Gibberellic-acid-stimulated Ca$^{2+}$ accumulation in endoplasmic reticulum of barley aleurone: Ca$^{2+}$ transport and steady-state levels

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Abstract. The steady-state levels of Ca$^{2+}$ within the endoplasmic reticulum (ER) and the transport of $^{45}$Ca$^{2+}$ into isolated ER of barley (Hordeum vulgare L. cv. Himalaya) aleurone layers were studied. The Ca$^{2+}$ level in the ER was measured using the Ca$^{2+}$-sensitive dye indo-1. Endoplasmic reticulum was isolated and purified from indo-1-loaded protoplasts, and the Ca$^{2+}$ level in the lumen of the ER was determined by the fluorescence-ratio method to be at least 3 $\mu$M. Transport of $^{45}$Ca$^{2+}$ into the ER was studied in microsomal fractions isolated from aleurone layers incubated in the presence and absence of gibberellic acid (GA$_3$) and Ca$^{2+}$. Isopycnic sucrose density gradient centrifugation of microsomal fractions isolated from aleurone layers or protoplasts separates ER from tonoplast and plasma membranes but not from the Golgi apparatus. Transport of $^{45}$Ca$^{2+}$ occurs primarily in the microsomal fraction enriched in ER and Golgi. Using monensin and heat-shock treatments to discriminate between uptake into the ER and Golgi, we established that $^{45}$Ca$^{2+}$ transport was into the ER. The sensitivity of $^{45}$Ca$^{2+}$ transport to inhibitors and the $K_m$ of $^{45}$Ca$^{2+}$ uptake for ATP and Ca$^{2+}$ confirm that the ER is the principal site of Ca$^{2+}$ transport in the microsomal fraction of barley aleurone cells. The rate of $^{45}$Ca$^{2+}$ transport is stimulated several-fold by treatment with GA$_3$. This effect of GA$_3$ is mediated principally by an effect on the activity of the Ca$^{2+}$ transporter rather than on the amount of ER.

Key words: Aleurone – Calcium transport – Endoplasmic reticulum (Ca$^{2+}$ levels) – Gibberellin and Ca$^{2+}$ transport – Golgi apparatus – Hordeum (Ca$^{2+}$ transport) – Microsome

Introduction

Cytosolic Ca$^{2+}$ in plant cells is maintained at 50–500 nM, and organelles, such as chloroplasts, endoplasmic reticulum (ER), mitochondria and vacuoles, are thought to play an important role in Ca$^{2+}$ homeostasis (see reviews by Hepler and Wayne 1985; Poovaiah and Reddy 1987). There is increasing evidence that the ER and vacuoles can accumulate Ca$^{2+}$ above the level found in the cytoplasm by the activity of ATP-driven pumps located on their membranes (Buckhout 1984; Shuemaker and Sze 1985, 1987; Bush and Sze 1986; Giannini et al. 1987). The ability of the ER and tonoplast to accumulate Ca$^{2+}$ has led to the speculation that these organelles modulate cytosolic Ca$^{2+}$ levels. We here report evidence that the ER of the barley aleurone cell accumulates Ca$^{2+}$ to levels above that found in the cytoplasm.

Gibberellic acid (GA$_3$)-enhanced synthesis of $\alpha$-amylase by isolated barley aleurone layers or protoplasts requires millimolar concentrations of extracellular Ca$^{2+}$ (Chrispeels and Varner 1967; Jacobsen et al. 1970; Bush et al. 1986). The requirement for high concentrations of Ca$^{2+}$ has been explained in terms of the stability of the $\alpha$-amylase molecule (Chrispeels and Varner 1967; Jacobsen et al. 1970). Barley $\alpha$-amylase is a Ca$^{2+}$-containing metalloenzyme that, like other $\alpha$-amylases (Fisher and Stein 1960; Thoma et al. 1970), requires Ca$^{2+}$ for its activity and stability (Jacobsen et al. 1970). Cytoplasmic Ca$^{2+}$ in the aleurone
Material and methods

