Journal of Life Science 2009 Vol. 19. No. 12. 1685~1689 ⓒJLS / ISSN 1225-9918

Heterologous Expression of Fission Yeast Heavy Metal Transporter, SpHMT-1, Confer Tolerance to Cadmium in Cytosolic Phytochelatin-Deficient *Saccharomyces cerevisiae*

Sangman Lee*

Division of Applied Biology and Chemistry, School of Applied Biosciences, College of Agriculture and Life Sciences, Kyungpook National University, Daegu 702-701, Korea

Received September 14, 2009 / Accepted October 20, 2009

Phytochelatins (PCs) are small polypeptides synthesized by PC synthase (PCS). They are present in various living organisms including plants, fission yeast, and some animals. The presumed function of PCs is the sequestration of cytosolic toxic heavy metals like cadmium (Cd) into the vacuoles via vacuolar membrane localized heavy metal tolerance factor 1 (HMT-1). HMT-1 was first identified in fission yeast (SpHMT-1), and later in *Caenorhabdtis* (CeHMT-1). Recently, its homolog has also been found in PC-deficient *Drosophila* (DmHMT-1), and this homolog has been shown to be involved in Cd detoxification, as confirmed by the heterologous expression of DmHMT-1 in fission yeast. Therefore, the dependence of HMT-1 on PC in Cd detoxification should be re-evaluated. I heterologously expressed SpHMT-1 in cytosolic PC-deficient yeast, *Saccharomycea cerevisiae*, to understand the dependence of HMT-1 on PC. Yeast cells expressing SpHMT-1 showed increased tolerance to Cd compared with control cells. This result indicates that SpHMT-1 is not strictly correlated with PC production on its function. Moreover, yeast cells expressing SpHMT-1 showed increased tolerance to exogenously applied glutathione (GSH) compared with control cells, and the tolerance to Cd was further increased by exogenously applied GSH, while tolerance in control cells was not. These results indicate that the function of SpHMT-1 in Cd detoxification does not depend on PCs only, and suggest that SpHMT-1 may sequester cytosolic GSH-Cd complexes into the vacuole.

Key words : Cadmium, glutathione, heavy metal tolerance factor, phytochelatin, vacuole, yeast

Introduction

Heavy metal contaminated soils and water have been serious problems threatening human health and environmental ecology. The metals can be categorized into two groups. One group consists of essential metals such as copper (Cu), zinc (Zn), and iron (Fe), which are necessary for growth and the development of living organisms throughout their life. The other group consists of non-essential metals such as cadmium (Cd), lead (Pb), and mercury (Hg), these are toxic and deleterious even though they are present in low concentrations [3].

Plants and some other organisms have developed several mechanisms to detoxify the non-essential metals and to maintain homeostasis of the essential metals. One important mechanism is the production of phytochelatins (PCs). PCs are enzymatically synthesized small polypeptides with the general structure $(\gamma\text{-Glu-Cys})_n\text{-Gly}$, where n is 2 to 11 [11]. PC synthase (PCS, EC 2.3.2.15) utilizes glutathione (GSH) as a substrate, and transfers the γ-Glu-Cys moiety of GSH to either another GSH or a growing PC, resulting in the production of PCs [4]. Genes encoding PCS have been identified from various organisms including *Arabidopsis thaliana* (*AtPCS*), *Schizosaccharomycea pombe* (*SpPCS*), *Caenorhabditis elegans* (*CePCS*), and other species [1,5,15,16].

PCs can make stable complexes with heavy metals in the cytosol, and these complexes can be subsequently sequestered into the vacuole [2]. There has been research showing that plants can transport the PC-metal complexes into the vacuole using an ABC (ATP-binding cassette)-type transporter even though the corresponding gene has not been cloned yet [12]. In fission yeast, *S. pombe*, the *SpHMT-1* (heavy metal tolerance factor 1) gene was first identified and its homologs were further identified from *C. elegans*, *Clamydomonas reinhardtii*, and *Drosophilia* [9,10,14,17].

HMT-1 is a vacuolar membrane localized family of ABC-type transporters [9]. It has been suggested that the function of HMT-1 in Cd tolerance is correlated with the sequestration of cytosolic PC-Cd complex into the vacuole

***Corresponding author**
Tel : +82-53-950-7345, Fax : +82-53-953-7233
E-mail : sangman@knu.ac.kr

in fission yeast [10]. However, PC-deficient animals such as *Drosophilia* also contain a HMT-1 homolog, and there have been suggestions that the tolerance to Cd by HMT-1 is not strictly correlated with PC production [14,17]. Therefore, this study aims to determine whether the function of HMT-1 in Cd detoxification is strictly correlated with PC production.

