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The Crystal Structure of Δ^3-Δ^2-Enoyl-CoA Isomerase

Anu M. Mursula[1], Daan M. F. van Aalten[2], J. Kalervo Hiltunen[1] and Rik K. Wierenga[1]*

[1]*Biocenter Oulu and Department of Biochemistry University of Oulu, P. O. Box 3000, FIN-90014, Finland*

[2]*Wellcome Trust Biocentre Department of Biochemistry University of Dundee, Dundee DD1 5EH, UK*

**Corresponding author*

The active-site geometry of the first crystal structure of a Δ^3-Δ^2-enoyl-coenzyme A (CoA) isomerase (the peroxisomal enzyme from the yeast *Saccharomyces cerevisiae*) shows that only one catalytic base, Glu158, is involved in shuttling the proton from the C2 carbon atom of the substrate, Δ^3-enoyl-CoA, to the C4 atom of the product, Δ^2-enoyl-CoA. Site-directed mutagenesis has been performed to confirm that this glutamate residue is essential for catalysis. This Δ^3-Δ^2-enoyl-CoA isomerase is a hexameric enzyme, consisting of six identical subunits. It belongs to the hydratase/isomerase superfamily of enzymes which catalyze a wide range of CoA-dependent reactions. The members of the hydratase/isomerase superfamily have only a low level of sequence identity. Comparison of the crystal structure of the Δ^3-Δ^2-enoyl-CoA isomerase with the other structures of this superfamily shows only one region of large structural variability, which is in the second turn of the spiral fold and which is involved in defining the shape of the binding pocket.

Keywords: coenzyme A; isomerase; oxyanion hole; peroxisome; yeast

Δ^3-Δ^2-Enoyl-coenzyme A (CoA) isomerase (referred to here as enoyl-CoA isomerase; EC 5.3.3.8.) is involved in an auxiliary pathway of fatty acid degradation, converting 3-*cis*-enoyl-CoA or 3-*trans*-enoyl-CoA into 2-*trans*-enoyl-CoA[1,2] (Figure 1), which subsequently is metabolized further by the enzymes of the β-oxidation pathway. All characterized enoyl-CoA isomerases are sequence-related members of the hydratase/isomerase superfamily, but they can be grouped into three classes: monofunctional mitochondrial, monofunctional peroxisomal and multifunctional enzymes. The sequence homology between these classes is low. The monofunctional enoyl-CoA isomerases occur as trimers or hexamers. Mitochondrial monofunctional enoyl-CoA isomerase has been purified from the rat,[3,4] bovine tissue,[5] and human.[6] Peroxisomal monofunctional isomerases from plant,[7] yeast,[8,9] and recently human and mouse[10] sources have also been characterised. Aligned sequences of peroxisomal and mitochondrial enoyl-CoA isomerases are shown in Figure 2.

Multifunctional enzymes with enoyl-CoA isomerase activity are referred to as MFE-1 and they include proteins from bacteria and the peroxisomes of multicellular eukaryotes.[11] MFE-1 enzymes have two domains; the N-terminal domain, which is sequence-related to the hydratase/isomerase superfamily, and catalyzes the isomerase reaction as well as the hydratase reaction, and the C-terminal domain, which is a 3-hydroxyacyl-CoA dehydrogenase.[12]

Structures are known for four other members of the hydratase/isomerase superfamily. Two domains can be recognized in the structure of the enzymes of this superfamily: the N-terminal core domain, with a spiral fold, and the C-terminal helical domain. The spiral fold has four turns; each turn consists essentially of three subsequent secondary structure elements, two β-strands and an α-helix. The first structures found to have this fold were the mitochondrial enoyl-CoA hydratase from rat, referred to here as crotonase (liganded and unliganded)[13,14] and 4-chlorobenzoyl-CoA dehalogenase from *Pseudomonas* sp. (dehalogenase, liganded).[15] Other known structures are the peroxisomal dienoyl-CoA isomerase from rat (unliganded)[16] and the methylmalonyl-CoA decarboxylase from *Escherichia coli* (decarboxylase, liganded and unliganded).[17]

Abbreviations used: CoA, coenzyme A; Mes, 2-(*N*-morpholino)ethanesulfonic acid; MAD, multiwavelength anomalous dispersion.

