The Peroxisomal Acyl-CoA Thioesterase Pte1p from Saccharomyces cerevisiae Is Required for Efficient Degradation of Short Straight Chain and Branched Fatty Acids*

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The role of the Saccharomyces cerevisiae peroxisomal acyl-coenzyme A (acyl-CoA) thioesterase (Pte1p) in fatty acid β-oxidation was studied by analyzing the in vitro kinetic activity of the purified protein as well as by measuring the carbon flux through the β-oxidation cycle in vivo using the synthesis of peroxisomal polyhydroxyalkanoate (PHA) from the polymerization of the 3-hydroxyacyl-CoAs as a marker. The amount of PHA synthesized from free fatty acid and 8-methyl-nonanoic acid was equivalent or slightly reduced in the pte1Δ strain compared with wild type. In contrast, a strong reduction in PHA synthesized from heptanoic acid and 8-methyl-nonanoic acid was observed for the pte1Δ strain compared with wild type. The poor catalabilism of 8-methyl-nonanoic acid via β-oxidation in pte1Δ negatively impacted the degradation of 10-cis-heptadecenoic, tridecanoic, undecanoic, or nonanoic acids was equivalent or slightly reduced in the pte1Δ strain compared with wild type. In contrast, a strong reduction in PHA synthesized from heptanoic acid and 8-methyl-nonanoic acid was observed for the pte1Δ strain compared with wild type. The poor catalabilism of 8-methyl-nonanoic acid via β-oxidation in pte1Δ negatively impacted the degradation of 10-cis-heptadecenoic acid and reduced the ability of the cells to efficiently grow in medium containing such fatty acids. An increase in the proportion of short chain 3-hydroxyacid monomers was observed in PHA synthesized in pte1Δ cells grown on a variety of fatty acids, indicating a reduction in the metabolism of short chain acyl-CoAs in these cells. A purified histidine-tagged Pte1p showed high activity toward short and medium chain length acyl-CoAs, including butyryl-CoA, decanoyl-CoA and 8-methyl-nonanoyl-CoA. The kinetic parameters measured for the purified Pte1p fit well with the implication of this enzyme in the efficient metabolism of short straight and branched fatty acyl-CoAs by the β-oxidation cycle.

Degradation of fatty acids is mediated by the β-oxidation cycle, which is located exclusively in the peroxisome in Saccharomyces cerevisiae. Numerous enzymes involved in the degradation of both saturated and unsaturated fatty acids have been identified and characterized. These include the three core enzymes of the β-oxidation cycle, namely the acyl-CoA oxidase Pox1p, the multifunctional enzyme Fox2p, harboring both an enoyl-CoA hydratase II activity and an (R)-3-hydroxyacyl-CoA dehydrogenase activity, and the 3-ketothiolase Fox3p. Furthermore, the roles of the auxiliary enzymes enoyl-CoA isomerase Eci1p, dienoyl-CoA isomerase Dci1p, and 2,4-dienoyl-CoA reductase Sps19p in the degradation of unsaturated fatty acids have also been defined (1).

YJR019C (PTE1, TES1) has been identified in the S. cerevisiae genome as a gene encoding an acyl-CoA thioesterase (2, 3). Acyl-CoA thioesterases are a group of enzymes catalyzing the hydrolysis of acyl-CoAs to fatty acids and unesterified CoA (CoASH). Two types of acyl-CoA thioesterases have been found in Escherichia coli (4). The acyl-CoA thioesterase II (TesB) of E. coli is a tetramer with a molecular mass of 120 kDa and cleaves C6–C18 acyl-CoA esters as well as 3-hydroxyacyl-CoA esters (5). TesB-like acyl-CoA thioesterases have also been found in eukaryotes, including yeast, mammals, and plants (2, 3, 6, 7). The S. cerevisiae Pte1p is a TesB-like acyl-CoA thioesterase and contains the Asp, Ser/Thr, and Gln catalytic triad expected for thioesters. The protein contains a peroxisomal targeting signal type I at the carboxy-terminal end and was shown to be located in the peroxisome (2, 3). Amino acid sequences of acyl-CoA thioesterases belonging to the TesB family show good conservation among the animal, plant, fungi, and bacterial kingdoms (7). The existence of various acyl-CoA thioesterases having activities toward either a narrow or broad range of substrates could provide important control points in the oxidation of many peroxisomal substrates as well as regulate intraperoxisomal levels of CoA esters and CoASH.

