The degradation of fatty acids in yeast was observed to be confined to the peroxisome that contains a complete fatty acid β-oxidation system, an acyl-CoA oxidase, a multifunctional enzyme (MFE) type 2 possessing 2-enzyme-CoA hydratase 2 and (3R)-hydroxyacyl-CoA dehydrogenase (HADH) activities and a 3-ketoacyl-CoA thiolase (1, 2). Because multifunctional enzyme type 1 (MFE-1), which metabolizes 3-ketoacyl-CoA thiolase (1, 2). Because multifunctional enzyme type 2; MFE-2) has two N-terminal domains belonging to the short chain alcohol dehydrogenase/reductase superfamly (8), whereas the mammalian MFE-2 has only one. The (3R)-HADH activities of MFE-2 have been assigned to the short chain alcohol dehydrogenase/reductase domains in both the yeast (2) and mammalian enzymes (Refs. 9 and 10 and Fig. 1). An interesting question arises from what the physiological functions of the two domains are or even whether both of them show enzymatic activities. To answer this question, wild type human and yeast MFE-2, as well as their mutated variants, were tested for complementation in vivo. Wild type and mutated dehydrogenase domains of yeast MFE-2 were overexpressed, purified, and characterized in vitro. Our data show that the domains A and B have different enzymatic properties and that both domains play a functional role in the β-oxidation of fatty acids in yeast peroxisomes.

EXPERIMENTAL PROCEDURES

Strains, Growth Conditions, and Media—All bacterial manipulations were performed with Escherichia coli strain DH5α. S. cerevisiae fox-2 (ura3–52, trp1, leu2–3, 112, ade, fox2) has been described previously (2). For routine culture, yeast cells were grown at 30 °C on either rich medium (YPD; 1% yeast extract, 2% bactopeptone, and 2% D-glucose) or synthetic medium (SD/uracil, 0.67% Bacto-yeast nitrogen base without amino acids, 0.2% dropout powder without uracil, 2% D-glucose). For enzyme assays, the cells from stationary phase cultures in YPD or SC/uracil were harvested, washed with H2O, and diluted in YPDT (0.5% yeast extract, 0.5% Bactopeptone, 0.1% oleic acid, 0.1% glucose, 0.5% Tween 40 at pH 7.0) to an absorbance of A660nm = 0.2 and grown for an additional 18 h to induce peroxisome proliferation.

Growth Curve—The strain was grown at 30 °C for 18 h in YPD medium or SC/uracil. Cells were harvested, washed with H2O, and used to inoculate 10 ml of YNO medium (0.1% yeast extract, 0.67% yeast nitrogen base with amino acids, 0.2% dropout powder without uracil, 2% D-glucose). For enzyme assays, the cells from stationary phase cultures in YPD or SC/uracil were harvested, washed with H2O, and diluted in YPD (0.5% yeast extract, 0.5% Bactopeptone, 0.1% oleic acid, 0.1% glucose, 0.5% Tween 40 at pH 7.0) to an absorbance of A660nm = 0.2 and grown for an additional 18 h to induce peroxisome proliferation.

Construction of Plasmid pYE352::HsMFE-2, pYE352::ScMFE-2, and Its Mutant Variants—The open reading frame of human MFE-2 cDNA was obtained from total RNA isolated from human fibroblasts by reverse transcription with Moloney murine leukemia virus reverse transcriptase (Life Technologies, Inc.), amplified by PCR using human MFE-2 specific primers 5’-primer, 5’-gacctgtaag ATG GCC TCA CCC CTG AGG TTC GA-3’ and 3’-primer, 5’-ctgag TCA GAG TCT GGT GAA AAG A-3’. The primers contained SacI and XhoI sites (with lowercase letters indicating mismatches to HsMFE-2 gene) for subsequent cloning into pUC18 vector using the Sure Clone Ligation kit (Amersham Pharmacia Biotech). Following SacI and XhoI digestion,
production and purification of (3r)-hydroxyacyl-coa dehydrogenase—mzeb medium supplemented with carbenicillin (50 µg/ml) and chloramphenicol (34 µg/ml) was used for expression experiments. 10 ml of an overnight culture of the E. coli cells containing the plasmid pET3a::CtMFE-2(h2Δ) or its mutated variants were used to inoculate 1 liter of culture. The cells were allowed to grow at 37 °C under aerobic conditions until an A600 of 0.6 was reached. The expression of the recombinant protein was induced by addition of isopropyl-1-thio-β-D-galactopyranoside to a final concentration of 0.4 mM. After 2 h of induction at 33 °C, the cells were harvested and washed with phosphate-buffered saline (10 mM sodium phosphate, 2 mM potassium phosphate, 140 mM NaCl, 3 mM KCl, 5 mM β-mercaptoethanol, pH 7.4). The pellet was stored at −70 °C until use.

