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Biochemistry

cDNA cloning, sequence analysis, and chromosomal localization of the gene for human carnitine palmitoyltransferase

(mitochondria/β-oxidation/polymerase chain reaction)

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ABSTRACT **We have cloned and sequenced a cDNA encoding human liver carnitine palmitoyltransferase (CPTase; palmitoyl-CoA:L-carnitine *O*-palmitoyltransferase, EC 2.3.1.21), an inner mitochondrial membrane enzyme that plays a major role in the fatty acid oxidation pathway. Mixed oligonucleotide primers whose sequences were deduced from one tryptic peptide obtained from purified CPTase were used in a polymerase chain reaction, allowing the amplification of a 0.12-kilobase fragment of human genomic DNA encoding such a peptide. A 60-base-pair (bp) oligonucleotide synthesized on the basis of the sequence from this fragment was used for the screening of a cDNA library from human liver and hybridized to a cDNA insert of 2255 bp. This cDNA contains an open reading frame of 1974 bp that encodes a protein of 658 amino acid residues including 25 residues of an NH_2-terminal leader peptide. The assignment of this open reading frame to human liver CPTase is confirmed by matches to seven different amino acid sequences of tryptic peptides derived from pure human CPTase and by the 82.2% homology with the amino acid sequence of rat CPTase. The NH_2-terminal region of CPTase contains a leucine-proline motif that is shared by carnitine acetyl- and octanoyltransferases and by choline acetyltransferase. The gene encoding CPTase was assigned to human chromosome 1, region 1q12–1pter, by hybridization of CPTase cDNA with a DNA panel of 19 human–hamster somatic cell hybrids.**

A major source for energy production in the cell is the mitochondrial β-oxidation of long-chain fatty acids. In order to cross the mitochondrial membranes, long-chain fatty acids are first activated by coenzyme A and then reversibly conjugated with L-carnitine, a reaction that is catalyzed by the enzyme carnitine palmitoyltransferase (CPTase; palmitoyl-CoA:L-carnitine *O*-palmitoyltransferase, EC 2.3.1.21) (1). One part of CPTase activity, conventionally referred to as CPTase I, is associated with the outer mitochondrial membrane, and another significant pool of CPTase activity, conventionally referred to as CPTase II, is in close relationship with the inner mitochondrial membrane (2). However, all the attempts to purify CPTase led to the identification of a single protein with a subunit of $M_r \approx 70$ kDa (3–5, 42).

An important role in the regulation of CPTase activity is played by malonyl-CoA, an intermediate in fatty acid biosynthesis that inhibits the activity of outer (CPTase I) but not of inner (CPTase II) CPTase (6). The malonyl-CoA binding domain has been proposed to be located either on CPTase I (4) or on a regulatory protein without CPTase activity (7).

CPTase activity is also under hormonal control of estrogen (8), insulin, and glucagon. Diabetes causes increased CPTase activity and decreased affinity for malonyl-CoA, both reversed by insulin (9). Glucagon has been reported to stimulate CPTase through phosphorylation by a cAMP-dependent protein kinase (10). Interestingly, hypoglycemic agents, such as sulfonylureas, exercise their pharmacological action by strongly inhibiting CPTase activity (11).

CPTase seems also to play a role in the regulation of the hepatic synthesis of very low density lipoproteins (VLDL), since its inhibition increases VLDL production, whereas activation following depletion of malonyl-CoA levels decreases VLDL synthesis (12, 13). Accordingly, the hypolipidemic drug lovastatin causes a 2-fold increase in the activity of hepatic CPTase (14).

From these observations it appears that CPTase has a role in the development of metabolic alterations in very common diseases such as diabetes and hyperlipoproteinemias. Furthermore, recessively inherited deficiency of CPTase in man has been described with two distinct phenotypes. In adults, CPTase deficiency causes a muscle disease with weakness, cramps, and myoglobinuria (15). In infants, CPTase deficiency is characterized by hyperammonemia, increased levels of serum transaminases and plasma-free fatty acids, hepatomegaly, nonketotic hypoglycemia, and coma (16). This life-threatening and often fatal disease is one of the recently recognized defects of mitochondrial β-oxidation associated with hypoglycemia and infant death (17).

The clinical heterogeneity of CPTase deficiency may be caused by different mutations in the same allele, by alterations of tissue-specific forms of CPTase, or by mutations affecting either CPTase I or CPTase II. However, the existence of CPTase I and CPTase II as distinct proteins is questioned (18). It has been shown recently that tetradecylglycidyl-CoA, a CPTase inhibitor interacting with the same site as malonyl-CoA on the mitochondrial membrane (19), binds to 94- and 86-kDa proteins in rat liver and muscle, respectively, both immunologically unrelated with purified inner-membrane CPTase of M_r 70 kDa (4). These proteins have been proposed to be tissue isoforms of CPTase I, but they have not been demonstrated to catalyze the formation of acylcarnitines.