E-mail address of the corresponding author: rik.wierenga@oulu.fi

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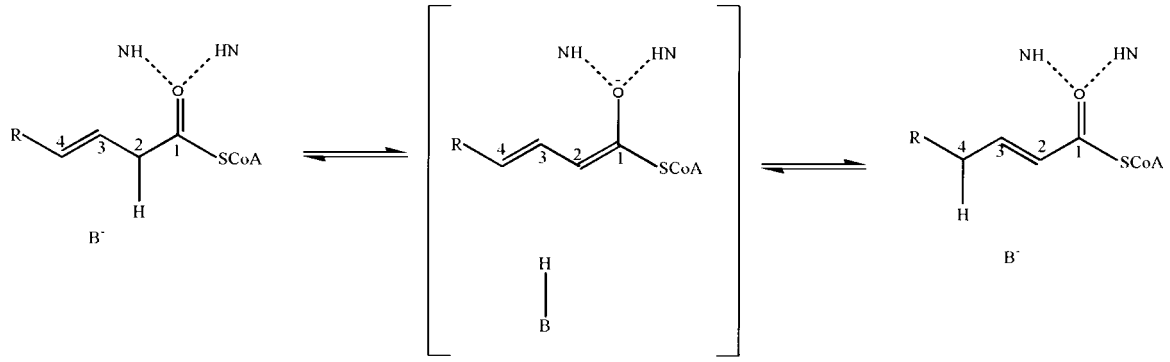

Figure 1. The reaction mechanism catalyzed by enoyl-CoA isomerase. The fatty acid tail can be of variable length. The two NH groups are peptide NH groups making an oxyanion hole. The reaction intermediate (shown in brackets) is stabilized by hydrogen-bonding interactions of the thioester oxygen atom in the oxyanion hole (see the text). From the structure of enoyl-CoA isomerase it can be deduced that there is only one base (B) which is Glu158. The enoyl-CoA isomerase studied here is from yeast (*S. cerevisiae*) peroxisomes. The enzyme, expressed in *E. coli*, was encoded by the full-length isomerase gene (ECI1)[8]. The isomerase-activity assays were performed according to published methods[26] using 60 μM *trans*-3-hexenoyl-CoA as substrate. The mutagenesis of Glu158 to alanine was done using the QuickChange™ mutagenesis kit (Stratagene) following the manufacturer's instructions. The expression and purification of the E158A variant were performed as for the wild-type.[8,25] Gel-filtration studies showed similar elution patterns for the E158A variant and the wild-type, in good agreement with the hexamer.[8]

Yeast peroxisomal monofunctional enoyl-CoA isomerase has only been characterized rather recently, after its gene had been discovered in the genome of *Saccharomyces cerevisiae*.[8,9] A yeast strain in which the isomerase gene is deleted is not able to grow on unsaturated fatty acids. This suggests that the peroxisomal monofunctional isomerase is the only enoyl-CoA isomerase in yeast.[8] Yeast isomerase is active at mildly basic pH values and utilizes 3-*trans*-hexenoyl-CoA and 3-*cis*-octenoyl-CoA as substrate at rates of 11 units/mg and 16 units/mg, respectively.[8,9] In addition, it has been shown that yeast isomerase lacks any detectable enoyl-CoA hydratase activity.

Members of the hydratase/isomerase superfamily are known to catalyze a wide variety of reactions on acyl chains, of very different structure, but always covalently linked to CoA *via* a thioester bond. In all known liganded structures of members of this superfamily, the carbonyl oxygen atom of the thioester is bound in an oxyanion hole, which activates the substrate for the conversion catalyzed by the active-site residues of the respective enzymes. Two members of this superfamily with known structure are enzymes involved in fatty-acid metabolism, namely dienoyl-CoA isomerase and crotonase. The two other members, dehalogenase and decarboxylase, are involved in other pathways. In dienoyl-CoA isomerase and crotonase the acyl chain of the CoA substrate is an extended carbon chain, of variable length, with double bonds in different places. Dienoyl-CoA isomerase shifts the position of two double bonds, catalyzing the conversion of 3,5-dienoyl-CoA to 2,4-dienoyl-CoA.[18] Crotonase catalyzes the hydration of a *trans*-2-double bond."[19] The substrate acyl chains of dehalogenase and decarboxylase are 4-chlorobenzoyl[20] and methylmalonyl,[21] respectively.

In enoyl-CoA isomerase the catalyzed reaction concerns the shifting of a double bond by proton abstraction (from atom C2) and subsequent proton donation (to atom C4; Figure 1)."[22] This reaction is similar to the reaction catalyzed by dienoyl-CoA isomerase; however, for dienoyl-CoA isomerase the proton abstracted from C2 is subsequently transferred to C6 of the fatty-acyl chain, which is further away from the common thioester moiety. Also in crotonase a proton has to be exchanged from the C2 carbon atom. In crotonase and dienoyl-CoA isomerase the C2 proton is abstracted by Glu164 and Glu196,[14,16] respectively, whereas it has been postulated that Asp204 is involved in proton exchange at C6 in dienoyl-CoA isomerase.[16] The catalytic residue Asp145 of dehalogenase is equivalent to Asp204 of dienoyl-CoA isomerase. Despite common features of the catalytic steps, the catalytic residue known to be involved in proton exchange at C2 in crotonase (Glu164) and dienoyl-CoA isomerase (Glu196) is not conserved in the yeast enoyl-CoA isomerase. However, Asp204 of dienoyl-CoA isomerase is conserved in enoyl-CoA isomerase as Glu158. Here, the crystal structure of enoyl-CoA isomerase is described and compared with the other known structures of enzymes of the hydratase/isomerase superfamily.