Bacterial cell pellet (wet weight, 5.0 g) was suspended in 50 ml of 30 mM sodium phosphate, 90 mM NaCl, 1.0 mM phenylmethylsulfonyl fluoride, 0.5 mM benzamidine hydrochloride hydrate, and 0.5 mM dithiothreitol at pH 7.0 (buffer A). The bacteria were lysed by adding lysozyme (125 µg/ml), and the viscosity was reduced by adding DNase (25 µg/ml) and RNase (25 µg/ml) in the presence of 10 mM MgCl2 for 30 min at 30 °C. The insoluble material was removed by centrifugation at 30,000 × g for 45 min at 4 °C. The supernatant was applied to a 2.5 × 12.5 cm DEAE-Sepharose (Amersham Pharmacia Biotech) column equilibrated with buffer A. The bound proteins were eluted with a 50-ml linear gradient of 0–0.3 M NaCl. The pooled active fractions from the Resource Q column were concentrated and dialyzed against 20 mM MES, pH 6.1 (buffer C), and applied to a 1-mL Resource Q column (Amersham Pharmacia Biotech) equilibrated with buffer C. The dehydrogenase was eluted with a 25-ml linear gradient of 0–0.2 M NaCl. Purification of the recombinant (3r)-hADH was completed after applying the protein to a Superdex 200 HR10/30 column (Amersham Pharmacia Biotech) equilibrated with 200 mM po-

The HsMFE-2 insert was ligated into a similarly digested pYE352 behind the catalase A1 promoter (11), resulting in pYE352::HsMFE-2. The cDNA encoding ScMFE-2 was obtained from S. cerevisiae genomic DNA by PCR with Pfu polymerase, using 5′- primer, tctagaag ATG CCT GGA AAT TTA TCC TTCCGT GAT CAA AAC-3′ and 3′-primer, GCA GGA GGT GGT C-3′, respectively (with lowercase letters indicating mismatches to ScMFE-2 gene). The 2.7-kilobase PCR fragment was first cloned into the pUC18 vector and subsequently digested by XbaI and Xhol, respectively (with lowercase letters indicating mismatches to ScMFE-2 gene). The 2.7-kilobase PCR fragment was then inserted into a similarly digested pYE352 (11), resulting in pYE352::ScMFE-2. Both pYE352::HsMFE-2 and pYE352::ScMFE-2 were transformed into fos-2 cells by the lithium acetate method (12) and selected on SD/uracil plates.

Site-directed Mutagenesis of (3r)-Hydroxyacyl-CoA Dehydrogenase—The HsMFE-2 insert in pUC18 was used as a template to generate a G16S mutation. Site-directed mutagenesis was performed according to the instructions of the QuikChange™ site-directed mutagenesis kit (Stratagene). The ScMFE-2 insert in pUC18 was taken as a template for generating pUC18::ScMFE-2(Δa) or pUC18::ScMFE-2(Δb). The primers designed for generating the G16S mutation in Sc MFE-2(aΔ) were 5′-GGT GTA ATC ACG TCT GCT GGA GGG GG-3′ (5′-primer) and 5′-CC CCC TCC TGC AGA GTA GAT TAC AAC-3′ (3′-primer). Primers 5′-GGT GTA ATC ACG TCT GCT GGA GGG GG-3′ (5′-primer) and 5′-G ACC TTC TGC AGA GTG AAC TAC AAT-3′ (3′-primer) were used in generating the G329S mutation in Sc MFE-2(bΔ). pET3a::ScMFE-2(ΔaΔ) was used as a PCR template to obtain pUC18::ScMFE-2(ΔaΔ) with the primers designed for mutation G329S. All of the mutated DNA inserts were cloned into pYE352 (11), resulting in pYE352::HsMFE-2(ΔaΔ), pYE352::ScMFE-2(ΔaΔ), pYE352::ScMFE-2(ΔbΔ), and pYE352::ScMFE-2(ΔaΔ). The nucleotide sequences of all the mutated variants were verified.

