**IDP3 Encodes a Peroxisomal NADP-dependent Isocitrate Dehydrogenase Required for the β-Oxidation of Unsaturated Fatty Acids**

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In **Saccharomyces cerevisiae** the metabolic degradation of saturated fatty acids is exclusively confined to peroxisomes. In addition to a functional β-oxidation system, the degradation of unsaturated fatty acids requires auxiliary enzymes, including a Δ2,Δ3-enoyl-CoA isomerase and an NADPH-dependent 2,4-dienoyl-CoA reductase. We found both enzymes to be present in yeast peroxisomes. The impermeability of the peroxisomal membrane for pyrimidine nucleotides led to the question of how the NADPH needed by the reductase is regenerated in the peroxisomal lumen. We report the identification and functional analysis of the IDP3 gene product, which is a yeast peroxisomal NADP-dependent isocitrate dehydrogenase. The newly identified peroxisomal protein is homologous to the mitochondrial Idp1p and cytosolic Idp2p, which both are yeast NADP-dependent isocitrate dehydrogenases. Yeast cells lacking Idp3p grow normally on saturated fatty acids, but growth is impaired on unsaturated fatty acids, indicating that the peroxisomal Idp3p is involved in their metabolic utilization. The data presented are consistent with the assumption that peroxisomes of **S. cerevisiae** contain the enzyme equipment needed for the degradation of unsaturated fatty acids, including an NADP-dependent isocitrate dehydrogenase, a putative constituent of a peroxisomal NADPH-regenerating redox system.

Peroxisomes harbor variable metabolic pathways that differ depending on cell type, developmental stage, and food supply (1, 2). In reference to the multiplicity of cellular functions and to the ability of cells to adjust the enzymatic equipment as well as the size and number of these organelles in response to the cellular demand, peroxisomes are appropriately called multipurpose organelles (3). The importance of peroxisomes for cellular function is especially emphasized by a number of inherited diseases in humans that are caused by peroxisomal dysfunction and usually have profound clinical consequences (4).

A typical metabolic pathway of peroxisomes is the β-oxidation of fatty acids (5, 6). In fact, whereas the presence of a mitochondrial β-oxidation system is restricted to mammalian cells and a few protists (7), the fatty acid oxidation in peroxisomes is nearly ubiquitous among eukaryotic cells (7, 8). The peroxisomal and the mitochondrial degradation of fatty acids is performed by functionally comparable but genetically distinct proteins (8, 9). In fungi and plants, the degradation of fatty acids exclusively takes place in peroxisomes, and growth on fatty acids results in proliferation of peroxisomes accompanied by a massive induction of peroxisomal proteins including the β-oxidation enzymes (7, 10).

In addition to the chain shortening β-oxidation system, the oxidation of unsaturated fatty acids requires auxiliary enzymes for the elimination of the double bonds (8). Degradation of unsaturated fatty acids with odd-numbered double bonds requires a Δ2,Δ3-enoyl-CoA isomerase (Fig. 1B) (11). For the degradation of unsaturated fatty acid with even-numbered double bonds, an NADPH-dependent 2,4-dienoyl-CoA reductase is needed in addition to the isomerase (Fig. 1A) (12). Recently, a novel pathway for the degradation of unsaturated fatty acids with double bonds at odd-numbered carbon atoms has been described that also requires the NADPH-dependent reductase described above (Fig. 1C) (13, 14). The successive reduction and isomerization of double bonds by these auxiliary enzymes results in the formation of intermediates that can reenter the β-oxidation spiral (8). The presence of both the Δ2,Δ3-enoyl-CoA isomerase and the NADPH-dependent 2,4-dienoyl-CoA reductase has been demonstrated in all peroxisomes studied so far (8). As the peroxisomal membrane has been suggested to be impermeable for small solutes (15), the requirement of the peroxisomal enoyl-CoA reductase for NADPH raises the question of the existence of an NADPH-regenerating system in peroxisomes.

We applied a reverse genetic approach to identify proteins essential for peroxisome function in **Saccharomyces cerevisiae**. Here we report the identification, characterization, and functional analysis of a peroxisomal NADP-dependent isocitrate dehydrogenase. Deficiency in this enzyme resulted in an impaired growth of **S. cerevisiae** on unsaturated fatty acids, whereas growth on saturated fatty acids was not affected. The peroxisomal Idp3p is suggested to be involved in the regeneration of the NADPH needed for the peroxisomal degradation of unsaturated fatty acids.

