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cDNA-derived amino acid sequence of rat mitochondrial 3-oxoacyl-CoA thiolase with no transient presequence: structural relationship with peroxisomal isozyme

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The sorting of homologous proteins between two separate intracellular organelles is a major unsolved problem. 3-Oxoacyl-CoA thiolase is localized in mitochondria and peroxisomes, and provides a good system for the study on the problem. Unlike most mitochondrial matrix proteins, mitochondrial 3-oxoacyl-CoA thiolase in rats is synthesized with no transient presequence and possesses information for mitochondrial targeting and import in the mature protein. Two overlapping cDNA clones contained an open reading frame encoding a polypeptide of 397 amino acid residues (predicted M_r = 41 868), a 5′ untranslated sequence of 164 bp, a 3′ untranslated sequence of 264 bp and a poly(A) tract. The amino acid sequence of the mitochondrial thiolase is 37% identical with that of the mature portion of rat peroxisomal 3-oxoacyl-CoA thiolase percursor. These results suggest that the two thiolases have a common origin and obtained information for targeting to respective organelles during evolution. Two portions in the mitochondrial thiolase that may serve as a mitochondrial targeting signal are presented.

Key words: non-cleavable signal/mitochondrial protein import/nucleotide sequence/3-oxoacyl-CoA thiolase/sequence homology

Introduction

Mammalian tissues have four known types of thiolase and these differ in intracellular localization. Cytosolic acetoacetyl-CoA thiolase catalyzes the formation of acetoacetyl-CoA required for cholesterol biosynthesis (Middleton, 1973). Two types of thiolase in the mitochondrial matrix have been identified. One is a mitochondrial acetoacetyl-CoA thiolase, which seems to function in ketone body metabolism (Middleton, 1973), and the other is a mitochondrial 3-oxoacyl-CoA thiolase (acetyl-CoA acyltransferase), which catalyzes the last step of the fatty acid β-oxidation cycle (Seubert *et al.*, 1968; Middleton, 1973; Staack *et al.*, 1978). Additionally, peroxisomes have a discrete β-oxidation system and a discrete 3-oxoacyl-CoA thiolase (peroxisomal 3-oxoacyl-CoA thiolase) (Lazarow, 1978; Miyazawa *et al.*, 1980). Use of these enzymes will facilitate study on the intracellular transport of proteins in higher species of animals. After synthesis, the cytosolic thiolase remains in the cytosol, whereas mitochondrial and peroxisomal thiolases are transported across the organelle membranes. Most mitochondrial proteins are synthesized initially as larger precursors with amino-terminal presequence in the cytosol. These precursors are subsequently transported into the mitochondria and cleaved to their mature subunits (for reviews, see Doonan *et al.*, 1984; Felipo and Grisolía, 1984; Hay *et al.*, 1984; Reid, 1985). The presequences of imported mitochondrial proteins share several characteristics, although they do not show extensive sequence homology. They are rich in positively charged amino acids (i.e. arginine and lysine), devoid of acidic residues, and have no hydrophobic amino acid stretch. Gene fusion experiments show that attachment of the presequences of imported mitochondrial proteins is sufficient to direct non-mitochondrial 'passenger' proteins into the mitochondria (Hurt *et al.*, 1984; Horwich *et al.*, 1985; Emr *et al.*, 1986; Nguyen *et al.*, 1986; van Loon *et al.*, 1986). Cleavable presequences thus contain sufficient information for mitochondrial targeting and import.

We carried out an *in vitro* synthesis of the mitochondrial and peroxisomal thiolases of rat liver. Like most mitochondrial matrix proteins, mitochondrial acetoacetyl-CoA thiolase is synthesized as a precursor which is larger than the mature subunit, whereas the *in vitro* product of mitochondrial 3-oxoacyl-CoA thiolase apparently is of the same size as the mature subunit (Ozasa *et al.*, 1984). Therefore, we assumed that 3-oxoacyl-CoA thiolase has a non-cleavable signal which is recognized by the mitochondria (Mori *et al.*, 1985). On the other hand, unlike most peroxisomal proteins, peroxisomal 3-oxoacyl-CoA thiolase is synthesized as a larger precursor and undergoes proteolytic processing during maturation, although function of the pre-peptide portion of the larger precursor in peroxisomal targeting is not known (Furuta *et al.*, 1982; Miura *et al.*, 1984). Molecular cloning of cDNA sequences for these thiolases should facilitate further examination of the synthesis, intracellular transport and processing of these enzymes. The cloning will aid in identifying the protein and gene structures of these enzymes.

