RESEARCH COMMUNICATION

cDNA cloning and amino acid sequence of human mitochondrial $\Delta^3\Delta^2$ -enoyl-CoA isomerase: comparison of the human enzyme with its rat counterpart, mitochondrial short-chain isomerase

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We report the isolation of a cDNA encoding a mature human monofunctional $\Delta^3\Delta^2$ -enoyl-CoA isomerase and the determination of its nucleotide sequence. The purified uncleaved protein, as well as several internal tryptic and CNBr fragments, were subjected to N-terminal peptide sequencing. The deduced amino acid sequence of the mature protein consists of 260 amino acids

with a predicted M_r of 28735. The human mitochondrial isomerase exhibits a 74% (78%) sequence identity with the corresponding rat counterpart at amino acid (nucleotide) level(s). Many basic amino acid residues in rat isomerase have been changed to acidic or neutral residues in human enzyme, explaining the differences observed between these proteins.

INTRODUCTION

$\Delta^3\Delta^2$ -Enoyl-CoA isomerase (EC 5.3.3.8) catalyses the transfer of both *cis* and *trans* double bonds from the Δ^1 position to *trans*- Δ^2 of enoyl-CoAs, and thus participates in the β -oxidation of unsaturated fatty acids with odd-numbered double bonds [1]. Since 2,4-dienoyl-CoA reductase (EC 1.3.1.34), the key enzyme for the metabolism of even-numbered double bonds, produces *trans*- Δ^3 -enoyl-CoA as the end product in mammals and in yeasts [2,3], $\Delta^3\Delta^2$ -enoyl-CoA isomerase is also required for the metabolism of unsaturated fatty acids with double bonds initially at even-numbered positions. Rat peroxisomal isomerase, which is an integral part of a multifunctional hydratase-dehydrogenase-isomerase polypeptide, is well characterized [4–6] and its mitochondrial counterpart, mitochondrial short-chain isomerase, has been purified [7,8], cloned [9] and crystallized [10]. Interestingly, the mitochondrial multifunctional β -oxidation enzyme, at least from human beings, is devoid of isomerase activity [11].

In view of the failure of our attempt to isolate human mitochondrial isomerase cDNA with rat antibody or cDNA probes we recently purified a mitochondrial monofunctional isomerase from human liver [12]. In the present work, degenerative oligonucleotide probes were synthesized on the basis of amino acid sequences obtained by cleaving the purified protein. Several overlapping clones were isolated, and the nucleotide sequences were found to cover a sequence for the monofunctional $\Delta^3\Delta^2$ -enoyl-CoA isomerase from human.

MATERIALS AND METHODS

Materials

The Mono Q (PC 1.6/5), Superdex 200 HR (10/30), μ RPC (C2/C18 PC 3.2/3) and μ RPC (C2/C18 SC 2.1/10) chromatography columns and Sure Clone Ligation Kit were purchased from Pharmacia Biotech, Uppsala, Sweden; *Taq* DNA polymerase I was from Perkin-Elmer/Cetus, Norwalk, CT, U.S.A.; [α - 32 P]dCTP from Amersham; T4 DNA ligase from NEB, Beverly, MA, U.S.A.; the Random Primed Labeling Kit and trypsin (bovine pancreas), modified sequencing grade, from

Boehringer, Mannheim, Germany; Sequenase 2.0 Enzyme from United States Biochemical Corp., Cleveland, OH, U.S.A.; murine-Moloney-leukaemia-virus (MMLV) reverse transcriptase from GIBCO BRL, Gaithersburg, MA, U.S.A.; rRNasin and deoxynucleotides from Promega Corporation, Madison, WI, U.S.A.; Ultrafree-Dunapore 0.45 μ m tubes from Millipore, Bedford, MA, U.S.A.; the human heart λ ZAP II and liver Uni-ZAP II cDNA libraries from Stratagene, La Jolla, CA, U.S.A.; human liver λ gt11, liver 5'-stretch λ gt11, hepatoma λ gt11, colon λ gt11, placental 5'-stretch λ gt11, lung 5'-stretch λ gt11, fetal liver λ gt10 and fetal heart λ gt10 cDNA libraries from Clontech, Palo Alto, CA, U.S.A.

