Deletion of the Conserved First 18 N-Terminal Amino Acid Residues in Rat Liver Carnitine Palmitoyltransferase I Abolishes Malonyl-CoA Sensitivity and Binding†

Jianying Shi, Hongfa Zhu, Dennis N. Arvidson, James M. Cregg, and Gebre Woldegiorgis*

Department of Biochemistry and Molecular Biology, Oregon Graduate Institute of Science and Technology, Portland, Oregon 97291-1000

Received February 11, 1998; Revised Manuscript Received April 21, 1998

ABSTRACT: To assess the role of the 130 N-terminal amino acid residues of rat liver carnitine palmitoyltransferase I (L-CPTI) on malonyl-CoA sensitivity and binding, we constructed a series of mutants with deletions of the 18, 35, 52, 73, 83, or 129 most N-terminal amino acid residues. The deletion mutants were expressed in the yeast *Pichia pastoris*. We determined the effects of these mutations on L-CPTI activity, malonyl-CoA sensitivity, and binding in isolated mitochondria prepared from the yeast strains expressing the wild-type and deletion mutants. The mutant protein that lacked the first 18 N-terminal amino acid residues, Δ18, had activity and kinetic properties similar to wild-type L-CPTI, but it was almost completely insensitive to malonyl-CoA inhibition (I_{50} = 380 μM versus 2.0 μM). In addition, loss of malonyl-CoA sensitivity in Δ18 was accompanied by a 70-fold decrease in affinity for malonyl CoA (K_D = 70 nM versus 1.1 nM) compared to wild-type L-CPTI. Deletion of the first 35, 52, 73, and 83 N-terminal amino acid residues had a similar effect on malonyl-CoA sensitivity as did the 18-residue deletion mutant, and there was a progressive reduction in the affinity for malonyl-CoA binding. By contrast, deletion of the first 129 N-terminal amino acid residues resulted in the synthesis of an inactive protein. To our knowledge, this is the first report to demonstrate a critical role for these perfectly conserved first 18 N-terminal amino acid residues of L-CPTI in malonyl-CoA sensitivity and binding.

Mammalian mitochondrial membranes contain two active but distinct carnitine palmitoyltransferases (CPTs),[1] a malonyl-CoA-sensitive, detergent-labile CPTI and a malonyl-CoA-insensitive, detergent-stable CPTII (*1*−*3*). CPTI, a rate-limiting enzyme in β-oxidation, catalyzes the conversion of long-chain acyl CoAs to acylcarnitines in the presence of L-carnitine. As an enzyme that catalyzes the first rate-limiting step in fatty acid oxidation, CPTI is tightly regulated by its physiological inhibitor malonyl-CoA (*1, 2*). This is an important regulatory mechanism in fatty acid oxidation, because malonyl-CoA is the first intermediate in fatty acid synthesis and suggests coordinated control of fatty acid oxidation and synthesis. Regulation of CPTI by malonyl-CoA provides a mechanism for cellular fuel sensing based on the availability of fatty acids and glucose (*4*). Understanding the molecular mechanism of the regulation of CPTI by malonyl CoA is important in the design of drugs for control of excessive fatty acid oxidation in diabetes mellitus (*4*) and in myocardial ischemia, where accumulation of acylcarnitines has been associated with arrhythmias (*5*).

Mammalian tissues express two isoforms of CPTI—a liver isoform, L-CPTI, and a heart/skeletal muscle isoform, M-CPTI—that are 62% identical in amino acid sequence (*6*−*12*). We have developed a novel, high-level expression system for rat L-CPTI and human heart M-CPTI in the yeast *Pichia pastoris*, an organism devoid of endogenous CPT activity (*3, 9, 13*). Using this system, we have demonstrated conclusively that L-CPTI and M-CPTI are active, distinct, malonyl-CoA-sensitive CPTIs that are detergent labile. Previously, L-CPTI and M-CPTI were presumed to be irreversibly inactivated by detergents (*2*). However, our reconstitution studies with yeast-expressed L-CPTI and M-CPTI demonstrate that both enzymes are reversibly inactivated by detergents (*9, 13*−*15*). Our development of a good expression system for CPTI in *P. pastoris* has enabled us to begin to map the malonyl-CoA binding site by mutational analysis as described below.

Amino acid sequence alignment of human and rat heart M-CPTI, human and rat L-CPTI, and human and rat liver CPTII sequences (*9*−*12, 16*−*20*) reveals a conserved N-terminal sequence of 124 residues with two putative transmembrane domains (Figure 1, shaded area) that is present in all known CPTI sequences, but is absent from CPTII. Based on limited proteolysis studies of intact rat liver mitochondria and activity studies with immobilized impermeable substrate and inhibitor, a model for the membrane topology of L-CPTI has been proposed that predicts exposure of the N- and C-termini, domains crucial for activity and malonyl-CoA sensitivity of L-CPTI, on the cytosolic side of the outer mitochondrial membrane (*21*). Furthermore, it

† This research was supported by Grant HL-52571 from the National Institutes of Health (to G.W.).