We isolated partial cDNA clones for rat mitochondrial 3-oxoacyl-CoA thiolase and measured the size and amount of the mRNA for the enzyme, using the cDNA as a probe (Miura *et al.*, 1986). We also isolated the overlapping cDNA clones coding for most of the thiolase mRNA. The present report is a description of the cDNA-derived entire amino acid sequence of rat mitochondrial 3-oxoacyl-CoA thiolase and comparison with that of the peroxisomal isozyme of 3-oxoacyl-CoA thiolase. The non-cleavable targeting signal of the mitochondrial thiolase is discussed.

Results

Isolation of cDNA clones

We isolated a cDNA clone (pT1-19) for rat 3-oxoacyl-CoA thiolase with an insert of ~1500 bp from a cDNA library constructed from enriched mRNA for the thiolase (Miura *et al.*, 1986). Nucleotide sequencing of the cDNA revealed that the insert contained a 3′ untranslated sequence, a poly(A) segment and most of the protein-coding sequence (corresponding to C-terminal 393 amino acid residues), but lacked the extreme 5′ end of the coding sequence (Figure 1). Therefore, we rescreened ~1 × 10^5 clones of a rat liver cDNA library in λgt11, using the 191-bp *Rsa*I fragment of the clone pT1-19 as a hybridization probe. Eight

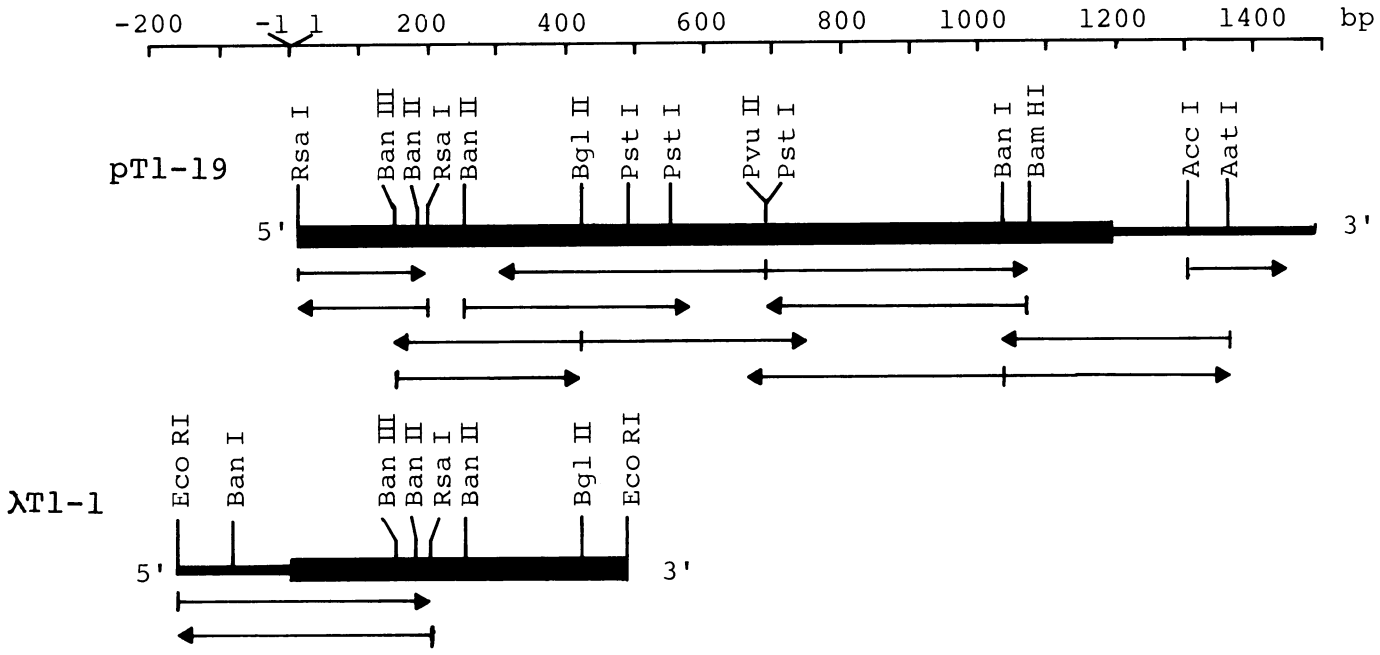

Fig. 1. Restriction endonuclease map and sequencing strategy of cDNAs (pT1-19 and λT1-1) of rat 3-oxoacyl-CoA thiolase. Protein coding sequences are indicated by the thick lines and untranslated sequences by the solid lines. The direction and extent of sequence determinations are shown by the horizontal arrows.