To understand the molecular basis of CPTase alterations in human metabolic disorders, it is important to know the primary structure of human CPTase. Very recently the cDNA encoding the rat enzyme has been cloned and sequenced (20). We have purified CPTase from human liver and determined partial amino acid sequences from several tryptic peptides and from the NH_2-terminal region (42). On the basis

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Abbreviations: CPTase, carnitine palimitoyltransferase; PCR, polymerase chain reaction.

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        *                                                                                                 M      1
1     CC TGA CGC AGT GTC TTG GGC GCT AAC GGC GGC GGC GGC CTT GTG TTT AGA CTC CAG AAC TCC CCA CTT GCC GCG TTC TCG CCG CCG CAG GCT CCC GGG ACG ATG
                                                                                      ↓
      V   P   R   L   L   L   R   A   W   P   R   G   P   A   V   G   P   G   A   P   S   R   P   L   S   A   G   S   G   P   G   Q   Y   L   Q     36
105   GTG CCC CGC CTG CTG CTG CGC GCC TGG CCC CGG GGC CCC GCG GTT GGT CCG GGA GCC CCC AGT CGG CCC CTC AGC GCC GGC TCC GGG CCC GGC CAG TAC CTG CAG
      R   S   I   V   P   T   M   H   Y   Q   D   S   L   P   R   L   P   I   P   K   L   E   D   T   I   R   R   Y   L   S   A   Q   K   P   L     71
210   CGC AGC ATC GTG CCC ACC ATG CAC TAC CAG GAC AGC CTG CCC AGG CTG CCT ATT CCC AAA CTT GAA GAC ACC ATT AGG AGA TAC CTC AGT GCA CAG AAG CCT CTC
      L   N   D   G   Q   F   R   K   T   E   Q   F   C   K   S   F   E   N   G   I   G   K   E   L   H   E   Q   L   V   A   L   D   K   Q   N    106
315   TTG AAT GAT GGC CAG TTC AGG AAA ACA GAA CAA TTT TGC AAG AGT TTT GAA AAT GGG ATT GGA AAA GAA CTG CAT GAG CAG CTG GTT GCT CTG GAC AAA CAG AAT
      K   H   T   S   Y   I   S   G   P   W   F   D   M   Y   L   S   A   R   D   S   V   V   L   N   F   N   P   F   M   A   F   N   P   D   P    141
420   AAA CAT ACA AGC TAC ATT TCG GGA CCC TGG TTT GAT ATG TAC CTA TCT GCT CGA GAC TCC GTT GTT CTG AAC TTT AAT CCA TTT ATG GCT TTC AAT CCT GAC CCA
      K   S   E   Y   N   D   Q   L   T   R   A   T   N   M   T   V   S   A   I   R   F   L   K   T   L   R   A   G   L   L   E   P   E   V   F    176
525   AAA TCT GAG TAT AAT GAC CAG CTC ACC CGG GCA ACC AAC ATG ACT GTT TCT GCC ATC CGG TTT CTG AAG ACA CTC CGG GCT GGC CTT CTG GAG CCA GAA GTG TTC
      H   L   N   P   A   K   S   D   T   I   T   F   K   R   L   I   R   F   V   P   S   S   L   S   W   Y   G   A   Y   L   V   N   A   Y   P    211
630   CAC TTG AAC CCT GCA AAA AGT GAC ACT ATC ACC TTC AAG AGA CTC ATA CGC TTT GTG CCT TCC TCT CTG TCC TGG TAT GGG GCC TAC CTG GTC AAT GCG TAT CCC
      L   D   M   S   Q   Y   F   R   L   F   N   S   T   R   L   P   K   P   S   R   D   E   L   F   T   D   D   K   A   R   H   L   L   V   L    246
735   CTG GAT ATG TCC CAG TAT TTT CGG CTT TTC AAC TCA ACT CGT TTA CCC AAA CCC AGT CGG GAT GAA CTC TTC ACT GAT GAC AAG GCC AGA CAC CTC CTG GTC CTA
      R   K   G   N   F   Y   I   F   D   V   L   D   Q   D   G   N   I   V   S   P   S   E   I   Q   A   H   L   K   Y   I   L   S   D   S   S    281
840   AGG AAA GGA AAT TTT TAT ATC TTT GAT GTC CTG GAT CAA GAT GGG AAC ATT GTG AGC CCC TCG GAA ATC CAG GCA CAT CTG AAG TAC ATT CTC TCA GAC AGC AGC
      P   G   P   E   F   P   L   A   Y   L   T   S   E   N   R   D   I   W   A   E   L   R   Q   K   L   M   S   S   G   N   E   E   S   L   R    316
945   CCC GGC CCC GAG TTT CCC CTG GCA TAC CTG ACC AGT GAG AAC CGA GAC ATC TGG GCA GAG CTC AGG CAG AAG CTG ATG AGT AGT GGC AAT GAG GAG AGC CTG AGG