Although it seems likely that the yeast Pte1p would be implicated in some aspect of fatty acid β-oxidation, its physiological function and enzymatic properties have not yet been clearly defined. Although one study has shown a reduction in the capacity of the pte1Δ mutant to grow in media containing oleic acid as the main carbon source, a separate study does not reveal any significant differences in phenotype between wild type and pte1Δ mutant (2, 3). In this work, we have studied the role of the Pte1p protein in peroxisomal β-oxidation by analyzing the kinetic activity of the purified protein in vitro, as well as by measuring the carbon flux through the β-oxidation cycle in vivo using the synthesis of peroxisomal polyhydroxyalkanoate (PHA) from the polymerization of the 3-hydroxyacyl-CoAs as a marker.

**EXPERIMENTAL PROCEDURES**

**Strains, Plasmids, and Culture Conditions**—The wild type S. cerevisiae strain BY4742 (mata his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0) and the isogenic mutant pte1Δ (pte1Δ::kan) were obtained from EUROSCARF.

The plasmid Yiplac128-PHA containing the gene for the PHAC1 synthase from Pseudomonas aeruginosa, modified at the carboxy end by the addition of a peroxisomal targeting sequence and placed under

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the control of the catalase A (CTA1) promoter and terminator, has been previously described (8). The plasmid Yiplac128-PHA was transformed into the wild type BY4742 as well as the isogenic pte1Δ by the lithium acetate procedure (9).

*S. cerevisiae* strains harboring the PHA synthase gene were maintained in leucine-deficient medium (0.67% yeast nitrogen base without amino acids (Difco), 0.5% ammonium sulfate, 2% glucose, and 0.69 g/liter of the appropriate amino acid drop-out supplement (Q-Biogen, Basel, Switzerland). For experiments analyzing PHA synthesis in cells growing on medium containing fatty acids, a stationary phase culture was harvested by centrifugation, the cells were washed once in water and resuspended at a 1:10 dilution in fresh medium containing 1% (w/v) glucose, 2% Pluronic-127 (w/v) (Sigma), and between 0.01 to 1% (w/v) of various fatty acids. The cells were grown for an additional 4 days before harvesting them for PHA analysis. The 10-cis-heptadecenoic, tridecanoic, undecanoic, nonanoic, and heptanoic fatty acids were purchased from Nu-Check-Prep (Elysian, MN), and 9-methyl-undecanoic acid and 10-methyl-undecanoic acid were purchased from Larodan Fine Chemicals (Malmö, Sweden), whereas 8-methyl-nonanoic acid was purchased from Sigma.

For experiments aimed at analyzing the growth of yeast in medium containing fatty acids, BY4742 and the isogenic pte1Δ were first grown in YPD (1% yeast extract, 2% bacto-peptone, and 2% dextrose) until the exponential phase. The cells were then washed once in water before harvesting them for PHA analysis. The 10-cis-heptadecenoic, tridecanoic, undecanoic, nonanoic, and heptanoic fatty acids were purchased from Nu-Check-Prep (Elysian, MN), and 9-methyl-undecanoic acid and 10-methyl-undecanoic acid were purchased from Larodan Fine Chemicals (Malmö, Sweden), whereas 8-methyl-nonanoic acid was purchased from Sigma.

**Results**

Analysis of PHA—PHA was analyzed as previously described (8). Briefly, cells were harvested by centrifugation, washed with water, and lyophilized in a glass tube. The dried material was washed with warm methanol (65 °C) to remove free fatty acids, lipids, and acyl-CoAs, and the remaining material, including PHA, was trans-esterified at 94 °C for 4 h with 1 ml of methanol/chloroform mixture (1:1) containing 3% sulfuric acid. One ml of 0.9% NaCl was added to the cooled mixture, vortexed vigorously, and centrifuged at 5000 × g for 5 min. The chloroform phase containing the methyl esters of 3-hydroxy-acids was then analyzed and quantified by gas chromatography/mass spectrometry using a Hewlett-Packard 5890 gas chromatograph (HP-SMS column) coupled to a Hewlett-Packard 5972 mass spectrometer.

