Journal of Plant Physiology **163** (2006) 405—416

Available online at www.sciencedirect.com

SCIENCE DIRECT®

JOURNAL OF PLANT PHYSIOLOGY

www.elsevier.de/jplph

Contributions of PIP_2-specific-phospholipase C and free salicylic acid to heat acclimation-induced thermotolerance in pea leaves

Hong-Tao Liu, Wei-Dong Huang*, Qiu-Hong Pan, Fang-Hua Weng, Ji-Cheng Zhan, Yan Liu, Si-Bao Wan, Yan-Yan Liu

College of Food Science & Nutritional Engineering, China Agricultural University, Beijing 100083, China

Received 4 March 2005; accepted 26 April 2005

KEYWORDS
Heat acclimation;
Neomycin;
PIP_2-PLC;
Salicylic acid;
Thermotolerance

Summary

The relationship between the accumulation in endogenous free salicylic acid (SA) induced by heat acclimation (37 °C) and the activity of PIP_2-phospholipase C (PIP_2-PLC; EC 3.1.4.3) in the plasma membrane fraction was investigated in pea (*Pisum sativum* L.) leaves. We focused our attention on the hypothesis that positive SA signals induced by heat acclimation may be relayed by PIP_2-PLC. Heat acclimation induced an abrupt elevation of free SA preceding the activation of PLC toward PIP_2. Immunoblotting indicated a molecular mass with 66.5 kDa PLC plays key role in the development of thermotolerance in pea leaves. In addition, some characterizations of PLC toward PIP_2 isolated from pea leaves with two-phase purification containing calcium concentration, pH and a protein concentration were also studied. Neomycin sulfate, a well-known PIP_2-PLC inhibitor, was employed to access the involvement of PIP_2-PLC in the acquisition of heat acclimation induced-thermotolerance. We were able to identify a PIP_2-PLC, which was similar to a conventional PIP_2-PLC in higher plants, from pea leaves suggesting that PIP_2-PLC was involved in the signal pathway that leads to the acquisition of heat acclimation induced-thermotolerance. On the basis of these results, we conclude that the involvement of free SA may function as the upstream event in the stimulation of PIP_2-PLC in response to heat acclimation treatment.

Abbreviations: APX, ascorbate peroxidase; CAT, catalase; CDPK, calcium-dependent protein kinase; DAG, diacylglycerol; IP_3, inositol-1, 4, 5-trisphosphate; MAPK, mitogen-activated protein kinase; MDA, malondialdehyde; PAL, phenylalanine ammonia-lyase; PI, phosphatidylinositol; PIP, phosphatidylinositol monophosphate; PIP_2, phosphatidylinositol 4, 5-bisphosphate; SOD, superoxide dismutase

*Corresponding author. Tel.:+86 10 62737024.
E-mail address: huanggwd@263.net (W.-D. Huang).

0176-1617/$ - see front matter © 2005 Elsevier GmbH. All rights reserved.
doi:10.1016/j.jplph.2005.04.027

Introduction

Higher plants are often subjected to either biotic stresses or abiotic ones like temperature variations. Compared with animals, plants can neither move to avoid the effects caused by temperature change nor maintain thermal balance in their bodies. They can only adapt to temperature changes by enhancing their own abilities against extreme temperature. For instance, some specific heat shock protein (HSPs) families have been identified in response to a sublethal heat treatment (Srikanthbabu et al., 2002; Vierling, 1991) that could reinforce thermotolerance in subsequent more severe heat adversity.

