FEBS 27978

FEBS Letters 557 (2004) 81–87

Structural studies on Δ^3-Δ^2-enoyl-CoA isomerase: the variable mode of assembly of the trimeric disks of the crotonase superfamily

Anu M. Mursula, J. Kalervo Hiltunen, Rik K. Wierenga*

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

Received 15 October 2003; revised 26 November 2003; accepted 1 December 2003

First published online 16 December 2003

Edited by Irmgard Sinning

Abstract **Subunits of the enzymes in the crotonase superfamily form tight trimeric disks. In most members of this protein superfamily these disks assemble further into hexamers. Here we report on the 2.1 Å structure of a tight hexameric crystal form of the yeast peroxisomal Δ^3-Δ^2-enoyl-CoA isomerase (Eci1p). A comparison of this structure to a previously solved crystal form of Eci1p and other structures of this superfamily shows that there is much variability with respect to the relative distance between the disks and their relative orientations. In particular helices H2 and H9 are involved in the inter-trimer contacts and there are considerable structural differences in these helices in this superfamily. Helices H2 and H9 are near the catalytic cavity and it is postulated that the observed structural variability of these helices, stabilized by the different modes of assembly, has allowed the evolution of the wide range of substrate and catalytic specificity within this enzyme superfamily.**

Key words: Δ^3-Δ^2-Enoyl-CoA isomerase; β-Oxidation; Thioester; Crotonase superfamily; Assembly of trimers

1. Introduction

Trans-2-enoyl-CoA is the only unsaturated intermediate in the classical β-oxidation pathway, and therefore auxiliary enzymes, such as Δ^3-Δ^2-enoyl-CoA isomerase, 2,4-dienoyl-CoA reductase and $\Delta^{3,5}$-$\Delta^{2,4}$-dienoyl-CoA isomerase, are needed for metabolizing additional double bonds in unsaturated fatty acids [1]. Enoyl-CoA isomerase (EC 5.3.3.8) catalyzes the reaction in which *cis*-3-enoyl-CoA or *trans*-3-enoyl-CoA is converted into *trans*-2-enoyl-CoA which is the substrate of 2-enoyl-CoA hydratase (crotonase) of the β-oxidation cycle. In eukaryotes, these isomerases are located either in mitochondria or in peroxisomes.

All characterized enoyl-CoA isomerases are sequence-related members of the hydratase/isomerase superfamily, also called the crotonase superfamily of enzymes [2]. CoA-dependent enzymes of this superfamily are known to catalyze a wide range of reactions [2]. Six structures of these enzymes have now been solved by X-ray crystallography. The known structures include the rat mitochondrial enoyl-CoA hydratase (crotonase) [3,4], the 4-chlorobenzoyl-CoA dehalogenase from *Pseudomonas* sp. strain CBS-3 [5], the rat $\Delta^{3,5}$-$\Delta^{2,4}$-dienoyl-CoA isomerase [6], the methylmalonyl-CoA decarboxylase from *Escherichia coli* [7] and the human RNA-binding AUH protein [8], now also known to be a 3-methylglutaconyl-CoA hydratase [9,10]. In addition, recently, the structure of peroxisomal Δ^3-Δ^2-enoyl-CoA isomerase from *Saccharomyces cerevisiae* (Eci1p) [11] has been solved [12]. The sequence similarities within this superfamily is rather low (Fig. 1) but still all these enzymes share a similar overall structure. They have an N-terminal core domain (in Eci1p residues 1–200) with a spiral fold topology and a C-terminal α-helical trimerization domain (in Eci1p residues 201–280). The spiral fold has four turns, each of which consists of three successive secondary structure elements, namely two β-strands and an α-helix. The active site is at the N-terminal end of the α-helix H3 in the third spiral turn. In Eci1p the peptide NH(Leu126) (helix H3) together with NH(Ala70) (just before H2) forms an oxyanion hole for the thioester oxygen atom of the substrate. In Eci1p the proton shuttling is catalyzed by Glu158 [12]. Glu158 is in the fourth turn of the spiral, after B4 and before H4 (Fig. 1). In all these enzymes three subunits assemble into a characteristic trimeric disk with tight interactions between the three subunits. In most of these enzymes the disks dimerize to form hexamers, although the trimer–trimer contacts are not as tight as the intra-trimer contacts. In the previously reported structure of Eci1p [12] the inter-trimer contacts were found to be rather loose.

In this study we report on a second crystal form of Eci1p in which the inter-trimer contacts are found to be much tighter. In addition, the hexameric assembly of the Eci1p structure is compared with the structures of other CoA-dependent enzymes of the hydratase/isomerase superfamily and it is found that the mode of assembly of the trimeric disks into hexamers is not conserved within this superfamily of enzymes.