Peptide sequencing

The mitochondrial isomerase was purified from human liver samples obtained during forensic autopsies by a modification of methods published recently [12]. The isomerase from a hydroxyapatite column was equilibrated with 20 mM Tris/HCl, pH 8.0 (buffer A) and applied to a Mono Q column equilibrated with buffer A. The bound proteins were eluted with a linear NaCl gradient from 0 to 0.3 M at a flow rate of 100 μ l/min using a SMART micropurification system (Pharmacia). The fractions containing isomerase were applied to a Superdex 200HR (10/30) column in equilibrium with 200 mM potassium phosphate/1 mM EDTA/1 mM EGTA, pH 7.4. The column was eluted at a flow rate of 500 μ l/min. The active fractions were pooled and applied to a μ RPC (C2/C18 PC 3.2/3) reverse-phase column in equilibrium with 0.1% trifluoroacetic acid/2% acetonitrile and a linear acetonitrile gradient from 2 to 98% was developed. SDS/PAGE analysis and immunoblotting with antibody to rat short-chain mitochondrial isomerase were carried out to identify fractions containing isomerase. The sample containing 10 μ g of purified isomerase was evaporated, dissolved in 50 μ l of 100 mM Tris/HCl, pH 8.5, and cleaved with 2 μ g of trypsin according to the protocol provided by the manufacturer. Another sample of 10 μ g of purified human isomerase was cleaved with CNBr in 70% formic acid by incubation for 24 h at room temperature. After evaporation the sample was dissolved in 45 μ l of 0.5 M

Abbreviations used: MMLV, murine-Moloney-leukaemia virus; poly(A)⁺, polyadenylated; oligo(s), oligonucleotide(s).

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

Key: R = A + G, Y = C + T, S = G + C, W = A + T/U, M = A + C, N = A + C + G + T

Name	Nucleotide sequence	Basis of the oligonucleotide
Oligo A	5'-ATGAARTTYAARAAYCCNCCNGTNA-3'	The amino acid sequence MKFNPPVN
Oligo B	5'-CCCTTYTCTTCCTTSAGCKTTCCARRTA-3'	The amino acid sequence YLERLKKEEG
Oligo C	5'-AAYCCNCCNGTNAAYAGYCT-3'	The amino acid sequence NPPVNSL
Oligo D	5'-ATGAASSWSACGAAGTTCTGSAC-3'	The amino acid sequence VQNFVSI
Oligo E	5'-GACCTGACGGAGATGTGT-3'	Human isomerase cDNA bp 200-217
Oligo F	5'-GACTCGAGTCGACATCG-3'	Adapter primer PCRII for RACE [14]
Oligo G	5'-GTCATCAGCCTGGAGAAGCTGGA-3'	Human isomerase cDNA bp 110-132
Oligo H	5'-CCACTATGCCACCTGCAGGGCCTC-3'	Human isomerase cDNA bp 521-545
Oligo I	5'-GGTGGCGACGACTCCTGGAGCC-3'	Vector arm: λ gt 11 forward
Oligo J	5'-TCTTTCAACCAGAAAGGGGCGATGA-3'	Human isomerase cDNA bp 423-447
Oligo K	5'-TGAGTCATGACAGTACCTGGG-3'	Human isomerase cDNA bp 383-404
Oligo L	5'-CCGGAYGGCGSCMGSCCSTT-3'	The signal sequence for rat isomerase*

* A part of the signal sequence for rat isomerase (VDGARFF) [15].

Figure 1 Isolation of human mitochondrial Δ^3, Δ^2 -enoyl-CoA isomerase cDNA

The clone PCR-1 was isolated by amplification with oligos A and B as primers for the first PCR and oligos C and D for the second PCR (see the Materials and methods section), using oligo(dT)-primed human skin fibroblast cDNA as a template. The clone RACE-PCR-3 was isolated by PCR with oligos E and F (PCR II) as primers and oligo(dT)₁₇-primed human skin fibroblast cDNA as a template. The library PCR with different human cDNA libraries listed in the Materials and methods section was performed using specific oligos G and H, yielding a product of expected size. Human colon and placental libraries were rescreened with a vector-arm oligo, oligo I (λ gt11 forward), as a sense primer and a specific oligo, oligo J, for the library PCR-5, and oligo K for a nested library, PCR-6. The clone containing the N-terminal region was obtained by another RACE-PCR-7 using the same oligo(dT)₁₇-primed cDNA as a template and oligo L and a specific antisense oligo, oligo J, as primers for the first amplification. The second nested PCR, RACE-PCR-8, was performed using oligo L and the specific antisense oligo, oligo K, as primers.