positive clones were isolated and inserts of these clones were subcloned into a plasmid vector pUC9. One clone, designated λT1-1, possessed a cDNA insert which overlapped that of pT1-19 and extended to the approximate 5′ end of the thiolase mRNA (Figure 1).

Nucleotide sequences and predicted amino acid sequence

The restriction maps of cDNAs and sequence analysis strategy are shown in Figure 1. The sequence was determined on both strands of the cDNAs, crossing restriction fragment junctures, except for the region around the 3′ terminus. The nucleotide sequence and predicted amino acid sequence are shown in Figure 2. The translation initiation site was assigned to the methionine codon ATG at nucleotide positions 1–3, because this was the first ATG triplet downstream of the in-frame nonsense codon TAA at positions −15 to −13. The sequence upstream from the ATG initiation codon at positions −8 to −1, GCTTCGTC, resembles the consensus sequence GTACCRCC for eukaryotic initiation sites (Kozak, 1984). Sequence analysis of puified rat 3-oxoacyl-CoA thiolase showed that the amino-terminal amino acid residue was blocked, as deduced from its resistance to Edman degradation. The cDNA insert contains the 5′ untranslated sequence of 164 bp, the protein coding sequence of 1191 bp and the 3′ untranslated sequence of 264 bp. The 5′ untranslated sequence contains a short open reading frame that begins with ATG at positions −123 to −121, terminates at position −114 to −112, and may encode for three amino acid residues. The 5′ untranslated sequence also contains GC-rich regions. The extreme 5′ region contains an unusual poly(dG) tract of 15 residues, although the possibility of cloning artifact was not ruled out. Putative polyadenylation signals, AATAAA, were present 14 and 112 nucleotides upstream from the poly(A) tract.

The amino acid composition predicted from the nucleotide sequence agrees well with that determined by amino acid analysis of the purified enzyme (Table I). The calculated mol. wt is 41 868, including the initiator methionine; a value in good agreement with the value (43 000) estimated by SDS–PAGE (Mori *et al.*, 1985).

Comparison with peroxisomal 3-oxoacyl-CoA thiolase

In addition to the mitochondrial fatty acid β-oxidation cycle, peroxisomes have a discrete β-oxidation system and a discrete 3-oxoacyl-CoA thiolase with catalytic, molecular and immunological properties which differ from those of the mitochondrial thiolase (Miyazawa *et al.*, 1980, 1981). Unlike most peroxisomal proteins, peroxisomal 3-oxoacyl-CoA thiolase is synthesized as a larger precursor with a transient presequence of ~3000 daltons, and undergoes proteolytic processing during maturation (Furuta *et al.*, 1982; Miura *et al.*, 1984). Recently, the primary structure of the peroxisomal thiolase precursor has been determined (T.Osumi and T.Hashimoto, Shinshu University, personal communication). Figure 3 shows a comparison of the amino acid sequences of the mitochondrial and peroxisomal 3-oxoacyl-CoA thiolases. To increase the homology, three gaps in the mitochondrial thiolase and two gaps in the peroxisomal thiolase precursor were introduced. The protein sequence of the mitochondrial thiolase is substantially homologous to that of the mature portion of the peroxisomal thiolase precursor. Without considering the gaps, the sequences of the mitochondrial and peroxisomal thiolases are 37% identical. COOH-half portions of the two thiolases are more homologous than NH_2-half portions. In the COOH-half portions, successive 11 amino acid residues match perfectly in one block and successive 4–6 residues match in six blocks. There are several highly homologous regions; the regions corresponding to the residues 160–188, 220–257 and 282–388 of the mitochondrial thiolase are 55, 58 and 54% identical respectively.

