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Involvement of glutathione peroxidase 1 in growth and peroxisome formation in *Saccharomyces cerevisiae* in oleic acid medium

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ABSTRACT

Saccharomyces cerevisiae is able to use some fatty acids, such as oleic acid, as a sole source of carbon. β-oxidation, which occurs in a single membrane-enveloped organelle or peroxisome, is responsible for the assimilation of fatty acids. In *S. cerevisiae*, β-oxidation occurs only in peroxisomes, and H_2O_2 is generated during this fatty acid-metabolizing pathway. *S. cerevisiae* has three *GPX* genes (*GPX1*, *GPX2*, and *GPX3*) encoding atypical 2-Cys peroxiredoxins. Here we show that expression of *GPX1* was induced in medium containing oleic acid as a carbon source in an Msn2/Msn4-dependent manner. We found that Gpx1 was located in the peroxisomal matrix. The peroxisomal Gpx1 showed peroxidase activity using thioredoxin or glutathione as a reducing power. Peroxisome biogenesis was induced when cells were cultured with oleic acid. Peroxisome biogenesis was impaired in *gpx1*Δ cells, and subsequently, the growth of *gpx1*Δ cells was lowered in oleic acid-containing medium. Gpx1 contains six cysteine residues. Of the cysteine-substituted mutants of Gpx1, $Gpx1^{C36S}$ was not able to restore growth and peroxisome formation in oleic acid-containing medium, therefore, redox regulation of Gpx1 seems to be involved in the mechanism of peroxisome formation.

1. Introduction

Lipids are one of the energy storage forms in cells. Fatty acids are constituents of lipids, from which an enormous amount of energy can be drawn. The process to draw energy from fatty acids is referred to as β-oxidation. In mammalian cells, β-oxidation occurs in mitochondria and peroxisomes [1]. In the first step of β-oxidation, fatty acyl-CoA is generated from fatty acid, ATP, and CoA by an action of acyl-CoA synthetase in the cytoplasm, and is transported to the mitochondrial matrix with the aid of carnitine. After which, fatty acyl-CoA is oxidized to *trans*-Δ^2-enoyl-CoA by acyl-CoA dehydrogenase. In this reaction, FAD is used as an acceptor of an electron from acyl-CoA, and the $FADH_2$ formed enters the electron transfer chain in mitochondria to produce ATP. The mitochondrial β-oxidation is responsible for moderately long chain fatty acids (~C_{18}); whereas, the β-oxidation in peroxisomes is responsible for very long chain fatty acids (>C_{20}) [1]. The budding yeast *Saccharomyces cerevisiae* is also able to use fatty acids as a sole source of carbon. Unlike mammalian cells, *S. cerevisiae* shows β-oxidation only in peroxisomes, the biogenesis of which is markedly induced when yeast cells are cultured in a medium containing fatty acids. The first step of β-oxidation in the yeast peroxisome is carried out by acyl-CoA oxidase. In this enzymatic reaction, molecular oxygen (O_2) is used as the final acceptor of electrons from acyl-CoA, and consequently, H_2O_2 is generated in the peroxisome. In the final step of β-oxidation, 3-ketoacyl-CoA thiolase liberates acetyl-CoA which results in 3-ketoacyl-CoA esters being converted into a C_2-shortened acyl-CoA. Acetyl-CoA generated from the β-oxidation of fatty acids is entered into the glyoxylate cycle. Enzymes constituting the glyoxylate cycle are located on both sides of peroxisomal membrane [2]. Succinate is released from the glyoxalate cycle, and transported into mitochondria. Alternatively, acetyl-CoA is transported from peroxisomes to mitochondria in a carnitine-dependent manner. Recent studies have revealed that there are several options for transferring acyl groups from peroxisomes to mitochondria (for review, see Ref. [3]). Consequently, ATP is produced in mitochondria [4]. In this way, the process to draw energy from fatty acids is aerobic, and so has the potential to produce a large amount of reactive oxygen species (ROS). Indeed, H_2O_2 is generated in the first step of β-oxidation in yeast, and the respiration process in mitochondria is also capable of generating ROS. To reduce the risk of oxidative stress caused by ROS during the metabolism of fatty acids, *S. cerevisiae* has a peroxisomal catalase, encoded by *CTA1*, whose expression is induced in medium containing fatty acids [5].