The refined structures

The structure has been solved using multiwavelength anomalous dispersion (MAD) phase information from a perrhenate derivative (Table 1). A perrhenate ion is bound in the active site and this structure will be referred to here as the structure of the complex, which has been refined at 2.15 Å resolution. The structure of the unliganded enzyme, refined at 2.5 Å resolution, is also

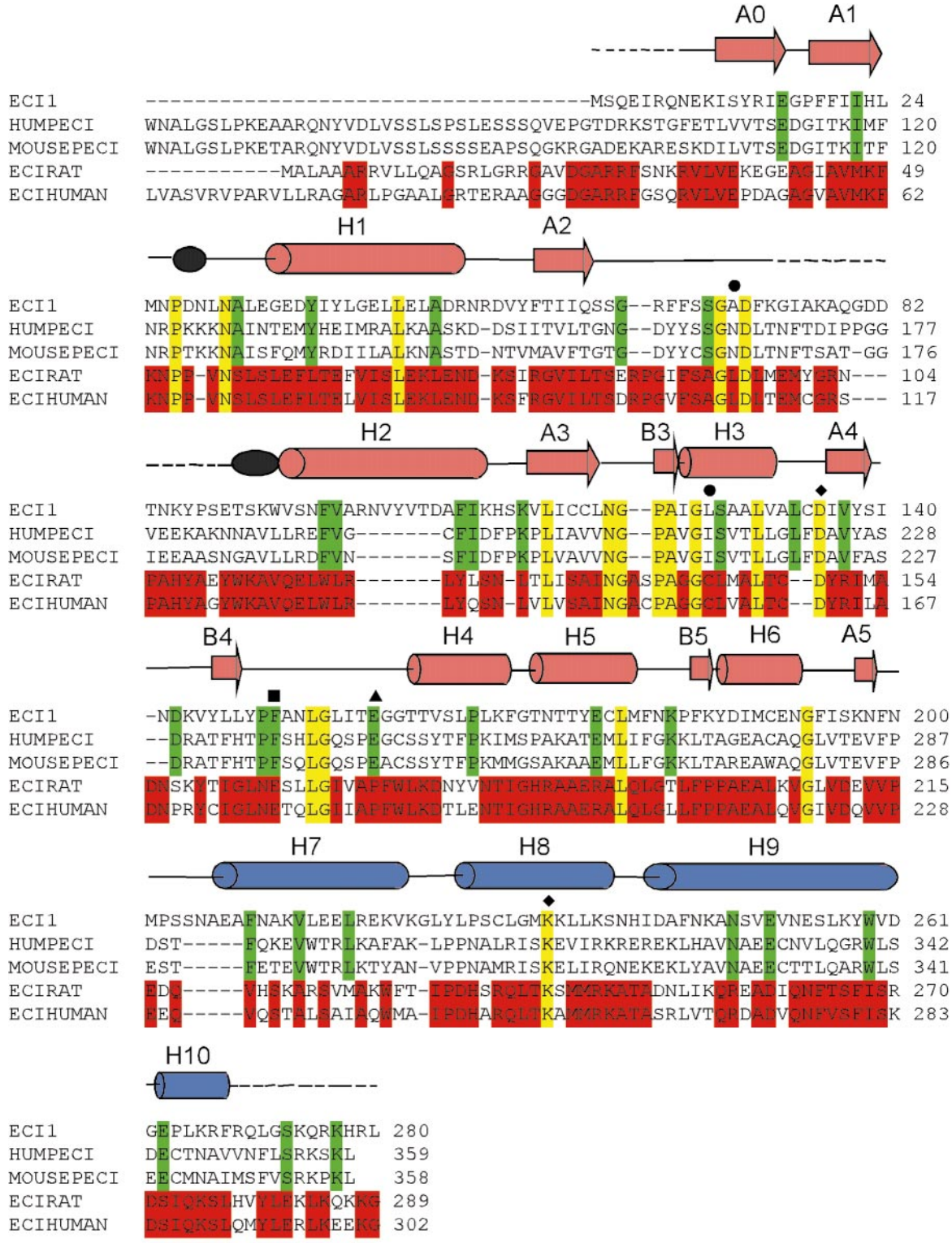

Figure 2. The sequences of peroxisomal and mitochondrial enoyl-CoA isomerases. The sequences of yeast peroxisomal enoyl-CoA isomerase (ECI1), human peroxisomal isomerase (HUMPECI), mouse peroxisomal isomerase (MOUSEPECI), rat mitochondrial isomerase (ECIRAT) and human mitochondrial isomerase (ECIHUMAN) are aligned. Residues conserved in all sequences are highlighted in yellow. Residues conserved in either the peroxisomal or the mitochondrial sequences are highlighted in green and red, respectively. The secondary structure of yeast enoyl-CoA isomerase, as defined by DSSP,[37] is shown above the sequences. Broken lines indicate the disordered part; arrows, black ovals, and cylinders indicate the presence of β-strands, 3_{10}-helices, and α-helices, respectively. The secondary-structure elements colored in red and blue are for the core domain and the trimerization domain, respectively. The nomenclature for the β-strands and α-helices is taken from the crotonase structure.[13] The following features are also highlighted: residues contributing to the oxyanion hole (●), catalytic glutamate residue in isomerase (▲), catalytic glutamate residue in crotonase (Glu164; see also Figure 4; ■), and the residues involved in the conserved salt bridge between the domains (◆; see also Figure 3). The sequence alignment was made using ClustalX.[38]