**Experimental Procedures**

**Strains, Growth Conditions, and General Methods**—The yeast strains used in this study were **S. cerevisiae** wild-type UTL-7A (MATa, ura3–52, trp1, leu2–3,112, pex7Δ (MATa, pex7::LEU2, ura3–52, trp1)) (16), idp3Δ (MATa, ura3–52, trp, leu2–3,112, idp3::kanMX4) (this paper), idp1ΔΔ (MATa, leu2–3,112, his3–1, trp1–289, idp1::URA3) (17), idp1, idp3Δ (MATa, leu2–3,112, his3–1, trp1–289, idp1::URA3, idp3::kanMX4) (this paper), idp1/idp2Δ (MATa, leu2–3,112, his3–1, trp1–289, idp1::URA3, idp3::kanMX4) (this paper), and idp1/idp2/idp3Δ (MATa, leu2–3,112, his3–1, trp1–289, idp1::URA3, idp2::URA3, idp3::kanMX4) (this paper).

Yeast strains were grown at 30 °C in YPD or in minimal medium.
NADP-dependent 2,4-dienoyl reductase is also needed for the degradation of fatty acids with odd-numbered double bonds. 

Pathways of the peroxisomal degradation of unsaturated fatty acids. In addition to the β-oxidation spiral, auxiliary enzymes are required to degrade unsaturated fatty acids. A, fatty acids with double bonds at even-numbered carbon atoms require an NADP-dependent 2,4-dienoyl reductase and Δ2,3-enoxy-CoA isomerase (8). B, degradation of fatty acids with double bonds extending from odd-numbered carbon atoms at least requires the activity of the Δ2,3-enoxy-CoA isomerase (11). C, in the alternative pathway discovered by Smeland et al. (13), the NADP-dependent 2,4-dienoyl reductase is also needed for the degradation of fatty acids with odd-numbered double bonds.

**Fig. 1.** Pathways of the peroxisomal degradation of unsaturated fatty acids. In addition to the β-oxidation spiral, auxiliary enzymes are required to degrade unsaturated fatty acids. A, fatty acids with double bonds at even-numbered carbon atoms require an NADP-dependent 2,4-dienoyl reductase and Δ2,3-enoxy-CoA isomerase (8). B, degradation of fatty acids with double bonds extending from odd-numbered carbon atoms at least requires the activity of the Δ2,3-enoxy-CoA isomerase (11). C, in the alternative pathway discovered by Smeland et al. (13), the NADP-dependent 2,4-dienoyl reductase is also needed for the degradation of fatty acids with odd-numbered double bonds.
Antibodies and Immunoblots—For generating antibodies against Idp3p, the IDP3-fragment IDP3* (144–210 amino acids) was amplified by PCR using GenBank™ plasmid YEp13-IDP3 and oligonucleotides KU188 (5'-CCGGAATTCGGCGATCTCAAGATTAAAAAGA-3') and KU189 (5'-TGCCTCTAGACCTAGCACTGCTGGATGAAAGATTACCGTAG-3'). The PCR product was digested with EcoRI and XhoI and inserted into pBluescript SK(+) (Stratagene, La Jolla, CA) to create pSKIDP3*. The BamHI/HindIII fragment of pSKIDP3* was subcloned into pET21b (Novagen), leading to plasmid pET-IDP3*. The plasmid was transformed into Escherichia coli BL21-DE3, resulting in an iso-propyl-1-thio-β-D-galactopyranoside-inducible expression of HIS6-tagged Idp3p*.

The tagged Idp3p* was purified by affinity chromatography on a nickel-nitrilotriacetic acid resin (Quiagen, Hilden, Germany) according to the manufacturer's protocol. Rabbit polyclonal antibodies to HIS6-tagged Idp3p* were produced by Eurogentec (Seraing, Belgium) according to standard methods (29).

Anti-thiolase (Fox3p), anti-Kar2p, anti-Pcs60p, anti-Pex14p, and anti-Pex3p antibodies have been described previously (30–34). Anti-rabbit or anti-mouse IgG-coupled horseradish peroxidase (Amersham Corp.) were used as the second antibody, and blots were developed using the ECL system (Amersham). Western blot analyses were performed according to standard protocols (29).

Construction and Expression of Idp3pΔCKL—To construct a Idp3pΔCKL, the IDP3 gene was amplified by PCR using plasmid YEp13-IDP3 (see above) and oligonucleotides KU308 5'-CGCGGATCCTTTAC-3' and KU311 5'-CGCGGATCCTTTACTGCAGCTACTCGAGTGTAAAGAAT-3', resulting in an iso-propyl-1-thio-β-D-galactopyranoside-inducible expression of HIS6-tagged Idp3p. The tagged Idp3p was purified by affinity chromatography on a nickel-nitrilotriacetic acid resin (Quiagen, Hilden, Germany) according to the manufacturer's protocol.

