Archives of Biochemistry and Biophysics 481 (2009) 157–168

Contents lists available at ScienceDirect

Archives of Biochemistry and Biophysics

journal homepage: www.elsevier.com/locate/yabbi

A tomato ER-type Ca^{2+}-ATPase, LCA1, has a low thapsigargin-sensitivity and can transport manganese

Neil A. Johnson [a,1], Fengli Liu [a,1], Phillip D. Weeks [a], Audrey E. Hentzen [b], Hilary P. Kruse [a], Jennifer J. Parker [a], Mette Laursen [c], Poul Nissen [c], Charles J. Costa [a], Craig Gatto [a,*]

[a] Division of Biomedical Sciences and Cell Biology & Physiology Section, School of Biological Sciences, Illinois State University, 210 Julian Hall, Campus Box 4120, Normal, IL 61790-4120, USA

[b] Department of Health Sciences, Illinois State University, Normal, IL 61790, USA

[c] Center for Membrane Pumps in Cells and Disease – PUMPKIN, Danish National Research Foundation, University of Aarhus, Dept. Molecular Biology, Gustav Wieds Vej 10C, DK 8000 Aarhus C, Denmark

ARTICLE INFO

Article history:
Received 14 September 2008
and in revised form 3 November 2008
Available online 20 November 2008

Keywords:
P-type ATPase
Ca-pump
Thapsigargin
Membrane transport
Tomato
Yeast
Mn^{2+} transport
Homology modeling

ABSTRACT

Recombinant Ca^{2+}-ATPase from tomato (i.e. LCA1 for *Lycopersicon esculentum* [Since the identification and naming of LCA1, the scientific name for the tomato has been changed to *Solanum lycopersicum*.] Ca-ATPase) was heterologously expressed in yeast for structure–function characterization. We investigate the differences between plant and animal Ca pumps utilizing comparisons between chicken and rabbit SERCA-type pumps with *Arabidopsis* (ECA1) and tomato plant (LCA1) Ca^{2+}-ATPases. Enzyme function was confirmed by the ability of each Ca^{2+}-ATPase to rescue K616 growth on EGTA-containing agar and directly via *in vitro* ATP hydrolysis. We found LCA1 to be ~300-fold less sensitive to thapsigargin than animal SERCAs, whereas ECA1 was thapsigargin-resistant. LCA1 showed typical pharmacological sensitivities to cyclopiazonic acid, vanadate, and eosin, consistent with it being a P_{IIA}-type Ca^{2+}-ATPase. Possible amino acid changes responsible for the reduced plant thapsigargin-sensitivity are discussed. We found that LCA1 also complemented K616 yeast growth in the presence of Mn^{2+}, consistent with moving Mn^{2+} into the secretory pathway and functionally compensating for the lack of secretory pathway Ca-ATPases (SPCAs) in plants.

Published by Elsevier Inc.

Introduction

Plants, like all higher eukaryotes, regulate a variety of cellular functions using calcium-mediated processes [1–4]. Calcium can be elevated in plants by such different physiological stimuli such as red light, touch, temperature and salinity [3]. Plant cells, similar to animals, produce a distinct range of cytoplasmic Ca^{2+} signals in order to achieve the diverse spectrum of responses observed [5–7]. Consequently, it is paramount that cytoplasmic $[Ca^{2+}]$ be kept low if these signaling events are going to be controlled; this tight $[Ca^{2+}]_{cyt}$ regulation is achieved by active Ca^{2+} sequestration within intracellular compartments (e.g. endoplasmic reticulum and Golgi) or extruded to the extracellular space [8,9], similar to the molecular mechanisms observed in animal cells [10]. However, the underlying cellular and biochemical processes that intercalate to produce these Ca^{2+} dynamics in plants remain largely unresolved. Many Ca^{2+} transporters, including Ca^{2+} channels, Ca^{2+} pumps, and H^+/Ca^{2+} antiporters are proposed to work together to regulate diverse Ca transients and oscillations required for plant development [7,11]. However, only a small portion of these Ca^{2+} transporters have been molecularly characterized, and the vast majority of those have been solely from *Arabidopsis*.