Figure 4 shows hydropathy profiles of rat mitochondrial and peroxisomal 3-oxoacyl-CoA thiolases and distribution of charged amino acid residues. The hydropathy profiles of the mitochondrial and peroxisomal enzymes are aligned so that the

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-164   TAACCTTGGCCCCGGCGGCGCGGGGGGGGGGGGGGGCAGGGATGGAAAAGTAGGGGAAAGGGCAGGGGCGCGGC

CTCTCGGGGCGCCGCCACACTATTAAGGCTGGGCTAGGGCTGGTGGCCGAGTGAAGCTGTGAGAGACCCGTGGAATAACTGAGCTTCGTC

        10         20         30         40         50         60         70         80         90
ATGGCGCTGCTACGAGGTGTGTTTATCGTTGCTGCGAAGCGAACACCCTTTGGAGCTTATGGGGGTCTTCTCAAGGACTTCACTGCCACT
MetAlaLeuLeuArgGlyValPheIleValAlaAlaLysArgThrProPheGlyAlaTyrGlyGlyLeuLeuLysAspPheThrAlaThr

       100        110        120        130        140        150        160        170        180
GACTTAACTGAATTTGCTGCCAGGGCTGCCCTGTCTGCTGGCAAAGTTCCACCGGAAACCATCGATAGTGTCATCGTGGGCAATGTCATG
AspLeuThrGluPheAlaAlaArgAlaAlaLeuSerAlaGlyLysValProProGluThrIleAspSerValIleValGlyAsnValMet

       190        200        210        220        230        240        250        260        270
CAGAGCTCTTCAGATGCGGCGTACCTGGCAAGGCATGTGGGTTTACGTGTGGGAGTCCCGACGGAGACTGGGGCCCTCACCCTCAACAGA
GlnSerSerSerAspAlaAlaTyrLeuAlaArgHisValGlyLeuArgValGlyValProThrGluThrGlyAlaLeuThrLeuAsnArg

       280        290        300        310        320        330        340        350        360
CTCTGTGGCTCTGGTTTCCAGTCCATCGTGAGCGGATGTCAGGAAATCTGCTCGAAAGACGCTGAGGTCGTCTTATGTGGAGGAACCGAG
LeuCysGlySerGlyPheGlnSerIleValSerGlyCysGlnGluIleCysSerLysAspAlaGluValValLeuCysGlyGlyThrGlu

       370        380        390        400        410        420        430        440        450
AGCATGAGCCAGTCCCCCTACTCTGTCAGAAATGTGCGCTTCGGAACCAAATTTGGGTTAGATCTCAAGCTGGAAGATACTTTGTGGGCA
SerMetSerGlnSerProTyrSerValArgAsnValArgPheGlyThrLysPheGlyLeuAspLeuLysLeuGluAspThrLeuTrpAla

       460        470        480        490        500        510        520        530        540
GGATTAACGGATCAACACGTGAAGCTCCCCATGGGGATGACTGCAGAGAACCTGGCTGCAAAATACAACATAAGCAGAGAAGACTGCGAC
GlyLeuThrAspGlnHisValLysLeuProMetGlyMetThrAlaGluAsnLeuAlaAlaLysTyrAsnIleSerArgGluAspCysAsp

       550        560        570        580        590        600        610        620        630
AGATACGCCCTGCAGTCCCAGCAGAGGTGGAAAGCCGCTAACGAGGCTGGCTACTTTAATGAGGAGATGGCCCCCATTGAGGTGAAGACC
ArgTyrAlaLeuGlnSerGlnGlnArgTrpLysAlaAlaAsnGluAlaGlyTyrPheAsnGluGluMetAlaProIleGluValLysThr

       640        650        660        670        680        690        700        710        720
AAGAAGGGCAAACAGACCATGCAAGTGGATGAGCACGCCCGGCCCCAAACGACCCTGGAGCAGCTGCAGAACCTCCCGCCAGTGTTCAAG
LysLysGlyLysGlnThrMetGlnValAspGluHisAlaArgProGlnThrThrLeuGluGlnLeuGlnAsnLeuProProValPheLys

       730        740        750        760        770        780        790        800        810
AAAGAGGGGACGGTCACAGCAGGGAACGCCTCGGGCATGTCTGACGGTGCTGGGGTCGTCATCATAGCCAGCGAAGATGCTGTCAAAAAA
LysGluGlyThrValThrAlaGlyAsnAlaSerGlyMetSerAspGlyAlaGlyValValIleIleAlaSerGluAspAlaValLysLys