Analytical Procedures—NADP-specific isocitrate dehydrogenase activity was determined as described by Loftus et al. (18). Catalase and fumarase were assayed as described by Moreno de la Garca et al. (38). A3,32-Enoyl-CoA isomerase was assayed spectrophotometrically at 340 nm in a coupled assay (39) with 3-trans-decanoyl-CoA as substrate. 2,4-Dienoyl-CoA reductase activity was determined spectrophotometrically at 340 nm with 2-trans-4-trans-decanoyl-CoA as substrate according to Kunau and Dommes (12). Total protein was measured by the BCA method (Pierce) using bovine serum albumin as a standard.

RESULTS

Isolation and Identification of Idp3p—Peroxisomes were isolated from oleic acid-induced S. cerevisiae cells and successively extracted by low and high salt treatments. The proteins salt-extracted peroxisomes were solubilized with SDS and separated by HPLC and SDS-PAGE (Fig. 2). Lys-C-derived internal fragments of the 45-kDa protein marked in Fig. 2 were subjected to amino-terminal protein sequencing in preparation for DNA cloning and sequencing of the corresponding gene (see “Experimental Procedures”). The open reading frame of the isolated DNA fragment encoded a new protein with a calculated molecular mass of 48 kDa (Fig. 3) that later on also appeared in the yeast genome database as open reading frame YNL009w. A search of the GenBank™ data base with the predicted amino acid sequence of IDP3 revealed the yeast genes IDP1 (17) and IDP2 (18) as close relatives of the newly identified gene, hereafter referred to as IDP3. The overall identity of Idp3p with Idp1p and Idp2p is 68 and 70%, respectively (Fig. 4). Idp1p and Idp2p represent the two NADP-dependent isocitrate dehydrogenases reported for S. cerevisiae to date. Idp3p is localized in the yeast cytosol, and Idp1p is a mitochondrial isoenzyme that differs from the other two proteins by an N-terminal extension, which functions as a mitochondrial targeting signal (Fig. 4) (18, 40). Idp3p lacks the mitochondrial targeting signal and instead is characterized by an additional nine amino acids at the extreme C terminus. These terminal amino acids of Idp3p comprise the tripeptide cysteine-lysine-leucine (CKL; Fig. 3), a putative peroxisomal targeting signal 1 (PTS1) for S. cerevisiae (41, 42). The prominent presence in the HPLC profile of peroxisomal proteins (Fig. 2), the sequence similarity to Idp1p and to Idp2p (Fig. 4), and the presence of the putative peroxisomal targeting signal (PTS1; Fig. 3) suggested that Idp3p might be a peroxisomal NADP-dependent isocitrate dehydrogenase.

IDP3 Is Induced upon Growth on Oleic Acid—Antibodies were generated against an internal fragment of Idp3p comprising amino acids 144–210, which displays the lowest similarity of the protein to Idp1p and Idp2p (Fig. 4). A polyclonal antibody with the predicted molecular mass for Idp3p (48 kDa) was detected in wild-type but not in idp3Δ yeast extracts (Fig. 5A). Although binding of the antibodies to other proteins was observed under low stringency conditions, none of these bands disappeared in

Fig. 2. Preparative chromatographic separation of peroxisomal membrane proteins. High salt-extracted peroxisomal membranes (1 mg of protein) were solubilized in SDS and separated by reverse phase HPLC. Polypeptides of selected fractions were separated by SDS-PAGE and visualized by Coomassie Blue staining. The band of Idp3p is indicated by the arrowhead. The amount per lane corresponded to 5% of the total fraction. Molecular mass standards are indicated on the left.

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mutants lacking either Idp1p or Idp2p, indicating that the antibodies generated do not recognize Idp1p or Idp2p but are specific for Idp3p (Fig. 5A).

In *S. cerevisiae*, growth on oleic acid results in a massive proliferation of peroxisomes accompanied by the induction of peroxisomal enzymes involved in peroxisomal fatty acid degradation (7, 10). The oleic acid induction is mediated by the transcription activator Pip2p, which binds to a well defined oleic acid-responsive element at the promoter of several peroxisomal proteins (43). A perfect consensus sequence for Pip2p binding is also present upstream of the *IDP3* open reading frame (position 231 to 289; Fig. 3), and Idp3p was found to be highly inducible by oleic acid (Fig. 5B), supporting the assumption of Idp3p being involved in peroxisomal fatty acid degradation.