**Plasmid Construction, Expression, and Purification of Recombinant Acyl-CoA Thioesterase**—The YJR019C open reading frame (EMBL/DDBJ/GenBank™ accession number NC_001142) was amplified from genomic DNA of the BY4742 strain using Pfu1 DNA polymerase (Stratagene) and oligonucleotides to add 12- and 15-base single-stranded cohesive ends at the 5’ and 3’ ends of YJR019c, respectively. These cohesive ends were created by T4 DNA polymerase treatment of the PCR product in the presence of deoxyguanosine 5’-triphosphate, and the resulting fragment was cloned into the expression plasmid pET30 Xa/LIC (Novagen, Madison, WI). The resulting clone, designated pET30-YJR019c, created a Pte1 protein that was fused at the amino terminus to 6 histidine residues (Pte1p-His6). A control plasmid encoding only a histidine tag was also created, named pET30-His.

pET30-YJR019c and pET30-His were first sequenced to ensure the absence of mutations and then introduced into E. coli BL21(DE3)pLysS (Novagen). For polypeptide expression, cells were first cultivated at 37 °C until an A600 of 0.4, isopropyl β-D-thiogalactopyranoside was then added at a concentration of 0.5 mM, and cells were grown overnight at 25 °C. Polypeptides were extracted from cells immediately after the

overnight cultivation with the B-PER bacterial protein extraction reagent (Pierce). The extract was loaded onto a column of His-bind resin (Novagen) equilibrated with 50 mM NiSO4. Polypeptides were purified with a standard protocol of His-bind buffer kit (Novagen) by gravity flow.

**Acyl-CoA Thioesterase Enzyme Assay**—Acyl-CoA thioesterase activity was measured spectrophotometrically using 5,5’-dithio-bis-(2-nitrobenzoic acid) (7). Briefly, 1 µg of PTE1p-His6 was added to a 1-ml assay mixture composed of potassium phosphate buffer, pH 8.0, 5,5’-dithio-bis(2-nitrobenzoic acid), acyl-CoA, and bovine serum albumin (BSA) if necessary, and the change in A412 was measured for 1 min. The effect of CoASH on the enzyme activity was measured spectrophotometrically at 232 nm using octanoyl-CoA. Acetyl-CoA and butyryl-CoA were purchased from Sigma. Other acyl-CoAs were synthesized and purified from free acid and CoA hydrate trilithium salt according to the method of Kurosawa et al. (10). The purity of acyl-CoAs, as evaluated by HPLC, was 88% for octanoyl-CoA, 89% for lauroyl-CoA, 93% for oleyl-CoA, 91% for nonanoyl-CoA, 83% for decanoyl-CoA, and 90% for 8-methyl-nonanoyl-CoA.

Kinetic curves were fitted to the Hill equation (11), as follows: $v = V_{max} \cdot \frac{[S]^h}{[S]^h + [S]^h}$, where $v$ is the velocity, $V_{max}$ is the maximum velocity, $K_m$ is the Michaelis constant, and $h$ is the Hill coefficient. The Hill coefficient and the Michaelis constant were determined from data plot to the following: $\log(v/V_{max}(1 - v/V_{max})) = h \cdot \log[K_m] - h \cdot \log[V_{max}]$, which was determined so that the coefficient of determination ($R^2$) in the plot became closest to 1.

**Analytical Procedures**—Proteins were separated by SDS-PAGE and stained with Coomasie Brilliant Blue G-250. Gel filtration chromatography was performed with an HPLC system equipped with a TSK gel G3000SW column (7.5 × 300 mm; Tosoh, Inc., Tokyo, Japan). Protein samples were eluted with 20 mM Tris-HCl buffer, pH 7.5, including 0.5 mM NaCl at 1.0 ml min⁻¹. Acyl-CoAs were analyzed at A260 with an HPLC system equipped with a TSK gel ODS-80TM column (4.6 × 250 mm, 5-µm-diameter particle, Tosoh, Inc.). Elution was performed with an acetonitrile gradient of 10–90% (v/v) in 20 mM potassium phosphate buffer, pH 5.4, at 1.0 ml min⁻¹.