Tris/HCl, pH 8.5, containing 6 M guanidinium chloride. The peptides were separated on a μ RPC (C2/C18 SC 2.1/10) column with an acetonitrile gradient, employing the peak fractionation mode of the SMART system. The amino acid sequences of the peptides were determined by automated Edman degradation with an Applied Biosystems model 477A Protein Sequencer. The use of human tissue was approved by the Bureau of Medicine in Finland.

PCR and DNA sequencing

PCR was performed in a Perkin-Elmer/Cetus DNA cycler using the oligonucleotides (oligos) shown in Table 1. Poly(A)⁺ RNA (1 μ g) isolated from total RNA [13] from cultured human skin fibroblasts was used for the rapid amplification of cDNA ends (RACE)-d(T)₁₇-primed reverse transcription reaction [14] with MMLV reverse transcriptase. For PCR amplification of the resultant cDNA, an aliquot of 5 μ l of the reaction mixture was used as a template and specific primers and *Taq* DNA polymerase I were added. The first amplification was performed using oligo A and oligo B as the primers by means of two cycles of denaturation (1 min at 94 °C), annealing (1 min at 60 °C) and extension (2 min at 72 °C), after which the annealing temperature was reduced in 1 °C steps from 60 to 48 °C, allowing two cycles at each temperature, with an additional final extension for 10 min at 72 °C. The sample of 5 μ l from the first PCR was taken for nested amplification performed using oligo C and oligo D as the primers with a total of 36 cycles. The amplification conditions were the same as in the first PCR, except for the additional 12 cycles with an annealing temperature of 48 °C. The PCR product of approx. 650 bp was detected on a 1%-agarose gel. The fragment was extracted from the gel with Ultrafree-MC Dunapore tubes, subcloned into pUC18 and sequenced.

The 3'-flanking region was obtained by performing another PCR using the same oligo(dT)₁₇-primed cDNA as described earlier as the template. A PCR reaction was carried out using a specific oligo E and oligo F as primers with one cycle of denaturation at 95 °C for 2 min, annealing at 55 °C for 5 min and extension at 72 °C for 40 min, followed by 40 cycles of denaturation at 95 °C for 40 s, annealing at 55 °C for 1 min and extension at 72 °C for 2 min, and an additional final extension at 72 °C for 10 min. The resultant PCR product of 600 bp in length was subcloned into pUC18 and sequenced further. Then ten different human cDNA libraries were screened by PCR with

Figure 2 Nucleotide sequence and deduced amino acid sequence of the cDNA for the proposed mature human $\Delta^3\Delta^2$ -enoyl-CoA isomerase

Nucleotides are numbered in the 5' to 3' direction, beginning from the first nucleotide encoding the mature protein, which is termed '+1.' From this point on the cDNA contains an open reading frame of 780 nucleotides encoding for 260 amino acid residues. The nucleotides on the 5' side of residue +1 are designated by negative (—) numbers. The amino acid sequences were determined by Edman degradation from the uncleaved human isomerase (*****), and CNBr (-----) and trypsin fragments (——). The letter X indicates an uncertain amino acid residue in peptide sequencing. The putative consensus sequence for transcription termination is indicated (====) and the proposed cleavage site of the mitochondrial targeting sequence is indicated by the arrow. The region representing the signature for the hydratase-isomerase protein family is marked '+++++'. The GenBank accession number for human isomerase is L24774.

specific oligo G and oligo H by means of five cycles of denaturation (1 min at 94 °C), annealing (1 min at 70 °C) and extension (2 min at 72 °C), followed by 35 cycles of denaturation (1 min at 94 °C), annealing (1 min at 65 °C) and extension (2 min at 72 °C). Those libraries giving an amplified fragment of expected size were rescreened with vector-arm oligo (oligo I) specific for each library (T3 and T7, λ gt11 reverse and forward, or λ gt10 reverse and forward) as sense primers, and oligo J for the first PCR and oligo K for a nested PCR as specific antisense primers. All the PCR fragments obtained were isolated, subcloned and partially sequenced. However, the clones containing isomerase cDNA did not extend to the 5'-flanking region.

