Carnitine palmitoyltransferase (CPT) I catalyzes the conversion of long-chain fatty acyl-CoAs to acyl carnitines in the presence of l-carnitine, a rate-limiting step in the transport of long-chain fatty acids from the cytoplasm to the mitochondrial matrix. To determine the role of the 15 cysteine residues in the heart/skeletal muscle isoform of CPTI (M-CPTI) on catalytic activity and malonyl-CoA sensitivity, we constructed a 6-residue N-terminal, a 9-residue C-terminal, and a 15-residue cysteineless M-CPTI by cysteine-scanning mutagenesis. Both the 9-residue C-terminal mutant enzyme and the complete 15-residue cysteineless mutant enzyme are inactive, but the 6-residue N-terminal cysteineless mutant enzyme had activity and malonyl-CoA sensitivity similar to those of wild-type M-CPTI. Mutation of each of the 9 C-terminal cysteines to alanine or serine identified a single residue, Cys-305, to be important for catalysis. Substitution of Cys-305 with Ala in the wild-type enzyme inactivated M-CPTI, and a single change of Ala-305 to Cys in the 9-residue C-terminal cysteineless mutant resulted in an 8-residue C-terminal cysteineless mutant enzyme that had activity and malonyl-CoA sensitivity similar to those of the wild type, suggesting that Cys-305 is the residue involved in catalysis. Sequence alignments of CPTI with the acyltransferase family of enzymes in the GenBank™ led to the identification of a putative catalytic triad in CPTI consisting of residues Cys-305, Asp-454, and His-473. Based on the mutagenesis and substrate labeling studies, we propose a mechanism for the acyltransferase activity of CPTI that uses a catalytic triad composed of Cys-305, His-473, and Asp-454 with Cys-305 serving as a probable nucleophile, thus acting as a site for covalent attachment of the acyl molecule and formation of a stable acyl-enzyme intermediate. This would in turn allow carnitine to act as a second nucleophile and complete the acyl transfer reaction.

Carnitine palmitoyltransferase (CPT) I catalyzes the conversion of long-chain fatty acyl-CoAs to acyl carnitines in the presence of l-carnitine, a rate-limiting step in the transport of long-chain fatty acids from the cytoplasm to the mitochondrial matrix (1, 2). Mammalian tissues express two isoforms of CPTI, a liver isoform (L-CPTI) and a heart/skeletal muscle isoform (M-CPTI), which are 62% identical in amino acid sequence (3–8). As an enzyme that catalyzes the first rate-limiting step in fatty acid oxidation, CPT is regulated by its physiological inhibitor, malonyl-CoA, the first intermediate in fatty acid synthesis, suggesting a coordinated control of fatty acid synthesis and oxidation (1, 2). Previous studies by our laboratory and others have established that M-CPTI is more sensitive to malonyl-CoA inhibition than L-CPTI (3–8). The molecular/structural basis for the differences in malonyl-CoA sensitivity between M-CPTI and L-CPTI was established recently by our demonstration that substitution of the conserved C-terminal L-CPTI residue Glu-590 with alanine increased its malonyl-CoA sensitivity close to that observed with M-CPTI (9). Because of its central role in fatty acid metabolism, understanding the catalytic mechanism and regulation of the CPT system is an important first step in the development of treatments for diseases such as myocardial ischemia, diabetes, and obesity and for human-inherited CPTI deficiency diseases (9–13).

We have expressed human heart M-CPTI, rat liver L-CPTI, and CPTII in the yeast Pichia pastoris, an organism devoid of endogenous CPT activity (6, 14–16). Our recent deletion and point mutation analyses have demonstrated that Glu-3 and His-5 are necessary for malonyl-CoA inhibition and high-affinity binding of L-CPTI but not for catalysis (17, 18). For M-CPTI, our site-directed mutagenesis studies demonstrate that in addition to Glu-3 and His-5, Val-19, Leu-23, and Ser-24 are necessary for malonyl-CoA inhibition and high-affinity binding but not for catalysis (19, 20). It has been generally predicted that the catalytic and substrate binding sites in both L-CPTI and M-CPTI reside in the C-terminal region of the enzymes. Recent studies from our laboratory demonstrate that mutations of conserved arginine and tryptophan residues in the C-terminal region of L-CPTI abolish catalytic activity (21). Because the major effect of the mutations was on the V_{max}, we predict that the conserved arginine and tryptophan residues stabilize the enzyme-substrate complex by charge neutralization and hydrophobic interactions (21). Furthermore, our site-directed mutagenesis studies demonstrate that deletion of the conserved C-terminal M-CPTI residue Leu-764 or mutation to Arg inactivates the enzyme (22). CPTI is an active acyltransferase that...
belongs to the acyltransferase family of enzymes; however, the molecular mechanism by which CPTI transfers the acyl group from the acyl-CoA to carnitine remains to be elucidated. In this study, our cysteine-scanning mutagenesis demonstrates that a single substitution mutation of Cys-305 to alanine abolishes M-CPTI catalytic activity.