**RESULTS**

Analysis of Carbon Flux through β-Oxidation in pte1Δ Mutant—The quantity and monomer composition of PHA synthesized from intermediates of the β-oxidation cycle was first measured in the wild type and pte1Δ background for *S. cerevisiae* cells grown in medium containing either 0.1% 10-cis-heptadecenoic acid or 0.1% tridecanoic acid. The amount of PHA synthesized in the pte1Δ strain represented 57 and 62% of the amount produced in wild type for cells in medium with 10-cis-heptadecenoic acid and tridecanoic acid, respectively (Fig. 1, A and B). Furthermore, a significant increase in the proportion of the H5:0 monomer (the 3-hydroxy acid monomers found in PHA are defined with the prefix H followed by the number of carbon and the number of unsaturated bonds; see Table 1) was evident in the pte1Δ strain. Thus, for PHA produced from 10-cis-heptadecenoic acid, the H5:0 monomer increased from 19 ± 1 mol % in wild type to 26.3 ± 0.8 mol % in pte1Δ, whereas for PHA produced from tridecanoic acid, the H5 monomer increased from 9 ± 1 mol % in wild type to 15.7 ± 0.7 mol % in pte1Δ (Student’s t test, p < 0.0001). In contrast to the relatively modest decrease in PHA quantity produced in the mutant strain grown in the presence of 10-cis-heptadecenoic acid or tridecanoic acid, a drastic difference was observed for cells grown in medium containing 8-methyl-nonanoic acid. Wild type cells grown in medium containing 0.1% 8-methyl-nonanoic acid produced 10 × 10⁻¹⁵ g of PHA/g of cell dry.
weight, with the polymer containing only two monomers, namely 3-hydroxy,8-methyl-nonanoic acid (8M-H9:0) and 3-hydroxy,6-methyl-heptanoic acid (6M-H7:0). In contrast, pte1Δ cells grown in the same medium produced PHA near the detection limit at 0.02 × 10⁻⁶ g of PHA/g of cell dry weight (Fig. 1C). A large decrease in PHA synthesized from the β-oxidation of 8-methyl-nonanoic acid was also observed when the quantity of fatty acid in the medium was decreased to 0.01% (Fig. 1D). Thus, under these conditions, wild type produced 11 × 10⁻⁶ g of PHA/g of cell dry weight, whereas pte1Δ produced only 0.3 × 10⁻⁶ g of PHA/g of cell dry weight. The proportion of the 6M-H7:0 monomer in cells grown on 0.01% 10-methyl-undecanoic acid increased from 30 ± 0.3 mol % in wild type to 41 ± 4 mol % in pte1Δ strain (Student’s t test, p < 0.0005).

To determine whether the decrease in the catabolism of 8-methyl-nonanoic acid observed in the pte1Δ strain could negatively influence the catabolism of other fatty acids in the same cells, wild type and pte1Δ cells were grown in medium containing either only 0.05% 10-cis-heptadecenoic acid, only 0.01% 8-methyl-nonanoic acid, or the combination of both 0.05% 10-cis-heptadecenoic acid and 0.01% 8-methyl-nonanoic acid (Fig. 4). For all treatments, the amount of PHA synthesized from the degradation of each external fatty acid was determined separately. Although PHA synthesized from the degradation of 10-cis-heptadecenoic acid in the pte1Δ strain reached 66% of the wild type level for cells grown in medium containing only 10-cis-heptadecenoic acid, PHA synthesized from the catabolism of the same fatty acid in pte1Δ decreased to 19% when 8-methyl-nonanoic acid was added to the medium. Conversely, although PHA synthesized from the degradation of 8-methyl-nonanoic acid in the pte1Δ strain reached only 3% of wild type level for cells grown in medium containing only the branched chain fatty acid, PHA synthesized from the catabolism of the same fatty acid in pte1Δ reached 15% when 10-cis-heptadecanoic acid was added to the medium.

