Biochemical and Biophysical Research Communications 499 (2018) 836–842

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Osh6p, a homologue of the oxysterol-binding protein, is involved in production of functional cytochrome P450 belonging to CYP52 family in *n*-alkane-assimilating yeast *Yarrowia lipolytica*

Ryo Iwama, Mariho Hara, Aya Mizuike, Hiroyuki Horiuchi, Ryouichi Fukuda*

Department of Biotechnology, The University of Tokyo, Yayoi 1-1-1, Bunkyo-ku, Tokyo, 113-8657, Japan

ARTICLE INFO

Article history:
Received 15 March 2018
Accepted 1 April 2018
Available online 10 April 2018

Keywords:
Yarrowia lipolytica
n-alkane
P450
Oxysterol-binding protein
OSH6
Phospholipid

ABSTRACT

In this study, we investigated the role of *OSH6*, which encodes a homolog of the oxysterol-binding protein, in the assimilation of *n*-alkanes in the yeast *Yarrowia lipolytica*. The deletion mutant of *OSH6* showed growth defects on *n*-alkanes of 10–16 carbons. In the deletion mutant, production of the functional cytochrome P450 was not observed. However, transcription of *ALK1*, encoding a major P450 belonging to the CYP52 family that plays a critical role in *n*-alkane hydroxylation, and further translation of its transcript were noted in the deletion mutant as well as in the wild-type strain. The phospholipid composition was altered and, the ratio of phosphatidylserine (PS) was reduced by the deletion of *OSH6*. Residues involved in the transport of PS and phosphatidylinositol-4-phosphate in Osh6 of *Saccharomyces cerevisiae* are conserved in *Y. lipolytica* Osh6p and substitutions of these residues resulted in a defect in the *n*-alkane assimilation by *Y. lipolytica*. From these results, we propose a hypothesis that Osh6p provides an ideal endoplasmic reticulum membrane environment for Alk proteins to have a functional conformation via lipid transport activity in *Y. lipolytica*.

1. Introduction

A dimorphic yeast *Yarrowia lipolytica*, which is referred to as an *n*-alkane-assimilating yeast or an oleaginous yeast, can utilize *n*-alkane as the sole carbon source [1–4]. In the *n*-alkane assimilation pathway of *Y. lipolytica*, *n*-alkanes are transported to the endoplasmic reticulum (ER) and hydroxylated to fatty alcohols by the cytochrome P450 (P450 or CYP), belonging to the CYP52 family [5–8]. Subsequently, fatty alcohols are oxidized to fatty aldehydes by fatty alcohol dehydrogenase or fatty alcohol oxidase. These fatty aldehydes are then oxidized to fatty acids by fatty aldehyde dehydrogenase in the ER and/or the peroxisome [7,9]. The fatty acids are converted to acyl-CoA in the cytosol and/or the peroxisome [10], which is used to synthesize lipids or is metabolized through β-oxidation in the peroxisome [3,4,11–13].

In *Y. lipolytica*, the CYP52-family P450s are encoded by twelve *ALK* genes (*ALK1–ALK12*). *ALK1*, *ALK2*, and *ALK6* have important roles in the process of growth on *n*-alkanes [5,6,8,14]. Eukaryotic microsomal P450s are membrane-bound enzymes, which are localized in the ER membrane. Consistent with the general features of microsomal P450s, Alk proteins are predicted to possess single trans-membrane domains at their N-termini [8]. The study using mammalian microsomal P450s demonstrated that anionic phospholipids, such as phosphatidic acid (PA), phosphatidylserine (PS), and phosphatidylinositol (PI), increase the P450 insertion ratio into membranes and the P450 activities [15]. Therefore, the lipid composition of the ER membrane could play an important role in the functions of the Alk proteins.