       820        830        840        850        860        870        880        890        900
CATAACTTCACACCACTGGCCAGAGTCGTGGGCTACTTTGTGTCTGGATGTGACCCTGCTATCATGGGGATCGGTCCAGTCCCTGCCATC
HisAsnPheThrProLeuAlaArgValValGlyTyrPheValSerGlyCysAspProAlaIleMetGlyIleGlyProValProAlaIle

       910        920        930        940        950        960        970        980        990
ACTGGAGCATTGAAGAAAGCTGGGCTGAGCCTTAAGGACATGGATTTGATAGACGTGAATGAAGCATTTGCTCCTCAGTTCTTGGCTGTT
ThrGlyAlaLeuLysLysAlaGlyLeuSerLeuLysAspMetAspLeuIleAspValAsnGluAlaPheAlaProGlnPheLeuAlaVal

      1000       1010       1020       1030       1040       1050       1060       1070       1080
CAGAAGAGCTTGGATCTCGACCCCAGTAAAACCAACGTGAGTGGAGGTGCCATAGCCCTGGGTCACCCGCTGGGAGGATCTGGATCCAGA
GlnLysSerLeuAspLeuAspProSerLysThrAsnValSerGlyGlyAlaIleAlaLeuGlyHisProLeuGlyGlySerGlySerArg

      1090       1100       1110       1120       1130       1140       1150       1160       1170
ATCACCGCACACCTGGTTCACGAGTTAAGGCGTCGAGGTGGAAAATACGCAGTGGGATCAGCTTGCATTGGAGGTGGCCAAGGCATCTCC
IleThrAlaHisLeuValHisGluLeuArgArgArgGlyGlyLysTyrAlaValGlySerAlaCysIleGlyGlyGlyGlnGlyIleSer

      1180       1190
CTGATCATCCAGAACACAGCCTGAAGGGATTGCAAGCATCCTACCCACCCTCACTTGGCCAGGCTACGGAACACAGGCGACCTTTGAGTC
LeuIleIleGlnAsnThrAla***

AGCCCTGCTGTGACAGTAAATGCATTTGACCAAGCCTTGATGGGTTCTGTCTACTTCATACCTGCCTAGCGTGTTAGAATAAAAAGACCA

GCCATCAGAGGCCTTTAGAGATACCTACTGGTGTCATTGTCAGCAGCCACCACTGTATGCCTTACATGGTGAAATTACAAACTGAATAAA

TGTTGCCTTAACTCC   1455
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Fig. 2. Nucleotide sequence of cDNA for rat 3-oxoacyl-CoA thiolase and deduced amino acid sequence. Nucleotides are numbered in the 5′ to 3′ direction, beginning with the first residue of the ATG triplet encoding the initiator methionine, and the nucleotides on the 5′ side of residue 1 are indicated by negative numbers. The G-homopolymer in the 5′ untranslated sequence is underlined, and the putative polyadenylation signals are doubly underlined. The poly(dA) tract residues is not included.

corresponding region of the two enzymes in Figure 3 occupies the same position in each profile. Both thiolases have a number of relatively short hydrophobic and hydrophilic regions. According to Kyte and Doolittle (1982), when the hydropathy of a given 19 residue segment averages >1.6, there is a high probability that it will be a membrane-spanning sequence. There is no such region in these two thiolases. This is consistent with previous biochemical and histochemical findings that the mitochondrial

Table I. Amino acid composition of rat mitochondrial 3-oxoacyl-CoA thiolase

Residue	Number of residues	
	Deduced from cDNAs[a]	Determined from purified enzyme[b]
Ala	44	40.2
Arg	17	18.9
Asn	13	31.5[c]
Asp	19	
Cys	7	5.3
Gln	17	35.6[d]
Glu	19	
Gly	44	42.9
His	7	6.8
Ile	19	16.9
Leu	36	36.0
Lys	23	25.4
Met	9	9.2
Phe	13	12.8
Pro	17	16.9
Ser	27	26.9
Thr	24	24.2
Trp	2	1.4
Tyr	8	8.0
Val	31	35.3
Total	397	394.2

[a]Initiation Met not included in amino acid composition data.
[b]From Miyazawa *et al.* (1981). Calculated based on subunit M_r = 41 868.
[c]Value for Asn plus Asp.
[d]Value for Gln plus Glu.

and peroxisomal thiolases are localized in the matrix of each organelle. The hydropathy profiles revealed that the profiles of the COOH-half portions are very similar between the two thiolases, whereas those of the NH_2-half portions are much less similar. These results, as well as the results of the sequence homology, suggest that the COOH-half portions of the thiolases are important for catalytic functions common to both thiolases.