Materials and Methods

Yeast transformation and growth assays

The *Saccharomyces cerevisiae* strain INVsc1 (*his3△1/his3△1 leu2/leu2 trp1-289/trp1-289 ura3-52/ura3-52*) (Invitrogen, Carlsbad, CA) was used for transformation using the lithium acetate method [13]. Transformed yeast cells were grown in yeast nitrogen base supplemented with appropriate amino acids and either 2% galactose or 2% glucose. To assay for heavy metal sensitivity, cells grown for 24 hr were incubated in the presence of various concentrations of heavy metals either for 24 hr or more, at 30°C. And then, cell density was measured by spectrophotometer at 600 nm.

DNA manipulation

Escherichia coli strain DH5α was used for DNA manipulation. The HMT-1 cDNA was produced by PCR (polymerase chain reaction) using pDH35 as a template [9]. The forward primer was 5′-TTT AAG CTT ATG GTT CTA CGT TAC AAC AGC-3′ with *Hin*dIII site (underlined) and the reverse primer was 5′-TTT GAG CTC TTA ATG AGT TTC AGC AGA AGT-3′ with *Sac*I site (underlined). The PCR product was subcloned into *Hin*dIII/*Sac*I sites of pYES2 vector (Invitrogen, Carlsbad, CA) resulting in pYES2:HMT-1. The other vector, named pDH35-HMT-1, was originated from pDH35 by removing HMT-1 through *Bam*HI cutting and self-ligation, and this was used as a control. These vectors were used in yeast transformation.

Results and Discussion

To understand the role of HMT-1 in the Cd detoxification mechanism, I expressed *SpHMT-1* in cytosolic PC-deficient yeast, *S. cerevisiae*. The pDH35 is a pART1 vector containing *SpHMT-1* [10]. As a control vector, I used pDH35-HMT-1 where the functional *SpHMT-1* was removed from pDH35 through *Bam*HI cutting and self-ligation. Sensitivities to Cd were compared in yeast cells containing either pHD35 or the control vector, pDH35-HMT-1 (Fig. 1). SpHMT-1

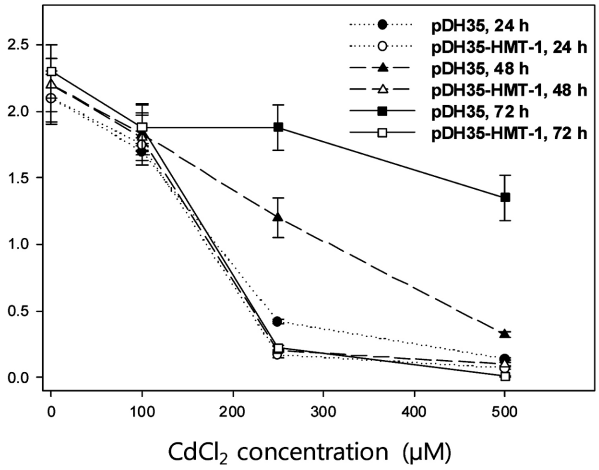

Fig. 1. Sensitivity to cadmium in *S. cerevisiae* cells expressing *SpHMT-1*. Yeast cells containing either pDH35 (pATR1 vector containing *SpHMT-1*; dark color) or pDH35-HMT-1 (pDH35 removed *SpHMT-1*; white color) were grown in media containing various concentrations of cadmium for 24 (circle), 48 (triangle), and 72 hr (square) at 30°C. Afterward, cell density was measured spectrophotometrically at 600 nm. Values are means±SE of three replicates.

conferred significant Cd tolerance in cytosolic PC-deficient *S. cerevisiae*. To confirm this fact, I expressed *SpHMT-1* under the control of galactose inducible promoter in pYES2, and compared Cd sensitivities between yeast cells containing pYES:HMT-1 and empty vector, pYES2 (Fig. 2). Consistently, SpHMT-1 conferred Cd tolerance in cytosolic PC-deficient *S. cerevisiae* when its expression was induced by galactose, while it did not when its expression was repressed by glucose.

In a time-dependent analysis of Cd sensitivity, the cells containing pYES2 showed severe growth retardation in media containing 500 μM $CdCl_2$ when incubated from 1 to 4 d, while the cells containing pYES2:HMT-1 showed Cd resistance and its cell density recovered to non-treatment levels in the 3 d incubation (Fig. 3).