discussed. The perrhenate complex and the unliganded structure have been refined to *R*-factors of 21.5 % and 20.1 %, respectively. The data collection and refinement statistics are given in Table 1. There are no Ramachandran outliers. The N-terminal core domain of enoyl-CoA isomerase is folded in the spiral-fold topology seen in the other structures of this superfamily of enzymes. The C-terminal domain is helical and consists of four helices, namely H7, H8, H9, and H10 (Figures 2

Table 1. Data collection and refinement statistics

	Complex with ReO4	Unliganded
A. *Data collection statistics*[a]		
Space group	$P6_322$	$P6_322$
Unit cell parameters *a*, *b* (Å)	116.14	116.01
c (Å)	123.28	122.93
Temperature (K)	100	100
Wavelength (Å)	1.1773	0.909
Resolution (Å)	30-2.15 (2.23-2.15)	50-2.5 (2.59-2.50)
R_{merge} (%)[b]	5.3 (47.4)	6.6 (34.9)
Completeness (%)	99.5 (99.7)	98.9 (98.8)
$I/\sigma I$	16.0 (2.9)	12.2 (4.9)
Unique reflections	27,191 (2651)	17,313 (1669)
Redundancy	4.8	4.8
Mosaicity (deg.)	0.25	0.34
B-factor from Wilson plot (Å^2)	41	44
B. *Refinement statistics*		
Resolution (Å)	20.0-2.15	20.0-2.5
Total number of reflections	26,907	17,271
Working set: number of reflections	25,560	16,394
R_{factor} (%)	21.5	20.1
Test set: number of reflections	1347	874
R_{free} (%)	25.7	25.1
Protein atoms	1986	1986
Water atoms	196	176
Ethylene glycol atoms	8	8
$ReO4^-$ atoms	10	-
Residues with two conformations	2	2
C. *Geometry statistics*		
rmsd (bond distance) (Å)	0.018	0.016
rmsd (bond angle) (deg.)	2.7	2.7
rmsd *B*:		
Main-chain bonded atoms (Å^2)	4.2	4.1
Side-chain bonded atoms (Å^2)	5.4	5.5
Average *B*:		
All atoms (Å^2)	45.0	50.0
Main-chain atoms (Å^2)	41.9	47.5
Side-chain atoms (Å^2)	48.0	51.5
Water molecules (Å^2)	57.3	61.2
Ramachandran plot[c]		
Most favored region (%)	93.2	90.9
Additionally allowed regions (%)	6.8	9.1
Generously allowed regions (%)	0	0
Disallowed regions (%)	0	0