Besides catalase, *S. cerevisiae* has several antioxidative enzymes that are able to scavenge H_2O_2. Peroxiredoxins are a growing family of antioxidants in many eukaryotic organisms. Since peroxiredoxins do not contain a redox active cofactor or prosthetic group, the redox

Abbreviations: ROS, reactive oxygen species; GPx, glutathione peroxidase; PTS, peroxisome targeting signal; ER, endoplasmic reticulum; SD, synthetic dextrose; PMSF, phenylmethylsulfonyl fluoride; PAGE, polyacrylamide gel electrophoresis; 3-AT, 3-amino 1,2,4-triazole; *t*-BHP, *tert*-butyl hydroperoxide

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status of cysteine residues plays crucial roles in peroxidase reactions [6]. Peroxiredoxins are classified into three groups depending on the number of conserved cysteine residues, i.e. 1-Cys, 2-Cys, and atypical 2-Cys peroxiredoxins [7]. *S. cerevisiae* has one 1-Cys peroxiredoxin (Prx1), and four 2-Cys peroxiredoxins (Tsa1, Tsa2, Ahp1, and Dot5) [8]. We have previously reported that *S. cerevisiae* has three glutathione peroxidases (Gpx1, Gpx2, and Gpx3), which are structural homologues of the mammalian glutathione peroxidase (GPx) [9]. After biochemical characterizations, these GPx homologues have been revealed to be atypical 2-Cys peroxiredoxins [10–12]. These peroxiredoxins use thioredoxin, a protein-disulfide oxidoreductase, as a reducing power to revert the critical oxidized cysteine(s) to retain the integrity of enzymatic activity. Gpx1 and Gpx2 are able to use glutathione also. Among yeast peroxiredoxins, Prx1 is distributed in mitochondria, Dot5 in the nucleus, Ahp1 in mitochondria and/or peroxisomes, and Tsa1/2 in the cytoplasm [8]. The intracellular distribution of GPx homologues has not yet been elucidated, except for Gpx2, which we have recently identified to be located in mitochondria [13]. In this study, we found that *GPX1* expression is induced by oleic acid, and a *gpx1*Δ mutant showed slow-growth phenotype in oleic acid-containing medium. We showed that Gpx1 is located in the peroxisomal matrix, and the peroxisomal Gpx1 exhibits peroxidase activity. Finally, we investigated the correlation between Gpx1 and peroxisome biogenesis.

2. Material and methods

2.1. Strains

All *S. cerevisiae* strains used in this study have the BY4741 background. The strains and PCR primers used are summarized in Supplementary Tables S1 and S2, respectively.

The *gpx1*Δ::*his5*$^+$ mutant was generated by replacing *kanMX4* of *gpx1*Δ::*kanMX4* mutant in the BY4741 background with a *his5*$^+$ cassette in pUG27. When combining other mutations constructed by the *kanMX4* marker with deletion of *gpx1*Δ::*his5*$^+$, PCR fragment amplified with primers, GPX1-F and GPX1-R was introduced to the mutant strains with the *kanMX4* marker. To construct *gpx3*Δ::*LEU2* and *msn2*Δ::*HIS3* mutants in the BY4741 background, each mutation allele in the YPH250-based mutant was amplified using primer sets as described before [9,14]. To construct a strain carrying GFP-tagged Vps1, PCR was done using primers VPS1-GFP-F and VPS1-GFP-R with the genomic DNA of VPS1-GFP (BY4742 background) [15] as a template. The resultant PCR fragment containing the C terminus half of Vps1 tagged with GFP and nourseothricin-resistant gene was introduced into the *VPS1* locus, and clones showing the resistance to nourseothricin were isolated.

2.2. Plasmids

The plasmids constructed in this study are summarized in Supplementary Table S3.

To construct pRS416 + GPX1-3HA, pSLF172-GPX1 [16] was digested with SalI and HindIII, and the *GPX1-3HA* cassette was inserted into the SalI–HindIII site of pRS416. To construct pRS306 + GPX1-3HA, PCR was done using primers GPX1-F-XbaI and GPX1-R-BamHI with pSFL172-GPX1 as a template, and the XbaI/BamHI fragment was inserted into the XbaI–BamHI site of pRS306. pRS306 + GPX1-3HA was digested with EcoRI, and integrated into the *GPX1* locus of BY4741. To construct pRS414-GPX1, the *GPX1* gene was amplified using GPX1-SalI-F and GPX1-PstI-R. SalI and PstI sites were designed in GPX1-SalI-F and GPX1-PstI-R, respectively (underlined). The PCR fragment was digested with SalI and PstI, and the resultant fragment was cloned into the SalI and PstI site of pRS414. To construct pRS411 + GPX1, pRS416 + GPX1 and pRS426 + GPX1, pRS414 + GPX1 as constructed above was digested with SalI and BamHI, and the DNA fragment containing *GPX1* was cloned into the SalI–BamHI site of pRS411, pRS416, and pRS426, respectively.