**EXPERIMENTAL PROCEDURES**

**Construction of Human Heart M-CPTI Cysteineless Mutants**—Mutants were constructed using the QuikChange™ multisite-directed mutagenesis kit (Stratagene, La Jolla, CA) with plasmid p12E (for N-terminal mutagenesis) and p13E (for C-terminal mutagenesis) as the templates. Plasmid p13E is a derivative of pTZ18U, containing wild-type N-terminal human M-CPTI from ATG downstream to 623 bp and a fragment of the vector pHWO10 in the extreme 5′-end, whereas plasmid p13E is a derivative of pTZ18U, containing the C-terminal end of M-CPTI from 623 bp to the stop codon. The mutagenesis was performed according to the manufacturer’s instructions. The mutants were in the vectors p12E and p13E. Mutations were confirmed by DNA sequencing.

For construction of the N-terminal 6-residue cysteineless mutant, plasmid 12E containing the substitution mutants C75A, C86K, C90T, C95G, C133S, and C155M was digested with AgeI-EcoRI and subcloned into AgeI-EcoRI-cut wild-type M-CPTI cDNA in the pGAP yeast expression vector (6). The EcoRI-cut 5′ M-CPTI cDNA fragment containing the six cysteine mutants was then ligated into an EcoRI-cut 3′ M-CPTI cDNA fragment of plasmid pTZ18U to generate the N-terminal cysteineless mutant M-CPTI, N6C. For construction of the C-terminal cysteineless M-CPTI, an EcoRI fragment of plasmid 13E containing the 9 mutated C-terminal M-CPTI cysteines (C305A, C448A, C504A, C526A, C548S, C562A, C586A, C608A, and C659A) was ligated to EcoRI-cut wild-type pGAP-M-CPTI to generate C9C, the 9-residue cysteineless mutant M-CPTI in pHWO10.

Mutations were confirmed by DNA sequencing. All the primers used for cysteine mutagenesis are listed in Table I, and the mutations are highlighted in bold. All the cysteine residues in human M-CPTI were either altered to alanine (if human L-CPTI has a cysteine residue at the corresponding position) or to the corresponding amino acid residue in human L-CPTI.

**Construction of the Single C-terminal M-CPTI Cysteine Mutants** C504A, C526A, C548S, C562A, C586A, and C608A—Mutants were constructed by the overlap extension PCR method using the primers shown in Table I with the wild-type and mutant M-CPTI cDNA (pGAP-M-CPTI) as template (23). For example, to construct the C562A mutant, the primers F2-C562AR and C562AF-R3 were used to generate 840- and 300-bp PCR products, respectively, using the wild-type M-CPTI cDNA as template. The two PCR products were purified, mixed, and used as template for a second-round PCR with the primer F2. The 1.1-kb PCR product was digested with SphI-BglIII and ligated into SphI-BglII-cut wild-type M-CPTI cDNA in the pGAP expression vector. The construction of C504A, C526A, C548S, and C562A was similar to that of C562A using the primers listed in Table I, F2 and R3.

The C305A mutant was constructed by the overlap extension PCR method using the primers F2-C305AR and C305AF-R3 with the wild-type M-CPTI cDNA (pGAP-M-CPTI) as template. The 1460-bp PCR product was digested with AluIII-SphI and ligated to AluIII-SphI-cut wild-type pGAP-M-CPTI.

Mutants C448A and C659A were constructed using the QuikChange™ multisite-directed mutagenesis kit with plasmid p13E (containing wild-type C-terminal human M-CPTI cDNA in pTZ18U vector) as the template. Primers C448A and C659AR were each used to screen the C448A and C659A mutants. The plasmid p13E carrying mutations in p13E were cut with EcoRI and used to replace the corresponding EcoRI fragment in the wild-type plasmid pGAP-M-CPTI.