2. Materials and methods

2.1. Solution studies

Yeast Eci1p was overexpressed in *E. coli* and purified as described before [13]. Gel filtration studies were done to establish the molecular mass of the native protein at pH 5.5 and pH 7.0. For the gel filtration with a Superdex 200 HR 10/30 gel filtration column (Pharmacia) six different buffers were used: 0.020 and 0.2 M phosphate buffer at pH 5.5 and pH 7.0; 0.1 M 2-[*N*-morpholino]ethanesulfonic acid (MES) buffer at pH 5.5 and 0.1 M triethanolamine buffer at pH 7.0. To verify that the oligomerization state of Eci1p is not concentration-

*Corresponding author. Fax: (358)-8-553 1141.
E-mail address: rik.wierenga@oulu.fi (R.K. Wierenga).

Abbreviations: Eci1p, yeast peroxisomal Δ^3-Δ^2-enoyl-CoA isomerase; ADA, *N*-[2-acetamido]-2-imino diacetic acid; BSA, bovine serum albumin; MES, 2-[*N*-morpholino]ethanesulfonic acid

0014-5793/03/$30.00
doi:10.1016/S0014-5793(03)01450-9

dependent, three samples with different concentrations (1.25 mg/ml, 0.375 mg/ml and 0.125 mg/ml) were analyzed by gel filtration. Similarly, duplicate Eci1p samples were analyzed in each buffer and the elution volumes of the samples were compared to those of the standards (chymotrypsinogen, 25 kDa; ovalbumin, 43 kDa; albumin, 67 kDa; aldolase, 158 kDa; catalase, 232 kDa; and ferritin, 440 kDa). To further analyze the properties of Eci1p at different pHs, the pH optimum of its enzyme activity was determined. The enzyme assays were performed according to a published method [14] using 80 μM *trans*-3-hexenoyl-CoA as substrate. The k_{cat} values of Eci1p were measured from duplicate samples at each of the following pH values: 7.0, 7.5, 8.0, 8.5, 9.0, 9.3, 9.5 and 9.8. A buffer consisting of 50 mM Tris, 50 mM KCl, 50 μg/ml bovine serum albumin (BSA) was used for the pH range 7.0–9.0 and 50 mM 2-[*N*-cyclohexenylamino]ethanesulfonic acid, 50 mM KCl, 50 μg/ml BSA was used for pH 9.0–9.8.

2.2. Crystallization, structure solution and refinement

For the crystallization experiments the enzyme was concentrated to 2.5 mg/ml in 20 mM potassium phosphate buffer, pH 7.2, 450 mM KCl, 1 mM EDTA, 1 mM EGTA, 0.5 mM benzamidine HCl by centrifugal filtration. The crystallization was carried out using the hanging drop vapor diffusion method by mixing 2 μl of protein solution with 2 μl of well solution [15]. The drop was equilibrated against the total volume of 1 ml of well solution. Bipyramidal-shaped crystals