Crystals were grown in 0.1 M Mes (pH 5.6), 5 % (w/v) 1,4-dioxane and 1.4 M ammonium sulfate. There is one subunit per asymmetric unit. All crystallographic data were collected at the EMBL/DESY in Hamburg from cryofrozen crystals protected with 20 % (v/v) ethylene glycol.[25] The crystal for the MAD experiment was prepared by soaking the crystal in mother liquor containing 1 mM potassium perrhenate ($KReO_4$) for four hours. The MAD data were collected using four wavelengths. The datasets were processed and scaled separately using the programs DENZO and SCALEPACK from the HKL package.[27] Further data processing was done using programs of the CCP4 package.[28] The MAD datasets collected at the peak and remote wavelengths were scaled to the dataset collected at the inflection-point wavelength with SCALEIT.[28] The initial heavy-atom positions were located with RSPS[28] and SOLVE.[29] The two heavy-atom positions were refined and the phases to 2.5 Å resolution were calculated with the program MLPHARE.[28] The final average figure of merit was 0.64. The initial maps were improved by solvent-flattening calculations and the phases were extended to 2.15 Å resolution with the program DM,[30] after which the maps were easily interpretable with the program O.[31] In O, residues 8-73 and 89-270, out of 280 residues, could be built. Subsequently, the model was subjected to restrained refinement, using the maximum-likelihood target, with REFMAC.[32] Individual restrained isotropic *B*-factors were refined for all atoms. Water molecules were added automatically by the solvent-building mode of the program ARP/wARP.[33] Ethylene glycol and perrhenate molecules were also added into the refinement. The geometry of the ReO_4^- ion was taken from entry ZURQIM of the Cambridge Structural Database."[34] The unliganded structure of yeast enoyl-CoA isomerase was solved by rigid-body refinement with REFMAC using the perrhenate complex structure as the starting model. Further restrained refinement to 2.5 Å resolution was carried out, also with REFMAC. The structures were analysed with O,[31] WHAT IF,[35] ICM[23] and PROCHECK.[36]

[a] The values in parenthesies are for the highest-resolution shell.

[b] $R_{merge} = \Sigma_h \Sigma_i |I - I_i| / \Sigma_h \Sigma_i I_i$.

[c] As defined by PROCHECK.[36]

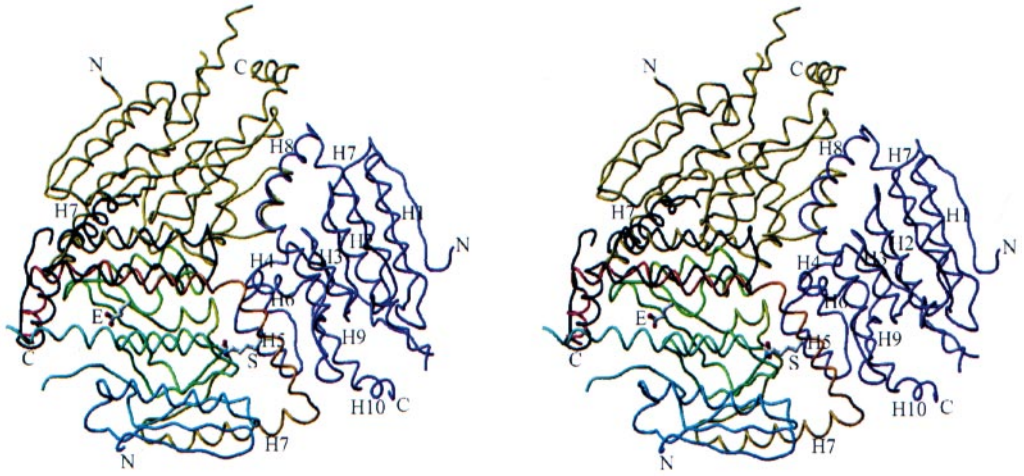

Figure 3. The C^{α}-traces of three subunits of enoyl-CoA isomerase are shown, viewed down the crystallographic 3-fold axis. The N and C termini of each subunit are labeled. Subunit 1 is shown color-coded from dark blue (N terminus) to dark red (C terminus). Subunit 2 is shown in purple and subunit 3 in yellow. In black is the helical C-terminal domain of the superimposed crotonase subunit. All the helices of subunit 2 are labeled. Also shown, as ball-and-stick models, are the catalytic glutamate residue, Glu158, (labeled E) and the conserved salt bridge (labeled S) between Asp135 (immediately after helix H3) and Lys233 (in helix H8). This Figure was produced using ICM.[23]

and 3). The N terminus (residues 1 to 7) and C terminus (residues 271 to 280), as well as region 74 to 88, could not be built due to the absence of any features in the electron density map. The C-terminal tripeptide, residues 278 to 280 (HisArgLeu), corresponding to the PTS-1 peroxisomal-targeting sequence, is therefore disordered. To the best of our knowledge this is a common feature of structures of peroxisomal proteins, the only exception being dienoyl-CoA isomerase."[16] Residues 74 to 88 are in spiral turn 2, after the second β-strand, before helix H2, near the active site. The other parts of the structure are well defined. There are no structural differences between the unliganded and the perrhenate complex structure; also the *B*-factor plots of these two structures show no differences (data not shown).