Oxysterol-binding protein (OSBP) and OSBP-related proteins (ORPs) comprise a family of lipid transfer proteins (LTPs) widely conserved in eukaryotes [16]. A common structural feature of ORPs is the presence of OSBP-related domains, which are involved in binding to lipid ligands. ORPs exhibit distinct specificities to various lipid ligands, including sterols, oxysterol, phosphatidylinositol 4-phosphate (PI4P), and PS. In the genome of the yeast *Saccharomyces cerevisiae*, seven genes, *OSH1–OSH7*, encoding OSBP homologs are present. Among the Osh proteins, it has been reported that Osh6 and Osh7 transport PS synthesized by PS synthase Pss1 in the ER to the plasma membrane (PM) and PI4P synthesized by PI-4-kinase Stt4 in the PM to the ER at the ER–PM contact site [17,18]. In *Y. lipolytica* genome, there are four genes that encode orthologs

* Corresponding author.
E-mail address: afukuda@mail.ecc.u-tokyo.ac.jp (R. Fukuda).

https://doi.org/10.1016/j.bbrc.2018.04.002
0006-291X/

of *S. cerevisiae* Osh proteins. In this study, we characterized these four genes in terms of *n*-alkane assimilation. Our results suggest that *YALI0A02354g*, which we named as *OSH6*, plays an important role in the production of functional Alk proteins in *Y. lipolytica*.

2. Materials and methods

2.1. Yeast strains and growth conditions

Y. lipolytica strain CXAU1 (*MATA ura3 ade1*) and CXAU/A1 (*MATA ura3 ade1*::*ADE1*), derived from CX161-1B (ATCC32338, *ade1*), were used as wild-type strains [5,19]. To delete *OSH* genes, *ADE1*-carrying fragments that were obtained by digestion of pBOSH1PT-ADE1 with EcoRV and SacII, and pBOSH3PT-ADE1, pBOSH4PT-ADE1, and pBOSH6PT-ADE1 with EcoRV and XbaI were used as deletion cassettes.

An appropriate carbon source was added to YNB [0.17% yeast nitrogen base without amino acids and ammonium sulfate (Difco), 0.5% ammonium sulfate] as follows: 2% (w/v) glucose (SD medium); 2% (v/v) *n*-dodecane; 2% (w/v) glucose plus 2% (v/v) *n*-dodecane; 0.1% (v/v) 1-dodecanol; 0.1% (v/v) dodecanal; 0.1% (w/v) dodecanoic acid. Uracil (24 mg l^{-1}) was added, if necessary. For solid media, *n*-alkanes (C10; *n*-decane, C12; *n*-dodecane, C14; *n*-tetradecane, C16; *n*-hexadecane and C18; *n*-octadecane) were supplied in the vapor phase to YNB solid media or SD solid media as described previously [20]. 1-Dodecanol, dodecanal, or dodecanoic acid was added to medium with 0.5% (v/v) Triton X-100. Yeast cells were grown at 30 °C.

2.2. Plasmids

Plasmids used in this study are shown in Table 1 and sequences of used primers are listed in Table 2.

The deletion cassettes for *OSH* genes were constructed as follows: The 5′- and 3′-adjacent regions of *OSH* genes were amplified from CXAU1 total DNA by PCR using primers OSH1P-F, OSH1P-R, OSH1T-F, and OSH1T-R for *OSH1*; OSH3P-F, OSH3P-R, OSH3T-F, and OSH3T-R for *OSH3*; OSH4P-F, OSH4P-R, OSH4T-F, and OSH4T-R for *OSH4*; and OSH6P-F, OSH6P-R, OSH6T-F, and OSH6T-R for *OSH6*. Amplified fragments were cloned into pBluescript II SK (+) to obtain pBOSH1PT, pBOSH3PT, pBOSH4PT, and pBOSH6PT, respectively. The plasmids were digested with BamHI and ligated with the *ADE1*-carrying BamHI fragment of pSAT4 to obtain pBOSH1PT-ADE1, pBOSH3PT-ADE1, pBOSH4PT-ADE1, and pBOSH6PT-ADE1, respectively.