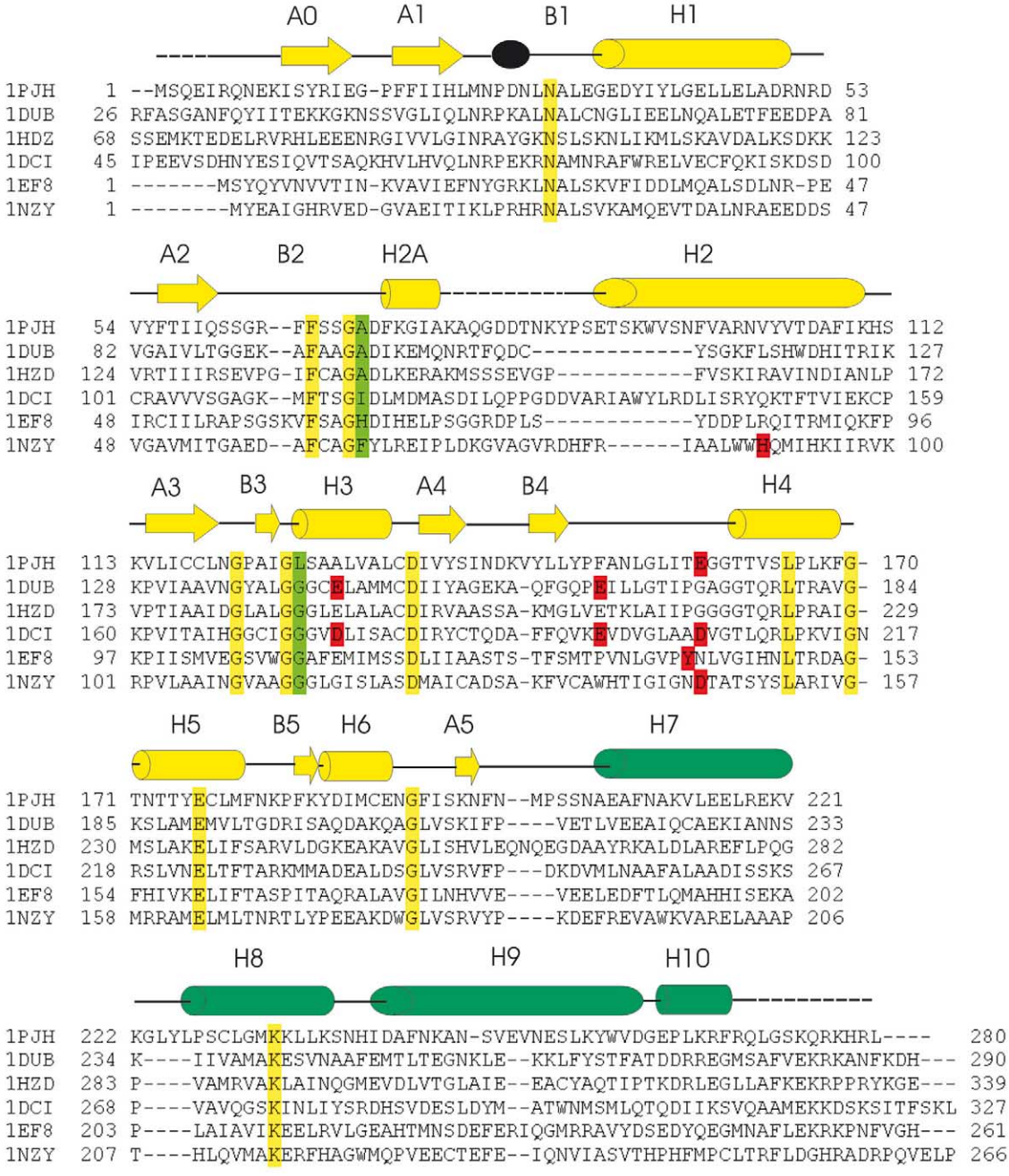

Fig. 1. The aligned sequences of Eci1p (1PJH), crotonase (1DUB), dienoyl-CoA isomerase (1DCI), dehalogenase (1NZY), AUH protein (1HZD) and decarboxylase (1EF8). The secondary structure of Eci1p is shown above the sequences. Dotted lines indicate the disordered part; arrows, black ovals and cylinders indicate the presence of β-strands, 3_{10}-helices and α-helices, respectively, as detected by DSSP [24]. The secondary structure elements colored yellow and green are for the core domain (residues 1–200) and the trimerization domain (residues 201–280), respectively. Residues highlighted in red are catalytic residues. Residues highlighted in green form the oxyanion hole. The residues completely conserved in all six sequences are highlighted in yellow. The sequence identities with Eci1p are 19% (for 1DUB), 16% (for 1HZD), 16% (for 1DCI), 14% (for 1EF8) and 15% (for 1NZY).

grew in about a week at 295 K and reached the maximum size of 0.4 mm in the longest dimension from a well solution consisting of 0.1 M *N*-[2-acetamido]-2-imino diacetic acid (ADA) pH 7.0, 0.1 M $MgSO_4$ and 1.7–2.2 M $(NH_4)_2SO_4$.

Crystals were flash-frozen in a stream of cold nitrogen gas before data collection. Before freezing the crystals were transferred for about 30 s into a solution consisting of 0.1 M ADA pH 7.0, 0.1 M $MgSO_4$, 2.2 M $(NH_4)_2SO_4$ and 20% glycerol. The crystals diffracted to 2.1 Å resolution using synchrotron radiation at DESY beamline X11 and belonged to the primitive tetragonal space group. The data were processed and scaled using the program XDS [16]. The data processing statistics are shown in Table 1. There is one trimer in the asymmetric unit.

The structure was solved by molecular replacement with the program AMoRe [17] using the previously solved Eci1p structure [12] (PDB entry 1HNU) as the search model. The new model was subjected to restrained refinement with REFMAC5 using loose non-crystallographic symmetry (NCS) restraints and TLS refinement [18]. O [19] was used for the model building in maps. The refinement statistics are shown in Table 1. In our previous structural studies it was found that Eci1p is packed loosely as a dimer of trimers [12]. The Eci1p structure solved here has, however, a different crystal form and in this form the assembly of the trimers into the hexamers is much tighter, i.e. the number of inter-trimer contacts is much higher. Therefore the earlier solved Eci1p structure is termed the loose hexamer form (PDB entry code 1HNU) and the here solved Eci1p structure the tight hexamer form.

