A Single Amino Acid Change (Substitution of Glutamate 3 with Alanine) in the N-terminal Region of Rat Liver Carnitine Palmitoyltransferase I Abolishes Malonyl-CoA Inhibition and High Affinity Binding*

Jianying Shi, Hongfa Zhu, Dennis N. Arvidson, and Gebre Woldegiorgis‡
From the Department of Biochemistry and Molecular Biology, Oregon Graduate Institute of Science and Technology, Portland, Oregon 97291-1000

We have recently shown by deletion mutation analysis that the conserved first 18 N-terminal amino acid residues of rat liver carnitine palmitoyltransferase I (L-CPTI) are essential for malonyl-CoA inhibition and binding (Shi, J., Zhu, H., Arvidson, D. N., Cregg, J. M., and Woldegiorgis, G. (1998) Biochemistry 37, 11033–11038). To identify specific residue(s) involved in malonyl-CoA binding and inhibition of L-CPTI, we constructed two more deletion mutants, Δ12 and Δ6, and three substitution mutations within the conserved first six amino acid residues. Mutant L-CPTI, lacking either the first six N-terminal amino acid residues or with a change of glutamic acid 3 to alanine, was expressed at steady-state levels similar to wild type and had near wild type catalytic activity. However, malonyl-CoA inhibition of these mutant enzymes was reduced 100-fold, and high affinity malonyl-CoA binding was lost. A mutant L-CPTI with a change of histidine 5 to alanine caused only partial loss of malonyl-CoA inhibition, whereas a mutant L-CPTI with a change of glutamate 6 to alanine had wild type properties. These results demonstrate that glutamic acid 3 and histidine 5 are necessary for malonyl-CoA binding and inhibition of L-CPTI by malonyl-CoA but are not required for catalysis.

Carnitine palmitoyltransferase I (CPTI)

Carnitine palmitoyltransferase I (CPTI) catalyzes the conversion of long chain acyl-CoA to acylcarnitines in the presence of L-carnitine, the first reaction in the transport of long chain fatty acids from the cytoplasm to the mitochondria, a rate-limiting step in β-oxidation (1, 2). 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 (GenBank accession number U62317; Refs. 3–7 and 9). As an enzyme that catalyzes the first rate-limiting step in fatty acid oxidation, CPTI is regulated by its physiological inhibitor malonyl-CoA (1, 2), the first intermediate in fatty acid synthesis, suggesting coordinated control of fatty acid oxidation and synthesis. 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 (10) and in myocardial ischemia where accumulation of acylcarnitines has been associated with arrhythmias (11).

We 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 (6, 12, 13). Using this system, we demonstrated conclusively that L-CPTI and M-CPTI are active, distinct, malonyl-CoA-sensitive CPTI isoforms that are reversibly inactivated by detergents. We recently showed that deletion of the conserved first 18 N-terminal amino acid residues of rat L-CPTI abolishes malonyl-CoA inhibition and high affinity malonyl-CoA binding (14). In this study, we have constructed and characterized rat L-CPTI deletion mutants of the first 12 and 6 N-terminal amino acid residues. To identify specific residue(s) involved in malonyl-CoA binding and inhibition of L-CPTI, we also constructed three substitution mutations within the conserved first 6 N-terminal amino acid residues (Glu3 → Ala, His5 → Ala, and Gln6 → Ala).