* Author to whom correspondence should be addressed at Department of Biochemistry and Molecular Biology, Oregon Graduate Institute of Science and Technology, P.O. Box 91000, Portland, OR 97291-1000. Telephone: 503-690-1676. Fax: 503-690-1464. E-mail: gwoldeg@bmb.ogi.edu.

[1] Abbreviations: CPT, carnitine palmitoyltransferase; L-CPTI, rat liver isoform of CPTI; M-CPTI, heart/skeletal muscle isoform of CPTI; Δ, deletion mutant; NIDDM, noninsulin-dependent diabetes mellitus; PCR, polymerase chain reaction.

S0006-2960(98)00342-0 CCC: $15.00
Published on Web 07/17/1998

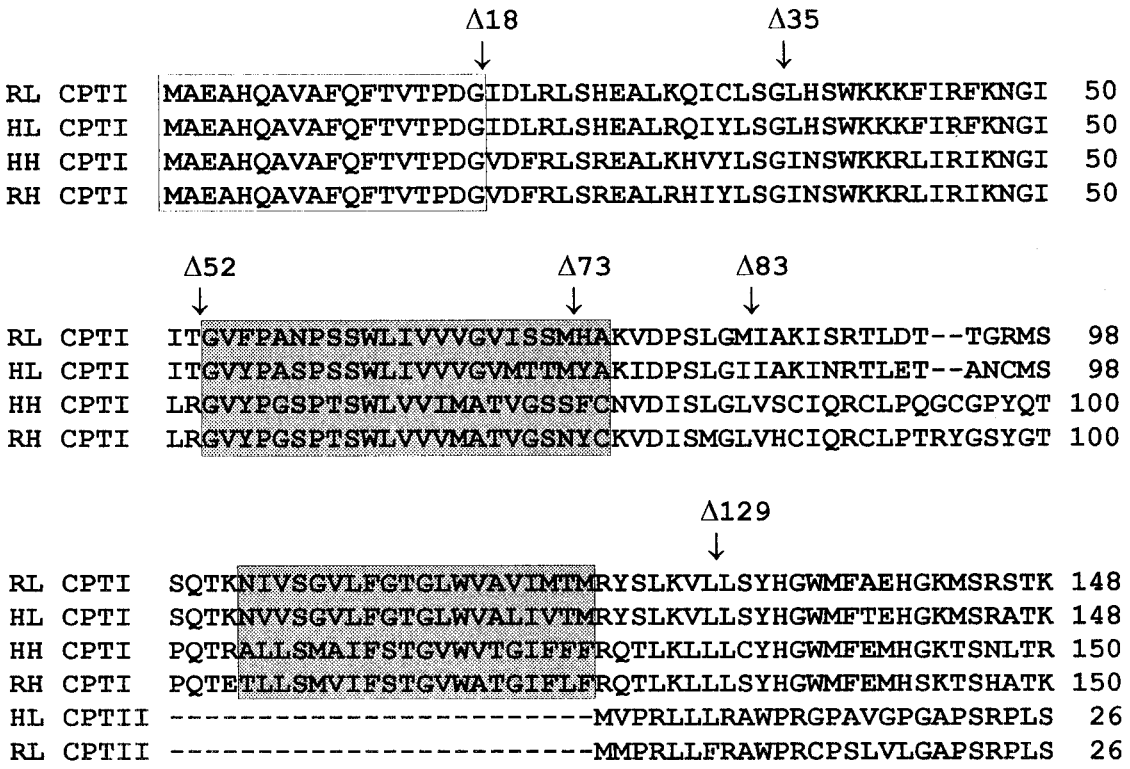

FIGURE 1: N-terminal amino acid sequence lineup of human and rat CPTs. The shaded areas represent the position of the two predicted membrane-spanning domains of all known CPTIs. The position of the conserved 18 N-terminal amino acid residues within the CPTIs is boxed. The position of each of the deletion mutants is shown by an arrow. RL, HL—rat, human liver; HH, RH—human, rat heart. Sources of the sequences from the data bank were from refs 9−12, and 16 as indicated in the text.

is predicted that the two transmembrane domains of L-CPTI are separated by a 30 amino acid linker (residues 76−103) that is located within the mitochondrial intermembrane space (Figure 1). It is hypothesized that malonyl-CoA-sensitivity of CPTI may reside in these 124 N-terminal amino acid residues, thus forming the structural basis for the malonyl-CoA sensitivity of CPTI. To test this hypothesis, we constructed six deletion mutants of the first 130 N-terminal amino acid residues of rat L-CPTI, ranging in size from 18 to 130 residues.