3. Results and discussion

3.1. Structural differences between the monomers of the tight hexamer form and the loose hexamer form of the yeast enoyl-CoA isomerase

The structure of the tight hexamer form of Eci1p has been refined at 2.1 Å resolution with an *R*-factor of 16.8% and free *R*-factor of 18.9% with good geometry statistics (Table 1). There are no structural differences between the three subunits of the trimer that forms the asymmetric unit (Table 1). In the loose hexamer form residues 74–88 were completely disordered; however, in the tight hexamer form a stretch of a new α-helix could be built. The new helix (residues 72–76) will be referred to as H2A (Fig. 1). In the current structure residues 78–84 of the A subunit, residues 78–84 of the B subunit and residues 77–85 of the C subunit are disordered. Structural differences concern not only the newly built residues (near helix H2), but the polypeptide chain after this region has also adopted a new conformation, such that the helix H2 is slightly bent upwards (Fig. 2A). In addition, structural changes are seen for helices H9 and H10, which are adjacent to helices H2 and H2A, respectively (Fig. 2A). From the comparison of the two hexameric crystal forms it is clear that the rearrangements of residues in helices H2A, H2, H9 and H10 change the shape of the putative binding pocket [12] and consequently are expected to be important for the substrate-binding properties of this enzyme.

Also in the catalytic site of Eci1p structural changes are seen, for example the reorientation of Arg100 and Asn101 of helix H2 (Fig. 2A). ND1 of Asn101 is hydrogen-bonded to OE2 of the catalytic glutamate, Glu158 and OD1 of Asn101 is hydrogen-bonded to both NH2 and NE of Arg100 (Fig. 2B). OE1 (Glu158) is hydrogen-bonded to wat19 and ND1 (Asn101) which is hydrogen-bonded to wat111 of a buried water cluster (Fig. 2B). This buried water cluster concerns a linear array of four hydrogen-bonded waters (wat19, wat47, wat111 and wat109; Fig. 2B) extending from wat19 (hydrogen-bonded to Glu158) to wat109 which is hydrogen-bonded to side chain atoms of Asn248 and Glu251. Asn248 and Glu251 are in helix H9; helices H9 and H10 shield the catalytic glutamate and the buried water cluster form bulk solvent (Fig. 2A). The carboxylate oxygens of the catalytic glutamate are also hydrogen-bonded to the O1 and O2 oxygen atoms of a glycerol molecule. This glycerol is hydrogen-bonded with its O3 atom to the peptide NH moieties of Leu126 and Ala70 of the active site oxyanion hole (Fig. 2B), possibly mimicking the mode of binding of the thioester oxygen atom of the substrate. In the observed active site geometry, the carboxylate oxygen atoms of the catalytic glutamate are pointing into the catalytic cavity allowing the catalysis of the proton transfer associated with the shift of the double bond [12]. The first step of the reaction concerns the proton abstraction of the C2 carbon atom of the substrate, indicating that for the active enzyme Glu158 should be deprotonated. Enzymatic assays done in the pH range of 7.0–9.8 showed a dependence of the k_{cat} on the pH, being low (0.11 s^{-1}) at pH 7 and maximal (6.5 s^{-1}) at pH 9.0.

3.2. The yeast enoyl-CoA isomerase hexamer is assembled from two tight trimers

The monomers of Eci1p are assembled into a tight trimer. Both in the loose hexamer form and in the tight hexamer form these trimers crystallize as hexamers with crystallographic 32 symmetry. However, the mode of assembly is rather different. In the tight hexamer form the two trimer disks interact tightly with each other (166 trimer–trimer contacts using a contacting distance of 3.5 Å) (Fig. 3) whereas in the loose hexamer form

Table 1
Data collection and refinement statistics

Data collection statistics[a]	
Temperature (K)	100
Wavelength (Å)	0.801
Space group	$P4_12_12$
Cell dimensions (Å)	$a = b = 116.24$, $c = 216.28$
Resolution range (Å)	20–2.1
Observed reflections	690 091
Unique reflections	86 026
Redundancy	8.0
$\langle I/\sigma I \rangle$	28.5 (10.5)
Completeness (%)	98.9 (93.4)
R_{merge} (%)	4.0 (19.9)
Refinement statistics	
Working set: number of reflections	81 748
R-factor (%)	16.8
Test set: number of reflections	4 278
Free *R*-factor (%)	18.9
Protein atoms	6 186
Solvent atoms	456
Geometry statistics	
Rmsd (bond distance) (Å)	0.011
Rmsd *B* all bonded atoms (Å^2)	1.2
Average *B* all protein atoms (Å^2)	22
Ramachandran plot[b]	
Most favored region (%)	92.6
Additionally allowed regions (%)	7.4
Generously allowed regions (%)	0
Disallowed regions (%)	0
Non-crystallographic symmetry	
Superposition rmsd for all Cα atoms (Å)	
Subunit A on subunit B (Å)	0.18
Subunit A on subunit C (Å)	0.15
Subunit B on subunit C (Å)	0.16