EXPERIMENTAL PROCEDURES

Construction of Plasmids for the N-terminal Point and Deletion Mutants of Rat L-CPTI—The cDNA used and the construction of the plasmids for the wild type, deletion, and point mutants for rat L-CPTI expression in P. pastoris was as described in our previous publications (12, 14). A HindIII-KpnI fragment (1–566 base pair; cDNA sequence; Refs. 12 and 15) was excised from pYGW9, a plasmid containing the full-length rat L-CPTI in pUC119 to generate the plasmid pYGW12. The Δ12 mutant was constructed by polymerase chain reaction amplification of a 662-base pair HindIII-EcoRI fragment using the plasmid pYGW9 as a template with the primers RLD65 (5′-CCACCAAGATTTAGGCT-3′) and RLD12 (5′-CAAGCTAAGTTCAATGACTGTCAACCCCAGATGGCAT-3′). HindIII and EcoRI enzyme restriction sites were introduced in primer RLD12. An ATG start codon (shown in bold) was added immediately after the EcoRI site and before the fourth amino acid glutamine. The polymerase chain reaction product was digested with HindIII and KpnI and then ligated into pYGW12 to generate plasmid pYGWD12. An EcoRI fragment of pYGWD12 containing the mutant Δ12 was then ligated into the EcoRI-cut P. pastoris expression vector pHW101 (12, 16). The DNA sequences of the deletion and point mutants were confirmed by sequencing.

Mutants Δ6 and Δ18 and point mutants Glu3 → Ala, His5 → Ala, and Gln6 → Ala were constructed in a similar manner as Δ12, using primer RL665 above and the following primers for each deletion and point mutant. The new translation start site or mutated amino acid codon is shown in bold: (RLD6, 5′-GGATCAAGCTTTTGATTTCCGCTCCATCTTCGCT-3′; RLD12, 5′-CTCTAAAGCAGTTCCGATGTGAGTCACCTCGGCTGAGC-3′; RLE3A, 5′-CCACCAAGTTCAATGACTGTCAACCCCAGATGGCAT-3′; RLE3B, 5′-CCACCAAGTTCAATGACTGTCAACCCCAGATGGCAT-3′; RLE3A, 5′-CCACCAAGTTCAATGACTGTCAACCCCAGATGGCAT-3′; and RLE7A, 5′-CCACCAAGTTCAATGACTGTCAACCCCAGATGGCAT-3′). All subsequent procedures were identical to those used elsewhere.

* This work was supported by Grant HL52571 from the National Institutes of Health (to G. W.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ To whom correspondence should be addressed: Dept. of Biochemistry and Molecular Biology, Oregon Graduate Inst. of Science and Technology, P.O. Box 91000, Portland, OR 97291-1000. Tel.: 503-748-1676; Fax: 503-748-1464; E-mail: gwoldeg@bmb.ogi.edu.

1 The abbreviations used are: CPTI, carnitine palmitoyltransferase I; L-CPTI, rat liver isoform of CPTI; M-CPTI, heart/skeletal isoform of CPTI.
for construction of Δ12.

The expression plasmids were linearized and integrated into the HIS4 locus of P. pastoris strain GS115 by electroporation (17). Histidine prototrophic transformants were selected on YND plates and grown on YND medium. Mitochondria were isolated from the wild type and mutant L-CPTIs as described previously (12).

CPT Assay—CPT activity was assayed by the forward exchange method using L-[3H]carnitine as described previously (12, 18). The Kₘ for palmitoyl-CoA was determined by varying the palmitoyl-CoA concentration in the presence of a fixed albumin concentration (1%) or a fixed molar ratio (6:1) of palmitoyl-CoA/albumin (19, 20). The Kₘ for malonyl-CoA inhibition of the yeast-expressed wild type and mutant (E3A) L-CPTIs was determined by assaying CPT activity with varying concentrations of palmitoyl-CoA (12.5, 25, 50, 75, and 100 μM) in the presence of 0, 1, 2, and 5 μM malonyl-CoA (wild type), or 0, 2, 25, and 200 μM malonyl-CoA (E3A), respectively. The concentration of the second substrate, carnitine, was fixed at 200 μM in all the assays. 106 ng (wild type) and 118 μg (E3A) of mitochondrial protein were used, and all incubations were performed at 30 °C for 3 min.