**Fig. 4.** Comparison of the deduced amino acid sequence of *S. cerevisiae* Idp3p with other isocitrate dehydrogenases from *S. cerevisiae*. The mitochondrial Idp1p (17) and the cytosolic Idp2p (18) are both NADP-dependent isocitrate dehydrogenases. The Idh1p (47) and Idh2p (48) are subunits of the heteromeric mitochondrial NAD-dependent isocitrate dehydrogenase. Alignment was performed with the BESTFIT program (EMBL, Heidelberg). Amino acids conserved in Idp3p and at least one of the other proteins are blocked.

**Fig. 5.** Immunological detection of Idp3p and time course of Idp3p induction by oleic acid. A, equal amounts of whole-cell lysates from oleic acid-induced wild-type and indicated single, double, or triple (*idp1*Δ, *idp2*Δ, or *idp3*Δ) mutant cells were subjected to Western blot analysis with rabbit antiserum against Idp3p. The amount loaded corresponds to 0.5% of extracts from 30 mg of cells. B, wild-type cells were precultured in 0.3% SD and subsequently shifted to oleic acid-containing medium. At the indicated time points, whole-cell extracts were prepared for immunological detection of oleic acid-inducible Fox3p (30), constitutively expressed Kar2p (31), and Idp3p. The amounts loaded correspond to 0.3 mg of cells.
was first analyzed by differential centrifugation of cell homogenates from oleic acid-induced wild-type yeast cells. More than 60% of the Idp activity was found in the supernatant fraction, suggesting that the cytosolic isofrom might be responsible for the majority of the endogenous enzyme activity (data not shown). Sedimented organelles were further fractionated by sucrose density gradient centrifugation. NADP-dependent isocitrate dehydrogenase activity was found in both the mitochondrial and the peroxisomal fractions (Fig. 6). The peroxisomal peak, however, comprised a smaller fraction of the total particular enzyme activity. To exclude the possibility that the activity found in the peroxisomal fraction was due to a mitochondrial contamination, the subcellular localization of the enzymes was also analyzed in an idp1Δ mutant strain, lacking the mitochondrial NADP-dependent isocitrate dehydrogenase. After differential centrifugation of cell homogenates of the idp1Δ mutant strain, about 15% of the total NADP-dependent isocitrate dehydrogenase activity was still localized to the organelar fraction. Subsequent sucrose density gradient fractionation confirmed the absence of the mitochondrial enzyme and demonstrated the activity to exclusively co-segregate with peroxisomal marker enzymes (Fig. 6), consistent with the presence of a peroxisomal isoenzyme of the NADP-dependent isocitrate dehydrogenases in S. cerevisiae.

The Peroxisomal NADP-dependent Isocitrate Dehydrogenase Activity Is Performed by Idp3p—As a first step to analyze whether the peroxisomal NADP-dependent isocitrate activity is due to Idp3p, we studied the subcellular localization of the protein. Immunological detection of Idp3p in fractions generated by differential centrifugation of yeast cell homogenates revealed that the protein is exclusively found in the organelar pellet (Fig. 7A). Immunoblot analysis of fractions gained by subsequent sucrose density gradient centrifugation of the organelar pellets demonstrated the protein to be exclusively localized in the peroxisomal fractions (Fig. 7B). In agreement with Idp3p being responsible for the peroxisomal NADP-dependent isocitrate dehydrogenase activity, the absence of this protein in idp3Δ mutant cells correlated with the disappearance of the enzyme activity in the organelar pellet and in the peroxisomal fractions of sucrose density gradients (Fig. 7).

The sequence similarity of Idp3p to the two NADP-dependent isocitrate dehydrogenases suggested that the protein Idp3p itself is an NADP-dependent isocitrate dehydrogenase. However, to confirm that the lack of the peroxisomal NADP-dependent isocitrate dehydrogenase activity upon IDP3 deletion is not caused indirectly, the protein was heterologously expressed in E. coli, and the enzyme properties of isolated Idp3p were analyzed. Transformation of E. coli with a plasmid carrying the IDP3 gene under the control of the bacterial promoter resulted in a massive increase in NADP-dependent isocitrate dehydrogenase activity in bacterial lysates accompanied by the appearance of immunoreactive Idp3p (Fig. 8A). Taking advantage of the C-terminal histidine tag, the protein was purified to apparent homogeneity as judged by SDS-PAGE. The isolated Idp3p showed a specific NADP-dependent isocitrate dehydrogenase activity of 1654 nanokatals/mg. Since expression of Idp3p did result in the concomitant appearance of NADP-dependent isocitrate dehydrogenase activity in bacterial extracts and since the enzyme activity was retained by purified Idp3p, these data confirmed Idp3p to be the yeast peroxisomal NADP-dependent isocitrate dehydrogenase (Fig. 8B).