EXPERIMENTAL PROCEDURES

Construction of Plasmids for the N-Terminal Deletion Mutants of Rat L-CPTI. The cDNA used and the construction of the plasmid (wild-type) for rat liver CPTI expression in *P. pastoris* is described in our previous publication (*3*). A *Hin*dIII-*Kpn*I fragment [1−566 bp; cDNA sequence (*3*, *18*)] was excised from pYGW9, a plasmid containing the full-length rat L-CPTI in pUC119 to generate the plasmid pGYW12. A 637-bp *Hin*dIII-*Eco*RI fragment was polymerase chair reaction (PCR) amplified using the plasmid pYGW9 as a template with the primers: RL655, 5′-CCACCAGGATTTTAGCT-3′, and RLD18, 5′-CTTCACAAGCTTGAATTC**ATG**ATTGACCTCCGCCTGAGC-3′. An ATG start codon (shown in bold) was added immediately after the *Eco*RI site. The PCR product was digested with *Hin*dIII and *Kpn*I, then ligated into pYGW12 to generate plasmid pYGW13. An *Eco*RI fragment of pYGW13 containing the deletion mutant RLD18 (Δ18) was then ligated into the *Eco*RI-cut *P. pastoris* expression vector pHW10 (*3*, *22*). The DNA sequences of the deletions were confirmed by sequencing.

Deletion mutants RLD35 (Δ35), RLD52 (Δ52), RLD72 (Δ72), RLD83 (Δ83), and RLD129 (Δ129) (Figure 1) were constructed in a manner similar to Δ18, using primer RL655 above and the following primers for each deletion mutant: Δ35, 5′-AAACAGAAGCTTGAATTCATGCTGCACTC-CTGGAAGAAG-3′; Δ52, 5′-CAAGAAGCTTGAATTC-ATGGGTGTGTTCCCCGCGAA-3′; Δ72, 5′-GTGGGT-AAGCTTGAATTCATGCATGCCAAAGTGGAC-3′; Δ83, 5′-GGACAAGCTTGAATTCATGATCGCAAAGATCA; and Δ129 5′-TGCGCAAGCTTGAATTCATGCTGCTCTCC-TACCACGGCT-3′, respectively, with plasmid pYGW9 as a template to generate 585-, 533-, 474-, 439-, and 299-bp *Hin*dIII-*Kpn*I PCR fragments. All subsequent procedures were identical to those used for construction of Δ18.

The expression plasmids were linearized and integrated into the HIS4 locus of *P. pastoris* strain GS115 by electrotransformation (*23*). Histidine prototrophic transformants were selected on YND plates and grown on YND medium, and mitochondria were isolated from both the wild-type and deletion mutant L-CPTIs as described previously (*3*).

CPT Assay. CPT activity was assayed by the forward exchange method using L-[^{3}H]carnitine as previously described (*24*). The standard assay reaction mixture contained in a total volume of 0.5 mL: 0.2 mM L-[^{3}H]carnitine (∼10 000 dpm/nmol), 50 μM palmitoyl CoA, 20 mM HEPES (pH 7.0), 1% fatty acid-free albumin, and 40−75 mM KCl, with or without malonyl-CoA as indicated in the figure legends and tables. Reactions were initiated by the addition of mitochondria. The reaction was linear up to 6 min, and all incubations were performed at 30 °C for 5 min. Reactions were stopped by addition of 6% perchloric acid and centrifuged at 2000 rpm for 7 min. The resulting pellet was

suspended in water, and the product, [^{3}H]palmitoylcarnitine, was extracted with butanol at low pH. After centrifugation at 2000 rpm for 2 min, an aliquot of the butanol phase was transferred to a vial for radioactive counting.

[^{14}C]Malonyl-CoA Binding Assay. [^{14}C]Malonyl-CoA binding was determined by a modified centrifugation assay as described previously (*25*). Isolated mitochondria from wild-type and deletion mutants were suspended in 0.5 mL of ice-cold medium composed of 72 mM sorbitol, 25 mM KCl, and 10 mM HEPES (pH 7.0). This was followed by addition of 0.1−1000 nM [2-^{14}C]malonyl-CoA, and the suspension was incubated at 4 °C for 30 min with periodic vortexing. After 30 min, bound and free malonyl-CoA were separated by centrifugation at 14 000 rpm for 30 min at 4 °C. The mitochondrial pellet was superficially washed twice (without resuspension by slow addition of ice-cold buffer to the wall of the tube) and centrifuged as described above. The pellet was then solubilized in 300 μL of 2% SDS and quantitatively transferred to a vial for radioactive counting. The final malonyl-CoA binding values for the wild-type and deletion mutants were corrected for background malonyl-CoA binding by the yeast control strain which carried the vector but without the CPTI insert. No correction for nonspecific binding was made, as suggested by Mendel and Mendel (*26*). Binding analysis was performed using Graph Pad software and a nonlinear regression procedure. The CPT activity and I_{50} values are given as a mean ± SD for at least three independent assays with different preparations of mitochondria.