In order to obtain the 5'-flanking region another RACE-PCR was performed using oligo(dT)₁₇-primed human fibroblast cDNA as a template with oligo L based on the published signal sequence

for rat isomerase [15], and a specific antisense primer oligo J; 10 μ l was taken from the PCR product and 100 μ l of water was added to the sample; 5 μ l from this dilution was taken for a nested PCR, which was carried out using oligo L and another specific antisense primer, oligo K. Both of these amplifications were performed with denaturation at 94 °C for 1 min, annealing at 63 °C for 1 min and extension at 72 °C for 2 min for a total of 30 cycles, followed by final extension at 72 °C for 10 min. On 1%-agarose gel a PCR fragment of approx. 400 bp was detected. This product was subcloned into pUC18, sequenced, and it turned out to contain the N-terminal part of the cDNA sequence. Figure 1 shows that the isolated PCR fragments overlapped several times, except the extreme 5' and 3' ends, which were obtained from only one PCR fragment. Both strands of the resultant cDNA for human isomerase were sequenced.

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rISO MALAAARRVLLQAGSRLGRRGAVDGARRFSN...KRVLVEKEG.EAGIAVMKKFNP.VNSLSLEFLTE 64
mISO MALAAARRLLHAGSRLGRRREAVDGARRPFAN...KRVLVETEG.PAGVAVMKLRNP.VNSLSLECLTE 64
hISO .....DGARRFGS...QRVLVEPDA.GAGVAVMKKFNP.VNSLLIEFLTE 41
rMFE .....AEYLRLPHSLAMIRLCNP.PVNAVSPTVIRE 30
rHYD ..MAALRALLPRACNSLLSPVRCPEFRFFASGANFQYIIEKKGKNSSVGLIQLNRKALNALCNGLIEE 68
hHYD ..MAALRVLLSCARGPLRPPVRCPAWRPFASGANFEYIIAEKRGKNTVGLIQLNRKALNALCDGLID 68

rISO FVISLEKLENDKSIRGVILTSERPGIFSAGLDLMEYGRNPAHYAEYWKAVQELWLRLYLSNLTISAIN 134
mISO FTISLEKLENDKSIRGVILTSECPGIFSAGLDLMEYGRNPAHYAEYWKAVQELWLRLYTSNMILVSAIN 134
hISO LVISLEKLENDKSIRVVILTSDRPGVFSAGLDLTEMCGRSPAHYAGYWKAVQELWLRLYQSNLVVSAIN 111
rMFE VRGLQKAGSDHTVKAIVICGANGN.FCAGMIRLCNP.PVNAVSPTVIREVRGLQKAGSDHTVKAIVIC 98
rHYD LNQALETFEEDPAVGAIVLTGGEKA.FAAGADIKEMQNRTFQDC..YSGKFLSHWDHITRIKPVIAAVN 135
hHYD LNQALKIFEEDPAVGIVLTGGDKA.FAAGADIKEMQNLSFQDC..YSSKFLKHWGHLTQVKKPVIAAVN 135

rISO GASPAGGCLMALTCDIRIMADNSKYTIGLNESLLGIVAPFWLKDNYVNTIGHRAAERALQLTLFPPAEA 204
mISO GASPAGGCLLALCDYRVMADNPKYTIGLNESLLGIVAPFWKDMYVNTIGHREADGALQLTLFSPAEA 204
hISO GASPAGGCLVALTCDIRILADNPRYCIGLNETQLGIAPFWLKDLENTIGHRAAESAPELGLFRPEA 181
rMFE GANGN.FCAGALGCHYRIANAKARV..GLPEVTLGILPGARGTQLLPRVVGPVALDLTIGDAISQDA 165
rHYD LVALGGGGCELAMCDIIYAGEKAQF..GQPEILLGTIPGAGGTQRLTRAVGKSLAMEMVLSGKYLSADE 203
hHYD GYPFGGGCELAMCDIIYAGEKAQF..AQPEILLGTIPGAGGTQRLTRAVGKSLEMVLTGDRISQDA 203

rISO LKVGLVDEVVPEDQVHSKARSVMAKWFTIPDHSRQLTK.....SMMRKATADNLKQREADIQNFTSFI 268
mISO LKVGVVDEVVPEDQVHSKARSVMTKWLAIPDHSRQLTK.....NMMRKATADNLKQREADIQNFTSFI 268
hISO LQVGIVDQVPEEQVQSTALSAIAQMAIPDHARQLTK.....AMMRKATASRLVTQRDADVQNFVSFI 245
rMFE KQAGLVSKICPVETLVEEAIQCAEKIASV.....SLPNMDSVFEAIAKVRKQYPGVLAPET 223
rHYD LRLGILDAVVKSDP.VEEAIKFAQKIIDKPIEPRIFNKP.....NSKIIVAMAK..... 252
hHYD KQAGLVSKIFPVETLVEEAIQCAEKIAN.....NSKIIVAMAK..... 241