**RESULTS**

*Ferreucination of isolated mitochondria from the yeast strain expressing human heart M-CPTI with 250 μM 5,5′-dithiobis(2-nitrobenzoic acid), an -SH-specific modifying reagent, at 4 °C for 30 min caused a 75% loss in CPTI activity, indicating that there are cysteine residues in M-CPTI that are important for catalysis and/or substrate binding. These preliminary chemical modification studies with 5,5′-dithiobis(2-nitrobenzoic acid) by us and others provided evidence that CPTI may contain a cysteine residue that is important for catalysis (27).*
proteins of predicted sizes with similar steady-state levels of expression.

Effect of Mutations on M-CPTI Activity and Malonyl-CoA Sensitivity—The C-terminal 9-residue cysteineless M-CPTI and the complete 15-residue cysteineless M-CPTI were inactive, despite the high level of protein expression observed for the mutants on the Western blot. The N-terminal 6-residue cysteineless mutant M-CPTI had activity and malonyl-CoA sensitivity similar to that of the wild type as shown in Table II, suggesting that 1 or more of the 9 cysteine residues in the C-terminal region of M-CPTI may be important for activity. Separate substitution mutation of C305A, C448A, C504A, C526A, C548A, C562A, C586A, C608A, and C659A showed that of the nine cysteine mutants, only the substitution mutant C305A was inactive, demonstrating that a single change of Cys-305 to Ala (or serine)\(^2\) resulted in complete loss in M-CPTI activity. As shown in Table II, a change of cysteine to either alanine or serine of the other 8 C-terminal cysteines did not affect activity and malonyl-CoA sensitivity. A single change of Ala-305 to Cys, A305C, in the 9-residue C-terminal cysteineless mutant resulted in a mutant enzyme with similar activity and malonyl-CoA sensitivity (Table II) as the wild-type M-CPTI, demonstrating that Cys-305 (but not the other 8 C-terminal cysteine residues) is essential for M-CPTI activity. In short, our studies identify for the first time that Cys-305 in the C-terminal region of M-CPTI is essential for catalytic activity because mutation of this residue to Ala inactivated M-CPTI.

\(^2\) H. Liu, G. Zheng, M. Treber, J. Dai, and G. Woldegiorgis, unpublished observations.
Cys-305 in M-CPTI Is Important for Catalysis

**TABLE II**  
CPTI activity and malonyl-CoA sensitivity of wild-type and mutant M-CPTI

Mitochondria (150 μg of protein) from the yeast strains expressing wild-type and human heart M-CPTI mutants were assayed for CPT activity and malonyl-CoA sensitivity as described under “Experimental Procedures.” The results are the means ± S.D. of at least two independent experiments with different mitochondrial preparations. N6C, 6-residue N-terminal cysteineless mutant; C9C, 9-residue C-terminal cysteineless mutant; Cys-less, cysteineless M-CPTI mutant; WT, wild type.

| Strain     | Activity | IC_{50}   |
|------------|----------|-----------|
| WT         | 2.90 ± 0.10 | 0.50 ± 0.04 |
| C305A      | Inactive |           |
| A305C      | 2.80 ± 0.03 | 0.52 ± 0.05 |
| N6C        | 2.82 ± 0.14 | 0.50 ± 0.04 |
| C9C        | Inactive |           |
| Cys-less   | Inactive |           |
| C448A      | 2.83 ± 0.20 | 0.55 ± 0.06 |
| C504A      | 2.75 ± 0.19 | 0.50 ± 0.02 |
| C526A      | 2.90 ± 0.15 | 0.20 ± 0.03 |
| C548S      | 2.80 ± 0.13 | 0.50 ± 0.04 |
| C562A      | 3.75 ± 0.14 | 0.20 ± 0.02 |
| C588A      | 3.75 ± 0.03 | 0.70 ± 0.04 |
| C659A      | 3.50 ± 0.37 | 0.24 ± 0.03 |

**TABLE III**  
Kinetic characteristics of yeast-expressed wild-type and mutant human M-CPTIs

Isolated mitochondria (150 μg of protein) from the yeast strains expressing the wild-type and A305C revertant mutant human M-CPTI were assayed for CPT activity in the presence of increasing concentrations of carnitine or palmitoyl-CoA. Values are the means ± S.D. of two independent experiments with different mitochondrial preparations. WT, wild type.