      K   V   D   S   A   V   F   C   L   C   L   D   D   F   P   I   K   D   L   V   H   L   S   H   N   M   L   H   G   D   G   T   N   R   W    351
1050  AAA GTG GAC TCG GCA GTG TTC TGT CTC TGC CTA GAT GAC TTC CCC ATT AAG GAC CTT GTC CAC TTG TCC CAC AAT ATG CTG CAT GGG GAT GGC ACA AAC CGC TGG
      F   D   K   S   F   N   L   I   I   A   K   D   G   S   T   A   V   H   F   E   H   S   W   E   D   G   V   A   V   L   R   F   F   N   E    386
1155  TTT GAT AAA TCC TTT AAC CTC ATT ATC GCC AAG GAT GGC TCT ACT GCC GTC CAC TTT GAG CAC TCT TGG GAA GAT GGT GTG GCA GTG CTC AGA TTT TTT AAT GAA
      V   F   K   D   S   T   Q   T   P   A   V   T   P   Q   S   Q   P   A   T   T   D   S   T   V   T   V   Q   K   L   N   F   E   L   T   D    421
1260  GTA TTT AAA GAC AGC ACT CAG ACC CCT GCC GTC ACT CCA CAG AGC CAG CCA GCT ACC ACT GAC TCT ACT GTC ACG GTG CAG AAA CTC AAC TTC GAG CTG ACT GAT
      A   L   K   T   G   I   T   A   A   K   E   K   F   D   A   T   M   K   T   L   T   I   D   C   V   Q   F   Q   R   G   G   K   E   F   L    456
1365  GCC TTA AAG ACT GGC ATC ACA GCT GCT AAG GAA AAG TTT GAT GCC ACC ATG AAA ACC CTC ACT ATT GAC TGC GTC CAG TTT CAG AGA GGA GGC AAA GAA TTC CTG
      K   K   Q   K   L   S   P   D   A   V   A   Q   L   A   F   Q   M   A   F   L   R   Q   Y   G   Q   T   V   A   T   Y   E   S   C   S   T    491
1470  AAG AAG CAA AAG CTG AGC CCT GAC GCA GTT GCC CAG CTG GCA TTC CAG ATG GCC TTC CTG CGG CAG TAC GGG CAG ACA GTG GCC ACC TAC GAG TCC TGT AGC ACT
      A   A   F   K   H   G   R   T   E   T   I   R   P   A   S   V   Y   T   K   R   C   S   E   A   F   V   R   E   P   S   R   H   S   A   G    526
1575  GCC GCA TTC AAG CAC GGC CGC ACT GAG ACC ATC CGC CCG GCC TCC GTC TAT ACA AAG AGG TGC TCT GAG GCC TTT GTC AGG GAG CCC TCC AGG CAC AGT GCT GGT
      E   L   Q   Q   M   M   V   E   C   S   K   Y   H   G   Q   L   T   K   E   A   A   M   G   Q   G   F   D   R   H   L   F   A   L   R   H    561
1680  GAG CTT CAG CAG ATG ATG GTT GAG TGC TCC AAG TAC CAT GGC CAG CTG ACC AAA GAA GCA GCA ATG GGC CAG GGC TTT GAC CGA CAC TTG TTT GCT CTG CGG CAT
      L   A   A   A   K   G   I   I   L   P   E   L   Y   L   D   P   A   Y   G   Q   I   N   H   N   V   L   S   T   S   T   L   S   S   P   A    596
1785  CTG GCA GCA GCC AAA GGG ATC ATC TTG CCT GAG CTC TAC CTG GAC CCT GCA TAC GGG CAG ATA AAC CAC AAT GTC CTG TCC ACG AGC ACA CTG AGC AGC CCA GCA
      V   N   L   G   G   F   A   P   V   V   S   D   G   F   G   V   G   Y   A   V   H   D   N   W   I   G   C   N   V   S   S   Y   P   G   R    631
1890  GTG AAC CTT GGG GGC TTT GCC CCT GTG GTC TCT GAT GGC TTT GGT GTT GGG TAT GCT GTT CAT GAC AAC TGG ATA GGC TGC AAT GTC TCT TCC TAC CCA GGC CGC
      N   A   R   E   F   L   Q   C   V   E   K   A   L   E   D   M   F   D   A   L   E   G   K   S   I   K   S   *
1995  AAT GCC CGG GAG TTT CTC CAA TGT GTG GAG AAG GCC TTA GAA GAC ATG TTT GAT GCC TTA GAA GGC AAA TCC ATC AAA AGT TAA CTT CTG GGC AGA TGA AAA GCT
2100  ACC ATC ACT TCC TCA TCA TGA AAA CTG GGA GGC CGG GCA TGG TGC TCA TGC CTG TAA TCC CAG CAT TTT GAG AGG CTG AGG CGG GTG GAT CAC TTG AGG TCA GGA
2205  GTT TGA GAC CAA CCT GGC CAA CAT GGT GAA ACC TTG TCT CTA CTA AAA ATG
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FIG. 1. Nucleotide sequence and deduced amino acid sequence of clone L60 encoding human CPTase. The nucleotides are numbered down the left-hand column (numbering begins with the first untranslated nucleotide), and the amino acids are numbered down the right-hand column. The sequenced peptides from human liver CPTase (42) that confirm the cloned sequence are indicated by underlines. *, Stop codon; ↓, NH_2 terminus of mature protein.

