Over-expression and characterization of active recombinant rat liver carnitine palmitoyltransferase II using baculovirus

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The cDNA encoding rat liver carnitine palmitoyltransferase II (CPT-II) was heterologously expressed using a recombinant baculovirus/insect cell system. Unlike *Escherichia coli*, the baculovirus-infected insect cells expressed mostly soluble active recombinant CPT-II (rCPT-II). CPT activity from crude lysates of recombinant baculovirus-infected insect cells was maximal between 50 and 72 h post-infection, with a peak specific activity of 100–200 times that found in the mock- or wild-type-infected control lysates. Milligram quantities (up to 1.8 mg/l of culture)

of active rCPT-II were chromatographically purified from large-scale cultures of insect cells infected with the recombinant baculovirus. The rCPT-II was found to be: (1) similar in size to the native rat liver enzyme (~ 70 kDa) as judged by SDS/PAGE; (2) immunoreactive with a polyclonal serum raised against rat liver CPT-II; and (3) not glycosylated. Kinetic analysis of soluble rCPT-II revealed K_m values for carnitine and palmitoyl-CoA of $950 \pm 27 \mu\text{M}$ and $34 \pm 5.6 \mu\text{M}$ respectively.

INTRODUCTION

In eukaryotic cells, the CoA esters of long-chain fatty acids (LCFA) undergo β -oxidation within the mitochondrial matrix but they do not readily cross the inner mitochondrial membrane. A specific mechanism has evolved for transporting LCFAs into the mitochondria which requires the transfer of the fatty acid moiety from the acyl-CoA ester to L-carnitine by carnitine palmitoyltransferase I (CPT-I). The acyl-carnitine conjugate is translocated across the inner mitochondrial membrane and then converted back to the acyl-CoA ester by carnitine palmitoyltransferase II (CPT-II) [1–3]. This transport process may be a rate-limiting step in fatty acid metabolism [3] with feedback regulation exerted on CPT-I by malonyl-CoA, an intermediate in fatty acid biosynthesis [3,4]. In addition, tissue-specific regulation of fatty acid oxidation in heart and liver indicates that there may be tissue-specific isoforms of CPT-I. This hypothesis is consistent with biochemical analyses of CPT-I from these tissues [5–7].

Interest in CPT has increased due to findings linking gluconeogenesis in non-insulin-dependent diabetes (NIDDM) to fatty acid oxidation [8]. First, CPT activity appears to be regulated by insulin [9–11]. In streptozotocin-treated diabetic rats, hepatic CPT-I activity was increased whereas the sensitivity of the enzyme to malonyl-CoA inhibition was decreased [9,10]. Administration of insulin reversed both the stimulatory effect of diabetes on CPT-I activity and the induced insensitivity of the enzyme to malonyl-CoA regulation [9,10]. Secondly, recessively inherited deficiencies in CPT activity (which are sometimes fatal) have been associated with non-ketotic hypoglycaemia [12–15]. Finally, hyperglycaemic diabetic animals and humans that have been treated with agents known to inhibit CPT-I activity, i.e. methyl 2-tetradecylglycidate, Clomoxir or Etomoxir, showed decreased blood glucose levels [16–19].

While the above CPT inhibitors are effective hypoglycaemic agents, their use in the treatment of NIDDM is limited by the finding of undesirable side-effects in animals [8,18]. Deleterious responses to Etomoxir and Clomoxir have been attributed to irreversible inhibition of CPT-I activity [18,20–22]. Reversible inhibition of CPT-I activity, on the other hand, may be a reasonable compromise in the development of safe and efficacious drugs for the treatment of NIDDM. As a first step in defining the biochemical and biophysical properties of CPT for drug development, recombinant CPT-II (rCPT-II) was over-expressed in insect cells, purified and characterized. CPT-II was chosen as a model for CPT-I because of the inherent difficulties involved in purifying and retaining CPT-I activity. Although biochemically similar to CPT-II, CPT-I appears to be an integral membrane-bound protein which loses activity upon solubilization [6,23]. On the other hand, CPT-II is loosely associated with the mitochondrial membrane and retains carnitine transferase activity when isolated in soluble form [6,23].