To construct pRS416 + GPX2, pRS416 + GPX3, pRS411 + GPX2, and pRS411 + GPX3, the *GPX2* and *GPX3* genes were amplified using GPX2-SalI-F and GPX2-BamHI-R, or GPX3-SalI-F and GPX3-BamHI-R using genomic DNA of BY4741 as a template. SalI and BamHI sites were designed in each forward primer (F), and reverse primer (R), respectively (underlined). Each PCR fragment was digested with SalI and BamHI, and the resultant fragment was cloned into the SalI and BamHI sites of pRS416 or pRS411.

To construct pRS411-based Gpx1-3HA cysteine-to-serine variants, pRS416-based Gpx1-3HA cysteine-to-serine variants [12] were digested with SalI and XbaI, and each DNA fragment containing *GPX1* was cloned into the XhoI–SpeI site of pRS411.

To construct *POT1-GFP* plasmid, the first PCR was done using POT1-F and POT1-R using the genomic DNA of BY4741 as a template. SacI and BamHI sites were designed in POT1-F and POT1-R, respectively (underlined). The PCR fragment was digested with SacI and BamHI, and the DNA fragment containing a part of *POT1* was cloned into the SacI–BamHI site of pKW430 [17]. The resultant plasmid was digested with SacI and XhoI, and the resultant fragment containing *POT1-GFP* was cloned in to the SacI–XhoI site of pRS306. pRS306 + POT1-GFP was digested with EcoRI, and integrated into the *POT1* locus of BY4741.

To construct *HA-POX1* plasmid, the first PCR was done with the following primer sets: POX1-F-SalI plus POX1-HA-R, and POX1-HA-F plus POX1-R + 900 using the genomic DNA of BY4741 as a template. SalI site was designed in POX1-F-SalI (underlined). The second PCR was done using the primers POX1-F-SalI and POX1-R + 900, with the mixture of the first PCR products as a template. The second PCR products were digested with SalI and BamHI, and the DNA fragment containing *HA-POX1* was cloned into the SalI–BamHI site of pRS306. pRS306 + HA-POX1 was digested with HindIII, and integrated into the *POX1* locus of BY4741. To construct pRS421 + POX1, the *POX1* gene was amplified using POX1-F-SalI and POX1-R-EagI. SalI and EagI sites were designed in POX1-F-SalI and POX1-R-EagI, respectively (underlined). The PCR fragment was digested with SalI and EagI, and the resultant fragment was cloned into the SalI–EagI site of pRS421.

2.3. Growth experiment

The media used were synthetic dextrose (SD) medium (2% glucose and 0.67% yeast nitrogen base without amino acids) with appropriate amino acids, YPOT medium (1% yeast extract, 2% peptone, 0.2% oleic acid, and 0.2% Tween 40), and YNBOT medium [0.67% yeast nitrogen base (without amino acids) with appropriate amino acids and bases, 0.15% oleic acid, and 0.15% Tween 40].

To monitor the growth of cells in oleic acid-containing medium, cells grown in SD medium until $A_{610} = 1.0$ were collected by centrifugation, washed once with a 0.85% NaCl solution, and suspended in YNBOT medium. Since oleic acid is difficult to dissolve in water, a detergent (Tween 40) was added to the medium. However, the A_{610} varied among experiments even though Tween 40 was added and the same yeast strain was used, which may be due to the difference in solubility of oleic acid in the medium in each experiment. To minimize the dispersion of the data, cell growth was expressed relative to the final A_{610} of the wild type in each experiment after a 216-h culture in YNBOT medium. The relative growth was expressed as the rate of A_{610} of each strain at each time *versus* A_{610} of wild type after 216-h culture, calculated with the following equation: $[(A_{610}$ of each strain at each culture time $- 1.0)/(A_{610}$ of wild type at 216-h culture $- 1.0)] \times 100$.

2.4. Western blotting

Cells cultured in YPOT or YNBOT medium were disrupted with glass beads in 10 mM Tris–HCl buffer (pH 7.0) containing 1 mM