The Role of Vacuole and Vacuolar H^+-ATPase in Tolerance to Cadmium in *Saccharomyces Cerevisiae*

Supat Jindarungraung, Sakkarin Bhubhanil, Choowong Auesukaree*.
Department of Biology, Faculty of Science, Mahidol University, Rama VI Rd., Payathai, Bangkok 10400
**Corresponding Author: sccar@mahidol.ac.th*

Abstract

Heavy metal is one of the major environmental pollutants. However, the protective cellular mechanisms in response to heavy metal stress are not well-understood yet. Previously, it has been found that the yeast deletion mutants lacking vacuolar H^+-ATPase (V-ATPase) activity, which functions in vacuolar acidification, exhibited growth defects under cadmium stress conditions. In addition, several genes involved in H^+-ATPase have been recently found to be required for resistance to aluminum. To investigate the roles of vacuolar H^+-ATPase, plasma membrane H^+-ATPase, and mitochondrial H^+-ATPase in tolerance to cadmium, we examined the growth of yeast deletion mutants lacking different types of H^+-ATPase in YPD media containing 80 μM of cadmium. Our results showed that a number of mutants lacking V-ATPase activity were sensitive to cadmium, suggesting the important role of V-ATPase in cadmium detoxification. We next examined the growth of mutants lacking genes responsible for different vacuolar functions in order to investigate the functional activities of vacuoles involved in cadmium detoxification mechanism. We found that the mutants lacking vacuolar protein-sorting genes exhibited high sensitivity to cadmium as well, suggesting the importance of protein transportation during cadmium stress.

Keywords: H^+-ATPase, V-ATPase, vacuole, cadmium, *Saccharomyces cerevisiae*

1. Introduction

Heavy metals have been used in many countries for a long time. The metals released into the environment by human activities, such as mining, municipal wastes, and phosphate fertilizers, is a worldwide problem that causes multiple harmful effects at different cellular levels [1]. Cadmium is one of the most toxic metals that contaminate soil and water, and is known to cause a severe disease called itai-itai [2]. Cadmium is a non-redox metal that induces oxidative stress by increasing reactive oxygen species (ROS), which are considered to lead to protein oxidation, lipid peroxidation and DNA damage [3-5]. Although toxicological effects of cadmium have been extensively studied, the mechanisms in response to cadmium stress are not completely understood.

H^+-ATPase or proton pump driven by the energy of ATP hydrolysis functions in extruding proton flux to create the electrochemical gradients. This pump is classified into 3 types, i.e. vacuolar H^+-ATPase (V-ATPase), mitochondrial H^+-ATPase (F-ATPase), and plasma membrane H^+-ATPase (P-ATPase) [6-8]. V-ATPase generates an electrochemical gradient across the vacuolar membrane by pumping proton into vacuolar lumen. V-ATPase is composed of V_1 complex containing ATP binding sites attached to V_0 complex of integral membrane proteins containing proton pore. Although the structure of F-ATPase is similar to V-ATPase, F-ATPase localizes to mitochondrial interior and acts as ATP syntheses [9-11]. The P-ATPase functions in pumping protons out of the cells, which contributes not only to intracellular pH regulation but also to generation of electrochemical gradient essential for nutrient uptake [12].

Previous study has shown that yeast mutants lacking vacuolar H^+-ATPase (V-ATPase) activity exhibited growth defect under heavy metal stress conditions [13]. Furthermore, vacuole has been shown to play an important role in detoxification through the regulation of cytosolic metal ion concentrations [14]. Recently, several genes involved in H^+-ATPase have been found to be required for resistance to aluminum [15]. It is therefore possible that H^+-ATPase may be also essential for tolerance to cadmium. In this work, we examined the role of V-ATPase, P-ATPase and F-ATPase in tolerance to cadmium, and screened for vacuolar genes that are required for cadmium tolerance.

2. Materials and Methods

2.1 Strain and growth conditions

The yeast strains used in study were the haploid *Saccharomyces cerevisiae* wild-type strain (BY4742) and its isogenic deletion mutants lacking subunits of H^+-ATPase and vacuolar proteins. Yeast strains were grown on YPD plate (1% yeast extract, 2% Bacto peptone, 2% glucose, and 2% agar) at 30°C.