This finding that SpHMT-1 can confer tolerance tor Cd in cytosolic PC-deficient *S. cerevisiae* suggests the role of HMT-1 in Cd detoxification is not strictly correlated with PC production. In previous reports of others, they suggest that SpHMT-1 and DmHMT-1 do not transport PC-Cd complex but are involved in Cd detoxification [14,17]. Therefore, the question of how HMT-1 can confer Cd tolerance without the involvement of PCs remains unanswered. There are several possible scenarios for the function of HMT-1 in Cd detoxification. One of them is that HMT-1 can sequester both

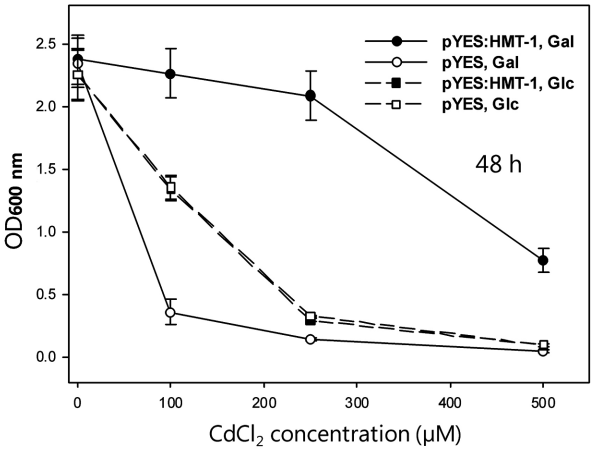

Fig. 2. Sensitivity to cadmium in *S. cerevisiae* cells expressing *SpHMT-1* under the control of galactose inducible promoter. Yeast cells containing either pYES:HMT-1 (dark color) or empty vector pYES2 (white color) were grown in media containing various concentrations of cadmium with either galactose (circle) or glucose (square) for 48 hr at 30°C. Afterward, cell density was measured spectrophotometrically at 600 nm. Values are means±SE of three replicates.

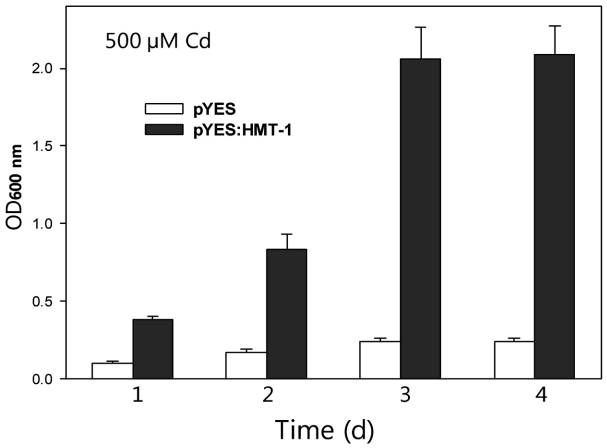

Fig. 3. Time-dependent sensitivity to cadmium in *S. cerevisiae* cells expressing *SpHMT-1*. Yeast cells containing either pYES:HMT-1 (dark color) or empty vector pYES2 (white color) were grown in media containing 500 μM $CdCl_2$ from 1 d to 4 d at 30°C. Afterward, cell density was measured spectrophotometrically at 600 nm. Values are means±SE of three replicates.

cytosolic PC-Cd complex as a minor and unknown something (e.g. GSH)-metal (e.g. Cd) complex as a major into the vacuole. If HMT-1 is involved in sequestration of GSH-Cd complexes into the vacuole like YCF1 (yeast cadmium factor protein), it is assumed that yeast cells containing pYES2: HMT-1 may be relatively tolerant for toxic levels of GSH, and the Cd tolerance may be further increased

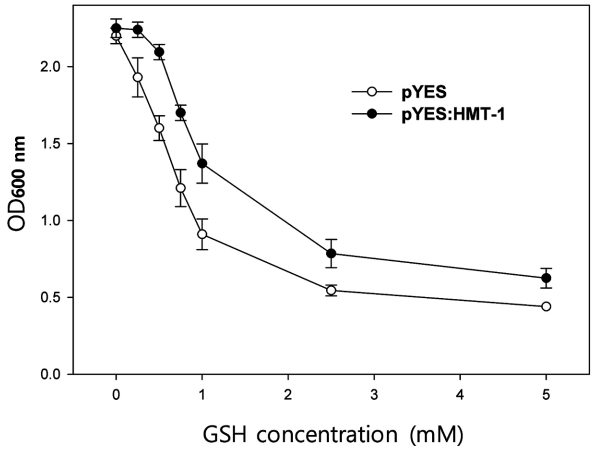

Fig. 4. Sensitivity to glutathione (GSH) in *S. cerevisiae* cells expressing *SpHMT-1*. Yeast cells containing either pYES: HMT-1 (dark color) or empty vector pYES2 (white color) were grown in media containing various concentrations of GSH for 24 hr at 30°C. Afterward, cell density was measured spectrophotometrically at 600 nm. Values are means±SE of three replicates.