| Strain     | Carnitine | Palmitoyl-CoA |
|------------|-----------|---------------|
|            | K_{m}  | V_{max}  | K_{m}  | V_{max}  |
| WT         | 824.2 ± 8.2 | 3.4 ± 0.3 | 73.0 ± 3.1 | 17.5 ± 0.5 |
| A305C      | 920.0 ± 10.5 | 2.9 ± 0.2 | 66.0 ± 1.9 | 15.8 ± 0.3 |

**DISCUSSION**

Our cysteine-scanning mutagenesis of M-CPTI demonstrated that both the 9-residue C-terminal and the complete 15-residue cysteineless mutant enzymes are inactive but that the 6-residue N-terminal cysteineless mutant enzyme had activity and malonyl-CoA sensitivity similar to those of wild-type M-CPTI. These results suggested that 1 or more of the 9 C-terminal cysteine residues was important for catalysis. Separation of each of the 9 C-terminal cysteines to alanine or serine identified a single residue, Cys-305, to be essential for catalysis. Substitution of Cys-305 with Ala in the wild-type enzyme inactivated M-CPTI, and a single change of Ala to 5-Cys in the 9-residue C-terminal cysteineless mutant resulted in an 8-residue C-terminal cysteineless mutant enzyme that had activity and malonyl-CoA sensitivity similar to those of the wild type. Cys-305 is a conserved residue within the family of enzymes that includes CPTI, carnitine acetyltransferase, and choline acetyltransferase, and choline acetyltransferase from different species, but it is not conserved in CPTII or carnitine octanoyltransferase, in which the corresponding Cys-305 is replaced by Asp as shown in Fig. 2. Of the remaining 8 C-terminal cysteine residues in M-CPTI, 7 are conserved in both M-CPTI and L-CPTI but not in the other acyltransferase-family enzymes. The 8th residue, Cys-548, is present in all CPTI enzymes, and Ser replaces Cys in wild-type L-CPTI. The M-CPTI mutant C548S has activity and malonyl-CoA sensitivity similar to those of the wild type. Human heart M-CPTI has 6 N-terminal cysteine residues that are absent in L-CPTI. Because M-CPTI is much more sensitive to malonyl-CoA inhibition than L-CPTI, we hypothesized that the differences in malonyl-CoA sensitivity observed between the two isoforms could be due to the presence of the 6 additional N-terminal cysteine residues in M-CPTI compared with L-CPTI. However, our cysteine-scanning mutagenesis demonstrated that the N-terminal cysteineless M-CPTI had activity and malon-
CPTM_HUMAN  human carnitine o-palmitoyltransferase I, mitochondrial muscle isoform (Q92523)
CPTM_RAT   rat carnitine o-palmitoyltransferase I, mitochondrial muscle isoform (Q63704)
CPTM_MOUSE mouse carnitine o-palmitoyltransferase I, mitochondrial muscle isoform (Q530150)
CPT1_RAT   rat carnitine o-palmitoyltransferase I, mitochondrial liver isoform (P32198)
CPT1_HUMAN human carnitine o-palmitoyltransferase I, mitochondrial liver isoform (P50416)
CPT1_MOUSE mouse carnitine o-palmitoyltransferase I, mitochondrial liver isoform (P67742)
CPT1_PIG    pig carnitine o-palmitoyltransferase I, mitochondrial liver isoform (AAR69171)
CPT2_HUMAN human carnitine o-palmitoyltransferase II (P23786)
CPT2_MOUSE mouse carnitine o-palmitoyltransferase II (P18886)
CPT2_PIG    pig carnitine o-palmitoyltransferase II (P52825)
CRTC_BOVIN bovin peroxisomal carnitine octanoyltransferase (O192094)
CRTC_HUMAN human peroxisomal carnitine octanoyltransferase (Q9UKG9)
CRTC_RAT   rat peroxisomal carnitine octanoyltransferase (P11466)
CPTA_PIG    pigeon carnitine o-acetyltransferase (P52826)
CPTA_MOUSE mouse carnitine o-acetyltransferase (P47734)
CPTA_HUMAN human carnitine o-acetyltransferase (P43155)
CPTB_MOUSE mouse choline o-acetyltransferase (Q03059)
CPTB_HUMAN human choline o-acetyltransferase (P28329)
CPTB_RAT   rat choline o-acetyltransferase (A48319)
CPTC_CHICKEN chicken choline o-acetyltransferase (AAC4673)
CPTC_PIG    pig choline o-acetyltransferase (P13222)