Western Blot Analysis. Proteins were separated by SDS−PAGE in a 7.5% gel and transferred onto nitrocellulose membranes. Immunoblots were developed by incubation with the CPTI-specific polyclonal antibody (1:2500 dilution) followed by an anti-rabbit IgG conjugated to horseradish peroxidase as described previously (*3*). The antigen−antibody complex was detected using an ECL-enhanced chemiluminescence detection kit (Amersham, Arlington Heights, IL).

Other Materials and Procedures. DNA sequencing was performed at the Oregon Regional Primate Research Center core facility using an automatic DNA sequencer (*27*). Protein was determined by the Lowry procedure (*28*). All restriction enzymes were from New England Biolabs (Beverly, MA). L-[^{3}H]Carnitine and [2-^{14}C]malonyl-coenzyme A were from Amersham (Arlington Heights, IL). Nucleotides were from Pharmacia (Piscataway, NJ), palmitoyl-CoA was from Boehringer Mannheim (Indianapolis, IN), and malonyl-CoA was from Sigma (St. Louis, MO).

RESULTS

Generation of Deletion Mutants and Expression of Wild-type and Deletion Mutants in P. pastoris. Construction of plasmids carrying the N-terminal deletion mutants of rat L-CPTI was performed as described in Experimental Procedures, and the deletions were confirmed by DNA sequencing. The positions of the N-termini of the L-CPTI deletion mutants are indicated in Figure 1. The deletions range from the smallest, 18, to the largest, 129 amino acid residues. *P. pastoris* was chosen as an expression system for L-CPTI and the deletion mutants because it does not have endogenous CPT activity (*3, 9, 13*). The *P. pastoris* expression plasmids

Table 1: Activity, Malonyl-CoA Sensitivity, and Malonyl-CoA Binding in Yeast Strains Expressing Wild-Type L-CPTI and N-Terminal Deletion Mutants[a]

strain	activity (nmol mg^{-1} min^{-1})	I_{50} (μM)	K_D (nM)	B_{max} (pmol/mg)
wild-type	7.8 ± 0.5[b]	2.0 ± 0.2	1.1	1.9
Δ18	5.3 ± 0.6	380 ± 30	70	2.2
Δ35	5.0 ± 0.2	200 ± 20	240	0.4
Δ52	5.4 ± 0.6	170 ± 20	420	2.6
Δ73	5.1 ± 0.4	180 ± 20	880	3.1
Δ83	5.2 ± 0.8	300 ± 30	no binding	
Δ129	no activity		no binding	

[a] Mitochondria were isolated from the yeast strains separately expressing L-CPTI, and the deletion mutants and were assayed for CPT activity, malonyl-CoA sensitivity, and binding as described in the Experimental Procedures. The I_{50} is the concentration of malonyl-CoA needed to inhibit 50% of the activity of the yeast-expressed L-CPTI, and results are mean ± SD of at least four independent experiments with different mitochondrial preparations. The K_D and B_{max} values are averages of two independent experiments with different mitochondrial preparations. [b] In-intact rat liver mitochondria, values for CPTI activity under similar assay conditions are 1.0−3.0 nm min^{-1} mg^{-1} protein (*24, 25*).

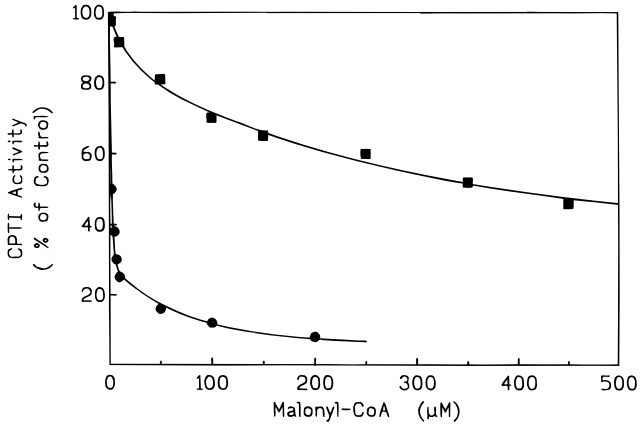

FIGURE 2: Effect of increasing concentrations of malonyl-CoA on the activities of yeast-expressed wild-type L-CPTI and Δ18. (●) wild-type, (■) Δ18. Approximately 150 μg of mitochondrial protein were used for the assay.

expressed L-CPTI under control of the *P. pastoris* glyceraldehyde-3-phosphate dehydrogenase gene promoter (*3, 22*). *P. pastoris* strains transformed with the wild-type L-CPTI gene and the deletion mutants were grown in liquid medium supplemented with glucose. As previously reported (*3*), no CPT activity was found in the control yeast strain with the vector, but without the CPT cDNA insert.