A set of kinetic properties of the Idp3p enzyme activity were studied with the recombinant yeast protein purified from E. coli extracts. The enzyme activity did strongly depend on the presence of NADP⁺ that could not be replaced by NAD⁺ (data not shown). The $K_m$ values for NADP⁺ and isocitrate were 0.02 and 0.05 mM, respectively (Fig. 9), and are in the range of those reported for the peroxisomal NADP-dependent isocitrate dehydrogenase of Candida tropicalis (0.016 mM for NADP and 0.11 mM for isocitrate) (44).

Idp3p Is Localized in the Peroxisomal Lumen—An organelar fraction isolated from spheroplasts of yeast wild-type cells was subjected to extraction by low salt, high salt, and carbonate at pH 11 according to Ref. 37. Idp3p was resistant to low salt extraction but was released by high salt and carbonate treatment of the organelles (Fig. 10A). These extraction properties distinguished Idp3p from two other peroxisomal proteins. Pex3p was resistant to all treatments, consistent with it being an integral membrane protein (34). As expected for a matrix protein, peroxisomal thiolase (Fox3p) (30) was extracted by all treatments. The extractability of Idp3p by carbonate treatment suggested that Idp3p does not span the peroxisomal membrane. Idp3p also does not seem to be tightly associated with the peroxisomal membrane, since part of the protein could be extracted with high salt. The extraction properties of Idp3p are similar to those observed for Pcs60p, a protein of the peroxisomal matrix that is also loosely associated with the peroxisomal membrane (32). To distinguish whether Idp3p is associated with the outer aspects of peroxisomes or whether the protein resides in the peroxisomal lumen, we analyzed the sensitivity of organelar Idp3p to externally added proteases. In the presence of detergents and proteases, all proteins were rapidly degraded. When detergents were present, degradation of proteins was also observed without the addition of protease, presumably due to the liberation of endogenous proteases (data not shown). However, in the absence of detergents, both the
intraperoxisomal thiolase (Fox3p) and Idp3p were protected against added proteases (Fig. 10B). Under the same conditions, Pex14p, which is located at the cytosolic face of the peroxisomal membrane (33), was rapidly degraded. Taken together, these results are consistent with an intraperoxisomal localization of Idp3p.

Peroxisomal Targeting of Idp3p Depends on the Presence of the Three C-terminal Amino Acids—The amino acids CKL at the extreme C terminus of Idp3p fit the consensus for a yeast PTS1 (41, 42). To analyze whether this putative PTS1 of Idp3p is functional, we analyzed the subcellular localization of a mutated Idp3pΔCKL lacking the last three amino acids. Idp3pΔCKL was expressed in an idp3Δ strain, and localization of the protein was determined by subcellular fractionation of whole-cell homogenates on sucrose density gradients. Idp3pΔCKL did not co-segregate with the peroxisomal markers but instead was exclusively found in the loading zone of the gradient, suggesting a cytosolic localization of the protein (data not shown). This result indicated that the last three amino acids of Idp3p are essential for the peroxisomal targeting of the protein. The presence of a functional PTS1 in Idp3p is in line with the observed protease resistance of the protein (Fig. 10B), since this signal sequence is known to target proteins to the peroxisomal matrix (41, 45).

Idp3p Is Required for the Peroxisomal Degradation of Unsaturated Fatty Acids—In search for the function of Idp3p in peroxisomal metabolism, we tested the growth abilities of idp3Δ cells on different carbon sources. Cells grew normally on medium containing glucose, glycerol, or stearate as a single carbon source (Fig. 11A). Also, on oleic acid plates, no significant growth differences between wild-type and idp3Δ mutant cells were observed (Fig. 11B). In liquid oleic acid medium, however, the generation time of idp3Δ mutant cells increased from 8 h determined for the wild type to 12 h for the mutant (Fig. 11B). Because the only difference between stearic acid and oleic acid is the presence of one double bond in position 9, the observed growth defect suggested that the peroxisomal Idp3p might play a role in the degradation of unsaturated fatty acids. This assumption was further supported by the complete inability of cells lacking Idp3p to grow on petroselinic acid, an unsaturated fatty acid that contains a double bond at position 6 (Fig. 11C). The observed growth defects on oleic acid and petroselinic acid medium were complemented upon transformation of the idp3Δ mutant with the wild-type IDP3 gene (Fig. 11, B and C). These results confirmed that the impaired growth of idp3Δ mutant cells on unsaturated fatty acids was indeed