14C/Malonoyl-CoA Binding Assay—[14C]Malonyl-CoA binding was determined by a modified centrifugation assay as described previously (14, 21). Isolated mitochondria from wild type and mutants were suspended in 0.5 ml of ice-cold medium composed of 72 mM sorbitol, 60 mM KCl, 25 mM Tris/HCl (pH 6.8), 1.0 mM EDTA, 1.0 mM dithiothreitol, and 1.3 mg/ml fatty acid-free bovine serum albumin (22). This was followed by addition of 0.1-1000 nm [2-14C]malonyl-CoA, and the suspension was incubated at 4 °C for 30 min with periodic vortexing. All subsequent procedures were as described previously (14). The C₅₀ values are given as the means ± S.D. for at least three independent assays with different preparations of mitochondria. The Kᵦ values are averages of at least two independent experiments.

Western Blot Analysis—Proteins were separated by SDS-polyacrylamide gel electrophoresis in a 7.5% gel and transferred onto nitrocellulose membranes. Immunoblots were developed by incubation with the CPTI-specific polyclonal antibody (1:4000 dilution) followed by an anti-rabbit IgG conjugated to horseradish peroxidase (1:10,000 dilution) as described previously (12). The antigen-antibody complex was detected using an ECL-enhanced chemiluminescence detection kit (Amersham Pharmacia Biotech).

Other Materials and Procedures—DNA sequencing was performed at the Oregon Regional Primate Research Center core facility using an automated DNA sequencer (23). Protein was determined by the Lowry procedure (24). All restriction enzymes were from New England Biolabs (Beverly, MA). L-[3H]Carnitine and [2-14C]malonyl-coenzyme A were from Amersham Pharmacia Biotech. Nucleotides were from Amersham Pharmacia Biotech, palmitoyl-CoA was from Boehringer Mannheim, and malonyl-CoA was from Sigma.

RESULTS

Generation of Deletion and Point Mutants and Expression in P. pastoris—Construction of plasmids carrying the N-terminal deletions Δ6 and Δ12 and point mutants Glu³ → Ala, His⁶ → Ala, and Gln⁶ → Ala of rat L-CPTI (Fig. 1) was performed as described under "Experimental Procedures." Lane 1, wild type L-CPTI; lanes 2–6, Δ12, Δ6, Glu³ → Ala, His⁶ → Ala, and Gln⁶ → Ala, respectively.

FIG. 2. Immunoblot showing expression of wild type, deletion, and point mutant L-CPTIs in the yeast P. pastoris. Mitochondria (40 μg of protein) from the wild type yeast strain and the strains expressing each of the deletion and the point mutants were separated on a 7.5% SDS-polyacrylamide gel electrophoresis and blotted onto a nitrocellulose membrane; the immunoblot was developed as described under "Experimental Procedures." Lane 1, wild type L-CPTI; lanes 2–6, Δ12, Δ6, Glu³ → Ala, His⁶ → Ala, and Gln⁶ → Ala, respectively.

FIG. 1. The amino acid sequence of the first 25 N-terminal residues of rat L-CPTI. The position of each of the deletion and point mutants is shown by an arrow. Sources of the sequences from the data bank were from Refs. 12 and 15 as indicated in the text.

Western blot analysis of wild type L-CPTI (88 kDa) and the mutants using a polyclonal antibody directed against a maltose-binding protein-L-CPTI fusion protein (12) is shown in Fig. 2. For the wild type and all the deletion and point mutants (Δ18, Δ12, Δ6, Glu³ → Ala, His⁶ → Ala, and Gln⁶ → Ala), proteins of predicted sizes were synthesized and were expressed at similar steady-state levels.