[a]The values in parentheses are for the highest resolution shell (2.2–2.1 Å).
[b]Defined by PROCHECK [23].

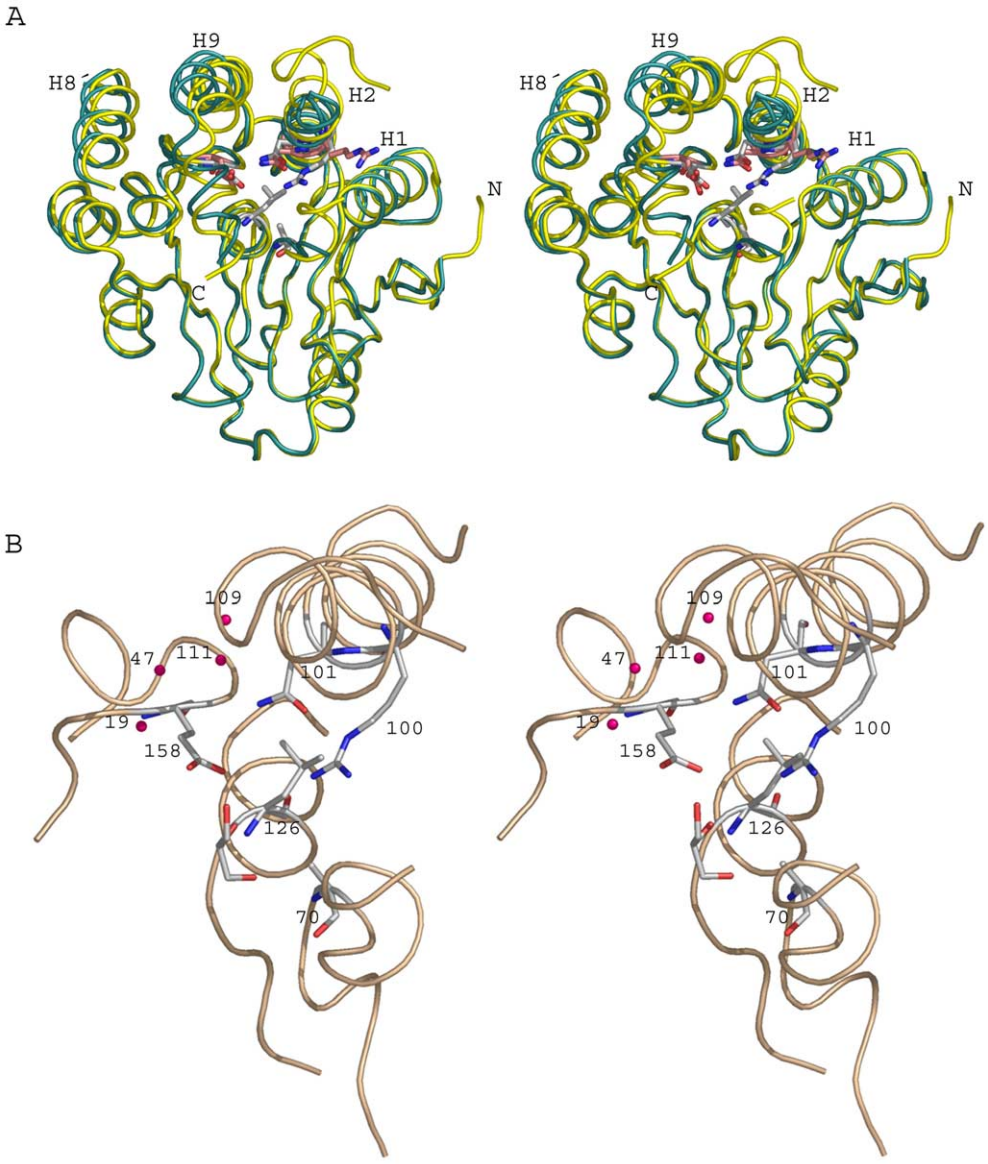

Fig. 2. A: The superposition of the monomer fold of the tight hexamer form (yellow) and the loose hexamer form (green) of Eci1p. The helices facing the inter-trimer space, H1, H2, H9 and H8′, are labelled. The helix H8′ is from the neighboring subunit. Also shown are the catalytic residue Glu158 as well as Arg100, Asn101 (helix H2) and the oxyanion hole residues Ala70 and Leu126. The carbon atoms of Glu158, Arg100 and Asn101 of the loose hexamer structure are colored pink. N and C identify the amino-terminus and carboxy-terminus, respectively. B: The catalytic site of the tight hexamer form of Eci1p (as seen in subunit A). The side chain atoms of the catalytic glutamate Glu158 are hydrogen-bonded to Asn101, a glycerol molecule and wat19 of a buried cluster of four water molecules. Also shown are Arg100 (extending into the active site and hydrogen-bonded to Asn101) and oxyanion hole residues Ala70 and Leu126. Leu126 is at the beginning of helix H3. The figures were made with the program Pymol [25].

there are only 30 such trimer–trimer contacts (Table 2). A superposition analysis shows that in the tight hexamer form the trimer disks are 5.6 Å closer as compared to the loose hexamer form. In addition, the second trimer disk has rotated 25° when the two crystal forms are compared (Table 2). The much tighter mode of assembly for the tight hexamer form is correlated with the structural differences of the subunit, in particular in helices H2 and H9 (see above) as these helices are much involved in inter-trimer contacts (Table 2).