of this information, we have cloned the cDNA encoding the entire CPTase and determined its chromosomal localization.‖

MATERIALS AND METHODS

Identification of a CPTase DNA Probe by Polymerase Chain Reaction (PCR). From sequences deduced from CPTase tryptic peptide no. 130a (42), two oligonucleotide pools were synthesized by using the Gene Assembler + (Pharmacia). The first pool (130-GPS) was a mixture of 1536 32-mers, corresponding to the amino acid sequence Gly-Ile-Ile-Leu-Pro-Glu-Leu-Tyr-Leu-Asp-Pro. The DNA sequence was as follows: GGSATYATYCTSCCHGARCTSTAYCTSGAYCC, where S = G or C, Y = T or C, H = A or C or T, and R = G or A. The antisense mixture of oligonucleotides (130-AP-AS) was derived from amino acid sequence Ala-Val-Asn-Leu-Gly-Gly-Phe-Ala-Pro and was a mixture of 65,536 27-mers with this sequence: NGGNGCRAANCCNCCNAGRTTNACNGC, where N = any nucleotide. Human genomic DNA (1 μg) and 780 pmol of primers were used for PCR (21) in the presence of 200 μM dNTPs, 3 mM $MgCl_2$, 50 mM KCl, 10 mM Tris·HCl (pH 8.3), 0.001% gelatin, and 2.5 units of *Thermus aquaticus* (*Taq*) DNA polymerase (Perkin-Elmer Cetus). After 4 min of denaturation at 94°C, 35 cycles of amplification were performed (94°C for 2 min, 55°C for 2 min, and 72°C for 1 min) in a DNA Thermal Cycler (Perkin-Elmer Cetus).

Identification of a cDNA Clone Encoding Human CPTase. The cDNA library from human fetal liver in the expression vector λgt11 was a gift from Jan P. Kraus (Health Sciences Center, Univ. of Colorado, Denver). A 60-base-pair (bp) oligonucleotide was synthesized on the basis of the sequence obtained from PCR-amplified DNA: GGACAGGACATTGTGGTTTATCTGCCCGTATGCAGGGTCCAGGTAGAGTTCAGGCAGAAT. The DNA was end-labeled by standard procedures (22) to a final specific activity of 1.8×10^8 cpm/μg of DNA. Replica filters from 12 plates (132-mm diameter) were prepared (22) and hybridized for 16 hr at 60°C in 6× SSC (1× SSC = 0.015 M NaCl/0.0015 M sodium citrate) containing 50 mM sodium phosphate (pH 6.8), 5× Denhardt's solution (0.10% bovine serum albumin/0.10% Ficoll/0.10% polyvinylpyrrolidone), and 0.1 mg of denatured salmon sperm DNA per ml. Filters were washed in 2× SSC/0.5% sodium dodecyl sulfate (SDS) at room temperature and 1× SSC/0.1% SDS at 37°C and were exposed overnight with Kodak XAR films.

Phage DNA was prepared as described (23), and inserts obtained after *Eco*RI digestion were purified from the agarose gel by GeneClean (Bio 101, La Jolla, CA) and subcloned in plasmid pGEM-7Zf(+) (Promega) by standard procedures (22). DNA sequences were obtained by the dideoxynucleotide method (24) using the phage T7 DNA polymerase kit (Pharmacia).

Total RNA from HeLa cells was prepared as described (25) and fractionated by electrophoresis in 0.8% agarose-formaldehyde gels. RNA was blotted onto a nylon membrane (GeneScreen*Plus*, Dupont/NEN), hybridized, and washed according to the protocol suggested by the manufacturer. The probe was labeled with [α-^{32}P]dCTP by random primers (26) to a final specific activity of 7.5×10^8 cpm/μg of DNA. The final wash was in 0.1× SSC/1% SDS at 65°C.