Effect of Deletions on L-CPTI Activity and Malonyl-CoA Sensitivity. All of the deletion mutants except Δ129 retained significant CPT activity, which was 64−69% of that observed with the wild-type yeast strain expressing L-CPTI (Table 1). Δ129 had no CPT activity. In agreement with our previous report (*3*), the I_{50} for malonyl-CoA inhibition of the wild-type strain expressing L-CPTI was 2.0 μM, while the I_{50} for the minimal deletion mutant 18 was 380 μM, representing a 190-fold decrease in malonyl-CoA sensitivity compared to the wild-type strain. Deleting 35, 52, 73, and 83 amino acid residues from the N-terminus increased the I_{50} for malonyl-CoA sensitivity of L-CPTI in each of the mutants from 2.0 μM in the wild-type strain to 170−300 μM in the deletion mutants, thus decreasing the malonyl-CoA sensitivity by 85−150-fold (Table 1). Δ18 showed

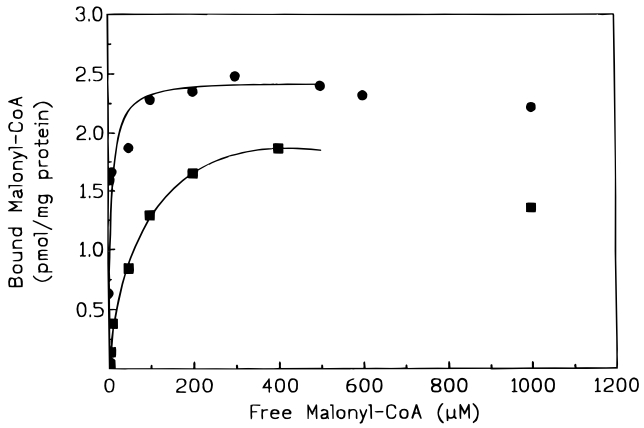

FIGURE 3: Binding of ^{14}C-malonyl-CoA to mitochondria isolated from the yeast strain expressing the wild-type and Δ18. Approximately 240 μg of protein were used for the binding assay. (●) wild-type, (■) Δ18. Malonyl-CoA binding values for the wild-type and deletion mutants were corrected for malonyl-CoA binding to the mitochondria from the yeast strain with the vector but no insert.

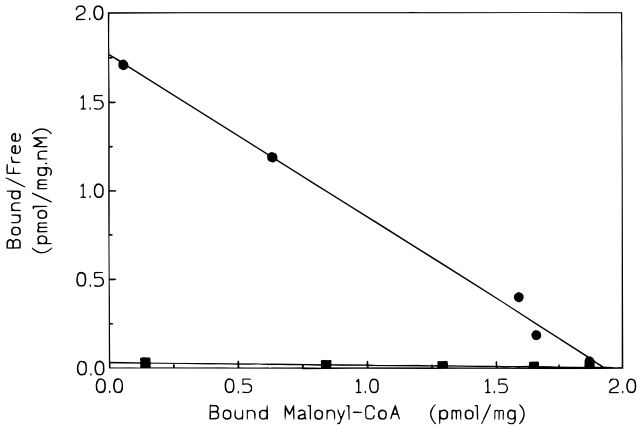

FIGURE 4: Scatchard plots for binding of ^{14}C-malonyl-CoA to mitochondria from yeast strains expressing wild-type (●) and Δ18 (■) for L-CPTI.

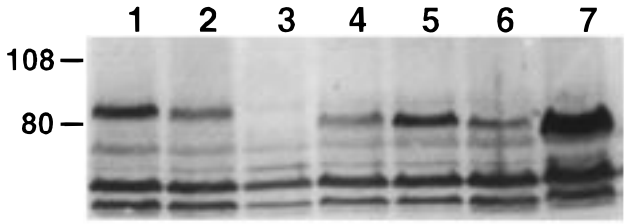

FIGURE 5: Immunoblot showing expression of wild-type and deletion mutant L-CPTIs in the yeast *P. pastoris*. Mitochondria (100 μg protein) from the wild-type yeast strain and the strains expressing each of the deletion mutants were separated on an 8% SDS−PAGE and blotted onto a nitrocellulose membrane; the immunoblot was developed as described in the experimental procedures. Lane 1, wild-type L-CPTI; lanes 2, 3, 4, 5, 6, and 7 represent Δ18, Δ35, Δ52, Δ73, Δ83, and Δ129, respectively.

