Isolation and characterization of cDNA for human 120 kDa mitochondrial
2,4-dienoyl-coenzyme A reductase

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INTRODUCTION

Our knowledge of the auxiliary enzyme systems required during the β-oxidation of (poly)unsaturated enoyl-CoAs [2,4-dienoyl-CoA reductase (EC 1.3.1.34), 2-enoyl-CoA hydratase 2, Δ⁹,Δ⁴- enoyl-CoA isomerase (EC 5.3.3.8) and Δ⁹,Δ⁴-dienoyl-CoA isomerase] has expanded during the past few years. New aspects include the observation that these enzymes exist in different isoforms distributed in different subcellular organelles, and occasionally within the same cell compartments (Hiltunen et al., 1993).

2,4-Dienoyl-CoA reductase, which catalyses the reaction:

$$\text{trans-2, cis/trans-4-dienoyl-CoA + NADPH + H}^+ \rightarrow \text{trans-3-enoyl-CoA + NADP}^+$$

in eukaryotes (Kunau and Dommes, 1978), has been purified from several sources: rat liver (Kimura et al., 1984), bovine liver (Dommes et al., 1982; Dommes and Kunau, 1984), the yeasts Candida lipolytica (Mizugaki et al., 1985) and Candida tropicalis (Dommes et al., 1983) and the bacterium Escherichia coli (Mizugaki et al., 1982). In contrast with eukaryotes, the end product of the bacterial reaction is trans-2-enoyl-CoA. Reductase activity in mammals has been shown to be associated with both mitochondria and peroxisomes (Dommes et al., 1981; Kimura et al., 1984; Hiltunen et al., 1986), and two mitochondrial isoforms with native molecular masses of 60 and 120 kDa have also been observed in rat liver (Hakkola and Hiltunen, 1993). The 120 kDa isoform contains four identical subunits, each with a molecular mass of 36 kDa. This subunit has been recently cloned and sequenced (Hirose et al., 1990). The necessity of 2,4-dienoyl-CoA reductase activity for β-oxidation of (poly)unsaturated fatty acids has been suggested by several observations: (a) The uncoupling of isolated liver mitochondria lowers the mitochondrial NADPH/NAD⁺ ratio and results in an accumulation of 2,4-dienoyl-CoA intermediates if polyunsaturated fatty acids are acting as the substrates for mitochondrial β-oxidation (Hiltunen et al., 1983). (b) Examination of solubilized peroxisome incubations has shown that the β-oxidation of docosahexaenoic acid was markedly stimulated by added NADPH (Hiltunen et al., 1986). (c) An E. coli mutant deficient in reductase activity was unable to utilize or grow on petrolsineic acid (cis-C₁₈:₁₄₆₆) (You et al., 1989). (d) Furthermore, one patient has been identified with an inborn 2,4-dienoyl-CoA reductase dysfunction. Urinary excretion of 2-trans,4-cis-decadienoylcarnitine, derived from incomplete oxidation of linoleic acid (cis,cis-C₁₈:₂₈:₋₂₋₁₂), was observed, and in spite of dietary therapy, the patient died at the age of a few months (Roe et al., 1990). In view of the arguments listed above and the important role which nutritional polyunsaturated fatty acids play in human well-being, we were prompted to undertake a project aimed at characterizing human 2,4-dienoyl-CoA reductases. We report in this paper on cDNA cloning of the mitochondrial 120 kDa isoform of human 2,4-dienoyl-CoA reductase.

MATERIALS AND METHODS

Materials

The SureClone ligation Kit and Quick-Prep Micro mRNA Purification Kit were purchased from Pharmacia Biotech, Upplands, Sweden; Taq DNA polymerase I (used in preparing of rat reductase cDNA) was from Perkin-Elmer/Cetus, Norwalk,
CT, U.S.A.; [α-32P]dCTP from Amersham International plc, Amersham, U.K.; the EcoRI- PstI, HindIII restriction enzymes from New England Biolabs (NEB), Beverly, MA, U.S.A.; the Random Primed Labelling Kit from Boehringer, Mannheim, Germany; Sequenase 2.0 Enzyme from United States Biochemical Corp., Cleveland, OH, U.S.A.; murine moloney leukaemia virus (M-MLV) reverse transcriptase (RT) from GIBCO BRL, Gaithersburg, MA, U.S.A.; Tag DNA polymerase, rRNasin, deoxynucleotides, pGEM-4Z plasmid and RNAagents (‘Total RNA Isolation Kit’) from Promega Corporation, Madison, WI, U.S.A.; Ultrafree MC Durapore 0.45 μm tubes from Millipore, Bedford, MA, U.S.A.; the human liver Agt11 cDNA library, 5'-AmpliFINDER RACE Kit and Multiple Tissue Northern (MTN) Blot from Clontech, Palo Alto, CA, U.S.A.; pBluescript vector from Stratagene, La Jolla, CA, U.S.A.; the nitrocellulose filter from Schleicher and Schuell, Dassel, Germany; goat anti-rabbit IgG from Bio-Rad Laboratories, Richmond, CA, U.S.A.; and 4-chloro-1-naphthol from Sigma Chemicals Co., St. Louis, MO, U.S.A.