rISO SRDSIQSLSHVYLEKLKQKG 289 (ends)
mISO SKDSIQSLSHMYLEKLKQKG 289 (ends)
hISO SKDSIQSLSQMYLERLKEKG 266 (ends)
rMFE CVRSIQSSVKHPYEVGIKEE 244 (continues)
rHYD ..ESVNAAFEMTLTEGNKLEK 271 (continues)
hHYD ..ESVNAAFEMTLTEGSKLEK 260 (continues)

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Figure 3 Comparison of amino acid sequences among members of the hydratase–isomerase family

Deduced amino acid sequence of human mitochondrial isomerase (hISO) and its alignment with rat short-chain isomerase (rISO) [9,15], mouse mitochondrial isomerase (mISO) [29], rat peroxisomal multifunctional enzyme (rMFE) [4], rat mitochondrial enoyl-CoA hydratase (rHYD) [30], and human mitochondrial enoyl-CoA hydratase 1 (hHYD) [31]. The one-letter amino acid notation is used. Gaps (.) have been inserted to achieve maximum homology. The identical amino acid residues with the human isomerase among all the shown members of hydratase–isomerase family are marked with **bold** letters.

Amino acid homology comparisons with the GenBank, EMBL, PIR and Swiss-Prot databases at The National Center for Biotechnology Information (NCBI) were performed using the BLAST network service [16] and the multiple sequence comparison was carried out using the Pileup software of the GCG package [17]. The search for functional patterns of amino acid sequences was carried out using the PROSITE database [18].

Northern-blot analysis

Total RNA extracted from cultured human skin fibroblasts was fractionated by gel electrophoresis, transferred to a nitrocellulose filter [13] and baked at 78 °C for 2 h. The membrane was prehybridized and then hybridized with the 650 bp cDNA insert as a probe, which was labelled with [α -³²P]dCTP by the random-primed method. The membrane was washed with 0.15 M NaCl/0.015 M sodium citrate (‘1 × SSC’)/0.1 % SDS for 1 h and exposed to X-ray film at –75 °C.

RESULTS

Peptide sequencing of the purified human liver $\Delta^3\Delta^2$ -enoyl-CoA isomerase and isolated peptides obtained from the cleaved enzyme yielded the total number of 81 amino acid residues (Figure 2). The first and second PCR amplifications (PCR-1 and PCR-2) were performed using degenerative oligonucleotide probes based on peptide sequences and human fibroblast-derived single-stranded DNA as a template (Figure 1 and Table 1). A DNA fragment of 650 bp was subcloned and sequenced. With the following RACE-PCR another PCR product of 600 bp was detected on 1 %-agarose gel. This clone was isolated, and it

proved to be an overlapping clone containing a termination codon and a poly(A)⁺ tail. The 3′ untranslated region had a putative AACAAA polyadenylation signal 21 bp upstream at the poly(A)⁺ tract instead of the common AATAAA [19]. The same putative polyadenylation signal has recently been reported in gastrin-binding protein [20].

To extend the cloned isomerase cDNA, ten different human cDNA libraries (human heart λ ZAP II, human liver Uni-ZAP II, human liver λ gt11, human liver 5′-stretch λ gt11, human hepatoma λ gt11, human colon λ gt11, human placental 5′-stretch λ gt11, human lung 5′-stretch λ gt10, human fetal liver λ gt10, and human fetal heart λ gt10 cDNA libraries) were screened by PCR with specific sense and antisense primers, oligo G and oligo H. Except for human hepatoma λ gt11 and fetal heart λ gt10 libraries, all the screened libraries yielded a PCR-fragment of expected size (bp 110–547). In order to obtain the 5′-flanking region of the isomerase cDNA, PCR was performed using a vector-arm oligo (oligo I) as sense primer, and oligo J for the first PCR and oligo K for a nested PCR as specific antisense primers. Only those PCR products which were obtained from human colon and placental cDNA libraries matched the amino acid sequences obtained from the isolated peptides of that region. However, the 5′-flanking cDNA of the insert from colon and placental libraries ended at nucleotide number +82.