decreased malonyl-CoA sensitivity at all levels of the inhibitor tested compared to the wild-type, as shown in Figure 2. Except for Δ129, which had no CPT activity (data not shown), this was also true for Δ35, Δ52, Δ73, and Δ83. Δ18 exhibited normal saturation kinetics when the carnitine concentration was varied relative to a constant second substrate, palmitoyl-CoA, a property identical to that of wild-type L-CPTI previously reported from our laboratory (*3*). For Δ18, the calculated K_m for carnitine was 31 μM and the V_{max} was 6.5 nmol min^{-1} mg^{-1} of protein which is similar to the wild-type L-CPTI (*3*). With respect to the second substrate, palmitoyl-CoA, Δ18 showed non-Michaelis−Menten saturation kinetics, characteristics similar to yeast-expressed wild-type L-CPTI (*3*). Thus, deletion of the first 18 N-terminal residues from L-CPTI appears to abolish malonyl-CoA sensitivity of L-CPTI with minimal effect on the catalytic activity of the enzyme.

^{14}C-Malonyl-CoA Binding in Yeast-Expressed L-CPTI and Deletion Mutants. A significant increase in K_D values was observed for all of the deletion mutants compared to the wild-type strain when [^{14}C]malonyl-CoA binding was determined in isolated mitochondria from the yeast strains expressing wild-type and mutant L-CPTI. The K_D value for Δ18 was 70-fold higher (1.1 nM versus 70 nM) than that of the wild-type (Table 1). Δ35, Δ52, Δ73, Δ83, and Δ129 had K_D values of 240−1000-fold higher than that of the wild-type (Table 1). The stepwise increase in K_D values for the observed deletion mutants suggests a direct effect of the deletions on malonyl-CoA binding.

Malonyl-CoA binding to the mitochondria from the yeast strains expressing the wild-type and deletion mutants clearly resolved into a high-affinity site (wild-type) and a low-affinity site (deletion mutants) as shown in Figures 3 and 4, and in both cases was saturable. Our studies also show small increases in B_{max} with an increase in the number of residues deleted, as shown in Table 1, except for Δ35 where a decrease in B_{max} was observed.

Effect of Deletions on Protein Expression. Western blot analysis of wild-type L-CPTI (88 kDa) and the deletion mutants using a polyclonal antibody directed against a maltose-binding protein-L-CPTI fusion protein *(3)* are shown in Figure 5. For Δ18, Δ51, Δ72, and Δ83, proteins of predicted sizes were synthesized, but they had 30% lower activity than the wild-type. Δ35 was barely detectable on the immunoblots, but it had 64% of the activity of the wild-type and was insensitive to malonyl-CoA inhibition. Δ129 was completely inactive, but was synthesized with the predicted size protein, as shown by the immunoblot.

DISCUSSION

Mitochondria from the yeast strain expressing Δ18 had 64% of the activity of the wild-type L-CPTI, but were insensitive to malonyl-CoA inhibition and had very low affinity for malonyl-CoA. Furthermore, the decrease in malonyl-CoA sensitivity (increase in I_{50}) was accompanied by a decrease in the affinity for malonyl-CoA (increase in K_D). No additional loss of malonyl-CoA sensitivity was detectable with increasing deletion size. However, increased loss of binding energy was observed with increasing deletion size. The 70-fold lower affinity seen in Δ18 corresponds to 2.3 kcal/mol in lost binding energy. The increasingly larger deletions exhibited additional, albeit smaller, losses of binding energy (Δ35, +0.7 kcal/mol; Δ52, +0.3 kcal/mol; Δ73, +0.4 kcal/mol). It should be noted that Δ73, when compared to Δ18, shows only 1.4 kcal/mol further reduction in binding energy. These results suggest that, while Δ18 abolishes malonyl-CoA sensitivity, significant affinity (K_D = 70 nM) for malonyl-CoA binding remains. Since the affinity for malonyl-CoA binding decreased incrementally with the number of residues deleted, we suggest that amino

acid residues between 19 and 129 may make weak contacts important for malonyl-CoA binding.

Earlier malonyl-CoA binding studies in isolated liver and heart mitochondria suggested the presence of two classes of malonyl-CoA binding sites, i.e., high- and low-affinity sites in each tissue (*29, 30*). Furthermore, inhibition of CPTI by malonyl CoA was proposed to be mediated by the high-affinity binding sites in both tissues, which were suggested to be separate from the catalytic sites. The possibility of multiple classes of malonyl-CoA binding sites or cooperativity between sites was also raised. A previous attempt to express a mutant L-CPTI that lacked the first 82 N-terminal residues was described by Brown et al. (*31*), but results were inconclusive due to low expression levels (*3*). Interestingly, the residual malonyl-CoA sensitivity shown by the deletion mutants is similar to that observed with yeast-expressed CPTII (*3*), suggesting that, for these mutants, malonyl-CoA inhibits via direct interaction with the active site.