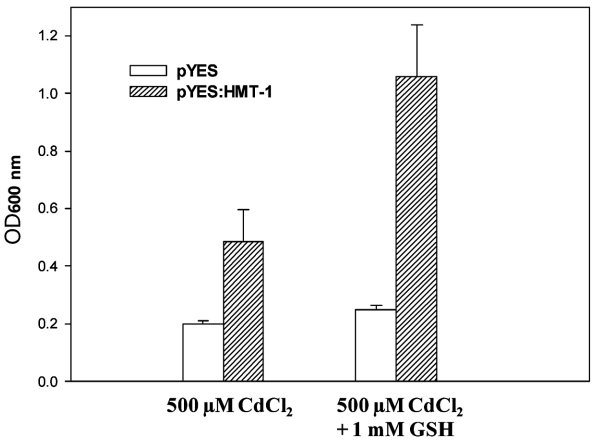

Fig. 5. Effect of glutathione (GSH) on sensitivity to cadmium in *S. cerevisiae* cells expressing *SpHMT-1*. Yeast cells containing either pYES:HMT-1 (dark color) or empty vector pYES2 (white color) were grown in media containing either 500 μM $CdCl_2$ or 1 mM GSH plus 500 μM $CdCl_2$ for 24 hr at 30°C. Afterward, cell density was measured spectrophotometrically at 600 nm. Values are means±SE of three replicates.

by exogenously applied GSH compared with the control cells [7,8].

Thiol-containing compounds, especially cysteine and GSH, are toxic to living organisms when they are present at supra-optimal concentrations [6]. In analysis of sensitivity to exogenously applied GSH, the yeast cells containing pYES2:HMT-1 were more resistant than control cells containing pYES2 (Fig. 4). This result suggests that SpHMT-1 may

be involved in transport of cytosolic GSH (or GSH-metal complex) into the vacuole. Furthermore, the tolerance for Cd by SpHMT-1 was more increased by exogenously applied 1 mM GSH as shown in yeast cells containing pYES2:HMT-1 grew 2 times well than without GSH, while, control cells did not (Fig. 5).

In conclusion, the function SpHMT-1 in the mechanism of Cd detoxification is not strictly correlated with cytosolic PC production, and it is suggested that SpHMT-1 may sequester cytosolic GSH-Cd complexes like YCF1 into the vacuole.

Acknowledgments

I thanks to Dr. David W. Ow (University of California, Berkeley, California) for his generous gift of pDH35. This research was supported by Kyungpook National University Research Fund, 2007.