The *Arabidopsis* genome has more than a dozen genes end coding Ca^{2+}-ATPases and many of these remain to be characterized. It is this abundance of high-affinity Ca^{2+}-ATPases in plants that makes characterization of an individual pump rather difficult in the endogenous tissue. Consequently, heterologous expression is usually exploited to biochemically address the functional mechanism of individual Ca^{2+} pumps. The two categories of plant Ca^{2+} pumps are (i) a plasma membrane (PM)-type Ca^{2+}-ATPase, which is stimulated by calmodulin [12,13], like animal PM-type Ca^{2+} pumps, and (ii) an ER-type which mimics the SERCA-type pumps of animals [3,14]. Both types of Ca^{2+} pumps belong to the P-type ATPase family with a conserved kinetic model for ion transport. This transport cycle has been described according to a model with two major conformational states, E1 and E2. The hydrolysis of ATP proceeds via a series of reactions where the γ-phosphate of ATP is transferred to the pump, forming an acid stable anhydride with an aspartate residue (Asp^{366} in LCA1). The resulting phosphoenzyme exists in two conformations (E_1P and E_2P). The first conformation, E_1P is rapidly dephosphorylated by ADP in the reverse direction; the second conformation, E_2P is dephosphorylated by the addition of a counter-

* Corresponding author. Fax: +1 309 438 3538.
E-mail address: cgatto@ilstu.edu (C. Gatto).

[1] These authors contributed equally to this work.

0003-9861/$ - see front matter Published by Elsevier Inc.
doi:10.1016/j.abb.2008.11.010

transported ion (likely H^+ in LCA1, but as yet undetermined) and is insensitive to ADP. Binding of the counter ion stimulates pump dephosphorylation (i.e. an E_2 autophosphatase activity) and a conformational change back to E_1 with the concomitant release of the counter ion [15,16].

Manganese is a common element in soil, which is utilized by plants for photosynthesis [17,18]. Since Mn^{2+} can compete with Ca^{2+} and Mg^{2+} for binding sites on proteins [19,20], cytosolic levels of Mn^{2+} must be kept low in plants. In particular, this appears to be a substantial burden in the tomato plant root system as it has been shown to accumulate Mn^{2+} from soil at a rate 10-fold higher than most plants [21]. The yeast Ca-ATPase, Pmr1, has been demonstrated to be both a Ca^{2+} and Mn^{2+} transporter [22]. Yeast Pmr1 and human SPCA pumps belong to a separate class of secretory pathway Ca^{2+}-ATPases that are localized to the Golgi and participate in protein maturation and sorting via the active transport of Ca^{2+} and Mn^{2+} [23,24]. Interestingly, to date there has not been a single secretory pathway-type Ca^{2+}-ATPase identified in plants. How then do plants accumulate the required divalent metal concentrations within Golgi? One hypothesis gaining support is that plant ER-type Ca^{2+}-ATPases have acquired this role as some *Arabidopsis* ER-type Ca^{2+} pumps have been shown to transport Mn^{2+}. Moreover, one isoform, ECA3, has been localized to the Golgi [25,26]. Whether these observations for ECA3 represent a ubiquitous adaptation retained in all plants remains to be determined.

Here, in order to study an individual tomato plant Ca^{2+} pump, we functionally expressed the *LCA1* gene in a yeast triple mutant defective in both a Golgi and vacuolar Ca^{2+} pump, K616 (*pmr1*, *pmc1*, *cnb1*) [27]. We show that LCA1 behaves as an ER-type Ca-ATPase that is highly sensitive to the specific inhibitor cyclopiazonic acid. Interestingly, this tomato plant Ca-ATPase is only mildly sensitive to the more potent ER-type pump inhibitor thapsigargin. This mild sensitivity uniquely separates LCA1 from both the extremely thapsigargin-sensitive animal SERCA pumps and the apparently completely insensitive *Arabidopsis* ER-type pump, ECA1. Moreover, our data suggest that LCA1 may also act as a Mn^{2+} pump, consistent with a possible role in secretory pathway divalent cation homeostasis. This latter role may be an evolutionary physiological compensation for the absence of SPCAs in plants. Preliminary reports of this work have been presented to the Biophysical Society and the Society of General Physiologists [28,29].