The trimer and the hexamer

Three enoyl-CoA isomerase monomers are related by a crystallographic 3-fold axis such as to form a tight trimer in which the subunit-subunit interface is extensive. As can be seen in Figure 3, the main contact region concerns the protruding helix H8 of one subunit fitting into a complementary docking site shaped by three regions of the adjacent subunit, these being (i) residues from helix H5, (ii) residues 151-172, including helix H4, and (iii) residues from helix H9. Within a contact distance of 3.5 Å there are ten hydrogen-bonding interactions and 33 additional van der Waals contacts. The center of the trimer, along the 3-fold axis, is a water-filled tunnel, traversing completely the trimer disk. Three peptide regions of each subunit, near His240, Gly193, and Gly170, contribute to the walls of this tunnel. Gly193 and Gly170 are fully conserved residues in the structures of other superfamily members. In the crystal, two trimers are packed such as to form hexamers with 32 crystallographic symmetry, although there are few intertrimer contacts.

Comparison with the related structures: the C-terminal domain

The C-terminal helices (helix H9 and H10) of the C-terminal helical domain are folded back on the core domain, instead of protruding out towards the adjacent subunit of the trimer, as is seen, for example, in crotonase (Figure 3). The comparison of enoyl-CoA isomerase with crotonase shows that this structural switch, located immediately after helix H8, positions helix H9 in the equivalent position of helix H9 of crotonase, such that it covers the active site of its own subunit (Figure 4). In this respect the structures of enoyl-CoA isomerase and decarboxylase are similar, whereas in the structures of crotonase, dienoyl-CoA isomerase and dehalogenase, helices H9 and H10 protrude away from the core domain and cover the active site of the adjacent subunit. A salt bridge between Lys233 in helix H8 of the C-terminal domain and Asp135 (just after helix H3; Figure 3) of the core domain, anchoring these domains together, is completely conserved in the five known structures of this superfamily. Sequence conservation suggests that this salt bridge is also present in the other isomerases (Figure 2).

The variable structure of the second spiral turn

The comparison of enoyl-CoA isomerase and crotonase reveals a large structural difference for the helix of the second spiral turn, helix H2. In crotonase this helix is split into two parts; the