Chromosomal Localization of the CPTase Gene. The somatic cell hybrids were obtained as described (27). Hybrids HY.90A and HY.85D3OT2 were obtained by fusing the YH.21 Chinese hamster ovary cell line with cells from the National Institute of

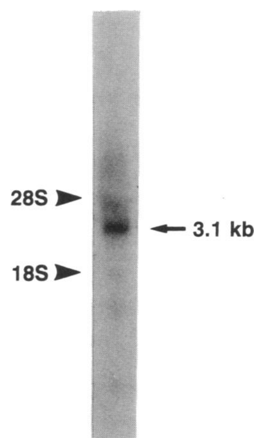

FIG. 2. Northern analysis of total RNA (30 μg) from HeLa cells. CPTase cDNA hybridized to a single RNA species that was calculated to be 3.1 kb based on RNA markers (not shown). Hybridization and washing conditions were as described. The film was exposed for 14 hr.

General Medical Sciences (NIGMS) Human Genetic Mutant Cell Repository (GMO4618 and GMOOO97, respectively) as described (28). Hybrid HY.90A retained the rearranged chromosome Xqter>p22.1::1q23>qter. Hybrid HY.85D30T2 retained the rearranged chromosome Xpter>q26::1q12>qter.

High molecular weight genomic DNA from somatic cell hybrids and human lymphoblastoid cell lines were prepared by standard procedures (22). Complete endonuclease digestions were performed by using a 4-fold excess of enzyme under the conditions suggested by the suppliers. Genomic DNAs were run in 0.8% agarose gels for 16–18 hr, denatured, and transferred to Hybond-N membranes (Amersham) with 20× SSC as buffer. Clone insert pL60 (50 ng) was labeled with [α-^{32}P]dCTP (Amersham, 800 Ci/mmol; 1 Ci = 37 GBq) by using random primers (26). The hybridization was carried out in 50% formamide/6× SSC/5× Denhardt's solution/0.5% SDS/10 mM EDTA/100 μg of sonicated herring sperm DNA per ml at 42°C for 16–18 hr. Final washing conditions were at 65°C in 0.1× SSC/0.1% SDS for 15 min.

RESULTS AND DISCUSSION

Since the screening of expression libraries with antibody probes or oligonucleotides was unsuccessful, we tried to synthesize a more specific probe by PCR. In spite of the high degree of primer degeneration, only one band of 0.12 kilobase (kb) was visible after gel electrophoresis of PCR products (not shown). Similar results were obtained with two other antisense primers of lesser degeneration. Sequence analysis demonstrated that the DNA region encompassed by the primers encodes a stretch of 18 amino acids, perfectly matching with the sequence of peptide 130a (42).

A 60-mer oligonucleotide from this sequence was used as a probe in the screening of a human liver cDNA library and identified only a single positive signal out of 6×10^5 plaques examined. The cDNA insert in this positive clone, named L60, was sequenced on both strands by using synthesized oligonucleotides. The complete sequence of the insert is shown in Fig. 1: it is 2255 bp long and contains a single *Eco*RI site at position 1461. The cDNA was used as a probe for Northern blot analysis of total RNA from HeLa cells** and identified a single RNA species of ≈3.1 kb (Fig. 2). The cloned cDNA has a 5′ untranslated region of 101 bp and a 3′ untranslated region of 180 bp that does not contain either the polyadenylylation signal or the poly(A)$^+$ tail. The ATG codon

‖The sequence reported in this paper has been deposited in the GenBank data base (accession no. M58581).

**Because of the difficulty in finding fresh human tissues for RNA analysis, we used RNA from HeLa cells in Northern blot analysis. It must be mentioned that the nucleotide sequence of a 410-bp cDNA fragment (from nucleotide 1476 to nucleotide 1886) amplified by PCR from HeLa cells was identical to the sequence of human liver CPTase. Therefore, it is likely that CPTases from human liver and HeLa cells are the product of the same gene.

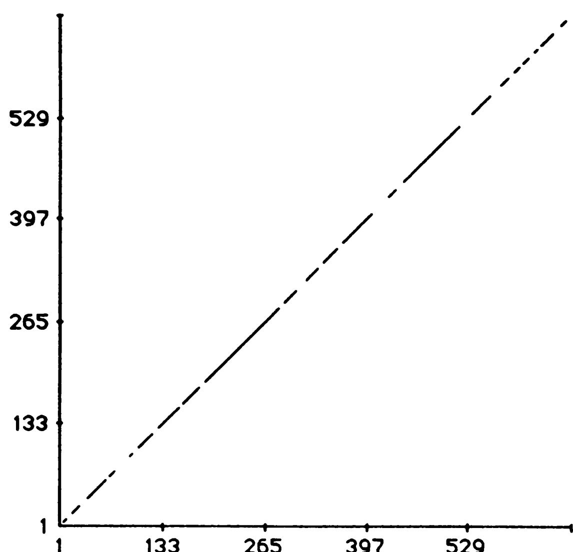

FIG. 3. Comparison between amino acid sequences of human and rat CPTase (horizontal and vertical axis, respectively). The matrix plot was obtained by using a DNA analysis software (MACMOLLY, Soft Gene, Berlin).