To assess whether the reduction in the catabolism of 10-cis-heptadecenoic acid observed in the pte1Δ strain by the addition of 8-methyl-nonanoic acid in the medium had an impact on the ability of the cells to grow using fatty acids as the external carbon source, wild type and pte1Δ cells that did not contain the peroxisomal PHA synthase were first grown to an exponential phase in medium containing glucose, 3.5 × 10⁵ cells/ml were then inoculated to medium containing 0.05% 10-cis-heptadecanoic acid alone or in combination with 0.01 or 0.02% 8-methyl-nonanoic acid, and the cells were counted after 4 days (Fig. 5). Although pte1Δ cells reached a density equivalent to 75% of wild type in medium containing only 10-cis-heptadecanoic acid, this level decreased to 40

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**TABLE 1**

**Names and abbreviations of PHA monomers**

| Monomer                                      | Abbreviation |
|----------------------------------------------|--------------|
| 3-Hydroxy,6-cis-tridecanoic acid             | H13:1        |
| 3-Hydroxy-tridecanoic acid                   | H13:0        |
| 3-Hydroxy-undecanoic acid                    | H11:0        |
| 3-Hydroxy-nonanoic acid                      | H9:0         |
| 3-Hydroxy-heptanoic acid                     | H7:0         |
| 3-Hydroxy-pentanoic acid                     | H5:0         |
| 3-Hydroxy,10-methyl-undecanoic acid          | 10M-H11:0    |
| 3-Hydroxy,8-methyl-nonanoic acid             | 8M-H9:0      |
| 3-Hydroxy,6-methyl-heptanoic acid            | 6M-H7:0      |
| 3-Hydroxy,9-methyl-undecanoic acid           | 9M-H11:0     |
| 3-Hydroxy,7-methyl-nonanoic acid             | 7M-H9:0      |
| 3-Hydroxy,5-methyl-heptanoic acid            | 5M-H7:0      |

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PHA was also analyzed for cells grown in medium containing 9-methyl-undecanoic acid and 10-methyl-undecanoic acid, both at a 0.025% concentration (Fig. 3). Deletion of Pte1 resulted in a significant decrease in PHA synthesized from the β-oxidation of the branched chain fatty acid, tested with the PHA amount in pte1Δ being 34 and 59% of wild type level for cells grown on 9-methyl-undecanoic acid and 10-methyl-undecanoic acid, respectively. There was also a large increase in the proportion of the smallest monomer observed for PHA produced from either branched chain fatty acids in the pte1Δ mutant. Thus, for PHA synthesized from 9-methyl-undecanoic acid, the 5M-H7:0 monomer increased from 8.7 ± 0.5 mol % in wild type to 24.5 ± 0.4 mol % in pte1Δ (Student’s t test, p < 0.00001). Similarly, for PHA synthesized from 10-methyl-undecanoic acid, the 6M-H7:0 monomer increased from 25.5 ± 0.5 mol % in wild type to 49.1 ± 0.5 mol % in pte1Δ (Student’s t test, p < 0.0001).

To examine whether the poor catabolism of 8-methyl-nonanoic acid through the β-oxidation observed in the pte1Δ strain could negatively influence the catabolism of other fatty acids in the same cells, wild type and pte1Δ cells were grown in medium containing either only 0.05% 10-cis-heptadecanoic acid, only 0.01% 8-methyl-nonanoic acid, or the combination of both 0.05% 10-cis-heptadecanoic acid and 0.01% 8-methyl-nonanoic acid (Fig. 4). All treatments, the amount of PHA synthesized from the degradation of each external fatty acid was determined separately. Although PHA synthesized from the degradation of 10-cis-heptadecanoic acid in the pte1Δ strain reached 66% of the wild type level for cells grown in medium containing only 10-cis-heptadecanoic acid, PHA synthesized from the catabolism of the same fatty acid in pte1Δ decreased to 19% when 8-methyl-nonanoic acid was added to the medium. Conversely, although PHA synthesized from the degradation of 8-methyl-nonanoic acid in the pte1Δ strain reached only 3% of wild type level for cells grown in medium containing only the branched chain fatty acid, PHA synthesized from the catabolism of the same fatty acid in pte1Δ reached 15% when 10-cis-heptadecanoic acid was added to the medium.
PAGE and gel filtration chromatography. A predominant protein migrating at 43.4 kDa was found by SDS-PAGE, whereas the molecular mass of PTE1p-His6 polypeptide was predicted to be 45.3 kDa (Fig. 6A).