Expression of Recombinant (3r)-Hydroxyacyl-CoA Dehydrogenase—The region of cDNA encoding amino acid residues 1–612 of C. tropicalis peroxisomal MFE-2 was amplified from the plasmid pMK22-HDE50 (13) by PCR with Pfu polymerase, using 5′-primer 5′-cat ATG TCT CCA GGT TAT TTA AAA-3′ and 3′-primer 5′-agaatct TTA TTC TTC TTC AAG AAT TTA-3′ containing NdeI and BsmHI, respectively. The resulting 1899-base pair PCR fragment was subcloned into pUC18. Following digestion by NdeI and BsmHI, the fragment was cloned into the pET3a expression vector (Novagen, Inc., Madison, WI) yielding pET3a::CtMFE-2(h2Δ), and the nucleotide sequence encoding the dehydrogenase domain was sequenced. Site-directed mutagenesis was performed in a similar way as for the pYE352·ScMFE-2 variants, using the CtMFE-2 specific primers. By using pET3a::CtMFE-2(ΔaΔ) as a PCR template, pET3a::CtMFE-2(ΔbΔ) was generated with 5′-primer, 5′-GTT ATC ACC AGT GCC GGT G-3′ and 3′-primer, 5′-ACC ACC GCC GGT CAT GAT CAC-3′, and pET3a::CIMFE-2(ΔbΔ) was generated with 5′-primer, 5′-GTT ATC ACC AGT GCC GGT G-3′ and 3′-primer 5′-ACC ACC GCC GGT CAT GAT CAC-3′, and pET3a::CtMFE-2(ΔaΔΔ) was generated with the primers designed for CtMFE-2(ΔaΔΔ) using pET3a::CtMFE-2(ΔbΔΔ) as a template. The pET3a::CtMFE-2(ΔaΔΔ) and its mutated variants were transformed into E. coli BL21(DE3) pLysS cells.

Production and Purification of (3r)-Hydroxyacyl-CoA Dehydrogenase—
tassium phosphate, 3 mM ethylene diaminetetraacetic acid, and 3 mM EGTA, pH 7.2.

Substrate Synthesis and Enzyme Assays—(3R)-Hydroxacyl-CoA esters were synthesized by the mixed anhydride method (14) and purified by high pressure liquid chromatography on a reversed phase μBondapak™ C18 column (Waters, Milford, MA) applying a linear acetonitrile gradient. The assay buffer contained 50 mM Tris/Cl, pH 8.0, 50 mM KCl, 1 μM of NAD’ (Merck), 1 mM sodium pyruvate (Sigma), 2.5 mM MgCl2, 10 μg (21 units) of lactate dehydrogenase, and 30 nmol of (3R)-hydroxybutyryl-CoA in 0.5 ml. The dehydrogenase reaction was started by adding the sample and monitored by formation of the magnesium complex of 3-ketobutyryl-CoA at 303 nm at 22 °C.

Circular Dichroism Spectroscopy—CD spectroscopy was carried out at 22 °C using a Jasco J710 spectropolarimeter. Adsorption at 280 nm was measured for all samples and used for fine adjustment of the protein concentration of the sample used for CD spectroscopy. The far-UV spectra of the proteins were measured from 200 to 250 nm in 80 mM potassium phosphate, pH 7.0, with the following instrument settings: response, 1 s; sensitivity, 100 mdeg; speed, 50 nm/min; average of 30 scans.

Determination of the k_on and K_m Values for Recombinant (3R)-Hydroxacyl-CoA Dehydrogenase—Kinetic constants for the (3R)-HADH activity were determined toward oxidation of (3R)-hydroxacyl-CoA using formation of the Mg2+ complex of 3-ketocarbonyl-CoA as detected at 303 nm. The substrate concentrations for (3R)-hydroxybutyryl-CoA were 10, 30, 50, 80, 100, 150, and 200 μM, for (3R)-hydroxydecanoyl-CoA and those for (3R)-hydroxyhexadecanoyl-CoA were 2, 4, 8, 10, 30, and 50 μM. (3R)-Hydroxoydecanoyl-CoA and (3R)-hydroxyhexadecanoyl-CoA were obtained by pre-incubating trans-2-decenoyl-CoA and trans-2-hexadecenoyl-CoA with recombinant 2-enoyl-CoA hydratase 2 (10). The equilibrium of the reaction is >90% on the side of the (3R)-hydroxy intermediate under the conditions used.6 The measurement was performed in a final volume of 0.5 ml at 22 °C. Kinetic data were transformed to Lineweaver-Burk plots by using the GraFit computer software (Sigma). The K_m values were calculated from the slopes of the curves, and the catalytic turnover numbers (k_on) were calculated by dividing the maximal velocities with the total amount of enzyme in the reaction.