at nucleotide 102 is preceded by a TGA codon at nucleotide 3 and is followed by an open reading frame of 1974 bp encoding a protein of 658 amino acids with a predicted molecular mass of 74,156 Da (Fig. 1). The open reading frame was confirmed by matches to seven different amino acid sequences (underlined in Fig. 1), including the NH_2-terminal amino acid sequence, obtained by automated protein sequencing of tryptic peptides from purified human liver CPTase (42). Furthermore, the predicted amino acid sequence of human liver CPTase and that of rat liver CPTase (20) were found to be 82.2% identical (Fig. 3). Comparison of the NH_2-terminal amino acid sequence of human CPTase with that of rat CPTase (20) showed 75% similarity, whereas no similarity was found with the putative NH_2-terminal sequence from rat CPTase described by Brady *et al.* (29).

Table 1. Sequence homologies among different acyltransferases

Acyltransferase	Amino acids	Sequence
hCarPamTase*	45–58	Y Q D S L P R L P I P K L E
rCarPamTase*	45–58	Y Q D S L P R L P I P K L E
rCarOcoTase	56–69	Y Q D S L P R L P V P S L E
hCarAcTase	NH_2-terminal	X Q D A L P R L X V P X L X
rChoAcTase	19–32	E E L D L P K L P V P P L Q
pChoAcTase	20–33	E E P G L P K L P V P P L Q
D.m. ChoAcTase	92–105	F P D T L P K V P V P A L D

h, Human; r, rat; p, pig; *D.m.*, *D. melanogaster*; Car, carnitine; Cho, choline; Pam, palmitoyl; Oco, octanoyl; Ac, acetyl; Tase, transferase. The NH_2-terminal sequence of human carnitine acetyltransferase was determined from the purified preparation by K. Williams and K. Stone (Department of Molecular Biochemistry and Biophysics, Yale University).
*CPTase in text.

Most mitochondrial proteins are encoded by nuclear genes and synthesized on cytosolic polyribosomes as larger precursors that contain cleavable NH_2-terminal targeting sequences (30). These sequences (20–70 amino acids long) are rich in positively charged and hydroxylated residues and generally lack acidic amino acid residues (31, 32). In human CPTase, the NH_2-terminal amino acid (serine at position 26 in the sequence shown in Fig. 1) is preceded by a 25-amino acid leader peptide containing four arginine residues whose spacing is consistent with the amphiphilicity required for the function of mitochondrial targeting presequences (33).

The comparison of human CPTase sequence with that of other acyltransferases, namely rat CPTase and carnitine octanoyltransferase (20, 34); rat, pig, and *Drosophila melanogaster* choline acetyltransferases (35–37); and human carnitine acetyltransferase (recently purified and characterized by us; ref. 38) revealed the highly conserved leucine-proline motif: Leu-Pro-(Arg or Lys)-Leu-Pro-(Val or Ile)-Pro-Xaa-Leu (Table 1). The search in the EMBL data base showed that this leucine-proline motif is not present in other proteins but acyltransferases. In particular, it is not present in enzymes interacting with CoA derivatives, suggesting that it