Methods

Microbial cultures and plasmid isolation

The pBKS-CPT II.4 clone was provided by Dr. J. Denis McGarry (Departments of Internal Medicine and Biochemistry, University of Texas Southwestern Medical Center) [24]. The pBKS(-) plasmid (Stratagene) containing CPT-II.4 cDNA was propagated in *Escherichia coli* XL-1 BLUE (Stratagene), isolated and purified using Magic Minipreps plasmid purification kit (Promega). The pVL-1393 baculovirus transfer vector (Invitrogen) was propagated in *E. coli* XL-1 BLUE, isolated and purified as described above.

Abbreviations used: LCFA, long-chain fatty acid; NIDDM, non-insulin-dependent diabetes; CPT-I, carnitine palmitoyltransferase I; CPT-II, carnitine palmitoyltransferase II; rCPT-II, recombinant carnitine palmitoyltransferase II; AcMNPV, *Autographa californica* nuclear polyhedrosis virus; SF-9, *Spodoptera frugiperda* cells; MOI, multiplicity of infection; PMSF, phenylmethanesulphonyl fluoride; pI, isoelectric point; DTNB, 5,5'-dithiobis(2-nitrobenzoic acid).

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Preparation of *Autographa californica* nuclear polyhedrosis virus (AcMNPV) transfer vectors

The CPT-II.4 cDNA was altered at the 5'- and 3'-ends by PCR (Perkin-Elmer). All 5' and 3' non-translated sequences were removed and *EcoRI* and *SmaI* sites were placed at the 5'- and 3'-ends respectively. Oligonucleotides were synthesized on an Applied Biosystems, Inc. 392 RNA/DNA synthesizer. The sequences of the 5' and 3' primers were 5'-CTG CAC CCG GGG ACG ATG ATG CCG-3' and 5'-CGA TGA ATT CAT TAC TAA GTT TTG ATG-3' respectively. The 2.0 kb PCR product was cloned into pBKS(-), sequenced and then subcloned into the baculovirus expression vector pVL-1393 using standard cloning procedures [25].

DNA sequencing of the CPT-II PCR products

DNA sequencing following the Sanger dideoxynucleotide chain-termination method [26] was performed on the pBKS-CPT-II PCR clone using Sequenase II [U.S. Biochemical Inc. (USB)] and [$\alpha^{32}\text{P}$]dATP (New England Nuclear). Universal primers complementary to the multiple cloning site of pBKS(-), and seven internal CPT-II primers based on the published sequence of CPT-II, were synthesized. The products of the sequencing reactions were separated in 6% acrylamide gels containing 8 M urea. Dried gels were exposed to Kodak XAR-5 film for 16 h at room temperature.

Construction of recombinant baculovirus, screening and plaque purification

Co-transfection of pVL-1393-CPT-II with AcMNPV DNA (Invitrogen or PharMingen) into *Spodoptera frugiperda* cells (SF-9) was performed as previously described [27]. Transfected cultures were maintained for at least 84 h at 27 °C and recombinant AcMNPVs were identified and purified by plaque assay [27,28]. Screening for rCPT-II expression was done using a rabbit anti-CPT-II polyclonal serum (obtained from Dr. J. Denis McGarry) diluted 1:500 as described elsewhere [27,28]. A recombinant baculovirus clone, CPTIIBG.2B, was identified, plaque-purified twice and used throughout these experiments.

Isolation of total cellular DNA from AcMNPV-infected SF-9 cells and Southern-blot analysis

Confluent SF-9 cells in three 25 cm² flasks were either mock-infected or infected with wild-type or recombinant CPTIIBG.2B AcMNPVs at a multiplicity of infection (MOI) of 10. The cells were incubated for 72 h at 27 °C. Total DNA from the infected SF-9 cells was isolated [28], digested with *EcoRI*/*SmaI* and separated on a 1% agarose gel. The DNA was Southern transferred [29] to a nylon membrane and probed with biotinylated CPT-II cDNA (Images kit, USB). Size marker, 1 kb ladder, was purchased from Gibco/BRL. The blot was exposed to Hyperfilm (Amersham) for 15 min.

Expression of rCPT-II

For the initial small-scale evaluation of rCPT-II expression, infected insect cells were incubated in 25 cm² flasks for 72 h at 27 °C, after which the cells were removed and pelleted at 500 g for 5 min at 4 °C. After decanting the supernatants, the cell pellets were immediately frozen in a dry-ice/ethanol bath and stored at -70 °C until assayed. For large-scale production and purification of rCPT-II, suspension cultures (500 ml, 3 × 10⁶ cells/ml in logarithmic growth, 27 °C) of SF-9 cells were inocu-

lated with recombinant baculovirus CPTIIBG.2B at an MOI of 10. After 72 h the cells were harvested by centrifugation (750 g, 4 °C), washed once with PBS and stored at -70 °C. For both small- and large-scale samples, SF-9 cells were lysed using a combination of freeze/thaw cycles and hypotonic lysis in 5 mM potassium phosphate buffer (pH 7.5; 16–35 ml/10¹⁰ cells) containing the protease inhibitors phenylmethanesulphonyl fluoride (PMSF) (1 mM), leupeptin (10 µM) and pepstatin (1 µM). n-Octyl glucoside (1% w/v) was added to each sample which was then mixed for 30 min at 4 °C. The large-scale sample was treated further by centrifugation at 140 000 g at 4 °C for 60 min. The clear portion of the supernatant was removed and stored at 4 °C. The pellet and denser phase were re-extracted with lysis buffer (44–70 ml/10¹⁰ cells) containing n-octyl glucoside (1% w/v) and re-centrifuged. The clear portion of the supernatant was pooled with the first.

Protein purification

A modified method of Miyazawa et al. [30] was used to isolate rCPT-II. Crude rCPT-II was batch-adsorbed to DEAE-cellulose equilibrated in 5 mM potassium phosphate/0.5% (v/v) Tween 20/1 mM PMSF, pH 7.5. The slurry was packed into a chromatographic column and washed with 5 mM potassium phosphate/0.5% (v/v) Tween 20, pH 7.5. The enzyme was eluted with 20 mM potassium phosphate/0.5% (v/v) Tween 20, pH 7.5. Fractions containing rCPT-II activity were pooled, adjusted to pH 6.5 with 1 M phosphoric acid and then batch-adsorbed to hydroxyapatite equilibrated in 40 mM potassium phosphate/0.2% (v/v) Tween 20, pH 6.5. The slurry was packed into a chromatographic column and washed with 40 mM potassium phosphate/0.2% (v/v) Tween 20, pH 6.5. rCPT-II was eluted with a linear salt gradient up to 400 mM potassium phosphate/0.20 (v/v) Tween 20, pH 6.5. Fractions containing rCPT-II activity were pooled, concentrated and dialysed against 5 mM potassium phosphate/0.5% (v/v) Tween 20, pH 7.5. As a final step, the rCPT-II sample was applied to DEAE-agarose equilibrated with 5 mM potassium phosphate/0.5% (v/v) Tween 20, pH 7.5. The column was washed with 5 mM potassium phosphate/0.5% (v/v) Tween 20, pH 7.5, and rCPT-II was eluted with a linear salt gradient up to 20 mM potassium phosphate/0.5% (v/v) Tween 20, pH 7.5. In some instances, fractions containing rCPT-II were pooled and subjected to another round of DEAE-agarose ion-exchange chromatography as described above. Purified rCPT-II was stored ($\approx$ 1 mg/ml) at -70 °C.

Carnitine transferase activity assays

rCPT-II activity was determined by: (1) the production of palmitoyl-[¹⁴C]carnitine from [¹⁴C]carnitine in the presence of 1% (w/v) BSA [4]; or (2) the reduction of 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) by CoASH in the absence of BSA [31]. *K_m* values for carnitine and palmitoyl-CoA were determined as described above [31] at 37 °C in the presence of 0.2 mM DTNB over a range of substrate concentrations (50–750 µM L-carnitine, 25–400 µM palmitoyl-CoA) in 150 mM Tris-HCl buffer (pH 7.2). *K_m* values were estimated by analysing the data from three experiments performed in triplicate using the *GraFit* program (version 2.0) [32]. All protein concentrations were determined using the DC protein assay system (Bio Rad) with BSA as the standard.

Protein characterization

SDS/PAGE, isoelectric focusing, silver staining and Western transfer were performed using a PhastSystem apparatus

(Pharmacia). SDS/PAGE and Western/immunodetection were also performed as described elsewhere [28]. An Ultrosan XL analyzer (Pharmacia) was used for densitometry. Glycosylation was investigated by enzyme-linked immunoassay of digoxigenin-labelled glycoconjugates (Glycan Detection Kit, Boehringer Mannheim Biochemica), using transferrin and α 1-acid glycoprotein as positive controls. Protein sequence was determined on an Applied Biosystem 477A Protein Sequencer after removal of Tween 20 using pre-packed 'Extracti-Gel D' columns (Pierce).

RESULTS

The CPT-II.4 cDNA clone [24] was altered by removing both 5' and 3' non-coding regions from the gene by gene amplification. Two additional in-frame stop codons along with an *Eco*RI site were introduced at the 3'-end of the gene in the same reaction. The newly constructed CPT-II gene, containing only the coding sequence for full-length CPT-II, was sequenced to assure that no base alterations were introduced during the amplification reaction. The gene was then subcloned into a baculovirus expression vector and transferred into the baculovirus genome by homologous recombination. A Southern blot of total DNA extracted from mock-, wild-type- and recombinant CPTIIBG.2B-infected SF-9 cells demonstrated successful gene transfer (Figure 1). Only the insect cells infected with recombinant AcMNPV CPTIIBG.2B contained the rat liver CPT-II gene. Little or no cross-hybridization was observed in the control infections. Heterologous expression of rCPTII was verified by SDS/PAGE and Western-immunodetection using an anti-CPT-II polyclonal serum. An immunoreactive protein band, similar in apparent molecular mass (~ 70 kDa) to that of the native rat liver CPT-II, was observed only in cell extracts infected with the recombinant AcMNPV CPTIIBG.2B (Figure 2).

CPT activity of rCPT-II was assayed initially using radio-labelled [14 C]carnitine under conditions supporting the forward addition of carnitine to palmitoyl-CoA. A time-course of intracellular CPT activity versus time post-infection with recombinant AcMNPV CPTIIBG.2B showed a dramatic increase in

Figure 1 Southern analysis of total DNA extracted from infected SF-9 cells

Total DNA was isolated from mock-, wild-type- and recombinant CPTIIBG.2B-infected SF-9 cells and probed for the CPT-II gene as described in the Methods section. Lane 1 contained the positive control CPT-II gene ($0.1 \mu\text{g}$) isolated from the pBKS-CPT-II.4 construct. Equivalent amounts of total cellular DNA from the mock, wild-type and CPTIIBG.2B infections were loaded into lanes 2, 3, and 4 respectively.

Figure 2 Western-immunodetection of total protein isolated from infected SF-9 cells

The total cellular protein from mock-, wild-type- and CPTIIBG.2B-infected-SF-9 cell lysates was separated by SDS/PAGE, transferred to nitrocellulose and probed with anti-CPT-II polyclonal antibodies. Samples from each of the lysates ($5 \mu\text{l}$) were loaded onto the gel as follows: lane 1, CPTIIBG.2B infection; lane 2, mock infection; lane 3, wild-type infection. Lane 4 contained $1 \mu\text{g}$ of partially purified native rat liver CPT-II.

Figure 3 Substrate specificity profile of rCPT-II in both soluble and insoluble fractions of cell extracts

rCPT-II activity was determined spectrophotometrically using the continuous assay system described in the Methods section. The data were plotted as absorbance at 405 nm/min per mg of protein versus carbon chain length of acyl-CoA substrate: ◆, CPTIIBG.2B soluble fraction; ▲, CPTIIBG.2B pellet; ▼, mock pellet; and ■, mock soluble fraction. The final concentration of all acyl-CoA substrates was 0.1 mM and of carnitine 5.0 mM. The data shown are the mean specific activities $\pm$ SD of a single assay performed in triplicate.

specific activity which peaked between 55 and 72 h (results not shown). At 72 h, the specific activity of crude soluble rCPT-II from SF-9 cell lysates was determined to be 100–200 times that observed in the two control samples (23.28 ± 1.62 , 0.11 ± 0.03 and 0.16 ± 0.02 nmol palmitoylcarnitine/min per mg of protein from CPTIIBG.2B, mock and wild-type infections respectively).

The carnitine transferase activity in soluble and insoluble fractions of insect cells expressing rCPT-II, as determined by the assay method of Bieber and Fiol [31] (see Methods section), revealed that in the absence of both detergent and BSA, soluble

Table 1 Summary of rCPT-II purification

CPT activity was determined spectrophotometrically at each of the purification stages. Specific activity is given in arbitrary units based on the change in A_{405}/min . Data are from purification that yielded 2 mg of rCPT-II and are representative of the specific activities, total units, percentage yield and fold purification of the various purification steps.

Purification step	Specific activity (units/min per mg protein)	Total units (units/min)	Yield (%)	Fold purification
SF9 -lysate	7	100 000	100	1.0
Ultracentrifugation supernatant	10	68 000	68	1.4
DEAE-cellulose	23	14 000	14	3.3
Hydroxyapatite	140	5600	6	20
DEAE-agarose	430	4600	5	61

rCPT-II was more active with acyl-CoAs of chain-length C_8 – C_{10} than with palmitoyl-CoA (Figure 3). The observed activity profile did not correlate directly with substrate solubility, since acyl-CoAs of carbon chain length C_8 – C_{10} were preferred over those of C_4 – C_6 . This type of activity profile for the forward reaction of CPT-II with acyl-CoAs of different chain-length is similar to that reported for mitochondrial rat liver, chicken embryo liver and bovine heart CPT-II [33–35]. Moreover, the C_8 and C_{10} transferase activities were partially inhibited by the addition of 1% n-octyl glucoside (results not shown). This type of inhibition has previously been reported for native CPT-II [36]. In contrast to C_8 and C_{10} substrates, the C_{16} transferase activity was identical in both the soluble and insoluble cell fractions and was not sensitive to octyl glucoside inhibition. Finally, no inhibition of enzyme activity was observed for any of the substrates in both the soluble and insoluble rCPT-II fractions upon the addition of 50 μM malonyl-CoA (inhibitor of CPT-I activity).

The production of rCPT-II was scaled-up for purification of the enzyme, based on the parameters described above. Table 1 summarizes representative specific activities, total units, percentage yield and fold-purification after the various purification steps. Typically, about 65–80% of the total CPT activity from the cell extract was adsorbed to the DEAE-cellulose matrix in the first step of purification. Different batches of purification yielded different amounts of soluble rCPT-II. The highest recovery of enzyme was 1.8 mg of soluble rCPT-II per litre of cell culture from batch rCPT-IIF. The rCPT-IIF batch of enzyme yielded a total of about 32 mg of purified soluble CPT-II and was used throughout this study. SDS/PAGE of purified rCPT-II showed a single protein band which migrated with an apparent molecular mass similar to that of native rat liver CPT-II (~70 kDa) (Figure 4; see Figure 2 for Western blot of crude enzyme preparation). Densitometric analysis of the silver-stained gel indicated that the rCPT-II was >95% pure. Furthermore, this material was immunoreactive with the anti-CPT-II polyclonal serum (Figure 4). To confirm the identity of rCPT-II, the purified enzyme was subjected to 15 cycles of N-terminal amino acid sequence analysis. The sequence information provided evidence authenticating rCPT-II and, at the same time, revealed the existence of two rCPT-II isoforms that were present in similar amounts. The N-terminal amino acids of the two isoforms were determined to be Leu-25 and Ser-26 of the deduced rat liver CPT-II amino acid sequence [24].

Isoelectric focusing in polyacrylamide gels revealed similar isoelectric points (pI) for both native and recombinant CPT-II, estimated to be in the range 6.4–6.5. However, an additional

Figure 4 Silver stained SDS/PAGE and Western blot of purified rCPT-II

Lanes 1 and 2 both contained about 50 ng of purified rCPT-II. Lane 1 shows the silver-stained gel and lane 2 shows the corresponding Western blot.

band (pI 6.2–6.4) was observed in the rCPT-II sample (results not shown). It is believed that the second isoelectric point can be explained by the different amino acids at the N-termini of the two proteins and/or by deamidation, because no evidence for glycosylation was seen in different preparations ($n = 3$) of rCPT-II or in samples of the native enzyme (results not shown).

The kinetic parameters of purified soluble rCPT-II were determined using the assay method of Bieber and Fiol [31] (see Methods section). Under the conditions used, K_m values were calculated to be $950 \pm 27 \mu\text{M}$ for carnitine and $34 \pm 5.6 \mu\text{M}$ for palmitoyl-CoA. rCPT-II activity was also assayed under different pH conditions and after various storage and handling procedures. No loss in activity was observed after six freeze/thaw cycles ($-70^\circ\text{C}/\text{room temperature}$) or after storage at room temperature (6.5 h). Moreover, soluble rCPT-II maintained activity in different buffers over the pH range 7–10. Storage in these buffers for 24 h at 4°C did not appear to affect enzyme activity.

DISCUSSION

Previous attempts to over-express active CPT-II in *E. coli* or in yeast have resulted in either low levels of expression (in yeast) or mainly insoluble recombinant protein (*E. coli*). In contrast, milligram quantities of soluble rat liver rCPT-II could be obtained using the baculovirus expression system. Interestingly, two isoforms of soluble rCPT-II, present in similar amounts, were produced in the insect cell system. One isomer had a pI which was similar to that of native rat liver CPT-II (pH 6.4–6.5) and the other had a pI which was slightly lower (pH 6.2–6.4). The two isoforms appeared to differ by only a single amino acid, Leu-25. Native rat liver CPT-II is believed to contain an N-terminal mitochondrial leader sequence that is cleaved during processing and translocation of the enzyme into the mitochondrial membrane [24]. The insect cells were capable of recognizing and processing the CPT-II rat liver mitochondrial leader sequence, since the first 24–25 amino acids were not found in the N-terminal sequence of the mature rCPT-II isoenzymes. Furthermore the microsequencing data showed no evidence of contamination by other non-related proteins. The difference in the N-termini of the two rCPT-II isoforms was most likely due to differential cleavage by the insect cells during post-translational modification or proteolytic degradation.

Together, the rCPT-II isoforms represented >95% of the total protein in the purified enzyme preparation. The purified material was of the same apparent size as rat liver CPT-II, showed similar activity over a wide pH range (pH 7–10) and

appeared to be stable at room temperature in phosphate buffer containing 0.5% Tween 20. In buffers of pH below the pI of rCPT-II (6.5), the rate of catalysis declined sharply. Histidine is the only titratable amino acid with an R group that has a pK_a in this pH range (pK_a of 6.0). Moreover, a histidine residue (CPT-II, His-372) is conserved among all members of the carnitine acyltransferase family of enzymes and a histidine residue is believed to be involved in carnitine acetyltransferase catalysis [37]. Based on this information, it is likely that a histidine residue is also involved in reactions catalysed by CPT-II.

Slightly different K_m values for mitochondrial CPT-II, isolated from various species and tissues, have previously been reported [33,38,39]. Although not specified, it is assumed that these kinetic analyses were performed with CPT-II and not CPT-I. Some of the kinetic variations observed may be attributed to species-specific isoforms of CPT-II. However, rat and human liver CPT-II are approximately 80% homologous [40], with near identity in certain regions of the protein that may be involved in substrate binding. Since tissue-specific isoforms of CPT-II have not been reported, and given the high degree of homology among species, variations in the kinetic characterization of this enzyme are more likely to be due to differences in experimental assay conditions and methods of purification. In general, however, the K_m values for carnitine and palmitoyl-CoA are around 1–1.5 mM and 10–60 μ M respectively. The kinetic parameters determined for rCPT-II were similar to those of mitochondrial CPT-II isolated from chick embryo liver [33], and in general, fell within the range determined for the various samples of CPT-II as described above. On the other hand, comparison of the kinetic values for rCPT-II with those determined for rat liver mitochondrial CPT-I indicated that the K_m for palmitoyl-CoA (CPT-I, K_m 30–60 μ M) is similar for both enzymes, while CPT-I has a much lower K_m for carnitine (K_m 32 ± 1.4 μ M) [41]. Also, when assayed under similar conditions (100 μ M palmitoyl-CoA/200 μ M carnitine/1% BSA) the specific activity of purified rCPT-II (460 nmol/min per mg of protein) was 50–70-fold greater than that of CPT-I from intact rat liver mitochondria.

Finally, the activity of rCPT-II associated with the soluble and insoluble fractions of insect cell lysates was assayed using acyl-CoA substrates of different chain length in the absence of detergent. The ratio of enzyme activity in the soluble to insoluble fractions varied depending on the specific acyl-CoA substrate tested, approaching unity only with butyryl- and palmitoyl-CoA substrates. These differential activities may reflect differences in the affinity of the rCPT-II for acyl-CoA substrates of varying chain-length, depending on the presence or absence of a membrane environment, and/or differential but direct effects of the acyl-CoA substrates on the physical environment of the enzyme in the two fractions. A similar sensitivity to environment has been observed with CPT-I, where the enzyme is sensitive to malonyl-CoA inhibition only when associated with the membrane [4].

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