Effect of Mutations on L-CPTI Activity and Malonyl-CoA Inhibition—All of the mutants retained significant CPT activity, which was 60–80% of that observed with the wild type yeast strain expressing L-CPTI (Table I). The IC₅₀ for malonyl-CoA inhibition of the wild type strain expressing L-CPTI was 2.0 μM, in agreement with our previous report (12), whereas the IC₅₀ for Δ6, Δ12, and the point mutant Glu³ → Ala was 200 μM, representing a 100-fold decrease in malonyl-CoA sensitivity compared with the wild type strain (Table I). The Kₘ for malonyl-CoA inhibition of the Glu³ → Ala mutant L-CPTI was approximately 10-fold higher than that of the wild type (32 μM versus 2.7 μM), a trend similar to the increase in IC₅₀ for malonyl-CoA inhibition observed in the Glu³ → Ala mutant. Mutation of histidine residue 5 to alanine increased the IC₅₀ for malonyl-CoA inhibition to 25 μM compared with the 2.0 μM value of the wild type strain, representing a mild 12.5-fold decrease in malonyl-CoA sensitivity, whereas mutation of glutamine residue 6 to alanine had no effect on malonyl-CoA sensitivity (Table I).

Glu³ in L-CPTI Is Essential for Malonyl-CoA Inhibition

The expression plasmids were linearized and integrated into the HIS4 locus of P. pastoris strain GS115 by electroporation (17). Histidine prototrophic transformants were selected on YND plates and grown on YND medium. Mitochondria were isolated from the wild type and mutant L-CPTIs as described previously (12).
Mitochondria were isolated from the yeast strains separately expressing L-CPTI, and the deletion and point mutants were assayed for CPT activity, malonyl-CoA sensitivity, and binding, as described under "Experimental Procedures." The IC₅₀ is the concentration of malonyl-CoA needed to inhibit 50% of the activity of the yeast-expressed L-CPTI, and the results are the means ± S.D. of at least three independent experiments with different mitochondrial preparations. The IC₅₀ values are averages of two independent experiments with different mitochondrial preparations.

Table I

| Strain     | Activity (nmol/mg-min) | IC₅₀ (µM) | Kᵢ₁ (nM) | Kᵢ₂ (nM) | Bₘ₃₃ (pmol/mg) | Bₘ₃₄ (pmol/mg) |
|------------|------------------------|-----------|----------|----------|----------------|----------------|
| Wild type  | 7.8 ± 0.5              | 2.0 ± 0.2 | 1.0      | 33       | 10             | 110            |
| L-CPTI     |                        |           |          |          |                |                |
| Mutants    |                        |           |          |          |                |                |
| Q6A        | 5.5 ± 0.5              | 2.0 ± 0.2 | 102      | 500      | 15.9           | 33.6           |
| H6A        | 5.1 ± 0.5              | 25 ± 3    | 3.7 × 10²| 103      |                |                |
| E6A        | 5.5 ± 0.5              | 200 ± 20  | 6.0 × 10²| 113      |                |                |
| ∆6         | 4.7 ± 0.7              | 197 ± 20  |          |          |                |                |
| Δ12        | 4.7 ± 0.7              | 210 ± 20  |          |          |                |                |

*In intact rat liver mitochondria, values for CPTI activity under similar assay conditions are 1.0–3.0 nmol/min/mg protein (18, 21).

**Fig. 3.** Effect of increasing concentrations of malonyl-CoA on the activities of yeast-expressed wild type and mutant L-CPTI. Approximately 150 µg of mitochondrial protein were used for the assay. ●, wild type; ■, ∆6; ▲, Glu³ → Ala; ●, His⁵ → Ala.

