bush, 1996) and barley (Gilroy and Jones, 1992). Cytoplasmic Ca\(^{2+}\) has been suggested to play an important role in the response to GA that induces the production and secretion of \(\alpha\)-amylases in cereal aleurone cells. However, the early event mediated by Ca\(^{2+}\) in GA action is not well known. Thus, it is important to identify and clarify the biological significance of the calcium-dependent type protein kinase in rice dry seeds.

References

Anderberg, R.J. and Walker-Simmons, M.K. 1992. Proc. Natl. Acad. Sci. USA 89 : 10183-10187.
Bewley, J.D. 1997. Plant Cell 9 : 1055-1066.
Bush, D.S. 1996. Planta 199 : 89-99.
Edelman, A.E. et al. 1987. Annu. Rev. Biochem. 56 : 567-613.
Gilroy, S. and Jones, R.L. 1992. Proc. Natl. Acad. Sci. USA 8 : 3591-3595.
Kanekatsu, M. et al. 1993a. FEBS Lett. 335 : 176-180.
Kanekatsu, M. and Ohtsuki, K. 1993b. Plant Cell Physiol. 34 : 627-631.
Kanekatsu, M. et al. 1995. Plant Cell Physiol. 36 : 1649-1655.
Koornneef, M. et al. 2002. Curr. Opin. Plant Biol. 5 : 33-36.
Hutty, A.K. and Phillips, A.L. 1995. Plant Mol. Biol. 27 : 1043-1052.
Lovegrove, A. and Hooley, R. 2000. Trends Plant Sci. 5 : 102-110.
Pinna, L.A. 1990. Biochem. Biophys. Acta 1054 : 267-284.
Sheen, J. 1996. Science 274 : 1900-1902.
Urao, T. et al. 1994. Mol. Gen. Genet. 244 : 331-340.