2.2 Screening for cadmium-sensitive genes

Each strain was precultivated overnight in YPD media at 30°C to the logarithmic phase. The preculture was then stamped onto YPD plates containing 80 μM $CdCl_2$ by 48 pin replicator. The plates were incubated at 30°C for 3 days.

2.3 Classification of genes by gene function

Genes were classified into functional categories based on the

database of Munich Information center for protein sequences (MIPs) and the Saccharomyces Genome Database (SGD).

3. Results and Discussion

3.1 Identification of cadmium-sensitive H^+-ATPase mutants

To determine the role of H^+-ATPase in tolerance to cadmium, we examined the growth of mutants lacking genes encoding components of V-ATPase, P-ATPase, and F-ATPase on YPD plates containing 80 μM $CdCl_2$. Our results showed that only mutants lacking genes encoding V-ATPase subunits, but not P-ATPase and F-ATPase subunits, were sensitive to cadmium (Table 1), suggesting the specific role of V-ATPase in cadmium tolerance. Among these, the *Δvma3*, *Δvma6*, and *Δvma16* mutants are mutants lacking components of proton pore V_0 complex, whereas the others are mutants lacking components of V_1 complex involved in ATP hydrolysis. Since it is known that V-ATPase functions in maintaining intracellular pH homeostasis through vacuolar acidification, it is likely that V-ATPase may be required for recovery from intracellular acidification caused by cadmium. In addition, it has been found that the mutants lacking V_1 subunit (*Δvma2)* and V_0 subunit (*Δvma3*) were acutely sensitive to oxidative stress [16], and Cd^{2+} is also known to induce oxidative stress level [2]. It is therefore possible that V-ATPase may be important for reduction of intracellular oxidative level.

Since not only V-ATPase but also vacuole itself is important for cadmium tolerance [14], we next investigated the functional activities of vacuole involved in cadmium tolerance. We screened 148 mutants lacking genes responsible for different vacuolar functions on YPD plates containing 80 μM $CdCl_2$, and found that 27 mutants were sensitive to cadmium. The genes deleted in these mutants were classified into functional categories by using the MIPS and the SGD database (Table 2). We found that a large number of genes required for $CdCl_2$ tolerance was classified into the category of vacuolar protein sorting involved in transportation, docking, and fusion of protein into endosome (Table 2). Our results therefore suggested that not only V-ATPase but also functional vacuole, especially the vacuolar protein sorting process, is important for cadmium tolerance.

Table 1.Classification of H^+-ATPase genes whose deletions result in $CdCl_2$ sensitivity

Function	Gene name
V-ATPase (10)	*VMA1, VMA2, VMA3, VMA4, VMA5, VMA6, VMA7, VMA8, VMA10, VMA16*
F-ATPase (0)	-
P-ATPase (0)	-

Table 2.Classification of vacuolar genes whose deletions result in $CdCl_2$ sensitivity

Function	Gene name
Vacuolar protein sorting (14)	*DID4, VPS1, VPS3, VPS8, VPS9, VPS15, VPS21, VPS30, PS34, VPS38, VPS41, VPS55, VPS63, VPS71*
Vacuolar protein (5)	*PHM7, VAM3, VAM7, VAM10, VAM14*
Vacuolar enzyme (3)	*CPS1, PRC1, YPT6*
Vacuolar protein transport (3)	*APL6, BSD2, MVB12*
Vacuolar membrane protein (2)	*MON2, RCR2*
Vacuolar membrane kinase (0)	-
Vacuolar transporter (0)	-

4. Conclusions

In this study, we examined the role of H^+-ATPase in cadmium tolerance. We found that 10 mutants lacking genes encoding V-ATPase subunits were sensitive to cadmium, suggesting the role of V-ATPase in cadmium tolerance. We also determined vacuolar functions that are required for cadmium tolerance and found that a number of genes involved in vacuolar protein sorting are important for cadmium tolerance. Our results suggested that not only V-ATPase but also functional vacuole, especially the vacuolar protein sorting process, is important for cadmium tolerance.

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