**FIG. 7.** Idp3p is localized in yeast peroxisomes and is required for the peroxisomal oxidative decarboxylation of isocitrate. A, immunoblot analysis and enzyme activity measurements of cell fractions that were obtained by differential centrifugation of cell-free extracts from oleic acid-induced idp1Δ and idp1/idp3Δ cells. Equal volumes of each fraction were immunologically analyzed for the presence of Idp3p. In parallel, the fractions were assayed for NADP-dependent isocitrate dehydrogenase activity. Idp3p was exclusively localized to the organelar fraction of idp1Δ cells but was absent in idp1/idp3Δ cells. In idp1Δ cells, about 80% of the total enzyme activity was found in the soluble fraction. In idp1/idp3Δ cells, the deficiency in Idp3p correlated with the disappearance of NADP-dependent isocitrate dehydrogenase activity from the organelar fraction. B, correlation of Idp3p presence and NADP-dependent isocitrate dehydrogenase activity in peroxisomal fractions obtained by isopycnic 20–53% (w/w) sucrose density gradient centrifugation of organelles of the 25,000 × g pellet from oleic acid-induced idp1Δ and idp1/idp3Δ cells. Equal volumes of each fraction were immunologically analyzed for the presence of Idp3p and thiolase. Relative amounts of NADP+–dependent isocitrate dehydrogenase and peroxisomal marker enzymes catalase and mitochondrial fumarase were monitored by enzyme activity measurements. Peroxisomes peaked at a density of 1.21 g/ml, and mitochondria peaked at a density of 1.17 g/ml. Idp3p was found predominantly in the peroxisomal peak fractions of idp1Δ cells but was absent in idp1/idp3Δ cells. No NADP-dependent isocitrate dehydrogenase activity was detected in peroxisomes lacking the Idp3p.
caused by the lack of Idp3p.

Yeast Peroxisomes Contain Auxiliary Enzymes Needed for the Degradation of Unsaturated Fatty Acids—The ability of S. cerevisiae to grow on unsaturated fatty acids as the single carbon source (Fig. 11), the presence of a NADP-dependent isocitrate dehydrogenase in peroxisomes (Fig. 6), and its suggested role of supplying NADPH for the degradation of unsaturated fatty acids encouraged us to search for auxiliary enzymes of this pathway. Both the Δ2,Δ3-enoyl-CoA isomerase and the NADP-dependent 2,4-dienoyl-CoA reductase activities were detected in whole-cell yeast lysates (data not shown). For subcellular localization of the activities, wild-type yeast homogenates were subjected to sucrose density gradient centrifugation, which did result in a clear separation of peroxisomes and mitochondria as judged by organelle-specific marker enzymes (Fig. 12). Both the Δ2,Δ3-enoyl-CoA isomerase and the NADP-dependent 2,4-dienoyl-CoA reductase activities co-segregated with the peroxisomal marker catalase, demonstrating that both enzymes are localized in peroxisomes of S. cerevisiae (Fig. 12). These data suggest that peroxisomes of S. cerevisiae harbor the entire enzyme equipment needed for the utilization of unsaturated fatty acids, including an NADP-dependent isocitrate dehydrogenase, a putative component of an NADPH-regenerating system.

DISCUSSION

Here we report on the molecular identification and functional characterization of a peroxisomal NADP-dependent isocitrate dehydrogenase of S. cerevisiae. In line with a role in the peroxisomal metabolism of unsaturated fatty acids, the Idp3p has been demonstrated to be exclusively peroxisomal, and the protein was shown to be essential for the growth of S. cerevisiae on unsaturated fatty acids but dispensable for growth on saturated fatty acids. The supposed function of the protein in peroxisomal fatty acid metabolism is the regeneration of NADPH that is needed by the NADPH-dependent 2,4-dienoyl-CoA reductase for the reductive elimination of double bonds of unsaturated fatty acids. This reductase and the Δ2,Δ3-enoyl-CoA isomerase, another auxiliary enzyme needed for the degradation of unsaturated fatty acids, have been localized to yeast peroxisomes (Fig. 12). The presence of these enzyme activities in peroxisomes has far reaching implications for our understanding of the peroxisomal metabolism and transport of metabolites across the peroxisomal membrane. The data presented are consistent with the assumption that peroxisomes of S. cerevisiae maintain the entire enzyme equipment needed for the degradation of unsaturated fatty acids, including an NADP-dependent isocitrate dehydrogenase, a putative constituent of a peroxisomal NADPH-regenerating redox system.