Therefore, this nickel affinity protein fraction was used for Pte1p kinetic analysis. In gel filtration chromatography, several peaks emerged on the chromatogram of the PTE1p-His6 fraction (Fig. 6B). From comparison of the retention time of peak 2 with those of molecular mass marker proteins, it was found that the peak 2 fraction contained a protein with the molecular mass of 45 kDa, which corresponded to the putative molecular mass of PTE1p-His6 polypeptide (Fig. 6A). PAGE and gel filtration chromatography. A predominant protein migrating at 43.4 kDa was found by SDS-PAGE, whereas the molecular mass of PTE1p-His6 polypeptide was predicted to be 45.3 kDa (Fig. 6A).

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Enzymatic Activity of Purified Pte1p—A recombinant protein fraction purified by nickel affinity chromatography was analyzed with SDS-PAGE and gel filtration chromatography. A predominant protein migrating at 43.4 kDa was found by SDS-PAGE, whereas the molecular mass of PTE1p-His6 polypeptide was predicted to be 45.3 kDa (Fig. 6A).

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activity with decanoyl-, lauroyl-, or oleoyl-CoA was enhanced with increasing BSA concentrations and reached a plateau at a fatty acid to BSA molar ratio of 50, 4.7, and 2.2, respectively (data not shown). Thioesterase activity for acyl-CoA ranging from acetyl- to nonanoyl-CoA and 8-methyl-nonanoyl-CoA was thus measured without BSA, whereas activity for decanoyl-, lauroyl-, and oleoyl-CoA was measured at a fatty acid to BSA ratio of 50, 4.7, and 2.2, respectively (Fig. 8). The velocity curves described by the Hill equation (see "Experimental Procedures") after substitution of the kinetic parameters fit well to the data plots (Fig. 8). Table 2 provides the calculated $K_m$ and $V_{max}$ values for the purified Pte1p. The highest specific activity was observed for butyryl-CoA, whereas the lowest specific activities were obtained with oleoyl-CoA. CoASH was found to inhibit thioesterase activity with an IC$_{50}$ of $\approx 30 \mu M$ (Fig. 7B).

**DISCUSSION**

Peroxisomal acyl-CoA thioesterase has been identified in mouse, human, plant, and fungi (2, 3, 6, 7). Numerous roles have been postulated for this enzyme in the metabolism of lipids, including the release of free fatty acids from acyl-CoA intermediates of the $\beta$-oxidation cycle followed by export of the fatty acids out of the peroxisome for either excretion into the urine or re-entry into the mitochondria for further $\beta$-oxidation, prevention of the accumulation of poorly metabolized CoA esters to promote efficient flux through $\beta$-oxidation, or catabolism of cholesterol via hydrolysis of bile acid-CoA (12). However, direct experimental evidence of the in vivo importance of the peroxisomal acyl-CoA thioesterase in these proposed activities has been generally lacking. In *S. cerevisiae*, although one study shows that deletion of the Pte1 gene leads to a small but significant decrease in the ability of cells to grow on oleic acid as the main carbon source, no obvious effects have been observed in a separate study with a similar mutant (2, 3). Overexpression of the human peroxisomal acyl-CoA thioesterase III (hACTEIII/PTE-1) in human and murine T-cell lines or in thymocytes of transgenic mouse results in an increase in peroxisome number (13).