CPTM_HUMAN  human carnitine o-palmitoyltransferase I, mitochondrial muscle isoform (Q92523)
CPTM_RAT   rat carnitine o-palmitoyltransferase I, mitochondrial muscle isoform (Q63704)
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CPT1_MOUSE mouse carnitine o-palmitoyltransferase I, mitochondrial liver isoform (P67742)
CPT1_PIG    pig carnitine o-palmitoyltransferase I, mitochondrial liver isoform (AAR69171)
CPT2_HUMAN human carnitine o-palmitoyltransferase II (P23786)
CPT2_MOUSE mouse carnitine o-palmitoyltransferase II (P18886)
CPT2_PIG    pig carnitine o-palmitoyltransferase II (P52825)
CRTC_BOVIN bovin peroxisomal carnitine octanoyltransferase (O192094)
CRTC_HUMAN human peroxisomal carnitine octanoyltransferase (Q9UKG9)
CRTC_RAT   rat peroxisomal carnitine octanoyltransferase (P11466)
CPTA_PIG    pigeon carnitine o-acetyltransferase (P52826)
CPTA_MOUSE mouse carnitine o-acetyltransferase (P47734)
CPTA_HUMAN human carnitine o-acetyltransferase (P43155)
CPTB_MOUSE mouse choline o-acetyltransferase (Q03059)
CPTB_HUMAN human choline o-acetyltransferase (P28329)
CPTB_RAT   rat choline o-acetyltransferase (A48319)
CPTC_CHICKEN chicken choline o-acetyltransferase (AAC4673)
CPTC_PIG    pig choline o-acetyltransferase (P13222)

FIG. 2. Sequence alignment of portions of the C-terminal region of various acyltransferases.

CPT active site residue (His-473) that may form a hydrogen-bonding network or a salt bridge to Asp-454, the loss in activity observed in the D454G mutant may be due to disruption of the hydrogen-bonding network or salt bridge to His-473. Asp-323 and Asp-567 may be too far from the conserved His-473 at the active site pocket and thus may not be ideally positioned for such an interaction due to their location. Furthermore, the recent three-dimensional structure of carnitine acetyltransferase, a membrane-associated enzyme that belongs to the acyltransferase family of enzymes, suggests that Asp-567 may hydrogen-bond with Glu-590 (32), and more recent data from our laboratory (31) show that a change of Asp-567 to Ala or His but not Glu resulted in a significant loss in CPTI activity. Our cysteine-scanning mutagenesis and the His-473 and Asp-454 mutation studies with CPTI suggest that there are at least two conserved residues, namely, Cys-305 and His-473, at the active site pocket of CPTI that are essential for catalysis because separate mutation of these residues to Ala inactivates CPTI. In addition, Asp-454 may interact with His-473 and indirectly facilitate catalysis because a mutation of this conserved residue that is located close to the active site

nyl-CoA sensitivity similar to those of the wild-type enzyme, suggesting that these residues do not play a role in M-CPTI activity and inhibitor sensitivity.

More recently, in a patient with CPTI deficiency disease, a complete loss in L-CPTI activity was reported, which was due to substitution of Cys-305 with Trp, the conserved cysteine residue corresponding to that of human L-CPTI and M-CPTI that we demonstrated to be essential for human heart M-CPTI activity by our cysteine-scanning mutagenesis (29). Members of the acyltransferase family of enzymes, including M-CPTI and L-CPTI, contain a highly conserved His residue (Fig. 2) at the active site pocket, His-473, a general acid/base that, when mutated to Ala in CPTI, was shown to cause complete loss in activity (30). As a general acid/base, His-473 may form a hydrogen-bonding network or a salt bridge to a nearby conserved aspartate residue. There are also three highly conserved Asp residues within the family of acyltransferases, namely, Asp-323, Asp-454, and Asp-567, which are present in both CPTI and CPTII, as shown in Fig. 2. In a patient with hepatic CPTI deficiency disease who was homozygous for a D454G missense mutation, the yeast-expressed mutant L-CPTI exhibited only 2% of the wild-type L-CPTI activity, demonstrating that a change of D454 to Gly in CPTI was the cause for the disease (31). Because the conserved Asp-454 is closer to the conserved CPTI active site residue (His-473) that may form a hydrogen-bonding network or a salt bridge to Asp-454, the loss in activity observed in the D454G mutant may be due to disruption of the hydrogen-bonding network or salt bridge to His-473. Asp-323 and Asp-567 may be too far from the conserved His-473 at the active site pocket and thus may not be ideally positioned for such an interaction due to their location. Furthermore, the recent three-dimensional structure of carnitine acetyltransferase, a membrane-associated enzyme that belongs to the acyltransferase family of enzymes, suggests that Asp-567 may hydrogen-bond with Glu-590 (32), and more recent data from our laboratory (31) show that a change of Asp-567 to Ala or His but not Glu resulted in a significant loss in CPTI activity. Our cysteine-scanning mutagenesis and the His-473 and Asp-454 mutation studies with CPTI suggest that there are at least two conserved residues, namely, Cys-305 and His-473, at the active site pocket of CPTI that are essential for catalysis because separate mutation of these residues to Ala inactivates CPTI. In addition, Asp-454 may interact with His-473 and indirectly facilitate catalysis because a mutation of this conserved residue that is located close to the active site