[¹⁴C]Malonyl-CoA Binding in Yeast-expressed Mutant L-CPTIs—Malonyl-CoA binding to the mitochondria from the yeast strains expressing ∆6 and point mutants Glu³ → Ala and His⁵ → Ala was significantly lower compared with that observed in the mitochondria from the wild type strain but was saturable (Fig. 5). Malonyl-CoA binding clearly resolved into a high affinity and a low affinity site in the mitochondria from the wild type and point mutant His⁵ → Ala as shown by the Scatchard plots in Fig. 6 (A and B), but only very low affinity binding was observed in the mitochondria from point mutant Glu³ → Ala and ∆6 (Fig. 6B). Deletion of the first 6 N-terminal residues or substitution of glutamic acid 3 with alanine completely abolished high affinity malonyl-CoA binding (Kᵢ₁) and further decreased the low affinity malonyl-CoA binding (Kᵢ₂) by 100-fold (Table I). Furthermore, the loss of high affinity malonyl-CoA binding observed with the Glu³ → Ala mutant correlates with the increase in IC₅₀ and Kᵢ for inhibition of L-CPTI by malonyl-CoA. Although the mutations increased the Kᵢ₂ for the low affinity malonyl-CoA-binding site (∆6 and Glu³ → Ala), there was no change in the calculated Bₘ₃₃ for the wild type, suggesting that the observed loss in malonyl-CoA sensitivity and binding is not due to decreased abundance of the second malonyl-CoA-binding entity of L-CPTI.

Substitution of histidine 5 with alanine showed a moderate loss in malonyl-CoA sensitivity of L-CPTI (12.5-fold), resulting in loss of the high affinity and a significant decrease in low affinity malonyl-CoA binding (Table I). The low affinity binding site in mutant His⁵ → Ala resolved into two classes of binding sites (Fig. 6B), with the Kᵢ₁ and Kᵢ₂ for the mutant being 100- and 16-fold higher, respectively, than the corresponding wild type values (Table I). The calculated Bₘ₃₃ for mutant His⁵ → Ala was close to the wild type value, but Bₘ₃₄ was 3-fold lower, indicating a decrease in the malonyl-CoA-binding entity of L-CPTI. These studies suggest that histidine 5 in L-CPTI is one of the residues involved in malonyl-CoA binding and inhibition of the enzyme.

**DISCUSSION**

To define the role of the first 130 N-terminal amino acid residues of rat L-CPTI in malonyl-CoA sensitivity and binding, we previously constructed a series of deletion mutants and demonstrated that a mutant lacking the first conserved 18 N-terminal amino acid residues had activity and kinetic properties similar to those of wild type L-CPTI but had completely lost malonyl-CoA sensitivity and high affinity binding (14). Based on these previous studies (14), we report here on deletion mutations of the conserved first 12 and 6 N-terminal residues of L-CPTI. Like ∆18, ∆12 and ∆6 had 60–70% of the wild type activity and showed loss of both malonyl-CoA sensitivity and high affinity malonyl-CoA binding, indicating that residue(s) essential for malonyl-CoA binding and sensitivity reside within the conserved first 6 N-terminal amino acids. Of these conserved first 6 N-terminal amino acids, including the start codon Met, residues 2 and 4 are Ala, residue 3 is Glu, residue 5 is His, and residue 6 is Gln. Therefore, we constructed mutants with substitutions of Glu³ with Ala, His⁵ with Ala, and Gln⁶ with Ala of L-CPTI.

The mutant L-CPTI with a replacement of Glu³ with Ala had a phenotype similar to that of the N-terminal deletion mutants. The mutation resulted in complete loss of malonyl-CoA sensitivity and high affinity malonyl-CoA binding and a decrease in the low affinity malonyl-CoA binding. In contrast, substitution of Glu³ with Ala did not have a significant effect on the kinetic properties of the enzyme, because there was no change in the Kᵢ₃₃ value for palmitoyl-CoA and only a slight increase in the Kᵢ₄₃ value for carnitine. The 29–40% loss in catalytic activity observed with the deletion and point mutants compared with the wild type could be due to a reduction in the expression level or lack of interaction of the N-terminal domain with the catalytic domain as a result of the N-terminal mutations. A protein of the expected size (88 kDa) was detected in the mitochondria of the Glu³ → Ala mutant strain on immunoblotting with L-CPTI specific antibodies. These results demonstrate clearly that Glu³ in the wild type L-CPTI is essential for malonyl-CoA inhibition and binding but not for catalysis, because the kinetic properties of the mutant enzyme are virtually indistinguishable from those of the wild type. This is the first report to demonstrate...
the critical role of Glu\textsuperscript{3} residue of L-CPTI for malonyl-CoA sensitivity and binding.