Other Analyses—Protein concentrations were measured with Bio-Rad protein assay reagent, and protein samples were analyzed on 12% SDS-polyacrylamide gels.

RESULTS

Amino Acid Sequence Alignment of Nucleotide-binding Sites of Human and Yeast MFE-2—The G16S variant of Hs MFE-2 has been shown to result in MFE-2 deficiency (6). Because this glycin is in the nucleotide-binding site of the dehydrogenase domain and is conserved in MFE-2 of yeast, it is expected to be a functionally important amino acid residue in MFE-2 of lower eukaryotes (Fig. 2).

Growth of Yeast Cells on Oleic Acid—When pYE352::ScMFE-2 was introduced into S. cerevisiae fox-2 cells, the transformed strain regained the ability to grow on fatty acids as a carbon source as indicated by the clear zone formation on the oleic acid plate (Fig. 3A). However, no clear zones developed if the fox-2 cells transformed with pYE352::ScMFE-2(b)D, which encodes the G16S mutation (Fig. 3A). When the enzyme activities of MFE-2 were measured from soluble extracts of transformed fox-2 cells, the hydratase 2 activity was observed in all cases, whereas the (3R)-HADH activity was abolished by the G16S mutation (Table I). The expression of the Hs MFE-2 was also confirmed by immunoblotting of samples from these yeast extracts with antibody to rat 2-enoyl-CoA hydratase 2 (9), which recognized a 79-kDa band corresponding to the predicted molecular mass of Hs MFE-2.

Amino acid sequence alignment of nucleotide-binding sites revealed that Gly16 and Gly329 of S. cerevisiae fox-2 and the corresponding amino acid residues in MFE-2 of lower eukaryotes (Fig. 2). The introduction of Gly → Ser mutations in Sc MFE-2 resulted in generation of variants Sc MFE-2(a), Sc MFE-2(b), and Sc MFE-2(a,b)D. When the corresponding plasmids pYE352::ScMFE-2, pYE352::ScMFE-2(a), pYE352::ScMFE-2(b), and pYE352::ScMFE-2(a,b)D were transformed into fox-2 cells, each of the transforms grew on oleic acid plates and formed clear zones, except for those transformed with pYE352::ScMFE-2(a,b)D (Fig. 3B).

To investigate the kinetics of growth, the number of cells was monitored during growth in the liquid oleic acid media over time (Fig. 3C). The growth rates of S. cerevisiae fox-2 cells transformed with either pYE352::ScMFE-2(a)D or pYE352::ScMFE-2(b)D were about 50% of that found with pYE352::ScMFE-2 transformed cells. In agreement with the plate assay, fox-2 or fox-2 transformed with pYE352::ScMFE-2(a,b)D did not show observable growth. To verify that S. cerevisiae MFE-2 and its variants were expressed, both 2-enoyl-CoA hydratase 2 and (3R)-HADH activities were measured in yeast cell homogenates (Table I). With the exception of the fox-2 cells, hydratase 2 activity was detected for all transformants. When compared with Sc MFE-2(h2D), the dehydrogenase activity was decreased for the a and b variants, and it was completely lost for the a,bD mutant.

Expression of C. tropicalis (3R)-Hydroxacyl-CoA Dehydrogenase and Its Mutants in E. coli—Our attempts to express and purify Sc MFE-2(h2D) as a recombinant protein resulted in the enzyme undergoing inactivation within a few days. However, C. tropicalis MFE-2(h2D) yielded a protein stable for at least one year under the conditions stated under "Experimental Procedures." Subsequent in vitro characterizations were carried out with C. tropicalis preparation.

When pET3a::CtMFE-2(h2D) was expressed in E. coli BL 21plysS cells, (3R)-specific HADH activity was 23 μmol × min⁻¹ × mg⁻¹ in the soluble cell extract when measured with (3R)-hydroxydecanoyl-CoA, whereas the activity was below the detection limit in the cells transformed with only the vector pET3a. The expressed Ct MFE-2(h2) was purified to apparent homogeneity by using two anion exchange columns: DEAE-Sephacel and Resource Q, followed by a cation exchange column, Resource S, and a size exclusion Superdex 200/30 HR column (Table II). It is worth of noting that the Ct MFE-2(h2) preparation is, in fact, a multifunctional (3R)-HADH, which is
an enzyme not commercially available and thus can potentially be used as a tool for investigating the metabolism of fatty acids and their derivatives. Ct MFE-2(h2ΔA), Ct MFE-2(h2ΔAh), and Ct MFE-2(h2ΔAhΔa) were each expressed and purified following the same protocol, yielding apparent homogeneities (SDS-polyacrylamide gel electrophoresis analysis; Fig. 4). In immunoblotting, the antibody to Ct MFE-2 (15) recognizes these polypeptides of 66,000 Da, which agree with the molecular mass of 66,379 Da calculated from the amino acid sequence (data not shown). Native molecular masses of the proteins were determined to be 140 kDa by size exclusion chromatography (Superdex 200), indicating that they are dimers (Fig. 4). CD spectra in the far-UV region (200–250 nm) were practically identical for both Ct MFE-2(h2ΔA) and its variants (Fig. 5).