**Isolation of RNA**

Male Sprague-Dawley rats were fed on a standard pellet diet supplemented with clofibrate (0.5 %, w/w) for 14 days before being killed. Total RNA was isolated from the livers of treated rats with RNAagents ‘Total RNA Isolation Kit’. Human mRNA was isolated from 50 mg of cultivated fibrosarcoma cells [HT-1080 (ATCC CCL 121)] with a Quick-Prep Micro mRNA Purification Kit.

**Preparation of the rat reductase cDNA clone**

The oligonucleotide primers were synthesized with an Applied Biosystems DNA synthesizer at the Department of Biochemistry, University of Oulu. Rat cDNA primers (Table 1) were synthesized on the basis of the published cDNA sequence for rat 2,4-dienoyl-CoA reductase (Hirose et al., 1990). Single-stranded DNA (ssDNA) was synthesized with M-MLV RT using 1.5 μg of total RNA from the liver of a clofibrate-treated rat as a template and oligo B as an antisense primer in the presence of 20 units (defined by manufacturer) of rRNasin, deoxynucleotides and Tag DNA polymerase buffer supplied by the manufacturer of Tag DNA polymerase I. The reaction proceeded for 60 min at 42 °C. The RT sample of 20 μl was added to a PCR reaction and amplification was carried out with the antisense oligo B and the sense oligo A as primers using Tag DNA polymerase I under the following conditions: 35 cycles of 1 min at 94 °C (denaturation), 0.5 min at 55 °C (annealing) and 2 min at 72 °C (extension). The incubation was continued for 10 min at 72 °C after the last cycle. The RT–PCR product was subjected to electrophoresis on a 0.8 % (w/v) agarose gel and the amplified fragment was purified by centrifugation with Ultrafree MC Durapore 0.45 μm tubes. The PCR product, predicted to contain a 628 bp EcoRI–PstI fragment (rRed), was digested with appropriate endonucleases and ligated to the polyconding site of the pGEM-4Z plasmid. The rRed was partially sequenced by the deoxynucleotide method (Sanger et al., 1977) with the Sequenase 2.0 enzyme.

**Screening for cDNA of human mitochondrial 2,4-dienoyl-CoA reductase**

A human liver Agt11 cDNA library was screened (Sambrook et al., 1989) with the rRed as a probe, which was labelled with [α-32P]dCTP using a Random Primed Labelling Kit. Three positive Agt11 clones were obtained (hRed1, -2 and -3), to characterize which PCR reactions with Taq DNA polymerase were carried out using a sense primer to one Agt11 arm (oligo C), an antisense primer to the second Agt11 arm (oligo D) and an Agt11 clone DNA as a template under the following conditions: 1 min at 94 °C (denaturation), 1 min at 50 °C (annealing) and 2 min at 72 °C (extension). After 25 cycles the incubation was continued for an additional 10 min at 72 °C. Analysis with 0.8 % (w/v) agarose gel electrophoreses showed the amplified PCR fragments to be of the same size. The inserts were partially sequenced using the PCR-oligos as primers (Casanova et al., 1990).