Therefore, in order to get the N-terminal region, another RACE-PCR was performed using the same oligo(dT)₁₇-primed cDNA as a template as described above, and oligo L and specific primers, oligo J and oligo K, for the first and the nested PCR. A DNA fragment of approx. 400 bp in length was cloned into pUC18 and sequenced. This clone proved to contain the N-

terminal part of the isomerase. A Northern blot of human skin fibroblast mRNA gave the estimated size of 1.3 kb for the transcript.

The cDNA of 798 bp encodes an open reading frame of 266 amino acids which ends at the termination codon TGA, and 79 bp after the coding region before the poly(A)⁺ tail. The cDNA-derived sequences accommodate the amino acid sequences obtained for the uncleaved isomerase and isolated cleaved peptide sequences obtained by protein sequencing (Figure 2). The inspection of the N-terminal part of the deduced amino acid sequence revealed that it contains one motif for the cleavage site of the mitochondrial targeting peptide (-Xaa-Arg-Xaa-Xaa-Ser-Xaa) [21], namely the residues -Arg-Arg-Phe-Gly-Ser-. This sequence can be cleaved by intramitochondrial proteinase between Phe and Gly residues. The proposal matched perfectly with the N-terminal peptide sequence of the uncleaved isolated human isomerase (Gly-Ser-Gln-Arg-...) (Figure 2). This means that the mature human isomerase is one amino acid residue shorter than its rat counterpart [9]. Comparison of the deduced amino acid sequence of the mature human isomerase with rat and mouse mitochondrial isomerases gave similarity percentages of 74 % for rat isomerase and 71 % for mouse isomerase at the amino acid level, and 78 % and 77 % at the nucleotide level, respectively (Figure 3).

DISCUSSION

The claim that the open reading frame of 780 bp in the cloned cDNA represents the mature human mitochondrial isomerase is supported by the following facts: (i) the amino acid sequences of the N-terminal end of the purified isomerase and the isolated peptides showed a match with those deduced from the nucleotide sequences; (ii) the sequence Val-Ser-Xaa-Ile-Asn-Gly-Xaa-Xaa-Xaa-Ala-Gly-Gly-Xaa-Leu-Xaa-Xaa-Xaa-Xaa-Cys-Asp-Tyr found in human isomerase matches perfectly with the signature for the enoyl-CoA hydratase/isomerase protein family [22]; and (iii) the predicted M_r of this polypeptide is 28735, which corresponds to the value 31000 determined earlier for this protein by SDS/PAGE analysis [12].

Comparison of their characteristics shows important dissimilarities, however. The human isomerase shows identities at the nucleotide (78 %) and at amino acid levels (74 %) relative to the corresponding rat enzyme, but these values are lower than those usually observed between the human being and the rat. The estimated pI value of 6.29 for the mature protein is over 3 pH units lower than that calculated for the rat mitochondrial monofunctional isomerase (pI of 9.74) [9]. The electrofocusing of the purified human liver isomerase showed the activity maximum at pH 6.1 [12], whereas for the rat counterpart a pI value of 9.5 was obtained [8]. Inspection of the deduced amino acid sequences revealed that many basic amino acid residues (lysines and arginines) in the rat enzyme have been replaced by neutral or, in some cases, acidic, residues. These changes are usually due to point mutations at the first nucleotide of the coding triplets.

Regarding the mechanism of isomerization it has been proposed that double bonds of Δ^3 -enoyl-CoA esters are switched to position Δ^2 via the same mechanism that has been shown to occur with Δ^5 -3-oxo steroids and cholesterol (e.g., the reaction catalysed by Δ^5 -3-oxosteroid isomerase and cholesterol oxidase) [23–27]. This reaction mechanism involves the participation of tyrosine and aspartate/glutamate residues. The sequence com-

parison of Δ^3 -enoyl-CoA isomerases and 2-enoyl-CoA hydratases points to one conserved tyrosine residue (tyrosine-121 in human isomerase) in all isomerization-catalysing proteins (monofunctional isomerases from human, rat and mouse, and peroxisomal multifunctional enzyme from rat). This tyrosine residue was changed to isoleucine in both rat and human mitochondrial 2-enoyl-CoA hydratases. In addition there were several conserved glutamate/aspartate residues, which could be involved in isomerase catalysis. However, the sequence alignment does not provide any information to elucidate the important question of why human isomerase does not show any clear chain-length specificity [12], in contrast with its rat counterpart, which shows a higher specific activity with short-chain Δ^3 -enoyl-CoA substrates [8].

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