Kinetic Constants of Wild Type and Mutant Dehydrogenases—Kinetic parameters were determined for the purified Ct MFE-2(h2ΔA) and its mutated variants (Table III). The Ct MFE-2(h2ΔA) showed the highest catalytic efficiency (kcat/Km) with the substrate (3R)-hydroxydecanoyl-CoA (C10). The Km value was lowest for the C10 substrate, being approximately one-fifth and one-tenth of the value of the C16 and C4 substrates, respectively. Interestingly, the (3R)-HADH activity of Ct MFE-2(h2ΔA) broke into two different profiles when the mutated variants were analyzed (Table III). For Ct MFE-2(h2ΔAh), the catalytic constant (kcat) of C4 was the same as for Ct MFE-2(h2ΔA) (29 ± 1 s⁻¹ versus 31 ± 2 s⁻¹), whereas that of Ct MFE-2(h2ΔAhΔa) was below the detection limit, indicating that domain B is solely responsible for the utilization of C4 substrate. The hcat values of Ct MFE-2(h2ΔAhΔa) for C10 and C16 were 17 ± 1 s⁻¹ and 12 ± 2 s⁻¹, respectively. Interestingly, for Ct MFE-2(h2ΔAhΔa), the hcat values were 33 ± 2 s⁻¹ and 36 ± 6 s⁻¹, suggesting that domain A contributes more than domain B in the metabolism of medium and long chain substrates. The activity of Ct MFE-2(h2ΔAhΔa) toward the substrates tested was below the detection limit of the assays used.
DISCUSSION

The amino acid sequence of yeast peroxisomal MFE-2 reveals that the polypeptide contains two domains sharing 40% amino acid identity, both within the N-terminal half of the protein. The domains are about 300 amino acid residues long (Fig. 1), and each of the domains contains a binding site for NAD (Rossmann fold) close to their N termini. At about 120 amino acid residues from their C termini, the domains also contain the motif Tyr-Xaa-Xaa-Xaa-Lys. These features are characteristic of the members of the short chain alcohol dehydrogenase/reductase superfamily (8). A previous in vitro experiment with a truncated version of S. cerevisiae MFE-2 (deletion of C-terminal 271 amino acid residues) indicated that the N-terminal domains were responsible for the (3R)-HADH activity in MFE-2 (2). The present work, applying site-directed mutagenesis to dissect the putative nucleotide-binding sites of domains A and B, shows that the growth rates of fox-2 cells transformed with pYE352::ScMFE-2(aD) or pYE352::ScMFE-2(bD) are slower than those cells transformed with pYE352::ScMFE-2. This suggests that domains A and B are important for utilization of fatty acids as a carbon source. The fact that the activity of hydratase 2 was not changed in the yeast extracts when measured with the C10 substrate indicates that the growth rates were not due to differences in the levels of MFE-2 expression.

![Figure 4. Size exclusion chromatography of CtMFE-2(h2Δ).](image)

A

B

**TABLE II**

Purification of the yeast recombinant (3R)-hydroxyacyl-CoA dehydrogenase [CtMFE-2(h2Δ)] from E. coli

| Step                  | Protein | Total activity | Specific activity | Yield | Purification |
|-----------------------|---------|----------------|-------------------|-------|--------------|
|                       | mg      | μmol × min⁻¹ | μmol × min⁻¹ ×    | %     | fold         |
| Soluble extract       | 155     | 3250          | 21                | 100   | 1.0          |
| DEAE-Sephacel         | 71      | 3000          | 42                | 92    | 2.0          |
| Resource Q            | 27      | 1890          | 70                | 58    | 3.3          |
| Resource S            | 10      | 1080          | 108              | 33    | 5.1          |
| Superdex 200 HR 0/30  | 0.19    | 23            | 125               |       | 5.9          |