The loose hexamer crystal form was grown at pH 5.6 whereas the tight hexamer form crystals grow at pH 7.0. To establish whether Eci1p could be a trimer at pH 5.6 and a hexamer at pH 7.0, gel filtration analysis on Eci1p at both pHs was performed. In all samples, also differing in ionic strength (see Section 2), Eci1p was found to elute in a volume corresponding to that of a hexamer (170 kDa) at both pHs. Also, the observed molecular mass is not dependent on the protein concentration, as the same molecular masses were found at protein concentrations of 0.125 mg/ml, 0.375 mg/ml and 1.25 mg/ml. Consequently, it can be concluded that the observation that the hexamer seems to be split into two trimers in the crystal form obtained at pH 5.6 (Fig. 3) is induced by the crystallization conditions. Apparently, these crystallization conditions, including 5% dioxane, 10 mM *N*-octyl-β-glucoside, 1.4 M $(NH_4)_2SO_4$ in 0.1 M MES at pH 5.6, induce a loosening of the hexamer, shifting the hexamer–trimer equilibrium from tight hexamers into loose hexamers. This observation suggests that the inter-trimer interactions in

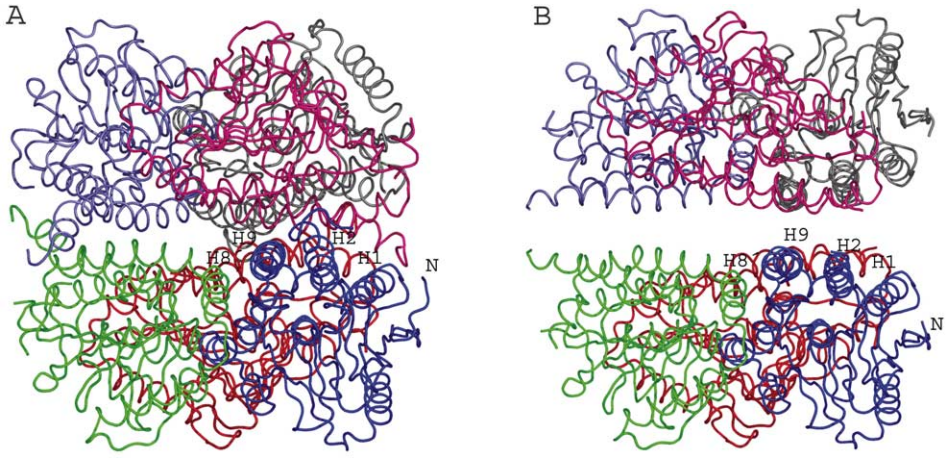

Fig. 3. Comparison of the assembly of the Eci1p hexamers. A: The tight hexamer form of Eci1p. The subunit shown in the standard view (as in Fig. 2) is labelled at the N-terminus; also labelled are helices H1, H2, H9 and H8′ which provide most of the inter-trimer contacts. B: The loose hexamer form of Eci1p [12]. The figures were made with the program Pymol [25]. Corresponding subunits in A and B are colored with the same colors, such as to visualize the relative rotation of the upper trimer with respect to the (stationary) lower trimer.