Idp3p was isolated from peroxisomes of oleic acid-induced yeast cells (Fig. 2), and peptide sequence data of the protein were instrumental in cloning the corresponding gene from a genomic yeast library (Fig. 3). Idp3p is exclusively localized in peroxisomes, and consistent with its function in peroxisomal fatty acid metabolism, Idp3p was highly induced upon growth on oleic acid (Figs. 5–7). Protease protection data suggested that the protein resides in the peroxisomal lumen (Fig. 10), which is further supported by the observation that peroxisomal targeting of Idp3p depends on the presence of a C-terminal type 1 peroxisomal targeting signal (data not shown), known to target proteins from the cytosol across the peroxisomal membrane barrier into the peroxisomal matrix (41, 45, 46). That the peroxisomal Idp3p indeed is an NADP-dependent isocitrate dehydrogenase was confirmed by the characterization of the enzymatic properties of purified, recombinant Idp3p (Fig. 8). Interestingly, an NADP-dependent isocitrate dehydrogenase has also been detected in peroxisomes of the n-alkane-utilizing yeast C. tropicalis (44).

Beside the peroxisomal Idp3p, three yeast isoenzymes of isocitrate dehydrogenase have been described that catalyze the oxidative decarboxylation of isocitrate to α-ketoglutarate. The NAD-specific mitochondrial isoenzyme is an octamer of two nonidentical subunits designated Idh1p and Idh2p (47, 48) and is believed to catalyze a key regulation step in the tricarboxylic cycle.
acid cycle. Less clear are the functions of the two NADP-specific isoenzymes located in mitochondria and the cytosol (17, 18). The glutamate auxotrophy upon deletion of both Idp1p and Idh1p suggest that both enzymes contribute to the anaerobic supply of \( \alpha \)-ketoglutarate for glutamate formation (40). Furthermore, as isocitrate and \( \alpha \)-ketoglutarate can traverse the mitochondrial membrane via specific transporters (49), it has been suggested that the proteins may participate in an inter-

The peroxisomal NADP-dependent isocitrate dehydrogenase Idp3p is required for growth on unsaturated fatty acids. The requirement of the peroxisomal degradation of fatty acids with even-numbered double bonds for NADPH is well established (8, 12). Degradation of these fatty acids in the \( \beta \)-oxidation spiral leads to 2,4-dienoyl-CoA intermediates that are reduced to 3-\( \text{trans} \)-enoyl-CoA in a redox reaction that requires NADPH and that is catalyzed by the peroxisomal NADPH-dependent 2,4-dienoyl-CoA reductase. The resulting 3-\( \text{trans} \)-enoyl-CoA is subsequently isomerized to 2-\( \text{trans} \)-enoyl-CoA, which can be reintroduced into the \( \beta \)-oxidation spiral (Fig. 1). The assumption that Idp3p provides the NADPH for this chain of reactions is supported by the observation that cells lacking the protein grow normally on stearate (C18:0) but have lost the ability to grow on petroselinic acid (C16:1; C18:1; Fig. 11).

Until recently, it was generally believed that unsaturated fatty acids with double bonds extending from odd-numbered carbon atoms are chain-shortened to 3-\( \text{cis} \)-enoyl-CoA esters, which after isomerization to 2-\( \text{trans} \)-enoyl-CoA are further degraded by the \( \beta \)-oxidation spiral (Fig. 1B) (11). According to this pathway, NADPH would not be needed for the metabolism of these unsaturated fatty acids (Fig. 1). However, Tserng and Jin (50) reported that in mammalian cells also the degradation compartmental exchange of reducing equivalents (18). This raises the question of whether Idp3p might play a comparable role in the peroxisomal metabolism. In the cytosol, NADPH is generated by, for instance, the pentose phosphate pathway. However, because of the impermeability of the peroxisomal membrane for pyrimidine nucleotides (15), the cytosolic NADPH pool cannot directly account for the peroxisomal need for NADPH. This emphasizes the necessity for an NADPH-regenerating system in the peroxisomal lumen. Because the formation of \( \alpha \)-ketoglutarate for the production of glutamate is primarily catalyzed by the yeast mitochondrial NAD-dependent and NADP-dependent isocitrate dehydrogenases (40), the most likely biological function of Idp3p is the regeneration of NADPH. The involvement of Idp3p in the intraperoxisomal regeneration of NADPH, which is necessary for the degradation of unsaturated fatty acids, is also more in agreement with the peroxisomal localization and with the oleic acid inducibility of the protein.