**Amplification of the N-terminus of 2,4-dienoyl-CoA reductase cDNA**

Because the isolated hRed clones did not contain the 5’-terminal sequence of the reductase (see the Results section), a method with the anchor primer PCR was employed using the 5’-AmpliFINDER RACE Kit. mRNA isolated from human fibrosarcoma cells (2 μg) was taken as a template and first-strand cDNA synthesis was carried out with oligo F (Table 1) using avian myeloblastosis virus (AMV) RT at 52 °C for 30 min. After synthesis of the first strand of the cDNA was complete, the mRNA was hydrolysed, the ssDNA was purified and the AmpliFINDER Anchor was ligated with T4 RNA ligase following the protocol recommended by the manufacturer. The anchor-ligated cDNA was taken as the template for PCR amplification, oligo G as the sense primer and oligo E as the nested antisense primer, under the following conditions: 35 cycles of 45 s at 94 °C, 45 s at 60 °C, and 2 min at 72 °C, with a

### Table 1 Oligonucleotide probes used for the isolation of human reductase cDNA clones

<table>
<thead>
<tr>
<th>Name</th>
<th>Nucleotide sequence</th>
<th>Basis of the oligonucleotide</th>
</tr>
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<tbody>
<tr>
<td>Oligo A</td>
<td>5'-TGACAACTTCTGTCACGCTG-3'</td>
<td>Rat reductase cDNA bp 224–246*</td>
</tr>
<tr>
<td>Oligo B</td>
<td>5'-TACATCCAGCCTCTCTGTGCA-3'</td>
<td>Rat reductase cDNA bp 930–972*</td>
</tr>
<tr>
<td>Oligo C</td>
<td>5'-GGTCCGAGCTGCTAGGAGCC-3'</td>
<td>Vector arm: Agt11 forward</td>
</tr>
<tr>
<td>Oligo D</td>
<td>5'-TTGACACCAAGCAGCCTGTGGT-5'</td>
<td>Vector arm: Agt11 reverse</td>
</tr>
<tr>
<td>Oligo E</td>
<td>5'-ATCTTTTACCAAGGCAAGTCC-3'</td>
<td>Human reductase cDNA bp 209–234</td>
</tr>
<tr>
<td>Oligo F</td>
<td>5'-GGCTGCTATCACGGACCTAGTGA-3'</td>
<td>Human reductase cDNA bp 254–280</td>
</tr>
<tr>
<td>Oligo G</td>
<td>5'-CTGGTGCAGCCACCTGAGGAGTCGAGATAGGACGAC-3'</td>
<td>Anchor primer sequence†</td>
</tr>
<tr>
<td>Oligo H</td>
<td>5'-GAGCTAAATGAGCTGAC-3'</td>
<td>Human reductase cDNA bp 1-21</td>
</tr>
<tr>
<td>Oligo I</td>
<td>5'-GAGTGAATGCTCAAGAGTAG-3'</td>
<td>Human reductase cDNA bp 1078–1101</td>
</tr>
</tbody>
</table>

* Hirose et al. (1990).
† Supplied with the AmpliFINDER RACE Kit (Clontech).
The fibrosarcoma cell line (see the Materials and Methods section), the PCR fragment obtained via amplification PCR (hRedT) and the RT–PCR fragment (hRed) are indicated in the figure. The mRNA for PCR amplification was obtained from cultivated fibrosarcoma cell line (see the Materials and Methods section). The arrows represent the direction and extent of the sequence determinations. *E* under the isolated reductase cDNA (RedcDNA) indicates an EcoRI restriction site.

**Figure 1** Isolation of cDNA for human mitochondrial 2,4-dienoyl-CoA reductase

The fragment isolated by screening the human liver Agt1 library with rat reductase (hRedT), the PCR fragment obtained via amplification PCR (hRedT) and the RT–PCR fragment (hRed) were subcloned into the subcloning vector. Total DNA was digested with EcoRI and HindIII. The digested DNA was then subjected to Southern blotting by standard methods (Sambrook et al., 1989). The blotted nitrocellulose filter was prehybridized for 4 h at 42 °C with 25 mM KH₂PO₄, 37.5 mM NaCl, 3.75 mM sodium citrate, 5 × Denhardt's solution, 50 μg/ml salmon sperm DNA and 50% (v/v) formamide. The hybridization was performed overnight in the above solution supplemented with 32P-labelled hRedT probe and 10% (w/v) dextran sulphate at 42 °C. The filter was washed twice with 0.15 M NaCl, 0.015 M sodium citrate, 0.1% (w/v) SDS at 42 °C for 15 min, and once at room temperature and once at 42 °C with 37.5 mM NaCl, 3.75 mM sodium citrate, 0.1% SDS for 15 min before autoradiographing at −70 °C for 48 h using Kodak XAR film with an intensifying screen.