The yeast strains expressing the deletion mutants synthesized proteins of the predicted size as shown by the immunoblot, except for Δ35 which was barely detectable on immunoblots. Δ35 had 64% of the wild-type activity, but the synthesized mutant protein may be unstable and quickly degraded due to improper folding. Δ129 had no measurable CPT activity and had extremely low malonyl-CoA binding, but the predicted size protein was synthesized as indicated by the strong signal on the immunoblot. The synthesized protein was located in the mitochondria of the yeast strain expressing each deletion mutant.

All of the deletion mutants except Δ129 had 65−70% of the activity of the wild-type, indicating that deletion of up to 83 residues from the N-terminus of L-CPTI had minimal effect on palmitoyl-CoA binding as a substrate, thus suggesting the existence of two separate acyl-CoA binding sites, a malonyl-CoA and palmitoyl-CoA binding site. The observed 30−35% loss in activity is likely due to changes in the tertiary structure of the enzyme, caused by lack of interaction of the N-terminal domain with the catalytic C-terminal domain as a result of N-terminal residue deletions or a reduction in the expression level.

The complete loss of CPT activity observed with Δ129 could be due to loss of the palmitoyl-CoA binding site or synthesis of an unstable, improperly folded mutant enzyme that is catalytically inactive. Alternatively, it could be due to change in the interaction of CPTI with the outer mitochondrial membrane as a result of the large deletion that includes the two predicted transmembrane domains (*21, 31*).

The B_{max} for binding of malonyl-CoA to the mitochondria from the yeast strains expressing the deletion mutants showed a slight increase with the number of residues deleted, a pattern similar to that observed in liver outer mitochondrial membranes from fasted and diabetic rats (*32*). We suggest that the small increase in B_{max} observed with the loss of the high-affinity site for malonyl-CoA in the deletion mutants may be due to an increase in the abundance of the low-affinity site. Of the mutants studied, only Δ35 had a lower B_{max} than wild-type L-CPTI. The decrease in B_{max} observed with Δ35 could be due to the extremely low level of synthesis of this mutant protein which was barely detectable on immunoblotting. Our studies suggest that the malonyl-CoA binding site is separate from the catalytic site and that the first conserved 18 N-terminal amino acid residues are critical for malonyl-CoA binding and inhibition.

Deletion of the first 18 N-terminal amino acid residues decreases binding at the high-affinity inhibitor binding site, but has no effect on the catalytic site. Further deletions decrease affinity for malonyl-CoA at the high-affinity malonyl-CoA binding site without further affecting the active site. Thus, deletion of the conserved 18 amino acid residues uncouples the high-affinity malonyl-CoA binding site from the catalytic site. Deletion of the conserved first 18 N-terminal residues of heart M-CPTI, which are identical to those of L-CPTI, also decreased the malonyl-CoA sensitivity of M-CPTI, indicating the same residues play a critical role in sensitizing heart and liver enzymes to malonyl-CoA (unpublished observation). However, the magnitude of the loss in malonyl-CoA sensitivity of M-CPTI, as a result of the 18 residue deletion, was lower than that of liver L-CPTI, suggesting the involvement of unknown additional residues in the high malonyl-CoA sensitivity of heart M-CPTI. Previous studies have demonstrated that mammals contain two isoforms of CPTI, referred to as liver L-CPTI and muscle M-CPTI, that are 62% identical in amino acid sequence (*9*−*12*). Isolated mitochondria from rat heart and yeast strains expressing human heart muscle M-CPTI exhibited a high K_m for carnitine and a low I_{50} for malonyl-CoA (*9, 16*). In adult rat heart, the total carnitine level is high; consequently, M-CPTI has a high K_m for carnitine (*8*). Although rat heart has a measurable level of malonyl-CoA, it is much lower than that of rat liver (*2*). Since the I_{50} for malonyl-CoA inhibition of heart mitochondrial M-CPTI is at least 30-fold lower than that of rat liver L-CPTI, the level of malonyl-CoA reported in heart (*2*) is sufficient to significantly inhibit fatty acid oxidation. It is estimated that about 60−80% of the energy requirement of the heart is derived from fatty acid oxidation (*35*). The important question in cardiac tissue metabolism then is how can fatty acid oxidation proceed in the heart in the presence of such high tissue levels of malonyl-CoA?

