Antioxidant system within yeast peroxisomes which protect organellar membrane

Yasuyoshi SAKAI

Division of Applied Life Sciences, Graduate School of Agriculture, Kyoto University

The methylotrophic yeast *Candida boidinii* had two anti-oxidant enzymes within peroxisomes, catalase (Cta1p) and CbPmp20. CbPmp20, a protein associated with the inner side of peroxisomal membrane, belongs to a recently identified protein family of antioxidant enzymes, the peroxiredoxins, which contain one cysteine residue. Pmp20 homologs containing the putative peroxisome targeting signal type 1 have also been identified in mammals and lower eukaryotes. However, the physiological function of these Pmp20 family proteins has been unclear.

In this study, we investigated the biochemical and physiological functions of recombinant CbPmp20 protein in methanol-induced peroxisomes of *C. boidinii* using the *PMP20*-deleted strain of *C. boidinii* (*pmp20Δ* strain). The His6-tagged CbPmp20 fusion protein was found to have glutathione peroxidase activity *in vitro* toward alkyl hydroperoxides and H_2O_2. Catalytic activity and dimerization of His6-CbPmp20 depended on the only cysteine residue corresponding to Cys53. The *pmp20Δ* strain was found to have lost growth ability on methanol as a carbon and energy source. Interestingly, the *pmp20Δ* strain had a more severe growth defect than the *cta1Δ* strain, which lacks catalase, another antioxidant enzyme within the peroxisome. During incubation of these strains in methanol medium, the *cta1Δ* strain accumulated H_2O_2, whereas the *pmp20Δ* strain did not. The *pmp20Δ* growth defect was rescued by CbPmp20, but neither CbPmp20 lacking the peroxisome targeting signal type 1 sequence nor CbPmp20 haboring the C53S mutation retrieved the growth defect. Therefore, it is speculated to be the main function of CbPmp20 is to decompose reactive oxygen species generated at peroxisomal membrane surface, e.g. lipid hydroperoxides, rather than to decompose H_2O_2. In addition, we detected a physiological level of reduced glutathione in peroxisomal fraction of *C. boidinii*. These results may indicate a physiological role for CbPmp20 as an antioxidant enzyme within peroxisomes rich in reactive oxygen species. And these biochemical nature of CbPmp20 will form the basis to establish the enzymatic method to determine unstable lipid hydroperoxides.

On the other hand, the *cta1Δ* strain showed marked growth inhibition on various peroxisome-inducing carbon sources, indicating an important non-specific role for Cta1p in peroxisomal metabolism. The peroxisomal import was shown to be necessary for their physiological function. Using GFP-fusion proteins, Cta1p is found to be bimodally distributed between cytosol and peroxisomes in methanol-grown cells, but is localized exclusively in peroxisomes in oleate- and D-alanine-grown cells. In contrast, the fusion protein GFP-AKL in the context of Pmp20, was shown to localize exclusively in peroxisomes. The low transport efficiency of the -NKF sequence of Cta1p was due to a lower level of interaction between the -NKF sequence and the PTS1 receptor than that of the -AKL sequence of Pmp20. The PTS1 sequence in peroxisomal catalases may have evolved in such a way as to give a higher priority for peroxisomal transport to other peroxisomal enzymes, which require a higher level of peroxisomal transport efficiency.

References

Hirofumi Horiguchi, Hiroya Yurimoto, Nobuo Kato and Yasuyoshi Sakai. Antioxidant System within Yeast Peroxisome: Biochemical and Physiological Characterization of CbPmp20 in the Methylotrophic Yeast *Candida boidinii*. *J. Biol. Chem.*, **276** (17) 14279–14288 (2001).

Hirofumi Horiguchi, Hiroya Yurimoto, Toh-Kheng Goh, Tomoyuki Nakagawa, Nobuo Kato and Yasuyoshi Sakai. Peroxisomal Catalase in the Methylotrophic Yeast *Candida boidinii*: Transport Efficiency and Metabolic Significance. *J. Bacteriol.*, **183** (21) 6372–6383 (2001). [cover story].