The coding region of *OSH6* with the 5′- and 3′-adjacent regions was amplified from CXAU1 total DNA using primers OSH6P-F and OSH6T-R2. The amplified fragment was cloned into pSUT5 to obtain pSOSH6. Plasmid to express Osh6^{L75D}p, Osh6$^{H163A/H164A}$p, or Osh6^{K358E}p under the control of native *OSH6* promoter was constructed as follows: The fragment containing the 5′-adjacent region and a part of ORF was amplified from CXAU1 total DNA using primers OSH6P-F and OSH6(L75D)-R, OSH6(H163A/H164A)-R, or OSH6(K358E)-R, respectively. The fragment containing the 3′-adjacent region and a part of ORF was amplified from CXAU1 total DNA using primers OSH6T-R2 and OSH6(L75D)-F, OSH6(H163A/H164A)-F, or OSH6(K358E)-F, respectively. The coding region of *OSH6* with the 5′- and 3′-adjacent regions was amplified using the two DNA fragments and primers OSH6P-F and OSH6T-R2 by fusion PCR [21]. The amplified fragment was cloned into pSUT5 to obtain pSOSH6^{L75D}, pSOSH6^{HHAA}, and pSOSH6^{K358E}.

The plasmid, pSALK1-EGFP, to express Alk1p fused with enhanced green fluorescent protein (EGFP) at its C-terminus from *ALK1* promoter was constructed as follows: The 5′-adjacent region with the ORF and the 3′-adjacent region of *ALK1* was amplified from CXAU1 total DNA by PCR using ALK1-EGFP_1 and ALK1-EGFP_2, and ALK1-EGFP_3 and ALK1-EGFP_4, and cloned into pSUT5 to obtain pSALK1pORt. The fragment carrying EGFP ORF obtained by digestion of pBS-EGFP with BamHI and StuI was cloned into the BamHI-StuI site of pSALK1pORt to obtain pSALK1-EGFP.

2.3. Transformation of Y. lipolytica

Y. lipolytica was transformed by electroporation as described previously [5].

2.4. Measurement of reduced CO-difference spectra

Reduced CO-difference spectra were measured as described previously [14].

2.5. β-Galactosidase activity assay

Measurement of β-galactosidase activity was performed as described previously [22].

2.6. Western blot analysis

Protein was separated by 10% SDS-polyacrylamide gel and transferred to a nitrocellulose membrane. Alk1p-EGFP was detected with Living Colors A.v. Monoclonal Antibody (JL-8) (Clontech). Anti-mouse IgG was used as a secondary antibody.

Table 1
Plasmids used in this study.

Plasmid	Descriptions	Reference or source
pBluescript II SK (+)	A cloning vector	Stratagene
pSAT4	A low copy YCp plasmid carrying *ADE1*	[5]
pSUT5	A low copy YCp plasmid carrying *URA3*	[28]
pSUT1642	pSUT5 carrying *lacZ* under the control of *ALK1* promoter	[29]
pBS-EGFP	A plasmid carrying *EGFP*	[7]
pBOSH1PT-ADE1	A deletion cassette of *OSH1*	This study
pBOSH3PT-ADE1	A deletion cassette of *OSH3*	This study
pBOSH4PT-ADE1	A deletion cassette of *OSH4*	This study
pBOSH6PT-ADE1	A deletion cassette of *OSH6*	This study
pSOSH6	pSUT5 carrying *OSH6*	This study
pSOSH6^{L75D}	pSUT5 carrying a mutated *OSH6*	This study
pSOSH6^{HHAA}	pSUT5 carrying a mutated *OSH6*	This study
pSOSH6^{K358E}	pSUT5 carrying a mutated *OSH6*	This study
pSALK1-EGFP	pSUT5 carrying *ALK1-EGFP*	This study