Distribution of charged amino acid residues is similar between the mitochondrial and peroxisomal thiolases at the COOH-half portions, but not at the NH_2-half portions. In the mitochondrial thiolase there are several regions with two or more basic amino acid residues and no acidic residue (see Discussion).

Discussion

Mitochondrial and peroxisomal proteins are synthesized on free ribosomes, released into the cytosol and then transported post-translationally into the respective organelles. Most mitochondrial proteins are synthesized as larger precursors with amino-terminal presequences, whereas most peroxisomal enzymes are synthesized with no transient presequence (for reviews, see Doonan *et al.*, 1984; Felipo and Grisolía, 1984; Hay *et al.*, 1984; Lazarow and Fujiki, 1985; Reid, 1985; Borst, 1986). Mitochondrial and peroxisomal 3-oxoacyl-CoA thiolase isozymes are quite unique in that the mitochondrial thiolase is synthesized with no transient presequence (Ozasa *et al.*, 1984; Mori *et al.*, 1985), whereas the peroxisomal thiolase is synthesized as a larger precursor and undergoes proteolytic processing during maturation (Furuta *et al.*, 1982; Miura *et al.*, 1984). The present results demonstrate that the amino acid sequence of the mitochondrial thiolase is highly homologous with that of the mature portion

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                                                          ↓
PT  -26-  5:                                MHRLQVVLGHLAGRSESSSALQAAPCSAGFP

T1    1- 49: MALLRGVFIVAAKRTPFG-AYGGLLKDFTATDLTEFAARAALSAGKVPPE
PT    6- 55: QASASDVVVVHGRRTPNRRAGRGGFKDTTPDELLSAVLTAVLQDVKLKPE

T1   50- 99: TIDSVIVGNVMQSSSDAAYLARHVGLRVGVPTETGALTLNRLCGSGFQSI
PT   56-104: CLGDISVGNVLQPGAGAAM-ARIAQFLSGIPETVPLSAVNRQCSSGLQAV

T1  100-149: VSGCQEICSKDAEVVLCGGTESMSQSPYSVRNVRFGTKFGLDLKLEDTLW
PT  105-143: ANIAGGIRNGSYDIGMACGVESMTLS----------ERGNPGNISSRLL

T1  150-199: AGLTDQHVKLPMGMTAENLAAKYNISREDCDRYALQSQQRWKAANEAGYF
PT  144-193: ENEKARDCLIPMGITSENVAERFGISRQKQDAFALASQQKAASAQSKGCF

T1  200-244: NEEMAP-----IEVKTKKGKQTMQVDEHARPQTTLEQLQNLPPVFKKEGT
PT  194-243: RAEIVPVTTTVLDDKGDRKTITVSQDEGVRPSTTMEGLAKLKPAFKDGGS

T1  245-294: VTAGNASGMSDGAGVVIIASEDAVKKHNFTPLARVVGYFVSGCDPAIMGI
PT  244-293: TTAGNSSQVSDGAAAVLLARRSKAEELGLPILGVLRSYAVVGVPPDIMGI

T1  295-344: GPVPAITGALKKAGLSLKDMDLIDVNEAFAPQFLAVQKSLDLDPSKTNVS
PT  294-343: GPAYAIPAALQKAGLTVNDIDIFEINEAFASQALYCVEKLGIPAEKVNPL

T1  345-393: GGAIALGHPLGGSGSRITAHLVHELRRRGGK-YAVGSACIGGGQGISLII
PT  344-393: GGAIALGHPLGCTGARQVVTLLNELKRRGRRAYGVVSMCIGTGMGAAAVF

T1  394-397: QNTA
PT  394-398: EYPGN
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Fig. 3. Comparison of amino acid sequence of rat mitochondrial 3-oxoacyl-CoA thiolase (T1) with that of rat peroxisomal 3-oxoacyl-CoA thiolase precursor (PT). The sequence of the peroxisomal thiolase precursor has been determined by Osumi, Hashimoto and others of Shinshu University (personal communication). The amino-terminal amino acid residue of the mature peroxisomal thiolase is indicated as residue 1 and the presequence by negative numbers. The arrow indicates the cleavage site. Gaps were introduced to increase the homology and matching amino acids are boxed.