Materials and methods

Construction of expression plasmids

pYES2-LCA and pYES2-SERCA1a

Avian SERCA1a and tomato LCA1 were amplified via PCR using *pfu* polymerase (Stratagene, La Jolla, CA) to increase fidelity. The unique restriction endonuclease sites (underlined) KpnI and NheI (LCA1), or HindIII and SphI (SERCA1a) were engineered via incorporation into the oligomeric primers used for amplification. Specifically, the primers used were:

LCAFwd: 5'-GGGGC**GGTACC**ATGGAAGAAAAACCATTCCCTGC-3'
KpnI

LCARev: 5'-GGGGC**GCTAGC**GCAGCTTTTAGTTTAGTTCTTC-3'
NheI

SERCA1aFwd: 5'- GGGGC**AAGCTT**ATGGAAAACGCGCACGCGAAAACG -3'
HindIII

SERCA1aRev: 5'- GGGGC**GCATGC**GCCTCCAGGTAATTCCGGGCGAC-3'
SphI

The PCR products were digested using the engineered restriction sites for directional cloning and ligated with the corresponding sites in the pYES2 vector (Invitrogen, Carlsbad, CA) to achieve the desired expression vector (NheI and XbaI share a "CTAG" overhang thus the XbaI site in pYES2 was utilized for LCA1 cloning). The null LCA1 mutant, LCA-D366A (changing the catalytic aspartic acid residue to alanine), was constructed by site-directed mutagenesis. Mutagenesis was performed by PCR using *Pfu* DNA polymerase via the Quick Change mutagenesis kit according to the manufacturer's protocol (Stratagene, La Jolla, CA). The fidelity, orientation, and junctions of all constructs were confirmed by DNA sequencing. We received cDNA clones of LCA1 from Dr. Alan Bennett, University California-Davis [30] and avian SERCA1 from Dr. Douglas Fambrough, Johns Hopkins University [31], which served as our PCR templates for subcloning into our yeast expression vectors.

Yeast

Plasmids were transformed into yeast *Saccharomyces cerevisiae* strains using the Liac/SS Carrier DNA/PEG method as described by Gietz and Woods [32]. Successful transformants were selected on SD-URA plates. Colonies were picked and the presence of the respective Ca-ATPase was confirmed by PCR using gene specific primers.

The pYES2 vector, which contains a galactose inducible (GAL) promoter and the URA 3-selectable marker, was used to express avian *SERCA1a, LCA1* and LCA1-D366A (null mutant) in yeast *S. cerevisiae*. For control experiments with the *Arabidopsis* Ca-ATPase, ECA1, the vector pYX112-ECA1 (gift from Dr. Harper, University Nevada-Reno) was used to express *ECA1* in *S. cerevisiae*, which contained a triose phosphate isomerase promoter (TPI) and the URA 3-selectable marker. The specific *S. cerevisiae* strains used in this paper were K601 (*MATa ade2-1, can1-100, his3-11,15, leu2-3,112, trp-1, ura3-1*); its triple mutant derivative, K616 (*MATa pmr1::HIS3 pmc1::TRP1 cnb1::LEU2, ura3*), which is devoid of endogenous Ca-ATPases (gift from Dr. K.W. Cunningham, MIT), and a protease deficient *S. cerevisiae* strain HI227 (*MATa, ura3-52, leu2-3112, trp1, his3-d200, lys2D, pep4-3, prb1, prc1*).

Media

Standard YPD medium containing: 1% Difco yeast extract, 2% Bacto-peptone, 2% glucose, was used to grow *S. cerevisiae* strains for preparation of competent cells for plasmid transformation. For *S. cerevisiae* strains, synthetic dextrose minimal medium (SD-URA) containing: 0.67% yeast nitrogen base (pH 5.5), 2% glucose, 0.008% adenine, 0.02% Arginine, 0.014% Histidine, 0.0247% Leucine, 0.044% Lysine, 0.01% Phenylalanine, 0.018% Serine, 0.008% Tryptophan, 0.003% Tyrosine, 0.038% Valine (2% bacto-agar was used in solid medium). This common medium was used to select for and maintain transformed plasmids. For protein expression experiments, the same synthetic medium was used except 2% galactose replaced 2% glucose (i.e. SG-URA).

Download English Version:

https://daneshyari.com/en/article/1926384

Download Persian Version:

https://daneshyari.com/article/1926384

Daneshyari.com