Table 2. Synteny analysis for human CPT

	Human chromosome present (+) or absent (−)																						
Hybrids	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X
Positive																							
HY.166T4	+	−	−	−	−	+	+	−	−	−	−	+	−	−	+	−	−	−	−	−	+	−	−
Y.173.5CT3	+	−	+	+	−	+	−	+	−	−	+	+	−	+	+	−	−	+	−	−	+	+	+
YC2T1	+	−	−	−	−	−	−	−	−	−	+	+	−	+	−	−	−	+	−	−	−	−	+
Negative																							
HY.19.16T3D	−	−	−	−	−	−	−	−	−	+	−	+	+	+	+	−	−	+	−	+	−	−	+
HY.22AZA1	−	−	−	−	+	−	−	−	−	−	−	+	−	+	−	−	+	+	+	−	−	−	+
HY.60A	−	−	−	−	+	+	−	+	−	−	−	−	+	+	−	−	−	+	−	+	−	−	+
HY.70B2	−	−	−	−	−	+	−	−	−	−	−	−	+	−	+	+	−	−	−	−	+	−	+
HY.75E1	−	−	−	−	+	−	−	−	+	−	−	+	−	−	−	−	−	−	−	−	−	−	+
HY.85D3OT2*	t	+	+	−	−	−	−	+	−	−	+	−	+	−	−	−	−	+	−	−	+	−	t
HY.90A*	t	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	+	−	t
HY.94A	−	−	−	−	−	+	+	+	−	−	−	−	−	−	−	+	−	−	−	−	−	+	+
HY.95A1	−	−	+	−	+	−	−	−	−	+	+	−	−	+	−	−	−	−	−	−	−	−	+
HY.95B	−	−	−	+	−	+	+	−	−	−	−	−	−	+	−	−	−	+	−	−	−	+	−
HY.95S	−	+	+	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	+	+	−	+
HY.137J	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	+	−	−	−	−	+	−
RJ.387.51T5	−	−	−	−	+	−	−	+	−	−	−	−	−	−	+	−	−	+	−	−	+	−	+
RJ.387.58	−	−	−	−	−	−	−	−	+	−	−	−	+	−	−	−	−	−	−	−	−	−	+
Y.XY.8F6	−	−	+	+	+	+	+	−	−	+	+	+	−	−	−	+	+	−	+	−	−	+	+
Y.XY.8FT7	−	−	−	−	−	+	−	−	−	+	+	−	−	−	−	−	−	−	−	−	−	−	−
% concordance	100	73	68	78	52	63	73	68	73	63	68	73	52	68	78	68	68	63	78	78	68	68	21

The percent concordance is calculated by dividing the number of concordant hybrids by the total number of hybrids. t, translocation.
*Hybrids HY.85D3OT2 and HY.90A contain rearranged chromosome 1 as described in *Materials and Methods*.

might be of functional relevance for the interaction with carnitine or choline, two chemically related compounds.

This probe was also used for the analysis of human–hamster somatic cell hybrids. It detected two bands of about 3.0 and 1.0 kb in human genomic DNA and three bands of about 3.7, 3.15, and 1.85 kb in hamster genomic DNA, after *Eco*RI digestion. The synteny analysis, performed on a panel of 19 somatic cell hybrids, reported in Table 2, assigned the CPTase gene to chromosome 1. The CPTase probe did not detect human bands in the hybrids HY.85D3OT2 and HY.90A. Thus, the mapping of the CPTase locus can be restricted to the region 1q12–1pter.

The availability of human CPTase cDNA offers the necessary tool for investigating whether the clinical heterogeneity of CPTase deficiency in man (39, 40) is caused by alterations of different forms of CPTase in tissues and/or subcellular compartments or by different mutations of one single gene. CPTase has a key role in the regulation of fatty acid metabolism: the interaction with CPTase of malonyl-CoA, either directly or through an intermediate regulatory protein, and the action of insulin and glucagon play major roles in this process (41). Site-directed mutagenesis and expression of CPTase cDNA, together with the identification of crucial regions for the regulation of CPTase gene expression, will help to understand the molecular basis of such roles. Furthermore, the availability of the primary structure of CPTase might help to design more effective drugs for the treatment of common disorders of human metabolism such as diabetes and hyperlipoproteinemias.