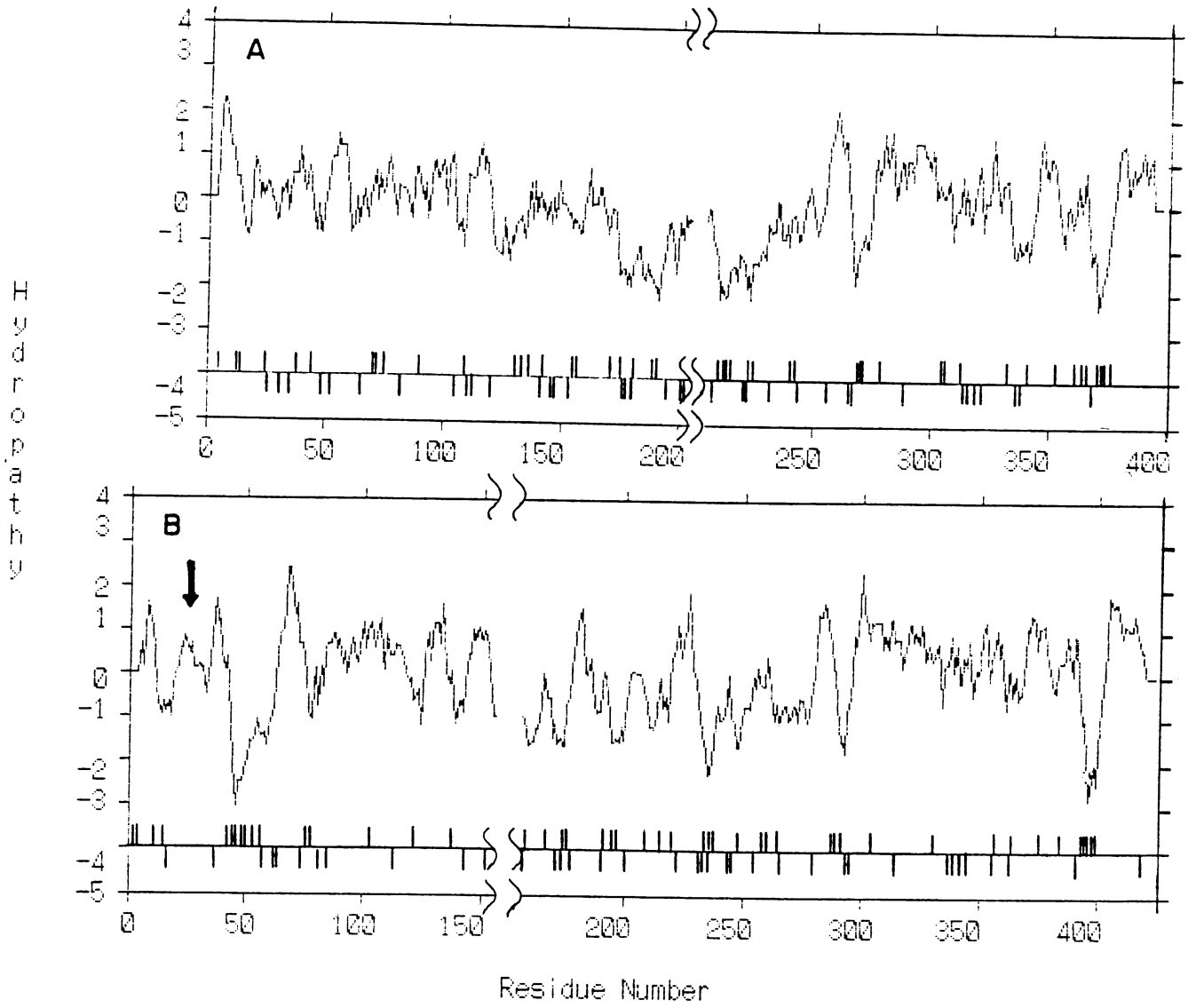

Fig. 4. Hydropathy profiles of rat mitochondrial **(A)** and peroxisomal **(B)** 3-oxoacyl-CoA thiolases and distribution of charged amino acid residues. Hydropathy was analyzed according to Kyte and Doolittle (1982). The hydropathy profiles of the mitochondrial and peroxisomal thiolases are aligned so that the corresponding region of the two enzymes in Figure 3 occupies the same position in each profile. Discontinuous regions correspond to the gaps in Figure 3. Distribution of charged amino acid residues is shown under the hydropathy profile; basic residues are shown by vertical bars above the horizontal line and acidic residues below the horizontal line. The arrow in B indicates the cleavage site.

of the peroxisomal thiolase. This suggests that these two thiolases have a common origin and obtained information for targeting to the respective organelles during evolution. We also determined most of the amino acid sequence of rat mitochondrial 3-hydroxyacyl-CoA dehydrogenase (Y.Amaya *et al.*, unpublished results) and found that the sequence is highly homologous to the sequence of the carboxy-terminal half of peroxisomal enoyl-CoA hydratase-3-hydroxyacyl-CoA dehydrogenase bifunctional protein (Osumi *et al.*, 1985).