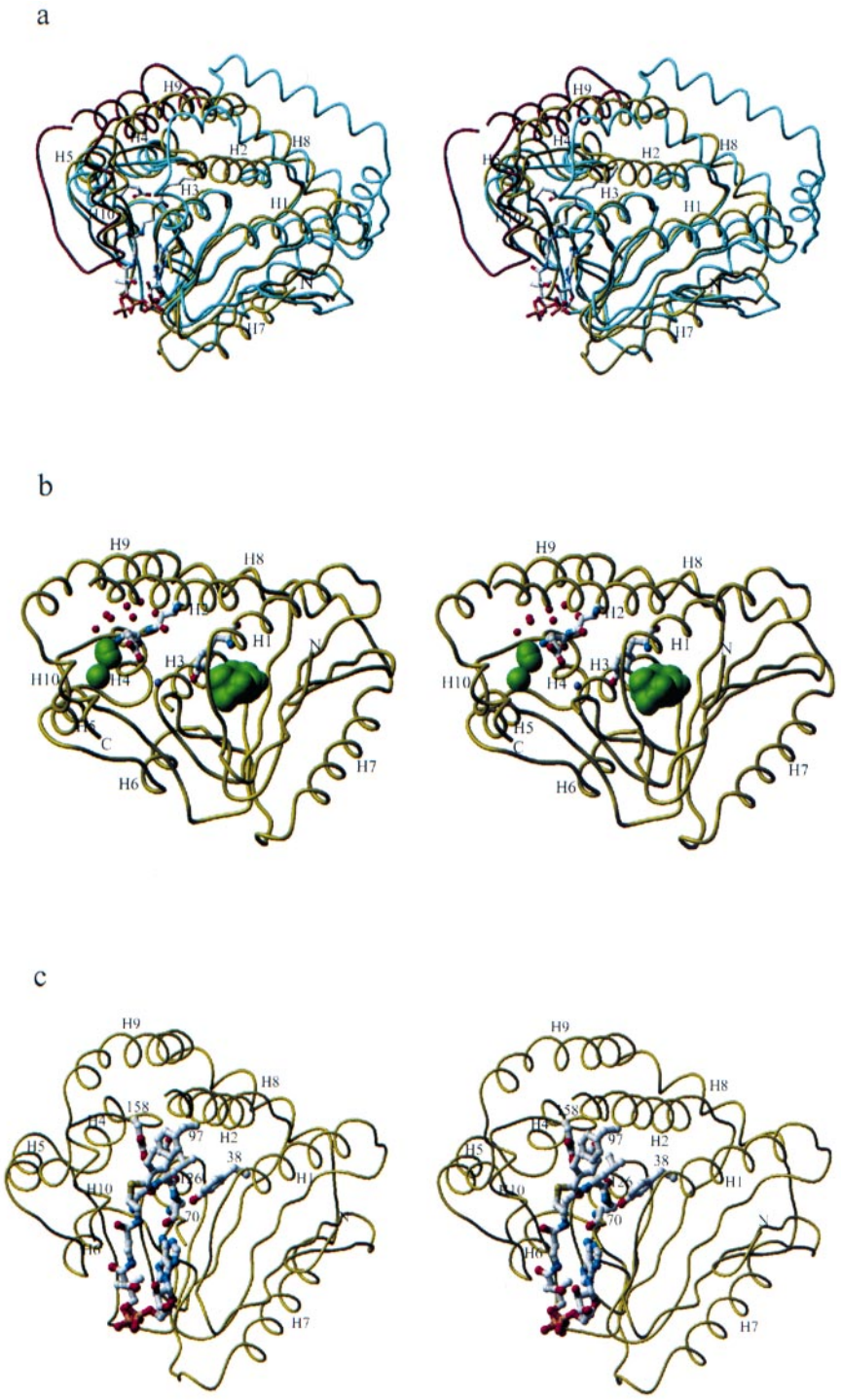

Figure 4. (a) A comparison of the C^{α}-trace of enoyl-CoA isomerase (yellow) and crotonase (cyan). Octanoyl-CoA, as bound to crotonase, and the catalytic residue Glu164 (PDB entry 2DUB) are also shown. Helices H8, H9, and H10 of the neighboring crotonase subunit are shown in red. All superimpositions were done with the lsq option in O, using the C^{α} atoms of the 25 residues which form the core of the four spiral turns (residues 19-23, 56-60, 115-119, 122-124, 136-139, and 145-147). This Figure was produced using ICM.[23] (b) Active-site geometry of enoyl-CoA isomerase. The catalytic site is near Glu158 and a water molecule (purple sphere), which is bound in the oxyanion hole. Also shown is the side-chain of Asn101 (helix H2), which is hydrogen bonded to Glu158. The empty cavity volumes are shown in green. The large cavity (177 $Å^3$) is separated from the catalytic site by Tyr38. A much smaller cavity (35 $Å^3$) is detected near Glu158. Glu158 is also hydrogen bonded to a buried water cluster of seven water molecules, shown as red spheres. (c) Proposed mode of substrate binding from the superimposed 4-hydroxybenzoyl-CoA as bound to the dehalogenase (PDB entry 1NZY). Also shown are the main-chain atoms of residues 125, 126, 127, and 70, as well as the side-chains of Tyr38, Phe97, Leu126, and Glu158. The thioester oxygen atom of the ligand binds in the oxyanion hole formed by N(Ala70) and N(Leu126). The substrate moiety is near Glu158, which is equivalent to the catalytic Asp145 of the dehalogenase. The extended, linear fatty-acyl chain, which is the substrate of enoyl-CoA isomerase, is proposed to bind between Phe97 and Leu126 and reach the large, empty cavity near Tyr38.

connecting peptide stretch (residues 114-118) has high *B*-factors in the structure liganded with a C4 fatty-acid CoA molecule. In the structure of the complex with a C8 fatty-acid CoA molecule, this connecting loop becomes completely disordered, allowing the fatty-acid tail to bind in an extended conformation (Figure 4), reaching the intratrimer space."[14] The comparison of the isomerase structure with other members of the hydratase/isomerase superfamily also highlights the structural and sequence variability of helix H2, as well as the loop preceding it. This variability correlates with the binding of rather different fatty-acid tails into the active sites of these enzymes.

The active site

This crystal form turned out to be not suitable for crystallographic binding studies with substrate or substrate analogues. Nevertheless, the mode of binding of the CoA moiety can be inferred from superimposition studies. Figure 4 shows the acyl-CoA ligand as seen in the dehalogenase structure when superimposed on the structure of enoyl-CoA isomerase. The thioester oxygen atom of the superimposed acyl-CoA points towards N(Ala70) and N(Leu126) which form the conserved oxyanion hole.