The high affinity site (\(K_{D1}, B_{max1}\)) for binding of malonyl-CoA to L-CPTI was completely abolished in the Glu\textsuperscript{3} → Ala, Δ6, and Δ18 mutants, suggesting that the >100-fold decrease in malonyl-CoA sensitivity observed in these mutants was due to the loss of the high affinity binding entity of the enzyme. Although low affinity malonyl-CoA binding was weakened, there was no change in the \(B_{max2}\) value between wild type L-CPTI and mutants Glu\textsuperscript{3} → Ala, Δ6, and Δ18, suggesting that the residual malonyl-CoA sensitivity observed in the mutants was due to the low affinity malonyl-CoA-binding entity of the enzyme. The results of this study provide strong evidence implicating Glu\textsuperscript{3} as one of the residues involved in high affinity malonyl-CoA binding. We hypothesize that the Glu\textsuperscript{3} → Ala substitution may disrupt a hydrogen bonding network or a salt bridge, perhaps to a residue near the active site of CPTI. As the high affinity site is abolished and binding to the low affinity site is weakened, the two sites may partially overlap. Alternatively, the possible loss of a salt bridge may weaken \(K_{D2}\) indirectly.

Replacement of His\textsuperscript{5} with Ala had a much less drastic effect on the IC\textsubscript{50} for malonyl-CoA inhibition of L-CPTI but severely diminished both high and low affinity malonyl-CoA binding. The \(B_{max1}\) for this mutant showed a slight increase, but \(B_{max2}\) showed a significant decrease compared with the wild type value, suggesting that the 10-fold lower IC\textsubscript{50} for malonyl-CoA inhibition observed with this mutant, compared with mutants Glu\textsuperscript{3} → Ala, Δ6, and Δ18, may be due to a slight increase in abundance of the high affinity binding entity with a lowered (100-fold) affinity for malonyl-CoA. The decrease in low affinity malonyl-CoA binding observed for the His\textsuperscript{5} → Ala mutant (\(\sim15\)-fold increase in \(K_{D2}\)) may be due, in part, to the decreased abundance of the low affinity binding entity of the enzyme (\(\sim3\)-fold decrease in \(B_{max2}\)). Because mutation of His\textsuperscript{5} → Ala reduced the malonyl-CoA sensitivity and binding, L-CPTI may be affected by pH. A pH-induced shift in malonyl-CoA sensitivity has been reported for CPTI (25, 26).

Our data clearly demonstrate that there are two classes of malonyl-CoA-binding sites in L-CPTI, namely, a high affinity and a low affinity binding site, similar to earlier studies in isolated rat liver and heart mitochondria (26, 27). A previous attempt to express a mutant L-CPTI that lacked the first 82 N-terminal residues was described by Brown \textit{et al.} (28), but results were inconclusive due to extremely low expression levels (12). The residual malonyl-CoA sensitivity shown by the deletion mutants is similar to that observed with yeast-expressed CPTII (12), suggesting that for these mutants malonyl-CoA may inhibit via direct interaction with the active site. Additional studies are needed to determine whether the active site acts as a low affinity malonyl-CoA-binding site, but our data suggest that there may be some overlap between the malonyl-CoA and palmitoyl-CoA binding sites. In the absence of malonyl-CoA, free CoA (50 \(\mu\)M) and acetyl-CoA (500 \(\mu\)M) inhibited the activities of both the wild type and the Glu\textsuperscript{3} → Ala
mutant L-CPTI by 50%. Because a total loss of the high affinity malonyl-CoA binding site was observed in the Glu<sup>3</sup>→Ala mutant, the results suggest that CoA and acetyl-CoA inhibit by binding to the active site or the low affinity malonyl-CoA-binding site. At high concentrations, both CoA and the substrate palmitoyl-CoA reduce the inhibition of L-CPTI by malonyl-CoA (18, 29), suggesting partial overlap between the malonyl-CoA and the substrate binding sites.