Functions of presequences of imported mitochondrial proteins have been studied in gene fusion experiments. Hurt *et al.* (1984) showed that the cleavable presequence of yeast cytochrome *c* oxidase subunit IV is sufficient to direct mouse cytosolic dihydrofolate reductase into the yeast mitochondria. Similar deductions have been made in case of presequences of human ornithine carbamoyltransferase (Horwich *et al.*, 1985), rat carbamoylphosphate synthetase (Nguyen *et al.*, 1986), yeast F_1-ATPase β-subunit (Emr *et al.*, 1986), yeast δ-aminolevulinate synthase (Keng *et al.*, 1986), yeast alcohol dehydrogenase III (van Loon *et al.*, 1986), and yeast cytochrome *c* (van Loon *et al.*, 1986). Critical regions and residues in presequences for mitochondrial targeting (Horwich *et al.*, 1986) and intramitochondrial sorting (van Loon *et al.*, 1986) have been investigated. Roise *et al.* (1986) and von Heijne (1986) propose a model in which mitochondrial presequences can form an amphiphilic α-helix which enables the presequences to interact directly with energized mitochondrial membranes (reviewed by Hurt and van Loon, 1986). More recenly, Allison and Schatz (1986) showed that an amphiphilic α-helix is not essential for mitochondrial targeting and that the targeting function may depend on the overall balance between basic, hydrophobic and hydroxylated amino acids.

On the other hand, a limited number of mitochondrial proteins, including outer membrane proteins, are synthesized with no transient presequence, and are expected to possess non-cleavable mitochondrial addressing signals. Regarding the non-cleavable signal, Hurt *et al.* (1985) showed that the first 12 amino acids of a yeast mitochondrial outer membrane protein, the 70-kd

protein, can direct an attached protein into the mitochondria. This portion contains three basic amino acid residues, no acidic residue and shares a common feature of mitochondrial protein presequences. The amino-terminal portion (14 amino acids) of 3-oxoacyl-CoA thiolase contains three basic residues and no acidic residue. The corresponding portion of the peroxisomal thiolase has three basic residues (two arginines and one histidine) and one acidic residue. Therefore, the amino-terminal portion of the mitochondrial thiolase may function as a mitochondrial targeting signal, although this portion cannot form an amphiphilic helix. It is also possible that the amino-terminal extension of the peroxisomal thiolase precursor might mask the adjacent 'putative' mitochondrial targeting signal, thus making it non-functional. Another portion that may function as the signal is that corresponding to the residues 123–138. This portion has three basic amino acid residues and no acidic residue, and the corresponding portion is missing in the peroxisomal thiolase. If this region is plotted on a helical wheel, all positive charges are clustered on one side (data not shown). However, it does not form an amphiphilic helix, because the opposite side is also hydrophilic. In addition to the two sequences, there are several other regions that have several basic amino acid residues and no acidic residue, and one of these regions may serve as the mitochondrial targeting signal. Gene fusion experiments to define the targeting signal are in progress.

Materials and methods

Cloning of cDNA carrying 5' terminal region

A rat liver cDNA library in λgt11 (Schwarzbauer *et al.*, 1983) was a generous gift from Dr R.O.Hynes (Massachusetts Institute for Technology, Cambridge,MA). The cDNA library was screened by plaque hybridization with the nick-translated 191-bp *Rsa*I fragment of the plasmid pT1-19 (Miura *et al.*, 1986) as a probe. *Eco*RI-excised cDNA inserts of the positive clones were subcloned into a plasmid vector pUC9 and analyzed by restriction mapping.

Nucleotide sequence analysis

Nucleotide sequence was determined by the dideoxynucleotide chain termination method (Sanger *et al.*, 1977) after subcloning into M13mp9 (Messing *et al.*, 1981).

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