In the present study, the role of the *S. cerevisiae* peroxisomal acyl-CoA thioesterase Pte1p in the metabolism of fatty acids via the $\beta$-oxidation cycle was analyzed using the synthesis of PHA via the polymerization of the $\beta$-oxidation intermediate 3-hydroxyacyl-CoA as a marker for the state of carbon flux through this pathway. Previous studies using PHA synthesis in *S. cerevisiae* peroxisomes have been used to reveal the existence of a futile cycle of intermediates of the fatty acid biosynthetic pathway toward the $\beta$-oxidation cycle, to monitor the substrate specificity of mutants of the multifunctional enzyme Fox2p, as well as to study the involvement of auxiliary enzymes in the degradation of transunsaturated and conjugated fatty acids (14–17).

PHA synthesized from the $\beta$-oxidation of fatty acids present in the medium was found to be slightly reduced (<2-fold) in *S. cerevisiae pte1Δ* mutant compared with wild type when cells were grown in medium containing 0.1% 10-cis-heptadecenoic acid or 0.1% tridecanoic acid. For tridecanoic acid, the difference in PHA amount between wild type and *pte1Δ* was abolished when the concentration of the fatty acid in the medium was decreased to 0.025%. No differences in PHA amount were also observed for wild type and *pte1Δ* cells grown in medium containing 0.025% undecanoic acid, whereas only 1.4-fold less PHA was observed.
normal level of carbon flux through the acyl-CoA thioesterase is not essential to maintain a normal or near acid. These data indicate that, although the presence of the peroxisomal with wild type for cells grown in medium containing 0.025% heptanoic acid. In contrast, 11-fold less PHA was observed for
8-methyl-nonanoic acid concentrations were 0.01 and 0.1%, respectively. In contrast, the reduction in PHA synthesized in
with wild type was 3-fold in the
It is possible that a CoA-ester derived from 8-methyl-nonanoic and CoASH is essential for the 3-ketoacyl-CoA thiolase step. Alternatively, a high level of such an acyl-CoA could potentially be inhibitory to an enzyme of the β-oxidation cycle (in this example, the acyl-CoA oxidase). Note that the equilibrium between free and CoA esterified fatty acids could occur also at the level of enoyl-CoA, 3-hydroxyacyl-CoA, or 3-ketoacyl-CoA. ACL, acyl-CoA ligase; PTE1, acyl-CoA thioesterase; AOX, acyl-CoA oxidase; MFE, multifunctional enzyme; KAT, 3-ketoacyl-CoA thiolase.

for pte1Δ compared with wild type for cells grown on 0.025% nonanoic acid. In contrast, 11-fold less PHA was observed for pte1Δ compared with wild type for cells grown in medium containing 0.025% heptanoic acid. These data indicate that, although the presence of the peroxisomal acyl-CoA thioesterase is not essential to maintain a normal or near normal level of carbon flux through the β-oxidation cycle when the medium or long straight chain fatty acids are metabolized, the same enzyme is important for efficient degradation of short chain fatty acids.

The effect of the absence of the peroxisomal acyl-CoA thioesterase on the β-oxidation of fatty acids was found to be even more drastic for the metabolism of 8-methyl-nonanoic acid. Thus, PHA synthesis from the β-oxidation of 8-methyl-nonanoic acid was reduced by ~30–500-fold in the pte1Δ mutant compared with wild type when the external 8-methyl-nonanoic acid concentrations were 0.01 and 0.1%, respectively. In contrast, the reduction in PHA synthesized in pte1Δ compared with wild type was 3-fold for 9-methyl-undecanoic acid and 1.5-fold for 10-methyl-undecanoic acid. These results indicate that short branched chain fatty acids are less well metabolized via β-oxidation than their straight chain counterpart in the pte1Δ mutant compared with wild type and that this difference is enhanced for shorter branched chain fatty acids such as 8-methyl-nonanoic acid.