**RT–PCR for the coding region of human mitochondrial 2,4-dienoyl-CoA reductase cDNA**

The RT–PCR (Sambrook et al., 1989) was carried out to obtain the full-length cDNA for the coding region of the human reductase using oligo I as the primer for the RT reaction and mRNA (1 μg) from fibrosarcoma cells as the template. The PCR reaction was run using oligo H as the sense primer and oligo I as the antisense primer for 25 cycles under the following conditions: 1 min at 94 °C, 1 min at 56 °C and 2 min at 72 °C. Thereafter the extensions were completed by incubating the reaction mixture for 10 min at 72 °C. The resulting PCR fragment (hRedT) was purified, subcloned to pUC18 with a SureClone Ligation Kit and both strands of the subcloned fragment were sequenced.

**Southern hybridization**

Total DNA was extracted from the blood of healthy men by the SDS–proteinase K method (Sambrook et al., 1989) and 15 μg of the isolated DNA was digested with EcoRI and HindIII. The digested DNA was then subjected to Southern blotting by standard methods (Sambrook et al., 1989). The blotted nitrocellulose filter was prehybridized for 4 h at 42 °C with 25 mM KH₂PO₄, 37.5 mM NaCl, 3.75 mM sodium citrate, 5 × Denhardt's solution, 50 μg/ml salmon sperm DNA and 50% (v/v) formamide. The hybridization was performed overnight in the above solution supplemented with 32P-labelled hRedT probe and 10% (w/v) dextran sulphate at 42 °C. The filter was washed twice with 0.15 M NaCl, 0.015 M sodium citrate, 0.1% (w/v) SDS at 42 °C for 15 min, and once at room temperature and once at 42 °C with 37.5 mM NaCl, 3.75 mM sodium citrate, 0.1% SDS for 15 min before autoradiographing at −70 °C for 48 h using Kodak XAR film with an intensifying screen.

**Figure 2** cDNA for human mitochondrial 2,4-dienoyl-CoA reductase and its deduced amino acid sequences

Nucleotides are numbered in the 5' to 3' direction, beginning with the first nucleotide encoding the initiation methionine, which is termed +1. The first nucleotide of the stop codon is in position +1006. The amino acid residues are numbered taking the initiation methionine as +1. The polyadenylation signal is underlined. The proposed cleavage site of the mitochondrial targeting signal is indicated by an arrow.

**Northern hybridization**

A nylon membrane on which 2 μg aliquots of poly(A)⁺ RNA from various human tissues had been blotted [Multiple Tissue Northern (MTN) Blot] was prehybridized at 42 °C for 4 h and hybridized with 32P-labelled hRedT at 42 °C for 20 h following the protocol provided by the supplier. The hybridized fragments were detected by exposing X-ray film at −70 °C for 17 h. The amount of mRNA on the nylon membrane was standardized with a human β-actin cDNA probe (supplied in the Kit).

**Immunoblotting**

Samples containing 100 μg of protein from rat and human liver homogenates were subjected to SDS/PAGE (Laemmli, 1970) followed by electrophoretic transfer of the proteins onto a nitrocellulose filter. Anti-(rat 2,4-dienoyl-CoA reductase) IgG (Hakkola et al., 1989) was used as the primary immunodetection antibody, and goat anti-(rabbit IgG) coupled to horseradish.
peroxidase as the second antibody (Towbin et al., 1979). 4-
Chloro-1-naphthol was used as the stain. The human liver
sample was obtained from forensic autopsies.

Others

DNA sequence analysis, sequence comparisons and restriction
mapping of the subclones were carried out using the HIBIO
DNASIS (V6.00) program package (Hitachi America, Ltd.
Brisbane, CA, U.S.A.), and the exposed films of the Northern
and Southern hybridizations were scanned for the optical
densities and sizes of the signals with a BioImage apparatus
(Millipore, Ann Arbor, MI, U.S.A.). The use of animals in
this project was approved by the University of Oulu Committee
on Animal Experimentation and the use of human tissue was
approved by the National Bureau of Medicine in Finland.