References

1. Clemens, S., E. J. Kim, D. Neumann, and J. I. Schroeder. 1999. Tolerance to toxic metals by a gene family of phytochelatin synthases from plants and yeast. *EMBO J.* **18**, 3325-3333.
2. Cobbett, C. S. 2000. Phytochelatin biosynthesis and function in heavy-metal detoxification. *Curr. Opin. Plant Biol.* **3**, 211-216.
3. Goyer, R. A. 1997. Toxic and essential metal interactions. *Annu. Rev. Nutr.* **17**, 37-50.
4. Grill, E., S. Loffler, E. L. Winnacker, and M. H. Zenk. 1989. Phytochelatins, the heavy-metal-binding peptides of plants, are synthesized from glutathione by a specific γ-glutamylcysteine dipeptidyl transpeptidase (phytochelatin synthase). *Proc. Natl. Acad. Sci. U.S.A.* **86**, 6838-6842.
5. Ha, S. B., A .P. Smith, R. Howden, W. M. Dietrich, S. Bugg, M. J. O'Conell, P. B. Goldsbrough, and C. S. Cob bett. 1999. Phytochelatin synthase genes from *Arabidopsis* and the yeast, *Schizosaccharomyces pombe*. *Plant Cell* **11**, 1153-1164.
6. Lee, S. and B. S. Kang. 2005. Phytochelatin is not a primary factor in determining copper tolerance. *J. Plant Biol.* **48**, 32-38.
7. Li, Z. S., Y. P. Lu, R. G. Zhen, M. Szczypka, D. J. Thiele, and P. A. Rea. 1997. A new pathway for vacuolar cadmium sequestration in Saccharomyces cerevisiae: YCF1-catalyzed transport of bis (glutathionato)cadmium. *Proc. Natl. Acad. Sci. U.S.A.* **94**, 42-47.
8. Li, Z. S., M. Szczypka, Y. P. Lu, D. J. Thiele, and P. A. Rea. 1996. The yeast cadmium factor protein (YCF1) is a vacuolar glutathione S-conjugate pump. *J. Biol. Chem.* **271**, 6509-6517.
9. Oritz, D. F., L. Kreppel, D. M. Speiser, G. Scheel, G. McDonald, and D. W. Ow. 1992. Heavy metal tolerance in the fission yeast requires an ATP-binding cassette-type vacuolar membrane transporter. *EMBO J.* **11**, 3491-3499.
10. Oritz, D. F., T. Ruscitti, K. F. McCue, and D. W. Ow. 1995. Transport of metal-binding peptides by HMT1, a fission yeast ABC-type vacuolar membrane protein. *J. Biol. Chem.* **270**, 4721-4728.
11. Rauser, W. E. 1990. Phytochelatins. *Annu. Rev. Biochem.* **59**, 61-86.
12. Salt, D. E. and W. E. Rauser. 1995. MgATP-dependent transport of phytochelatins across the tonoplast of oat roots. *Plant Physiol.* **107**, 1293-1310.
13. Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. Molecular Cloning: A Laboratory manual, Ed 2. Cold Spring Harbor Laboratory Press, N.Y.
14. Sooksa-nguan, T., B. Yakubov, V. Kozlovsky, C. M. Barkume, K. J. Howe, T. W. Thannhausen, M. A. Rutzke, J. J. Hart, L. V. Kochian, P. A. Rea, and O. K. Vatamaniuk. 2009. Drosophila ABC transporter, DmHMT-1, confer tolerance to cadmium. *J. Biol. Chem.* **248**, 354-362.
15. Vatamaniuk, O. K., S. Mari, Y. P. Lu, and P. A. Rea. 1999. AtPCS1, a phytochelatin synthase from *Arabidopsis*: isolation and *in vitro* reconstitution. *Proc. Natl. Acad. Sci. U.S.A.* **96**, 7110-7115
16. Vatamaniuk, O. K., E. A. Bucher, J. T. Ward, and P. A. Rea. 2001. A new pathway for heavy metal detoxification in animals. *J. Biol. Chem.* **276**, 20817-20820.
17. Vatamaniuk, O. K., E. A. Bucher, M. V. Sundaram, and P. A. Rea. 2005. CeHMT-1, a putative phytochelatin transporter, is required for cadmium tolerance in Caenorhabditis elegans. *J. Biol. Chem.* **280**, 23684-23690.

초록 : 분열효모 SpHMT1을 세포질 파이토킬레이트를 생성하지 않는 효모에서 발현으로 인한 카드뮴에 대한 저항성 증가

이 상 만*
(경북대학교 응용생명과학부)

파이토킬레이트(PC)는 PCS에 의해 생성되는 작은 폴리펩타이드로서 여러 생물에서 발견되고 있다. PC의 역할은 카드뮴과 같은 중금속을 세포질에서 결합하며 이는 액포막에 존재하는 HMT에 의해서 액포 안으로 이동된다. HMT1은 분열효모에서 처음으로 알려졌으며 이후 선충, 초파리 등에서도 발견되었으며 세포 내 역할은 카드뮴 같은 중금속 해독에 관여를 하고 있다. 하지만 액포가 존재하지 않고 PC를 생성하지 않는 초파리에서의 HMT1의 발견은 그 동안 알려진 HMT1의 역할을 재 조명하게 된다. 따라서 PC를 생성하지 못하는 출아효모에 PC를 생성하는 분열효모 유래 SpHMT1을 발현시켜 카드뮴에 대한 저항성을 분석하였다. SpHMT1을 발현하는 출아효모는 카드뮴에 대한 저항성이 현저하게 증가되었고 이는 SpHMT1이 PC가 존재하지 않는 조건에서도 카드뮴에 대한 해독작용을 하는 것을 암시한다. 또한 SpHMT1을 발현하는 출아효모는 GSH에 대한 저항성을 보였고 카드뮴에 대한 저항성도 GHS에 의해서 더 증가되는 결과를 보였다. 이러한 결과는 HMT1이 PC와 결합된 카드뮴을 액포안으로 이동시키는 가능성보다 GSH와 결합된 카드뮴을 액포 안으로 이동시켜 카드뮴에 대한 해독작용을 한다는 것을 암시한다.