The requirement of the peroxisomal degradation of fatty acids with even-numbered double bonds for NADPH is well established (8, 12). Degradation of these fatty acids in the \( \beta \)-oxidation spiral leads to 2,4-dienoyl-CoA intermediates that are reduced to 3-\( \text{trans} \)-enoyl-CoA in a redox reaction that requires NADPH and that is catalyzed by the peroxisomal NADPH-dependent 2,4-dienoyl-CoA reductase. The resulting 3-\( \text{trans} \)-enoyl-CoA is subsequently isomerized to 2-\( \text{trans} \)-enoyl-CoA, which can be reintroduced into the \( \beta \)-oxidation spiral (Fig. 1). The assumption that Idp3p provides the NADPH for this chain of reactions is supported by the observation that cells lacking the protein grow normally on stearate (C18:0) but have lost the ability to grow on petroselinic acid (C16:1; C18:1; Fig. 11).

Until recently, it was generally believed that unsaturated fatty acids with double bonds extending from odd-numbered carbon atoms are chain-shortened to 3-\( \text{cis} \)-enoyl-CoA esters, which after isomerization to 2-\( \text{trans} \)-enoyl-CoA are further degraded by the \( \beta \)-oxidation spiral (Fig. 1B) (11). According to this pathway, NADPH would not be needed for the metabolism of these unsaturated fatty acids (Fig. 1). However, Tserng and Jin (50) reported that in mammalian cells also the degradation compartmental exchange of reducing equivalents (18). This raises the question of whether Idp3p might play a comparable role in the peroxisomal metabolism. In the cytosol, NADPH is generated by, for instance, the pentose phosphate pathway. However, because of the impermeability of the peroxisomal membrane for pyrimidine nucleotides (15), the cytosolic NADPH pool cannot directly account for the peroxisomal need for NADPH. This emphasizes the necessity for an NADPH-regenerating system in the peroxisomal lumen. Because the formation of \( \alpha \)-ketoglutarate for the production of glutamate is primarily catalyzed by the yeast mitochondrial NAD-dependent and NADP-dependent isocitrate dehydrogenases (40), the most likely biological function of Idp3p is the regeneration of NADPH. The involvement of Idp3p in the intraperoxisomal regeneration of NADPH, which is necessary for the degradation of unsaturated fatty acids, is also more in agreement with the peroxisomal localization and with the oleic acid inducibility of the protein.
of unsaturated fatty acids with double bonds extending from odd-numbered carbon atoms requires NADPH. This observation gained support by the exploration of a novel pathway for the reductive removal of odd-numbered double bonds of fatty acids (Fig. 1C) (13). According to this pathway, a Δ3,5,Δ2,4-dienoyl-CoA isomerase, together with the NADPH-dependent 2,4-dienoyl-CoA reductase and the Δ3,Δ2-dienoyl-CoA isomerase facilitate the reduction of odd-numbered double bonds as illustrated in Fig. 1. Recently, it has been suggested that this novel pathway might also be responsible for the degradation of odd-numbered double bonded fatty acids in mammalian peroxisomes (8, 14). In this respect, it is interesting to note that also yeast cells lacking Idp3p are less capable than the wild type of growing on oleic acid (9-C18:1) as the single carbon source (Fig. 11).

The peroxisomal localization of the Idp3 protein leads to questions about the origin of the isocitrate and the fate of the α-ketoglutarate that is produced. The most simple explanation would be that α-ketoglutarate is exported directly in exchange for citrate as has been demonstrated for mitochondria (49). In principle, isocitrate could also form in peroxisomes from the citrate that is generated by the fusion of acetyl-CoA with oxaloacetate, catalyzed by the peroxisomal citrate synthase (Cit2p) (15, 51). However, despite efforts, an aconitase activity has not yet been detected in yeast peroxisomes, thus making the peroxisomal formation of isocitrate from citrate rather unlikely. The direct import of isocitrate from the cytosol into the peroxisomal lumen would predict the existence of a peroxisomal NADPH-regenerating redox system. The latter supports the notion of an involvement of peroxisomes in an intercompartmental exchange of reducing equivalents and predicts novel peroxisomal metabolite transporters as constituents of a redox shuttle across the peroxisomal membrane.

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