REFERENCES

1. Bieber, L. L. (1988) *Annu. Rev. Biochem. 57*, 261−283.
2. McGarry, J. D., Woeltje, K. F., Kuwajima, M., and Foster, D. W. (1989) *Diabetes/Metab. Rev. 5*, 271−284.
3. de Vries, Y., Arvidson, D. N., Waterham, H. R., Cregg, J. M., and Woldegiorgis, G. (1997) *Biochemistry 36*, 5285−5292.
4. Prentki, M., and Corkey, B. E. (1996) *Diabetes 45*, 273−283.
5. Corr, P. B., and Yamada, K. A. (1995) *Herz 20*, 156−168.
6. Weis, B. C., Esser, V., Foster, D. W., and McGarry, J. D. (1994) *J. Biol. Chem. 269*, 18712−18715.
7. Weis, B. C., Cowan, A. T., Brown, N., Foster, D. W., and McGarry, J. D. (1994) *J. Biol. Chem. 269*, 26443−26448.
8. Brown, N. F., Weis, B. C., Husti, J. E., Foster, D. W., and McGarry, J. D. (1995) *J. Biol. Chem. 270*, 8952−8957.
9. Zhu, H., Shi, J., de Vries, Y., Arvidson, D. N., Cregg, J. M., and Woldegiorgis, G. (1997) *Arch. Biochem. Biophys. 347*, 53−61.
10. Yamazaki, N., Shinhara, Y., Shima, A., and Terada, H. (1995) *FEBS Lett. 363*, 41−45.
11. Adams, M. D., Kerlavage, A. R., Fuldner, R. A., Philips, C. A., and Venter, J. C. (1996) Genbank Accession No. U62317.
12. Yamazaki, N., Shinhara, Y., Shima, A., Yamanaka, Y., and Terada, H. (1996) *Biochim. Biophys. Acta 1307*, 157−161.
13. Zhu, H., Shi, J., Cregg, J. M., and Woldegiorgis, G. (1997) *Biochem. Biophys. Res. Commun. 239*, 498−502.
14. Woldegiorgis, G., Fibich, B., Contreras, L., and Shrago, E. (1992) *Arch. Biochem. Biophys. 295*, 348−351.

15. Chung, C. H., Woldegiorgis, G., Dai, G., Shrago, E., and Bieber, L. L. (1992) *Biochemistry 31*, 9777−9783.
16. McGarry, J. D., and Brown, N. F. (1997) *Eur. J. Biochem. 244*, 1−14.
17. Britton, C. H., Schultz, R. A., Zhang, B., Esser, V., Foster, D. W., and McGarry, J. D. (1995) *Proc. Natl. Acad. Sci. U.S.A. 92*, 1984−1988.
18. Esser, V., Britton, C. H., Weis, B. C., Foster, D. W., and McGarry, J. D. (1993) *J. Biol. Chem. 268*, 5817−5822.
19. Woeltje, K. F., Esser, V., Weis, B. C., Sen, A., Cox, W. F., McPhaul, M. J., Slaughter, C. A., Foster, D. W., and McGarry, J. D. (1990) *J. Biol. Chem. 265*, 10720−10725.
20. Finocchiaro, G., Taroni, F., Rocchi, M., Martin, A. L., Colombo, I., Tarelli, G. T., and DiDonato, S. (1991) *Proc. Natl. Acad. Sci. U.S.A. 88*, 661−665.
21. Fraser, F., Corstorphine, C. G., and Zammit, V. A. (1997) *Biochem. J. 323*, 711−718.
22. Waterham, H. R., Digan, M. E., Koutz, P. J., Lair, S. V., and Cregg, J. M. (1997) *Gene 16*, 37−44.
23. Becker, D. M., and Guarente, L. (1991) *Methods Enzymol. 194*, 182−187.
24. Bremer, J., Woldegiorgis, G., Schalinske, K., and Shrago, E. (1985) *Biochim. Biophys. Acta 833*, 9−16.
25. Lund, H., and Woldegiorgis, G. (1987) *Biochim. Biophys. Acta 878*, 243−249.
26. Mendel, C. M., and Mendel, D. B. (1985) *Biochem. J. 228*, 269−272.
27. Sanger, F., Nicklen, S., and Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 5463−5467.
28. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) *J. Biol. Chem. 193*, 265−275.
29. Bird, M. I., and Saggerson, E. D. (1984) *Biochem. J. 222*, 639−647.
30. Mills, S. E., Foster, D. W., and McGarry, J. D. (1983) *Biochem. J. 219*, 601−608.
31. Brown, N. F., Esser, V., Foster, D. W., and McGarry, J. D. (1994) *J. Biol. Chem. 269*, 26438−26442.
32. Ghadiminejad, I., and Saggerson, E. D. (1991) *Biochim. Biophys. Acta 1083*, 166−172.
33. Whitmer, J. T., Idell-Wenger, J. A., Rovetto, M. J., and Neely, J. R. (1978) *J. Biol. Chem. 253*, 4305−4309.

BI9803426