the tight hexamer are not very strong. The lower pH, associated with the loose hexamer form, might contribute to the weakening of these interactions as there are 12 salt bridges across the inter-trimer space (involving the side chains of Lys110 (H2), Asp242 (H9), Lys246 (H9) and Glu254 (H9) of each subunit), which are expected to be destabilized at lower pH. Shifting the hexamer–trimer equilibrium in favor of independent trimers might also be of functional relevance, as it has been found that Eci1p can enter into the peroxisomes not only with its own PTS1 but also complexed to a partner, Dci1p [20]. Dci1p is the peroxisomal dienoyl-CoA isomerase [21] which also is a member of the crotonase superfamily; it has 50% sequence identity to Eci1p. As Dci1p and Eci1p are homologous proteins, one could speculate that they can form heterodimers, consisting of an Eci1p trimer and a Dci1p trimer, which would require the dissociation of an Eci1p hexamer, such that a Eci1p trimer can oligomerize with a Dci1p trimer.

3.3. The variable mode of assembly of the trimers in the crotonase superfamily

The largest structural differences between the monomers of the loose hexamer form and tight hexamer form of Eci1p are seen in helix H2 and the region preceding it (Fig. 2). A superposition analysis of other monomer folds of the hydratase/isomerase enzymes also highlights the large structural variability of this region [22]. Fig. 1, depicting the sequence alignment of these enzymes, emphasizes the high sequence variability in this region. For example, Eci1p and rat dienoyl-CoA isomerase have a 13 residue insertion as compared to crotonase.

Table 2
Comparison of the crystallographic hexamers

Known structure[a]	K[b] (°)	Δ[b] (Å)	Rms difference (monomer superposition)[b] (Å)	Rms difference (trimer superposition)[b] (Å)	Inter-trimer contacts[c]					Subunits/ crystallographic asymmetric unit	Physiological assembly
					All	H1	H2	H9	other		
Decarboxylase (1EF8)	37	−8.0	1.8	2.0	128	–	12	96	20[d]	3	6
Eci1p (loose hexamer form, 1HNU)	25	−5.6	0.1	0.2	30	24	–	6	–	1	6
Dienoyl-CoA isomerase (1DCI)	66	−4.4	0.9	1.1	156	–	65	91	–	3	6
Crotonase (1DUB)	65	−1.6	1.0	1.8	105	4	27	74	–	6	6
Dehalogenase (1NZY)	−25	−0.8	0.9	0.9	65	16	18	31	–	3	3
RNA-binding AUH protein (1HZD)	68	−0.3	1.1	1.4	22	–	–	22	–	6	6
Eci1p (tight hexamer form) (1PJH)	–	–	–	–	166	20	72	63	11[e]	3	6

[a]The PDB entries are listed in parentheses.
[b]Superpositions were done on the Eci1p structure (tight hexamer form) using 25 Cα atoms and 75 Cα atoms for the monomer and trimer superpositions, respectively. All superpositions were done with the lsq option in O, using the Cα atoms of 25 residues (19–23, 56–60, 115–119, 122–124, 136–139, 145–147 which are residues of β-strands A1, A2, A3, B3, A4, B4, respectively) as well as their equivalents in the other structures. Initially the first trimers of each hexamer were superimposed onto the Eci1p trimer. Subsequently the second trimers were superimposed; K (°) and Δ (Å), as defined in Fig. 4, are, respectively, the additional rotation and translation required for this second superposition. The negative sign of Δ refers to a shorter distance between the two trimers in Eci1p; in fact the tight hexamer form of Eci1p is the most compact hexamer of all known assemblies, whereas the decarboxylase hexamer is the most extended.
[c]All inter-trimer atom–atom contacts shorter than 3.5 Å have been counted.
[d]These contacts are from residues of H4.
[e]These contacts are from residue Arg6.

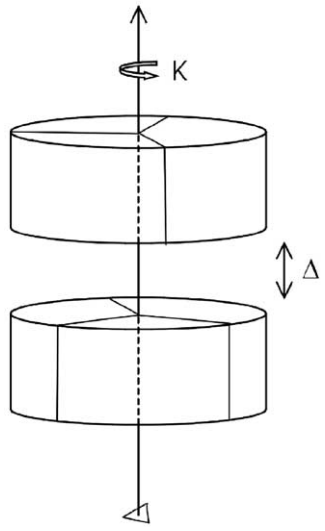

Fig. 4. Schematic diagram defining K and Δ (see Table 2) which describe the variable mode of assembly of the trimeric disks into hexamers. Each trimer is shown as a cylinder with three sectors. The threefold axis is marked with an arrow. K is a rotation around this axis and Δ is a shift along this axis.