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1. Bieber, L. L. & Farrell, S. (1983) in *The Enzymes*, ed. Boyer, P. D. (Academic, New York), Vol. 16, pp. 627–644.
2. Murthy, M. S. R. & Pande, S. V. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 378–382.
3. Clarke, P. R. H. & Bieber, L. L. (1981) *J. Biol. Chem.* **256**, 9861–9868.
4. Declercq, P. E., Falck, J. R., Kuwajima, M., Tyminski, H., Foster, D. W. & McGarry, J. D. (1987) *J. Biol. Chem.* **262**, 9812–9821.
5. Miyazawa, S., Ozasa, H., Osumi, T. & Hashimoto, T. (1983) *J. Biochem. (Tokyo)* **94**, 529–542.
6. McGarry, J. D., Leatherman, G. F. & Foster, D. W. (1977) *J. Biol. Chem.* **253**, 4128–4136.
7. Lund, H. & Woldegiorgis, G. (1986) *Biochim. Biophys. Acta* **878**, 243–249.
8. Weinstein, I., Cook, G. A. & Heimberg, M. (1986) *Biochem. J.* **237**, 593–596.
9. Cook, G. A. & Gamble, S. M. (1987) *J. Biol. Chem.* **262**, 2050–2055.
10. Harano, Y., Kashiwagi, A., Kojima, H., Suzuki, M., Hashimoto, T. & Shigeta, Y. (1985) *FEBS Lett.* **188**, 267–272.
11. Cook, G. A. (1987) *J. Biol. Chem.* **262**, 4968–4972.
12. Ide, T. & Ontko, J. A. (1981) *J. Biol. Chem.* **256**, 10247–10255.
13. Yamamoto, M., Fukuda, N., Triscari, J., Sullivan, A. C. & Ontko, J. A. (1985) *J. Lipid Res.* **26**, 1196–1204.
14. Cook, J. A., Khan, B. & Heimberg, M. (1988) *Biochem. Biophys. Res. Commun.* **150**, 1077–1082.
15. DiMauro, S. & DiMauro, P. M. M. (1973) *Science* **182**, 929–931.
16. Bougneres, P. F., Saudubray, J. M., Marsac, C., Bernard, O., Odievre, M. & Girard, J. (1981) *J. Pediatr.* **98**, 742–746.
17. Nyhan, W. L. (1988) *N. Engl. J. Med.* **171**, 1404–1406.
18. Bergstrom, J. D. & Reitz, R. C. (1980) *Arch. Biochem. Biophys.* **204**, 71–79.
19. Declercq, P. E., Venincasa, M. D., Mills, S. E., Foster, D. W. & McGarry, J. D. (1985) *J. Biol. Chem.* **260**, 12516–12522.
20. Woeltje, K. F., Esser, V., Weis, B. C., Sen, A., Cox, W. F., McPhaul, M. J., Slaughter, C. A., Foster, D. W. & McGarry, J. D. (1990) *J. Biol. Chem.* **265**, 10720–10725.
21. Saiki, R. K., Gelfand, D. H., Stoffel, S., Scharf, S. J., Higuchi, R., Horn, G. T., Mullis, K. B. & Erlich, H. A. (1988) *Science* **239**, 487–491.
22. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
23. Finocchiaro, G., Ito, M., Ikeda, Y. & Tanaka, K. (1988) *J. Biol. Chem.* **263**, 15773–15780.
24. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5463–5467.
25. Chomczynski, P. & Sacchi, N. (1987) *Anal. Biochem.* **162**, 156–159.
26. Feinberg, P. A. & Vogelstein, B. (1984) *Anal. Biochem.* **137**, 266–267.
27. Rocchi, M., Roncuzzi, L., Santamaria, R., Archidiacono, N., Dente, L. & Romeo, G. (1986) *Hum. Genet.* **74**, 30–33.
28. Couvreur, J. M., Azuma, T., Miller, D. A., Rocchi, M., Mohandas, T. R., Boudi, F. A. & Taggart, R. T. (1990) *Cytogenet. Cell Genet.* **53**, 137–139.
29. Brady, P. S., Feng, Y. & Brady, L. J. (1988) *J. Nutr.* **118**, 1128–1136.
30. Pfanner, N. & Neupert, W. (1990) *Annu. Rev. Biochem.* **59**, 331–353.
31. Hurt, E. C. & van Loon, A. P. G. M. (1986) *Trends Biochem. Sci.* **11**, 204–207.
32. Horwich, A. L., Kalousek, F., Fenton, W. A., Furtak, K., Pollock, R. A. & Rosenberg, L. E. (1987) *J. Cell Biol.* **105**, 669–678.
33. Roise, D., Theiler, F., Horvath, S. J., Tomich, J. M., Richards, J. H., Allison, D. S. & Schatz, G. (1988) *EMBO J.* **7**, 649–653.
34. Chatterjee, B., Song, C. S., Kim, J. M. & Roy, A. K. (1988) *Biochemistry* **27**, 9000–9006.
35. Itoh, N., Slemmon, J. R., Hawke, D. H., Williamson, R., Morita, E., Itakura, K., Roberts, E., Shively, J. E., Crawford, G. D. & Salvaterra, P. M. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 4081–4085.
36. Berrard, S., Brice, A., Lottspeich, F., Braun, A., Barde, Y. & Mallet, J. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 9280–9284.
37. Brice, A., Berrard, S., Raynaud, B., Ansieau, S., Coppola, T., Weber, M. J. & Mallet, J. (1989) *J. Neurosci. Res.* **23**, 266–273.
38. Bloisi, W., Colombo, I., Garavaglia, B., Giardini, R., Finocchiaro, G. & DiDonato, S. (1990) *Eur. J. Biochem.* **189**, 539–546.
39. Demaugre, F., Bonnefont, J., Mitchell, G., Nguyen-Hoang, N., Pelet, A., Rimoldi, M., DiDonato, S. & Saudubray, J.-M. (1988) *Pediatr. Res.* **24**, 308–311.
40. DiDonato, S., Castiglione, A., Rimoldi, M., Cornelio, F., Vendemia, F., Cardace, G. & Bertagnolio, B. (1981) *J. Neurol. Sci.* **50**, 207–215.
41. McGarry, J. D., Woeltje, K. F., Kuwajima, M. & Foster, D. W. (1989) *Diabetes Metab. Rev.* **5**, 271–284.
42. Finocchiaro, G., Colombo, I. & DiDonato, S. (1990) *FEBS Lett.* **274**, 163–166.