The very low amount of PHA synthesized from 8-methyl-nonanoic and heptanoic acids in the pte1Δ strain compared with wild type highlight the importance of the peroxisomal acyl-CoA thioesterase for the efficient metabolism of these short straight chain or branched chain fatty acids via the β-oxidation cycle. It is possible that one or several of the enzymes of the core β-oxidation cycle poorly metabolize such fatty acids. Accumulation of poorly metabolized CoA-esters would result in a block of the β-oxidation cycle, because adequate concentration of free CoASH is essential for the 3-ketoacyl-CoA thiolase step. Alternatively, it is possible that a CoA-ester derived from 8-methyl-nonanoic and heptanoic acids inhibits one of the enzymes of the β-oxidation cycle. Thus, whereas in the pte1Δ the inhibitory or poorly metabolized acyl-CoAs would accumulate and reduce the flux through the β-oxidation cycle, the presence of the acyl-CoA thioesterase in the wild type would decrease the level of such acyl-CoAs and allow β-oxidation to proceed (Fig. 9). A corollary of this hypothesis is that accumulation of the inhibitory or poorly metabolized acyl-CoA in the pte1Δ mutant grown in medium containing 8-methyl-nonanoic acid would not only block the metabolism of 8-methyl-nonanoic acid but also that of other fatty acid targeted for degradation via β-oxidation in the same cell. This was verified by showing that, although synthesis of PHA from 10-cis-heptade-
cenoic acid in wild type cells was not significantly affected by the addition of 8-methyl-nonalenonic acid in the medium, a 3.5-fold reduction in PHA synthesized from the degradation of 10-cis-heptadecenoic acid in *pte1*Δ was observed when 8-methyl-nonalenonic acid was added to the medium. The reduced catabolism of 10-cis-heptadecenoic acid in *pte1*Δ cells caused by the presence of 8-methyl-nonalenonic acid also impacted the ability of cells to grow in medium containing fatty acids as the main carbon source. This was revealed by the reduction in cell density in *pte1*Δ cells when either 0.01 or 0.02% 8-methyl-nonalenonic acid was added to medium containing 0.05% 10-cis-heptadecenoic acid.

Even for fatty acids that are relatively well metabolized in both wild type and *pte1*Δ, the reduced catabolism of short chain fatty acyl-CoAs in *pte1*Δ is revealed by the increased proportion of the H5:0 monomer in PHA synthesized in the mutant from straight chain fatty acids, as well as the 6M-H7:0 and 5M-H7:0 monomer in PHA synthesized from the catabolism of 10-methyl-undecanoic acid and 9-methyl-undecenoic acid, respectively. A similar increase in the proportion of short chain PHA monomers (H5:0 or H6:0) has been observed in a previous study, where the wild type Fox2p encoding the multifunctional enzyme (MFE-2) was replaced by a mutant variant with an inactivation in the B domain of the dehydrogenase (14). This MFE-2(Δ) mutant showed undetectable dehydrogenase activity toward (R)-3-hydroxybutyryl-CoA, whereas *kcat* values toward (R)-3-hydroxydecanoyl-CoA and (R)-3-hydroxyhexadecanoyl-CoA was reduced by <2-fold (18). It was reasoned that the reduced activity of the MFE-2(Δ) toward short chain (R)-3-hydroxyacyl-CoAs made them more available to the PHA synthase compared with the other intermediates generated by the wild type MFE-2. Thus, it can be also reasoned that increased proportion of thase compared with the other intermediates generated by the wild type *pte1*Δ and *pte1*Δ/H9004 strain reflects an increased concentration of the acyl-CoA and re-establish a pool of free CoASH required for maintaining a flux through the β-oxidation cycle.

Short chain fatty acids and methyl-branched fatty acids are relatively common fatty acids found in nature. Caproic (6:0) and caprylic (8:0) fatty acids are found in both animal and plant fats, such as the milk of cows and goats and the seeds of plants, such as coconut and oil palms. Although, to our knowledge, branched chain fatty acids have not been identified in *S. cerevisiae*, fatty acids with a methyl group in the iso or anteiso position are abundantly found in the membrane lipids of a wide variety of bacteria, including bacteria found in the gut of ruminants, causing the appearance of such fatty acids in the milk and meat of these animals (19, 20). Thus, the requirement of the peroxisomal acyl-CoA thioesterase for the efficient degradation of short straight chain and branched chain fatty acids could have implications as far the fitness of organisms, such as *S. cerevisiae*, which must grow and survive in an environment containing such fatty acids.

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