These residues shape the binding pocket of the acyl moiety and its structural and sequential variability correlates with the variability of the fatty acid moiety of the substrate. The superposition analysis of the enzyme monomers shows additional large structural rearrangements for helix H9. Like in the tight hexamer form of Eci1p also in the structures of the other superfamily members H2 and/or H9 provide most of the inter-trimer contacts (Table 2). As discussed below, the large structural variability of helices H2 and H9 correlates with a large variability in the mode of assembly of the trimeric disks into hexamers (Table 2) within the crotonase superfamily.

In all known structures of enzymes of the hydratase/isomerase superfamily crystallographic hexamers are observed. Also the dehalogenase trimers are packed in the crystal as hexamers although the dehalogenase is known to be a trimer [5]. The importance of the hexameric assembly for the function is not understood. Only for the RNA-binding protein AUH has a function been assigned to the hexameric assembly consisting of shaping an RNA-binding pocket formed by lysines of α-helices H1 belonging to two different disks [8]. Interestingly, a recent publication established a second important function for this protein, being an essential 3-methylglutaconyl-CoA hydratase, shown to be a key enzyme of the leucine metabolism [9,10]. In order to compare the hexameric assemblies of these structures the crystallographic packing information was used to construct hexamers. Subsequently the mode of assembly of the hexamers was analyzed after superposition. Initially trimers were superimposed on the first Eci1p trimer (tight hexamer form). Subsequently, the second trimers were superimposed on the second Eci1p trimer. For the superposition of the second trimer essentially only two parameters are required (Table 2, Fig. 4): a rotation (K) and a shift (Δ) along the axis of rotation. This analysis shows that the relative orientation of the two trimer disks with respect to each other (K) is not conserved. Also the distance between the disks (Δ) can vary substantially, even when the relative orientation of the two trimers is the same (crotonase, AUH and dienoyl-CoA isomerase) (Table 2). A relatively short distance between the trimer disks is observed for the dehalogenase, although it occurs as a trimer in solution, whereas the largest distance is observed for the decarboxylase, although this enzyme is a hexamer in solution. The tight hexamer form of Eci1p is the most compact assembly, having the shortest distance between the trimeric disks. Clearly, the mode of assembly of the trimer disks into hexamers is not a conserved feature of the crotonase superfamily. The helices facing the inter-trimer space are helices H1, H2, H9 and H8′ (of the adjacent subunit) (Fig. 3). From the data in Table 2 it can be seen that H2 and H9 contribute most to the inter-trimer contacts. Together with adjacent helices H2A and H10 these two helices define the binding pocket for the acyl moiety of the acyl-CoA substrate. The large structural variability of these helices, stabilized by the different assembly modes of the trimeric disks, has allowed the evolution of a wide range of substrate specificities and catalytic specificities in this superfamily of enzymes.

Acknowledgements: We thank Jukka Taskinen and Sanna Partanen for collecting the diffraction data and the staff of the beamline X11 at DESY, Hamburg, Germany for the help during data collection. We also thank Dr. Ylva Lindqvist for stimulating discussions. The Academy of Finland and Sigrid Juselius Foundation are thanked for the financial support. The structure and structure factors have been deposited at the RCSB as entry 1PJH.

References

[1] Hiltunen, J.K. and Qin, Y.-M. (2000) Biochim. Biophys. Acta 1484, 117–128.
[2] Holden, H.M., Benning, M.M., Haller, T. and Gerlt, J.A. (2001) Acc. Chem. Res. 34, 145–150.
[3] Engel, C.K., Mathieu, M., Zeelen, J.H., Hiltunen, J.K. and Wierenga, R.K. (1996) EMBO J. 15, 5135–5145.
[4] Engel, C.K., Kiema, T.R., Hiltunen, J.K. and Wierenga, R.K. (1998) J. Mol. Biol. 275, 847–859.
[5] Benning, M.M., Taylor, K.L., Liu, R.Q., Yang, G., Xiang, H., Wesenberg, G., Dunaway-Mariano, D. and Holden, H.M. (1996) Biochemistry 35, 8103–8109.
[6] Modis, Y., Filppula, S.A., Novikov, D.K., Norledge, B., Hiltunen, J.K. and Wierenga, R.K. (1998) Structure 6, 957–970.
[7] Benning, M.M., Haller, T., Gerlt, J.A. and Holden, H.M. (2000) Biochemistry 38, 4630–4639.
[8] Kurimoto, K., Fukai, S., Nureki, O., Muto, Y. and Yokoyama, S. (2001) Structure 9, 1253–1263.
[9] Ijlst, L., