High expression of a novel carnitine palmitoyltransferase I like protein in rat brown adipose tissue and heart: isolation and characterization of its cDNA clone

Naoshi Yamazaki, Yasuo Shinohara, Atsushi Shima, Hiroshi Terada*

Faculty of Pharmaceutical Sciences, University of Tokushima, Shomachi, Tokushima 770, Japan

Received 20 January 1995; revised version received 6 March 1995

Abstract To characterize energy metabolism in rat brown adipose tissue (BAT), we carried out differential screening of a cDNA library of BAT with a cDNA probe of white adipose tissue (WAT) and isolated one cDNA clone. It contained a single open reading frame of 2,316 bases which encodes a protein of 88.2 kDa. The predicted amino acid sequence showed the highest homology (62.6%) with that of rat carnitine palmitoyltransferase I (CPTI). The transcript corresponding to this cDNA was found to be abundantly expressed in BAT and heart. Therefore, the isolated clone is concluded to encode a CPTI like protein expressed in BAT and heart.

Key words: Brown adipose tissue; Differential screening; cDNA cloning; Mitochondrion; Carnitine palmitoyltransferase; Rat

1. Introduction

Two kinds of adipose tissues are known to exist in mammals; white and brown adipose tissues. The physiological role of WAT is storage of bioenergy in the form of fat. Unlike WAT, the role of BAT is dissipation of energy as heat, which prevents mammals from excess storage of energy. Therefore, BAT is important in maintenance of the body temperature and prevention of obesity (for reviews, see [1–4]). This unique function of BAT is mainly supported by uncoupling protein (UCP) present in the mitochondrial inner membrane (for reviews, see [5–7]). UCP is specifically expressed in BAT and acts as a H^+ conductor across the H^+-impermeable mitochondrial inner membrane. The proton conducting action of UCP results in dissipation of the proton motive force ($\Delta\mu H^+$) like protonophoric uncouplers of oxidative phosphorylation (for reviews, see [8,9]).

There could be some biological system(s) other than UCP to support the unique function of BAT. Therefore, in this study, we tried to identify a protein(s) specifically expressed in BAT but not in WAT by differential screening. As a result, we succeeded in isolating a cDNA clone which is expressed abundantly in rat BAT but not appreciably in rat WAT. This clone was found to encode a novel 88.2 kDa protein consisting of 772 amino acids. The deduced amino acid sequence was 62.6% homologous with that of rat liver CPTI (EC 2.3.1.21). The possible functional role of this protein in energy metabolism in BAT is discussed.

2. Materials and methods

2.1. Materials and general procedures

Restriction endonucleases and modification enzymes were obtained from Takara Shuzo (Kyoto), Nippon Gene (Tokyo) and Gibco BRL (Gaithersburg). An oligo(dT)-cellulose column, nitrocellulose membranes (type BA85) and a λZAP cDNA library synthesis kit were obtained from Becton Dickinson Labware (Bedford), Schleicher & Schuell (Dassel) and Stratagene (La Jolla), respectively. [α-^{32}P]dCTP (specific radioactivity, 111 TBq/mmol) was obtained from Amersham. Other reagents were of the highest grade commercially available. Recombinant experiments were carried out according to the standard method of Sambrook et al. [10].

2.2. Purification of RNA

Interscapular BAT, epididymal WAT and other tissues used were taken from 4-week-old male Wistar rats. Visible contaminating tissues were completely removed. Total RNA was extracted from these tissues with guanidine thiocyanate and purified by centrifugation in a solution of cesium chloride. Poly(A)$^+$ RNA was purified from total RNA by oligo(dT)-cellulose column chromatography. The concentration of RNA samples was determined from their absorbance at 260 nm in a Shimadzu spectrophotometer, model UV-160.

2.3. Differential screening

A cDNA library of rat BAT was prepared using a λZAP cDNA synthesis kit. This cDNA library consisted of about 1×10^5 independent clones. The phages of this cDNA library were plated at a final density of about 2,000 plaques/plate on 20 plates (150 mm diam.). The phages on these plates were transferred to nitrocellulose membranes for differential screening.

The first strand complementary DNAs synthesized by reverse transcription of poly(A)$^+$ RNA obtained from WAT with oligo(dT)$_{12-18}$ as a primer were used to detect the cDNA clones which were expressed in WAT. A cDNA fragment of UCP, prepared by the method reported previously [11], was used to detect cDNA clones encoding UCP.

2.4. Northern blotting

Samples of 10 μg of total RNA isolated from various tissues were analyzed. The cDNA fragments were prepared as follows: for analysis of the isolated clones, a DNA fragment of 1.0 kbp obtained by digestion of cDNA derived from the DS112 clone with *Eco*RI and *Xho*I or that of 650 bp obtained by digestion of cDNA derived from DS112-36 with *Eco*RI was used (for details, see Fig. 2). cDNA fragments encoding rat CPTI and CPTII were prepared by PCR as described previously [12] with use of first strand cDNA prepared by reverse transcription of poly(A)$^+$ RNA of rat liver as a template. The primers used for amplification of these DNA fragments were 5′-GGCAAATGATGTGGACCTGC and 5′-TCAAGTGCTTCCCAAAGCGG for CPTI (position 1620–2263 according to [13]) and 5′-ATGGCATTCAATCCGGACCC

*Corresponding author. Fax: (81) (886) 33-5195.

The nucleotide sequence data reported in this paper have been submitted to the GSDB, DDBJ, EMBL and NCBI nucleotide sequence databases under the accession number D43623.

Abbreviations: BAT, brown adipose tissue; WAT, white adipose tissue; UCP, uncoupling protein; CPTI, carnitine palmitoyltransferase I; CPTII, carnitine palmitoyltransferase II; PCR, polymerase chain reaction; bp, base pair(s).

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SSDI 0014-5793(95)00277-4

and 5′-TTCAGGGTTTCCTCATTGCC for CPTII (position 403–947 according to [14]). The PCR products obtained were subcloned into a plasmid vector and their nucleotide sequences were confirmed. cDNA fragments of lipoprotein lipase and β-actin were prepared as described previously [15]. These cDNA fragments were radiolabeled by the multi-priming method and used as probes.

3. Results and discussion

To isolate a cDNA clone that is expressed in BAT but not in WAT, we carried out differential screening by the method of Sambrook et al. [10] with slight modification as follows. First, plaques derived from the cDNA library prepared from poly(A)$^+$ RNA of rat BAT were transferred to nitrocellulose membranes. The DNAs in plaques on these membranes were hybridized with cDNA probes prepared from mRNA expressed in rat WAT (screened with the probe of WAT). After autoradiography, the membranes were washed and subsequently used for rehybridization with a cDNA fragment of UCP as a probe to eliminate cDNA clones that encoded rat UCP (screened with the UCP probe). By these two hybridization processes, we isolated 200 plaques that did not hybridize with the cDNA fragments prepared from mRNAs expressed in WAT and that of UCP.

From these 200 plaques, inserted fragments of cDNA were obtained as plasmids by in vivo automatic excision according to the method recommended by the supplier. Next the plasmids obtained from the plaques were analyzed with a cDNA probe prepared from poly(A)$^+$ RNA of WAT by Southern analysis. Sixty-nine clones showed 'negative' results in this hybridization experiment, suggesting that they are not expressed in WAT. We analyzed these 69 clones by Southern blotting with cDNA probes obtained from both BAT and WAT. Consequently, we obtained one clone (DS112) which hybridized with the probe of BAT, but not with the probe of WAT.

Next, we confirmed that the transcript corresponding to this cDNA clone (DS112) was observed in BAT but not in WAT by Northern analysis. To obtain the cDNA fragment, we digested the plasmid obtained from the DS112 clone with *Eco*RI and *Xho*I, their restriction sites being artificially inserted during preparation of the cDNA library. After these digestions, two DNA fragments of 1.0 kbp and 0.5 kbp were observed besides a plasmid vector of 3.0 kbp. We purified the longer fragment of 1.0 kbp and used it as a probe. As shown in Fig. 1, a large amount of transcript hybridizing with the cDNA probe was observed in an RNA sample from BAT, but an almost negligible amount in an RNA sample from WAT. The steady state transcript levels of lipoprotein lipase and β-actin, examined as controls, were almost the same in the RNA samples from BAT and WAT. These results clearly indicate that a transcript corresponding to cDNA clone DS112 is highly expressed in BAT but negligibly in WAT.

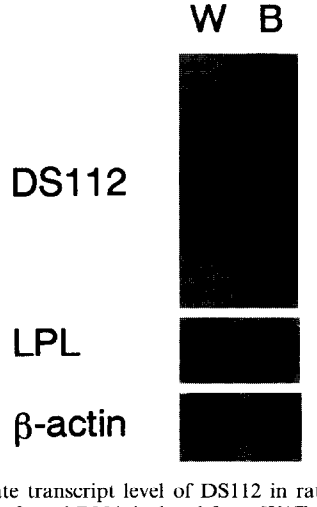

Fig. 1. Steady state transcript level of DS112 in rat WAT and BAT. Samples of 10 μg of total RNA isolated from WAT (W) and BAT (B) were analyzed. In addition, the transcript levels of lipoprotein lipase (LPL) and β-actin were examined.

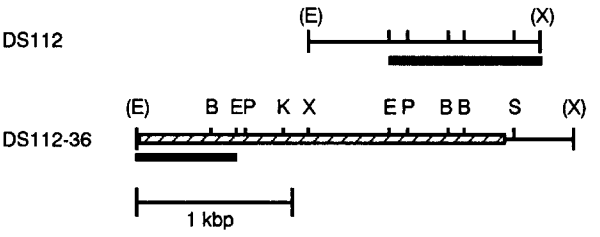

Fig. 2. Restriction maps of the cDNA fragments obtained from clones DS112 and DS112-36 derived from a cDNA library of rat BAT. The major restriction sites used for structural analysis are shown as follows: E, *Eco*RI; B, *Bgl*II; K, *Kpn*I; P, *Pst*I; S, *Sph*I; X, *Xho*I. E and X are additional restriction sites introduced for construction of the cDNA library. Black bars shown under the restriction maps of DS112 and DS112-36 represent the cDNA fragments used as probes in Northern blot analysis. The hatched box in the restriction map of DS112–36 is the open reading frame.

From the Northern blot analysis, the length of the transcript was estimated as about 3 kbp, indicating that the isolated clone DS112 did not contain the full-length DNA sequence corresponding to the observed transcript. Therefore, we rescreened the same cDNA library with the cDNA fragment obtained from the DS112 clone as a probe and isolated another clone, DS112-36, which contained a cDNA fragment of about 3 kbp. The restriction map of this clone and entire nucleotide and deduced amino acid sequences of the inserted cDNA fragment are shown in Figs. 2 and 3, respectively. In an homology search by BLAST [16] we found no entry with completely the same nucleotide sequence as that of DS112-36. However, the nucleotide sequence of DS112-36 showed the highest homology of about 60% with that of rat liver mitochondrial CPTI. One open reading frame was observed in this nucleotide sequence encoding an 88.2 kDa protein consisting of 772 amino acids, the amino acid sequence of which was 62.6% homologous with that of rat CPTI (Fig. 4). The presence of an in-frame stop codon, TGA, −15 bp upstream of the first translation initiation codon ATG excludes the possible extension of the open reading frame at the 5′-terminus of the cDNA clone (Fig. 3). Furthermore, the hydropathy profile of the deduced amino acid sequence of DS112-36 showed high similarity with that of CPTI including the presence of two hydrophobic putative membrane spanning domains in the N-terminal region (Fig. 5). In addition, DS112-36 was found to be 27.6% homologous in its amino acid sequence with the other isoform of CPT (CPTII), which catalyzes the formation of palmitoyl-CoA from CoA and transports palmitoylcarnitine in the matrix space of mitochondria [14].

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1                                                                CTGAGCTGTGCTGACTAAACCCCAGG
                                                                    ***
27   ATGGCGGAAGCACACCAGGCAGTAGCTTTCCAGTTCACTGTGACCCCAGACGGGGTCGACTTCCGGCTTAGTCGGGAGGCTCTGAGACAC
     MetAlaGluAlaHisGlnAlaValAlaPheGlnPheThrValThrProAspGlyValAspPheArgLeuSerArgGluAlaLeuArgHis   30

117  ATCTACCTGTCTGGAATCAACTCCTGGAAGAAACGCCTTATTCGAATCAAGAATGGTATCCTTAGGGGTGTGTACCCTGGCAGCCCTACC
     IleTyrLeuSerGlyIleAsnSerTrpLysLysArgLeuIleArgIleLysAsnGlyIleLeuArgGlyValTyrProGlySerProThr   60

207  AGCTGGCTGGTTGTTGTCATGGCAACAGTTGGTTCCAACTACTGCAAAGTGGACATCTCCATGGGGCTGGTCCATTGCATCCAGAGATGC
     SerTrpLeuValValValMetAlaThrValGlySerAsnTyrCysLysValAspIleSerMetGlyLeuValHisCysIleGlnArgCys   90

297  CTCCCGACAAGGTATGGCTCCTACGGGACCCCACAGACCGAGACACTTCTCAGTATGGTCATCTTCTCCACCGGAGTCTGGGCGACAGGC
     LeuProThrArgTyrGlySerTyrGlyThrProGlnThrGluThrLeuLeuSerMetValIlePheSerThrGlyValTrpAlaThrGly  120

387  ATTTTTTTATTCCGACAAACCCTGAAGCTGCTGCTTTCCTATCATGGGTGGATGTTCGAGATGCACAGCAAGACCAGCCATGCCACCAAG
     IlePheLeuPheArgGlnThrLeuLysLeuLeuSerTyrHisGlyTrpMetPheGluMetHisSerLysThrSerHisAlaThrLys  150

477  ATCTGGGCTATCTGTGTTCGTCTCCTGTCCAGCCGGCGGCCCATGCTCTATAGCTTCCAAACATCACTGCCCAAGCTTCCTGTCCCCAGT
     IleTrpAlaIleCysValArgLeuLeuSerSerArgArgProMetLeuTyrSerPheGlnThrSerLeuProLysLeuProValProSer  180

567  GTGCCAGCCACAATTCACCGGTACTTGGATTCTGTGCGGCCCTTGCTGGATGACGAAGCCTATTTCCGCATGGAGTCGTTGGCCAAAGAA
     ValProAlaThrIleHisArgTyrLeuAspSerValArgProLeuLeuAspAspGluAlaTyrPheArgMetGluSerLeuAlaLysGlu  210

657  TTCCAGGACAAGATTGCCCCCAGACTGCAGAAATACCTGGTGCTGAAGTCATGGTGGGCAACCAACTATGTAAGTGACTGGTGGGAAGAG
     PheGlnAspLysIleAlaProArgLeuGlnLysTyrLeuValLeuLysSerTrpTrpAlaThrAsnTyrValSerAspTrpTrpGluGlu  240

747  TACGTCTACCTCCGAGGCAGGAGCCCCATCATGGTGAACAGCAACTATTACGCCATGGATTTTGTGCTTATTAAGAACACGAGCCAACAA
     TyrValTyrLeuArgGlyArgSerProIleMetValAsnSerAsnTyrTyrAlaMetAspPheValLeuIleLysAsnThrSerGlnGln  270

837  GCAGCACGTTTGGGAAACACCGTTCACGCCATGATCATGTATCGCCGCAAACTGGACCGAGAAGAGATCAAGCCGGTAATGGCACTGGGT
     AlaAlaArgLeuGlyAsnThrValHisAlaMetIleMetTyrArgArgLysLeuAspArgGluGluIleLysProValMetAlaLeuGly  300

927  ATGGTACCCATGTGCTCCTACCAGATGGAGAGGATGTTCAACACTACACGCATCCCAGGCAAAGAGACAGACTTGCTACAGCACCTCTCA
     MetValProMetCysSerTyrGlnMetGluArgMetPheAsnThrThrArgIleProGlyLysGluThrAspLeuLeuGlnHisLeuSer  330

1017 GAGAGCAGGCACGTGGCTGTCTACCACAAAGGTCGCTTCTTCAAGGTTTGGCTCTATGAGGGCTCGTGCCTGCTCAAGCCCCGAGACCTC
     GluSerArgHisValAlaValTyrHisLysGlyArgPhePheLysValTrpLeuTyrGluGlySerCysLeuLeuLysProArgAspLeu  360

1107 GAGATGCAGTTCCAGAGAATCCTCGATGACACCTCCCCGCCTCAGCCTGGAGAGGAAAAGCTGGCAGCCCTCACCGCAGGAGGAAGGGTA
     GluMetGlnPheGlnArgIleLeuAspAspThrSerProProGlnProGlyGluGluLysLeuAlaAlaLeuThrAlaGlyGlyArgVal  390

1197 GAGTGGGCAGAAGCACGTCAGAAGTTCTTTAGCTCTGGCAAGAACAAGATGTCCCTGGATACCATCGAACGTGCTGCTTTCTTTGTGGCC
     GluTrpAlaGluAlaArgGlnLysPhePheSerSerGlyLysAsnLysMetSerLeuAspThrIleGluArgAlaAlaPhePheValAla  420

1287 CTGGACGAAGACTCTCACTGTTACAACCCTGATGACGAGGCCAGTCTCAGCCTCTACGGCAAATCCCTGCTGCACGGCAACTGCTATAAC
     LeuAspGluAspSerHisCysTyrAsnProAspAspGluAlaSerLeuSerLeuTyrGlyLysSerLeuLeuHisGlyAsnCysTyrAsn  450

1377 AGGTGGTTCGACAAATCTTTCACTCTCATCTCCTGCAAGAATGGCCAGCTGGGCCTCAACACAGAACACTCATGGGCAGATGCTCCCATC
     ArgTrpPheAspLysSerPheThrLeuIleSerCysLysAsnGlyGlnLeuGlyLeuAsnThrGluHisSerTrpAlaAspAlaProIle  480

1467 ATCGGTCACCTCTGGGAGTTCGTCCTGGCCACTGATACCTTTCACCTGGGCTACACGGAGACAGGACACTGTGTGGGTGAACCCAACACC
     IleGlyHisLeuTrpGluPheValLeuAlaThrAspThrPheHisLeuGlyTyrThrGluThrGlyHisCysValGlyGluProAsnThr  510

1557 AAGTTGCCGCCGCCTCAGCGGATGCAGTGGGACATTCCCGAGCAGTGCCAGACAGCCATCGAGAATTCGTACCAAGTAGCCAAGGCCCTG
     LysLeuProProProGlnArgMetGlnTrpAspIleProGluGlnCysGlnThrAlaIleGluAsnSerTyrGlnValAlaLysAlaLeu  540

1647 GCTGATGATGTGGAGTTATACTGCTTCCAGTTCTTACCCTTCGGCAAAGGCCTGATCAAGAAGTGTCGGACCAGCCCTGATGCCTTTGTG
     AlaAspAspValGluLeuTyrCysPheGlnPheLeuProPheGlyLysGlyLeuIleLysLysCysArgThrSerProAspAlaPheVal  570

1737 CAGATTGCCCTGCAGCTGGCTCATTTCCGGGACAAAGGCAAGTTCTGCCTGACCTATGAGGCCTCCATGACAAGAATGTTCCGAGAGGGG
     GlnIleAlaLeuGlnLeuAlaHisPheArgAspLysGlyLysPheCysLeuThrTyrGluAlaSerMetThrArgMetPheArgGluGly  600

1827 CGGACAGAGACTGTGCGTTCCTGTACTAGCGAGTCCACGGCCTTTGTGCGGGCCATGATGACGGGGTCCCATAAGAAACAAGACCTCCAA
     ArgThrGluThrValArgSerCysThrSerGluSerThrAlaPheValArgAlaMetMetThrGlySerHisLysLysGlnAspLeuGln  630

1917 GACCTCTTCCGGAAAGCCTCCGAAAAACACCAAAACATGTACCGCCTAGCCATGACAGGGGCTGGGATCGACAGGCACCTCTTCTGCCTC
     AspLeuPheArgLysAlaSerGluLysHisGlnAsnMetTyrArgLeuAlaMetThrGlyAlaGlyIleAspArgHisLeuPheCysLeu  660

2007 TACATCGTCTCCAAGTACTTAGGGGTTAGATCTCCTTTCCTGGACGAGGTGCTTTCGGAACCCTGGAGCCTCTCCACCAGCCAGATCCCC
     TyrIleValSerLysTyrLeuGlyValArgSerProPheLeuAspGluValLeuSerGluProTrpSerLeuSerThrSerGlnIlePro  690

2097 CAGTTCCAGATCTGCATGTTTGACCCAAAGCAGTACCCCAATCATCTGGGTGCTGGAGGTGGCTTTGGTCCTGTGGCCGACCACGGATAC
     GlnPheGlnIleCysMetPheAspProLysGlnTyrProAsnHisLeuGlyAlaGlyGlyGlyPheGlyProValAlaAspHisGlyTyr  720

2187 GGGGTTTCCTACATGATCGCAGGCGAAAACACAATGTTCTTCCATGTTTCCAGCAAGTTATCGAGTTCAGAAACGAACGCCCTGCGCTTC
     GlyValSerTyrMetIleAlaGlyGluAsnThrMetPhePheHisValSerSerLysLeuSerSerSerGluThrAsnAlaLeuArgPhe  750

2277 GGGAACCACATCCGTCAAGCACTGCTGGATATCGCCGACCTTTTCAAAATTTCCAAGACTGACAGCTGAGACCAGGAGACACACCAGCTG
     GlyAsnHisIleArgGlnAlaLeuLeuAspIleAlaAspLeuPheLysIleSerLysThrAspSer***                       772

2367 CCCTTTGGTCCCCACCTGGTGGAGGAAGAGGTCTGTGGCCAGTTCACAGGCATAAGGGGTGGCATGCACACGTGCCCAGTTCTGAGACCA
2457 GCTCCAGCGCAGGGGCTCCCCAGGCAGACACTGCTCCTCCAGGCCCGGTCGAGGTGGGATTGGAGTGGTGAGGGAACTTTGATCTTTTTT
2547 TTTCCCCCGGTCTTGGTAGATGCTAATAAAAATAAGGCTGTATAATTCTCTCTCAGCCCTTAGGTGCCTATGTTTGGTTAGAGAACTAGA
2637 AGGCCTTTCCCCTGCCCCTGCTCAGGTTAGGGTGGTGGCGACTGAAGGGCCGGGTGAATGTTCATAATGGCTTTTTACCTGCTTTGAAAT
2727 GTGTGCTTTTCCTGAATAATGCGGACTTCGAGAGTGCTGTCCAACCTCTCATGTGCACTTGGAATAAATTCTTACTTTAGAACCTTT (A) n
```

Fig. 3. Entire nucleotide sequence of the cDNA fragment of clone DS112-36. The deduced amino acid sequence of the protein is shown under the nucleotide sequence. The nucleotide and amino acid sequences are numbered on the left and right, respectively. Asterisks represent the stop codon. Two polyadenylation signals are underlined.

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DS112-36    1  MAEAHQAVAFQFTVTPDGVDFRLSREALRHIYLSGINSWKKRLIRIKNGILRGVYPGSPTSWLVVVMATVGSNYCKVDIS
CPTI        1  ******************I*L***H***KQ*C***LH****KF**F****IT**F*AN*S***I**VGVIS*MHA***P*

DS112-36   81  MGLVHCIQRCLPTRYGSYGTPQTETLLSMVIFSTGVWATGIFLFRQTLKLLLSYHGWMFEMHSKTSHATKIWAICVRLLS
CPTI       81  L*MIAK*S*T*D*--TGRMSS**KNIV*G*L*G**L*VAV*MTM*YS**V**********AE*G*M*RS****MAM*KV**

DS112-36  161  SRRPMLYSFQTSLPKLPVPSVPATIHRYLDSVRPLLDDEAYFRMESLAKEFQDKIAPRLQKYLVLKSWWATNYVSDWWEE
CPTI      159  G*K**********R*****A*KD*VS***E*****MKE*DFQ**TA**QD*AVNLG*K**W**K***************

DS112-36  241  YVYLRGRSPIMVNSNYYAMDFVLIKNTSQQAARLGNTVHAMIMYRRKLDREEIKPVMALGM-VPMCSYQMERMFNTTRIP
CPTI      239  *I*****G*L*********EMLY*TP*HI****A***I**ILL***T*****L**IRL**STI*L**A*W**L***S***

DS112-36  320  GKETDLLQHLSESRHVAVYHKGRFFKVWLYEGSCLLKPRDLEMQFQRILDDTSPPQPGEEKLAALTAGGRVEWAEARQKF
CPTI      319  *E***TI**IKD***IV***R**Y******HDGR**R**E**Q*M*Q****P*E*****A*******AD**P**KC**TY

DS112-36  400  FSSGKNKMSLDTIERAAFFVALDEDSHCYNPDD-EASLSLYGKSLLHGNCYNRWFDKSFTLISCKNGQLGLNTEHSWADA
CPTI      399  *AR****Q***AV*K*****T***SEQG*REE*P***IDS*A******R*FD******I*FVVF**SKI*I*A*******

DS112-36  479  PIIGHLWEFVLATDTFHLGYTETGHCVGEPNTKLPPPQRMQWDIPEQCQTAIENSYQVAKALADDVELYCFQFLPFGKGL
CPTI      479  **V*****Y*M***V*Q***S*D***K*DT*PNI*K*T*L*****GE**EV*DA*LSS*SL**N**D*HS*P*DS*****

DS112-36  559  IKKCRTSPDAFVQIALQLAHFRDKGKFCLTYEASMTRMFREGRTETVRSCTSESTAFVRAMMTGSHKKQDLQDLFRKASE
CPTI      559  **********I*L******YK*M**********L**********M**CN**Q***DPKSTAEQRLK**KI*C*

DS112-36  639  KHQNMYRLAMTGAGIDRHLFCLYIVSKYLGVRSPFLDEVLSEPWSLSTSQIPQFQICMFDPKQYPNHLGAGGGFGPVADH
CPTI      639  ***HL*****************V*****A*D****K*******R*****T**Q*VEL**FEKN*DYVSC*********D

DS112-36  719  GYGVSYMIAGENTMFFHVSSKLSSSETNALRFGNHIRQALLDIADLFKISKTDS
CPTI      719  ******I*V***FIH**I***F**P**DSH***K*L***MM**IT**GLTINSKK
```

Fig. 4. Alignment of the predicted amino acid sequence of DS112-36 with that of rat liver CPTI. The amino acid sequences are numbered on the left. To obtain maximum alignment of the two sequences, four gaps shown by (–) were inserted. Asterisks indicate identical residues.

Therefore, we conclude that the cDNA clone DS112-36 isolated from the cDNA library of rat BAT encodes a mitochondrial CPTI like protein.

As shown in Fig. 1, we observed marked expression of a transcript hybridizing with the cDNA fragment of DS112 in RNA of BAT, but not in RNA of WAT. We further examined the tissue distribution of transcripts of DS112-36, CPTI and CPTII by Northern blotting. As shown in Fig. 6, strong signals of the transcript hybridizing with the probe obtained from DS112-36 were observed in BAT and heart, weak signals in skeletal muscle and WAT, a faint signal in kidney and little if any in brain and liver. The transcript encoding rat CPTI was observed mainly in kidney, and much less in liver, heart and WAT. The transcript of rat CPTII, like that of the DS112-36 clone, was observed extensively in BAT and heart, much less in skeletal muscle and WAT, and only slightly in the other tissues. No cross-hybridizations of these three probes were observed on Southern analysis (data not shown).

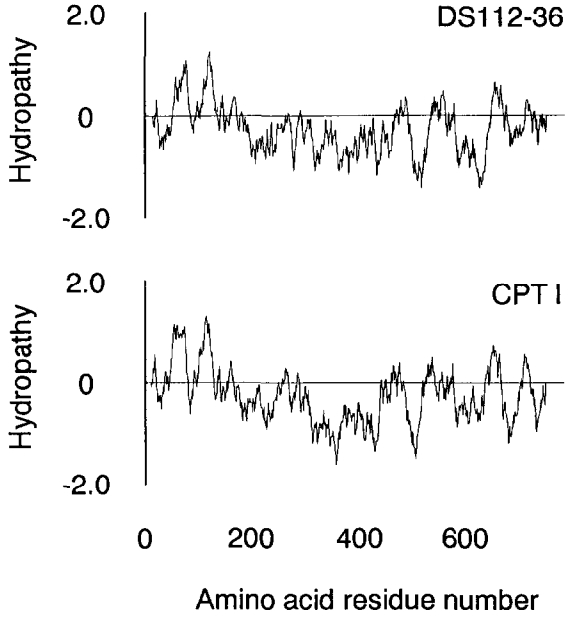

Fig. 5. Hydropathy profiles of DS112-36 and CPTI. The profiles of the two amino acid sequences were analyzed by the method of Kyte and Doolittle [25] with an interval of 30 amino acids.

CPTI catalyzes the formation of acylcarnitine esters from long-chain fatty acyl-CoA thioesters and carnitine in the intramitochondrial space, and CPTII catalyzes the conversion of acylcarnitine esters, which have been transported through the inner mitochondrial membrane via the acylcarnitine/carnitine antiporter, to acyl-CoA thioesters in the matrix space [17–21]. However, another possibility that CPTI is a regulatory protein of CPTII, which catalyzes these two types of conversions in both the intramitochondrial space and matrix space has been proposed [20,22]. Recently, cDNA clones encoding CPTI (M_r=88,150) and CPTII (M_r=74,119) were isolated from a cDNA library of rat liver [13,14]. However, as shown in Fig. 6, the transcript of the reported CPTI was mainly expressed in kidney and much less in liver, heart and WAT, and that of CPTII was mainly expressed in BAT and heart. For efficient oxidation of long-chain fatty acids in energy requiring tissues and organs such as BAT and heart, high and comparable amounts of CPTI and CPTII are expected to be required. The cDNA clone that we isolated was very similar in structural features to CPTI, and in tissue distribution to CPTII. Furthermore, the possible existence of an isozyme of CPTI has been suggested [23,24]. From these facts, we think that the cDNA clone that we isolated could encode an isozyme of CPTI mainly expressed in BAT and heart. Examination of the functional role of the protein encoded by the present cDNA clone in BAT and heart is underway.

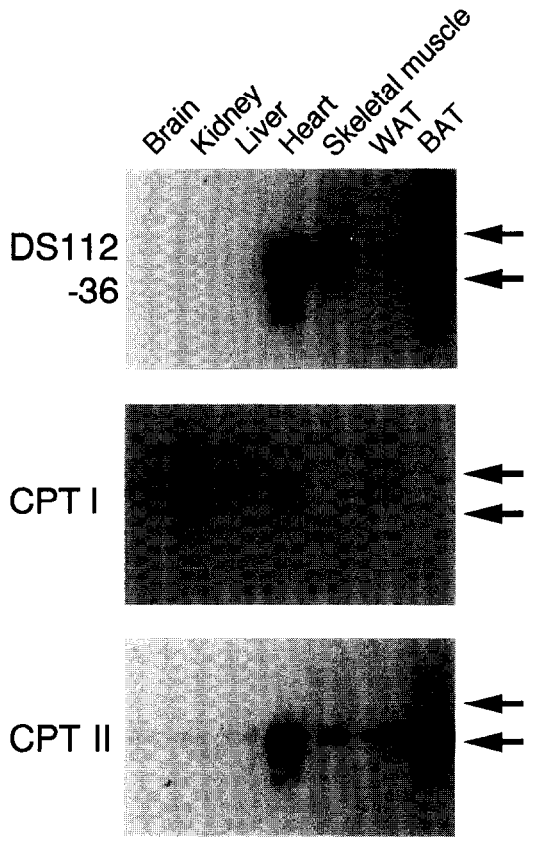

Fig. 6. Tissue distributions of transcripts of DS112-36, CPTI and CPTII. Samples of 10 μg of total RNAs obtained from rat brain, kidney, liver, heart, skeletal muscle, WAT and BAT were analyzed. The arrows indicate the positions of 28 S and 18 S ribosomal RNA.

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