RESULTS

Screening of $5 \times 10^4$ independent recombinants in the human
liver $\lambda g t1$ cDNA library using a cDNA fragment for rat
reductase (rRed) as the probe (see the Materials and methods
section) yielded three positive clones (hRed1, -2 and -3). The
PCR experiments, analysis of the amplified fragments by agarose
gel electrophoresis and partial sequencing of the inserted indicated
that the clones were identical, and thus only hRed1 was taken for
further characterization. To isolate the insert, the Agt1 clone
containing hRed1 was subjected to EcoRI digestion, as it was
this restriction site that was used in the construction of the library.
This resulted in three fragments of approx. 600, 200 and 130 bp,
originating from the insert. The fragments were subcloned to
Bluescript plasmids and both strands sequenced. Partial
sequencing of the PCR-amplified, undigested hRed1–3 inserts
(above) allowed the order of the EcoRI fragments to be deduced.

hRed1 contained a poly(A)+ tail and a signal for poly-
adenylation (AATAAA) 18 bp upstream of it. Because comparison of the hRed1 sequence with the published rat 120 kDa
reductase (reductase-120) nucleotide sequence (Hirose et al.,
1990) indicated that the 5' end was missing, mRNA was isolated from cultured human fibrosarcoma cells, ssDNA was synthe-
tsized and the anchor-primed PCR was carried out. The amplified
fragment was then sequenced after subcloning (see Materials and
methods section). The fragment (hRed4) overlapped with hRed1
and contained an additional 185 bp flanking to the 5' end (Figure
1).

To confirm that the cDNA sequences obtained really covered
the whole region coding for the reductase-120 nucleotide
sequence, an RT–PCR was carried out using mRNA isolated from
human fibrosarcoma cells as the template and taking the sense
oligo H and antisense oligo I as the primers. The amplified
cDNA fragment (hRedT) covered both hRed1 and hRed4 clones
(Figure 1).

The cloned cDNA was 1128 bp long and contained an open
reading frame with 1005 coding nucleotides and TAA as a stop
codon, 110 non-coding 3' flanking nucleotides and 10 non-
coding nucleotides flanking to the 5' end from the initiation ATG
(Figure 2). Adenine was in the –3 position, which is in the line
with the observation that there is a purine (usually A) in this
position in 97% of all vertebrate mRNAs (Kozak, 1991). The

Figure 3 Comparisons of the amino acid sequences of human and rat 2,4-dienoyl-CoA reductase

Identical amino acid residues are marked by a vertical line and amino acid residues which show similarities are marked by a point. There is a 82.7% similarity between the rat and human 2,4-dienoyl-CoA reductase amino acid sequences. The first amino acids of the mature proteins are shown in bold face, these being deduced from N-terminal peptide sequencing of the purified rat
enzyme (Hirose et al., 1990) and based on a prediction (see the Discussion section) in the case of the human enzyme.
deduced polypeptide included 335 amino acid residues, with a predicted molecular mass of 36066 Da. The human reductase-120 nucleotide sequence thus has exactly the same number of amino acid residues as its rat counterpart (Hirose et al., 1990). Comparison of the human and rat 2,4-dienoyl-CoA reductases showed 82.7% similarity in amino acid sequences (Figure 3) and 81.7% identity in the coding nucleotides. In immunoblotting the antibody against the purified rat liver enzyme (Hakkola et al., 1989) recognized only one band in human liver homogenate (Figure 4). However, the signal detected in human liver was slightly larger than in rat liver.

For Northern blotting, 2 μg aliquots of mRNA isolated from various human tissues (heart, brain, placenta, lung, liver, skeletal muscle, kidney and pancreas) were hybridized with hRedT as explained in the Materials and methods section. After 17 h of exposure, the signals for the brain and placenta tissues (Figure 5a, lanes 2 and 3) were not detectable, while all the observed signals were 1.2 kb, which is close to the size of the isolated cDNA (1128 bp). After normalizing the reductase signal intensity by reference to the signal for β-actin, the greatest abundance of reductase mRNA was observed in the heart, liver, pancreas and kidney (Figure 5a, lanes 1, 5, 7 and 8) with relative absorbances of 2.0, 2.0, 1.9 and 1.4 respectively. Northern hybridization with a β-actin probe showed that there are two enzymes of β-actin present in heart and skeletal muscle, namely a 2 kb one and a slightly smaller one of 1.6-1.8 kb (Giovanna et al., 1991; Lamballe et al., 1991). The other tissues have one mRNA of approx. 2 kb (Figure 5b).

When genomic DNA was digested and double-digested with EcoRI and HindIII and the products analysed by Southern blot hybridization with the hRedT as a probe, to obtain information on the gene(s) encoding the mitochondrial 2,4-dienoyl-CoA reductase, five fragments were detected in the case of both EcoRI (7.0, 6.0, 5.1, 3.1 and 2.3 kb) and HindIII (8.8, 4.6, 3.1, 2.7 and 1.3 kb), whereas double-digestion yielded six fragments (6.0, 4.9, 4.5, 2.4, 2.2 and 2.1 kb) (Figure 6). This restriction fragment pattern and the restriction map constructed by the DNASIS program suggest that the size of the reductase gene is approx. 22 kb.