It is generally recognized that salicylic acid (SA) is involved in systemic acquired resistance (SAR) in response to various pathogen attacks in plants (Alvarez, 2000; Klessig and Malamy, 1994). As a ubiquitous signal molecule, SA could also regulate physiological adaptation to some environmental stresses including oxidative damage (Borsani et al., 2001), cold injury (Janda et al., 1999) and ozone excess (Rao et al., 1996). Recently, a considerable body of evidences showed that both heat treatment at sublethal temperatures (i.e. heat acclimation) and exogenous application of SA could promote thermotolerance in plants (Dat et al.,1998a, b; Howarth and Skøt, 1994; Lopez-Delgado et al., 1998). However, any further relationship between the two methods mentioned above remains poorly understood, especially in the cellular signaling pathway. Although many important genes have been identified in signaling and shown to be induced by heat and/or SA treatment, the detailed pathways implicated in the perception and the transduction of heat or the SA signal into plant cells remain unclear.

PIP_2-phospholipase C (PIP_2-PLC; EC 3.1.4.3) is a crucial lipid-associated enzyme in intracellular signaling that hydrolyzes membrane bound phosphatidylinositol-4,5-bisphosphate (PIP_2) to produce inositol-1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG). These two second messengers play key roles in amplifying extracellular signals and regulating various intracellular processes triggered by abiotic and biotic stimuli (Cho et al., 1995; Dewald et al., 2001; Pical et al., 1999; Reggiani and Laoreti, 2000; Ruelland et al., 2002; Takahashi et al., 2001; Zhao et al., 2004). As such, the activation of PLC was usually regarded as an early signal event in response to external stresses.

The purpose of this research is to examine the linkages in heat acclimation at 37 °C, the transient increase in free SA content and the rapid activation of PIP_2-PLC in pea leaves. To our knowledge, this report is first inquiry into the function of PIP_2-PLC as a heat signal transduction mediated by dramatic changes in the free SA level in higher plants.

Materials and methods

Chemicals

Neomycin sulfate, deoxycholate (DOC) sodium, PEG 3350, leupeptine, L-α-PtdIns(4,5)P_2, 1-[6-((17β-3-methoxyestra-1,3,5-(10)-trien-17-yl)amino)hexyl]-1H-pyrrole-2,5-dione (U73122), calcium chloride were purchased from Sigma (St. Louis, MO, USA). Dextran T500 was purchased from Pharmacia (Sweden).

Plant materials and heat or SA treatment

Pea (*Pisum sativum* L. cv. ningxia) seeds were grown for eight days in prefertilized soil in a growth chamber, where the light intensity was maintained at 180 μmol/m^2/s, and the day/night temperature at 25/22 °C at 60% relative humidity. Heat treatment was performed by transferring the chambers into an intelligent climate incubator at 37 and 45 °C to mimic heat acclimation and stress conditions. A total of 100 μM SA solution was sprayed on the leaves of pea seedlings grown in normal conditions until drips formed as SA treatment. After the treatments were finished, the pea leaves were harvested and frozen in liquid nitrogen immediately and then kept at −80 °C until used.

Microsome and plasma membrane isolation

The pea leaves were homogenized at 4 °C with a mortar and pestle in 4 mL/g fresh weight of 30 mmol/L Tris buffered to pH 7.3 with HCl, containing 250 mmol/L sucrose, 2 mmol/L EDTA, 2 mmol/L dithiothreitol (DTT), 1 mmol/L phenylmethylsulfonyl (PMSF), and 5% glycerol. The microsome was obtained after two sequential centrifuge steps: the total homogenate was centrifuged at 10,000*g* for 30 min and the supernatant then spun at 100,000*g* for 50 min. The microsomal pellet was suspended in 0.5 mL of 5 mmol/L potassium phosphate buffered to pH 7.3, consisting of 250 mmol/L sucrose, 4 mmol/L KCl, 1 mmol/L DTT and 0.5 mmol /L PMSF using a hand-held Potter-Elvejhem homogenizer. Half of the suspension was put in reserve at −80 °C until needed for enzyme assay. The remaining microsome was added to a prepared phase mixture to yield an 8-g two-phase system [6.7%(w/w) Dextran T500, 6.4%(w/w) PEG3350, 1 mol/L