Plant material. Grains of barley (Hordeum vulgare L. cv. Himalaya, 1985 harvest; Agronomy Department, Washington State University, Pullman, USA) were de-embryonated, surface-sterilized, and allowed to imbibe sterile H2O for 4 d at 25 °C. Aleurone layers were isolated and unless otherwise indicated were incubated in GA3 (5 gM) and CaCl2 (10 mM) for 15 h at 25 °C as described by Jones and Jacobsen (1982). Protoplasts were isolated from aleurone layers and isolated in modifed Gamburg's B5 medium with or without GA3 (5 μM) and 10 mM CaCl2 for 36 h as described by Bush et al. (1986).

Monensin and heat-shock treatments. The sodium ionophore monensin (2-[5-ethyltetrahydro-5-(tetrahydro-3-methyl-5-{tetrahydro-3-methyl-5-[3H]-2-furyl]-2-furyl)-9-hydroxy-6-hydroxy-6-(hydroxymethyl)-3,5-dimethyl-2H-pyran-2-yl]2-furyl]-2-furyl)-9-hydroxy-f-methoxy-x,2,8-tetramethyl-1,6-dioxaspiro(4,5)decane-7-butyric acid) (Mon, 7.5 μM) was added to aleurone layers after 2.5 h of incubation in GA3 and CaCl2 by replacing the incubation medium with fresh GA3 and CaCl2 containing Mon and continuing the incubation for a further 3 h. Heat-shock treatment consisted of a 3-h incubation at 40 °C 15 h after the beginning of incubation in GA3 and CaCl2 at 25 °C.

Calcium measurement. Levels of Ca2+ in the ER were measured using the Ca2+-sensitive dye indo-1 (Molecular Probes, Eugene, Ore., USA). Protoplasts were loaded with indo-1 as described by Bush and Jones (1987). Fluorescence from indo-1 excited at 356 nm was measured using a modular fluorimeter (Fluorolog 2, model F2c; Spex Industries, Edison, N.J., USA) except in experiments with the calcium-sensitive dye indo-1 (Molecular Probes, Eugene, Ore., USA). Calcium transport buffer contained 25 mM Hepes (pH 7.4) and were buffered with 25 mM Hepes-BTP, pH 7.4.

Calcium transport. Net Ca2+ uptake into microsomal membrane fractions was monitored using the methods described by Bush and Sze (1986). Microsomal vesicles (100 μl, approx. 100 μg ml−1 protein) were mixed with 400 μl of a medium containing (final concentration) 25 mM Hepes adjusted to pH 7.4 with BTP, 10 mM potassium oxalate, 3 mM MgSO4, 10 mM CaCl2, 100 μM sodium azide, with or without 1 mM ATP and 45CaCl2 (3.6-103 Bq ml−1; specific activity 5.6-109 Bq mmol−1; Amersham, Arlington Heights, Ill., USA) except in experiments where the Km for ATP or Ca2+ were determined and the concentrations of ATP or Ca2+ were varied. In experiments where the Km for Ca2+ was determined, the Ca2+ concentration was adjusted by the addition of EGTA to the standard reaction mixture. The amount of EGTA required to set the Ca2+ level was calculated using a computer program we devised and then verified using a Ca2+-sensitive microelectrode (Radiometer Corp., West Lake, Oh., USA). Calcium transport proceeded at a constant rate for 20 min, after which time duplicate 200-μl samples were removed and filtered onto 0.45-μm filter discs (type HA; Millipore Corp., Bedford, Mass., USA) under vacuum. The filters were washed with 3.5 ml buffer containing 250 mM sucrose, 2.5 mM Hepes-BTP (pH 7.0) and 0.2 mM CaCl2, dried, and immersed in scintillation fluid (25% Triton X-114, 0.1 M NaCl, 0.01 M Tris-HCl, pH 7.4).