3 Y. L. Yang, M. Treber, and G. Woldegiorgis, unpublished observations.
**FIG. 3. Proposed catalytic mechanism of CPTI.** The reaction catalyzed by CPTI has five steps: 1) nucleophilic attack by the reactive thiolate anion of Cys-305 on the carbonyl carbon of the acyl-CoA substrate to form a tetrahedral intermediate; 2) hydrolysis of the tetrahedral intermediate to form an acyl-S-enzyme complex and free CoA; 3) nucleophilic attack by the reactive oxyanion of carnitine on the carbonyl carbon of the acyl-S-enzyme intermediate; 4) formation of a second tetrahedral acyl-carnitine-E-complex; and 5) hydrolysis of the tetrahedral intermediate to form acyl-carnitine and the regenerated active enzyme.
His-473 caused a significant loss in CPTI activity (33–38). As a rate-limiting enzyme that transports long-chain fatty acids from the cytosol to the mitochondrial matrix, CPTI in the presence of carnitine catalyzes the conversion of long-chain acyl-CoA to acylcarnitines. However, the molecular mechanism by which CPTII transfers the acyl group from the acyl-CoA to carnitine remains to be elucidated. We have hypothesized previously that the reaction catalyzed by CPTI at the active site, conversion of palmitoyl-CoA to palmitoylcarnitine in the presence of L-carnitine, involves deprotonation of the hydroxyl group of carnitine by the catalytic base, His-473, and attack by the resultant oxynion at the carbonyl of the thioester of palmitoyl-CoA to generate palmitoylcarnitine and free CoA (39). Based on our cysteine-scanning mutagenesis and acylation studies with CPTI and studies by others, we now propose a mechanism for the acyltransferase activity of CPTI that uses a catalytic triad composed of residues Cys-305, His-473, and Asp-454 as shown in Fig. 3. The cysteine residue forms a more stable acyl-enzyme intermediate that may allow the acceptor molecule, carnitine, to act as a second nucleophile and complete the acyl transfer reaction. We propose a mechanism for the catalysis whereby the active site catalytic base His-473 aided by Asp-454 abstracts a proton from the -SH group of Cys-305 to generate a reactive thiolate anion that carries out a nucleophilic attack on the substrate acyl-CoA, yielding a tetrahedral intermediate that is subsequently converted to a thioacyl-enzyme covalent intermediate (acyl-S-enzyme). It is envisaged that the negative charge from the carbonyl ion of Asp-454 is transferred to His-473 and then to Cys-305 to enhance the positive charge and relaying it to the cysteine residue, their mutation would be expected to destroy the charge relay system, thereby inactivating the enzyme. Whereas Cys-305 is important for CPTI activity, it is clear that none of the remaining 8 C-terminal cysteine residues are important for CPTI activity, and the disulfide bond formation involving these residues also must not be important for catalytic activity, although they may be involved in mediating the interaction of CPTI with other proteins. In short, our studies demonstrate that CPTI is a thiol acyltransferase and that Cys-305 is the essential nucleophilic residue critical for catalysis. In the presence of both substrates, carnitine and palmitoyl-CoA, CPTI acts primarily as an acyltransferase, but in the absence of either carnitine or CoA, CPTI may exhibit low hydrolase activity, resulting in the breakdown of palmitoyl-CoA or palmitoylcarnitine by a mechanism similar to that of the cysteine proteases and hydrolases, which utilize a Cys-His-Asp catalytic triad (33–38).

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