*Aliquot from Resource S containing 18.4 μmol × min⁻¹ (3R)-hydroxyacyl-CoA dehydrogenase activity measured with (3R)-hydroxydecanoyl-CoA for Superdex 200 HR 10/30 chromatography.*
lar ellipticities of the proteins are similar, indicating that the mutations do not cause a change in the composition of secondary structure elements. These data, together with the observation that Ct MFE-2(h2Δ) and its variants are dimeric proteins, strongly argues that the observed differences in kinetics were due to the mutations affecting catalytic amino acid residues but not due to a change in overall folding itself. The estimated $K_m$ values for domains A and B are between 5 and 50 $\mu M$, values that are within the same magnitude generally found for other $\beta$-oxidation enzymes (16). An interesting observation is that the sums of the $k_{\text{cat}}$ values for Ct MFE-2(h2Δ) and Ct MFE-2(h2ΔaΔ) are very close to $k_{\text{cat}}$ determined for Ct MFE-2(h2Δ).

According to the current literature, the physiological role of mammalian MFE-2 is to participate in metabolism of $\alpha$-methyl-CoA esters (5). This is supported by the observation that $\alpha$-methyl branched fatty acid intermediates accumulate in patients with MFE-2 deficiency (6, 17). However, an accumulation of straight very long chain fatty acids has also been reported in these patients (18). In mammalian MFE-2 is capable of catalyzing the cleavage of straight chain fatty acid intermediates that are within the same magnitude generally found for other $\beta$-oxidation enzymes (16). An interesting observation is that the sums of the $k_{\text{cat}}$ values for Ct MFE-2(h2Δ) and Ct MFE-2(h2ΔaΔ) are very close to $k_{\text{cat}}$ determined for Ct MFE-2(h2Δ).

An acquisition of two domains within a single polypeptide with different chain length specificities is a novel strategy among lipid binding proteins to overcome the problems related to the metabolism of a large variety of substrates. Previously described strategies include gene duplication, which lead to the evolution of separate enzymes, such as mammalian acyl-CoA dehydrogenases, which are presented as several paralogues (20). Adaptation can also occur via the development of adaptive substrate binding pocket, as is formed in both 2-enoyl-CoA hydratase 1 (crotonase) and the acyl-CoA binding protein. Mitochondrial hydratase 1, which catalyzes hydration reactions with $\text{trans}$-2-enoyl-CoA substrates from C4 to C20 in chain length, has an active site pocket in each of the subunits that is large enough to bind crotonyl-CoA (21). When a longer chain substrate is bound, the $\omega$ end of the acyl moiety clashes a flexible loop at the bottom of the pocket and the loop moves aside, resulting in the formation of tunnel traversing the whole subunit (22). The acyl-CoA binding protein binds the acyl-CoA

![Fig. 5. CD spectroscopy of recombinant yeast (3R)-hydroxyacyl-CoA dehydrogenase and its variants. Far-UV spectra of mutant variants: Ct MFE-2(h2Δ) (solid line), Ct MFE-2(h2ΔaΔ) (line with short dashes), Ct MFE-2(h2ΔaΔ) (line with long dashes), and Ct MFE-2(h2ΔaΔ) (line with long and short dashes).](image-url)
ester in a bent orientation anchoring the acyl chain in a hydrophobic cleft between two α-helices (23). The cleft in the bovine protein is large enough to engulf a carbon chain of up to C22 long.

The data of the experiments of yeast growing in liquid medium indicate that both domains A and B are required for optimal growth of yeast cells on fatty acid as the sole carbon source. Yeast peroxisomal MFE-2 provides an intriguing example of one polypeptide that has acquired two enzymatically active dehydrogenase domains with different chain length specificities.

Acknowledgments—PMK22/HDE50 plasmid is a gift from Dr. Richard A. Rachubinski (Department of Biochemistry, McMaster University, Hamilton, Ontario, Canada). The antibody to CtMFE-2 was kindly provided by Dr. Wolf-H. Kunau (Abteilung für Zellbiochemie, Medizinische Fakultät, Ruhr-Universität Bochum, Federal Republic of Germany). We are grateful to Ville Ratas, Tanja Kokko, and Marika Kamps for technical assistance and to Dr. Werner Schmitz and Anna-Leena Hietäärvi for substrate synthesis. We are especially thankful to Dr. Aner Gurvitz, Prof. Johan Kemmink, Prof. Rik Wierenga, and Sydney Higley for valuable comments.

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