**DISCUSSION**

Chromatographic characterization of 2,4-dienoyl-CoA reductase activity in the rat liver by subcellular fractionation, together with immunological experiments, have shown that the 120 kDa isoform of reductase in the rat originates from the mitochondria (Hakkola et al., 1989; Hakkola and Hiltunen, 1993). The conclusion that the human reductase cloned here represents the human counterpart of this same isoform is allowed by the observations of (a) a high degree of similarity between the nucleotide sequences of the rat and human 2,4-dienoyl-CoA reductase cDNAs, (b) the similar deduced amino acid sequences and hydrophobicity analysis blots (results not shown) for the rat and human reductases and (c) immunological cross-reactivity.

Sequencing of isolated undigested rat reductase has shown the N-terminal sequence of the mature enzyme to be Ser-Ile-Asp-Ala- (Hirose et al., 1990). Speculating on the observed size difference, one can note that the human polypeptide sequence Arg32-Phe24-Phe25-Ser26 agrees with one cleavage motif of the mitochondrial targeting peptides, Arg-X-Phe-Ser (Gavel and von Heijne, 1990), in which the cleavage occurs at -X-Phe-. The functionality of this model is demonstrated by the maturation pattern of rat mitochondrial short-chain Δ2,Δ2-enoyl-CoA isomerase, for example (Palosaari et al., 1991). This will result in a mature human enzyme 10 amino acids larger than that in the rat, and this estimation agrees with the results of immunoblotting of human and rat liver samples with the antibody to rat reductase, i.e. the detected signal in human sample was slightly larger than that in rat (Figure 4).
The helical wheel of the N-terminus of human reductase shows that the basic amino acid residues Lys², Arg⁸ and Arg¹⁴ are located on the same side of the wheel, the opposite side being occupied mainly by hydrophobic amino acid residues (results not shown). Furthermore, the predicted polypeptide does not contain acidic amino acid residues, and thus the structural composition of the predicted mitochondrial targeting signal follows the general topology of N-terminal intramitochondrial targeting polypeptides (von Heijne, 1986; Roise et al., 1986).

All the known 2,4-dienoyl-CoA reductases catalyse a NADPH-dependent, but not NADH-dependent, reduction of 2,4-dienoyl-CoAs, and the human reductase follows this rule (E. H. Hakkola and J. K. Hiltunen, unpublished work). The nucleotide-binding sites of NADP(H)⁺ in nicotinamide adenine dinucleotide-dependent enzymes show a highly conserved β₅α₉β₅ fold, which includes a motif -H-X-H-X-Gly-X-Gly-X-Gly-X-X-H-X-H... Asp/Glu- (H, hydrophobic amino acid residue) (Wierenga et al., 1983). The sequence that comes closest to this structure in human reductase is -Ala⁶₂-X-Ile⁶⁶-X-Gly⁶⁴-X-Gly⁴⁶-X-X-Leu⁹³-X-X-Met⁹⁴-X-Leu⁹⁵... Asp⁶⁵ -, which fits the consensus motif except that the third Gly is replaced by Leu⁹³. It has been shown, however, that the third Gly of the binding motif is not conserved at all in the NADPH-dependent enzyme (McKie and Douglas, 1991).

The 120 kDa reductase shows tissue-specific expression in the human being, its highest mRNA levels being in the myocardium, liver and pancreas, which are tissues known to be capable of oxidative degradation of fatty acids (Shipp et al., 1961; Lee et al., 1962; Weidemann and Krebs, 1969; Johnston, 1977). Interestingly, the reductase was also expressed at low level in the placenta, and we have made the same observation regarding the other auxiliary enzymes of β-oxidation, notably Δ⁹,Δ⁶-enoyl-CoA isomerase (H. M. Häyärinen and J. K. Hiltunen, unpublished work).

Over 20 inborn errors of either mitochondrial or peroxisomal β-oxidation have been identified in human beings and characterized to different extents, and these include one reported case of 2,4-dienoyl-CoA reductase deficiency. However, no inborn errors in the oxidative degradation of unsaturated fatty acids have been characterized at the molecular level so far. This work will therefore provide a tool for the screening and molecular characterization of inborn errors in unsaturated fatty acid metabolism.

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