Based on limited proteolysis studies of intact and outer membrane rat liver mitochondria, a model for the membrane topology of L-CPTI has been proposed that predicts exposure of 90% of L-CPTI, including N and C termini domains crucial for activity and malonyl-CoA sensitivity of the enzyme on the cytosolic side of the outer mitochondrial membrane (30). A more recent detailed deletion mutation analysis study of the 129 N-terminal amino acid residues of the yeast-expressed L-CPTI from our laboratory clearly demonstrated that residues critical for malonyl-CoA inhibition and binding of L-CPTI are located within the conserved first 18 N-terminal amino acid residues of the enzyme (14). In this study, we demonstrate that glutamic acid residue 3 and histidine 5 are essential for malonyl-CoA binding and inhibition.

Limited proteolysis of intact and outer membrane preparations of rat liver mitochondria result in a marked loss in L-CPTI activity and malonyl-CoA sensitivity (30, 31), accompanied by the cleavage of the extreme N terminus (<1 kDa) of L-CPTI (30). Mitochondria isolated from fasted and diabetic rat livers, metabolic conditions with increased fatty acid oxidation, exhibit increased L-CPTI activity and decreased malonyl-CoA sensitivity (32). Furthermore, insulin reverses the effects of diabetes on L-CPTI activity and malonyl-CoA sensitivity (32). Thus, fasting and diabetes, metabolic conditions that enhance protein degradation, reduce the sensitivity of CPTI to malonyl-CoA inhibition (32–34). The L-CPTI gene in INS-1 cells may be an early response gene like c-fos (35), suggesting that the enzyme may be subject to metabolic regulation by proteolysis (36), employing the cytosolic ubiquitin-proteasome system (36). Diabetes is a pathophysiologic condition associated with increased protein degradation, fatty acid oxidation, CPTI activity, and decreased malonyl-CoA inhibition of CPTI (33). Thus limited in vivo proteolysis of L-CPTI induced by diabetes, such as cleavage of the first 6 N-terminal residues, may decrease malonyl-CoA sensitivity and alter the normal control of hepatic fatty acid oxidation. Insulin inhibits proteasome activity resulting in decreased cellular protein degradation and con-

---

<sup>2</sup>J. Shi, H. Zhu, D. N. Arvidson, and G. Woldegiorgis, unpublished observation.
trolled fatty acid oxidation (8). We are currently conducting in vitro partial proteolysis studies with yeast-expressed L-CPTI to determine the role of protein degradation on malonyl-CoA sensitivity.

Acknowledgment—We are grateful to Dr. James M. Cregg (Oregon Graduate Institute of Science and Technology) for advice, helpful suggestions, and encouragement throughout these studies.