Molecular-surface calculations with ICM"[23] detected two cavities near the active site (Figure 4). One cavity, near Glu158, is almost completely filled with a water cluster, consisting of seven water molecules (Figure 4). The total volume of this cavity is 346 Å^3. The water cluster is connected to the polar atoms lining this cavity. However, the water cluster does not completely fill this cavity; for example, near the catalytic Glu158 there is a small empty space (35 Å^3) which is mainly lined by hydrophobic side-chains, in particular those of Phe150 and Leu153, but also one water molecule, and the side-chain atoms of Glu158. The other large cavity, with a volume of 177 Å^3, is lined by apolar residues, in particular the side-chain atoms of Leu24, Leu33, Tyr38, Leu41, Ile59, Phe66, Phe108, Cys117, Leu130, Val131, and Cys134, as well as Ser67 and Ser127. No solvent molecules could be detected in this cavity. This cavity is separated from the catalytic site by the side-chain of Tyr38 (Figure 4).

The oxyanion hole and the side-chain of Glu158 are at the center of the catalytic site. The Glu158 side-chain extends from a loop between β-strand B4 and helix H4. As can be seen in Figure 3, this region is part of the tight subunit-subunit interface, where it interacts with helix H8 of the other subunit. Other features near the active site concern Tyr38 (helix H1) and the adjacent, empty apolar pocket, Asn101 (in helix H2), and helix H3 (residues 125-134). The Glu158 side-chain is well defined, it is hydrogen bonded to only one protein atom, ND2 of Asn101. The side-chain of Asn101 is part of helix H2; it points down towards the catalytic site (Figure 4).

The catalytic site is accessible for acyl-CoA *via* an entrance lined by residues following the β-strand B2 and β-strand B4, as well as by residues of the C-terminal helix H10 (Figures 3 and 4). A major stretch of the residues after β-strand B2 (residues 74 to 88) is disordered; in some other structures of enzymes of the hydratase/isomerase superfamily this region is also disordered. The actual substrate of enoyl-CoA isomerase is an extended fatty-acyl chain (covalently connected to the CoA molecule), with a *cis* or *trans* double bond at carbon atom 3, as shown in Figure 1. It can be seen from Figure 4 that this double bond would be close to the side-chain of Glu158, which is in good agreement with the observation that no catalytic activity could be detected for the E158A variant.

Concluding remarks

Glu158 is seen to be the base, which in its deprotonated form can abstract a proton from the C2 atom of the substrate (Figure 1). Minor structural rearrangements of the Glu158 side-chain will be required such that the proton can be delivered to the C4 atom of the substrate (Figure 1). Such rearrangements seem entirely consistent with the structure of the active site, as the Glu158 side-chain is adjacent to a small, empty apolar pocket. The catalysis is facilitated by the interactions of the thioester oxygen atom with the two peptide NH groups of the oxyanion hole (N(Ala70) and N(Leu126)), which stabilize the transiently formed transition state with a negatively charged thioester oxygen atom (Figure 1). This transition-state stabilization has also been described for other enzymes of the hydratase/isomerase superfamily."[24] The role of the large, apolar, empty pocket near Tyr38 is unclear, but it seems likely that it is important for binding the fatty-acyl chain of the true substrate. From the mode of binding of the superimposed dehalogenase substrate (Figure 4) it can be seen that an extended fatty-acyl chain could reach this pocket. In this predicted mode of binding, two water molecules will be replaced by the fatty-acyl chain; also minor adjustments of the side-chains of Leu126 and Phe97 are required, as well as a conformational change for the Tyr38 side-chain. In the current structure the hydroxyl group of Tyr38 is completely buried but poorly hydrogen bonded; only weak hydrogen bonding with Ser127 exists. Small conformational changes of the side-chains of Tyr38 and Ser127 would be required to allow for better hydrogen bonding between OH(Tyr38) and OG(Ser127). Tyr38 and Ser127 are conserved in the sequences of monofunctional peroxisomal enoyl-CoA isomerases (Figure 2). This sequence alignment of the isomerases shows that two classes can be inferred: (i) peroxisomal enoyl-CoA isomerases with Glu158 (yeast enoyl-CoA isomerase numbering) which is equivalent to the catalytic aspartate residue in dehalogenase (Asp145) and in dienoyl-CoA isomerase (Asp204), and (ii) mitochondrial isomerases with a glutamic acid residue at position

150 (this is Phe150 in the yeast enoyl-CoA isomerase numbering). The latter residue is equivalent to Glu164 of crotonase (Figure 4) and Glu196 of dienoyl-CoA isomerase, which are known to be catalytic residues involved in the catalysis of proton exchange at C2 in these enzymes. The sequence conservation of Glu158 in the peroxisomal enoyl-CoA isomerases confirms its importance for catalysis.

Coordinates and structure factors

The coordinates and structure factors of the unliganded structure (1HNO) and the perrhenate complex structure (1HNU) have been deposited at the RCSB.

Acknowledgments

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