REFERENCES
1. Bieber, L. L. (1988) Annu. Rev. Biochem. 57, 261–283
2. McGarry, J. D., Wielage, K. F., Kawajima, M., and Foster, D. W. (1989) Diabetes Metab. Rev. 3, 271–284
3. Weis, B. C., Esser, V., Foster, D. W., and McGarry, J. D. (1994) J. Biol. Chem. 269, 18712–18715
4. Weis, B. C., Cowan, A. T., Brown, N., Foster, D. W., and McGarry, J. D. (1994) J. Biol. Chem. 269, 26443–26448
5. Brown, N. F., Weis, B. C., Husti, J. E., Foster, D. W., and McGarry, J. D. (1995) J. Biol. Chem. 270, 8952–8957
6. Zhu, H., Shi, J., de Vries, Y., Arvidson, D. N., Cregg, J. M., and Woldegiorgis, G. (1997) Arch. Biochem. Biophys. 347, 53–61
7. Yamazaki, N., Shinhara, Y., Shima, A., and Terada, H. (1995) FEBS Lett. 363, 41–45
8. Hamel, F. G., Bennett, R. G., Harmon, K. S., and Duckworth, W. C. (1997) Biochem. Biophys. Res. Commun. 234, 671–674
9. Yamazaki, N., Shinhara, Y., Shima, A., Yamazaki, Y., and Terada, H. (1996) Biochim. Biophys. Acta 1307, 157–161
10. Prentki, M., and Corkey, B. E. (1996) Diabetes 45, 273–283
11. Corr, P. B., and Yamada, K. A. (1995) Herz 20, 156–168
12. de Vries, Y., Arvidson, D. N., Waterham, H. R., Cregg, J. M., and Woldegiorgis, G. (1997) Biochemistry 36, 5285–5292
13. Zhu, H., Shi, J., Cregg, J. M., and Woldegiorgis, G. (1997) Biochem. Biophys. Res. Commun. 239, 498–502
14. Shi, J., Zhu, H., Arvidson, D. N., Cregg, J. M., and Woldegiorgis, G. (1998) Biochemistry 37, 11033–11038
15. Esser, V., Britton, C. H., Weis, B. C., Foster, D. W., and McGarry, J. D. (1993) J. Biol. Chem. 268, 5817–5822
16. Waterham, H. R., Digan, M. E., Koutz, P. J., Lair, S. V., and Cregg, J. M. (1997) Gene (Amst.) 16, 37–44
17. Becker, D. M., and Guarente, L. (1991) Methods Enzymol. 194, 182–187
18. Bremer, J., Woldegiorgis, G., Schalinske, K., and Shrago, E. (1985) Biochim. Biophys. Acta 833, 9–16
19. Pauly, D. F., and McMillin, J. B. (1988) J. Biol. Chem. 263, 18160–18167
20. Prip-Buus, C., Cohen, I., Kohl, C., Esser, V., McGarry, J. D., and Girard, J. (1998) FEBS Lett. 429, 173–178
21. Lund, H., and Woldegiorgis, G. (1987) Biochim. Biophys. Acta 876, 243–249
22. Ghadiminejad, I., and Saggerson, E. D. (1991) Biochim. Biophys. Acta 1083, 166–172
23. Sanger, F., Nicklen, S., and Coulson, A. R. (1977) Proc. Natl. Acad. Sci. U. S. A. 74, 5463–5467
24. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275
25. Stephens, T. W., Cook, G. A., and Harris, R. A. (1983) Biochem. J. 212, 521–524
26. Mills, S. E., Foster, D. W., and McGarry, J. D. (1984) Biochem. J. 219, 601–608
27. Bird, M. I., and Saggerson, E. D. (1984) Biochem. J. 222, 639–647
28. Brown, N. F., Esser, V., Foster, D. W., and McGarry, J. D. (1994) J. Biol. Chem. 269, 26438–26442
29. Cook, G. A., Mynatt, R. L., and Kashfi, K. (1994) J. Biol. Chem. 269, 8803–8807
30. Fraser, F., Corstorphine, C. G., and Zammit, V. A. (1997) Biochem. J. 323, 711–718
31. Kashfi, K., Mynatt, R. L., and Cook, G. A. (1994) Biochim. Biophys. Acta 1212, 245–252
32. Cook, G. A., and Gamble, M. S. (1987) J. Biol. Chem. 262, 2050–2055
33. Park, E. A., Mynatt, R. L., Cook, G. A., and Kashfi, K. (1995) Biochem. J. 310, 853–858
34. Kashfi, K., Cagen, L., and Cook, G. A. (1995) Lipids 30, 383–388
35. Assimacopoulos-Jeannet, F., Thumelin, S., Roche, E., Esser, V., McGarry, J. D., and Prentki, M. (1997) J. Biol. Chem. 272, 1659–1664
36. Varshavsky, A. (1997) Genes Cells 2, 13–28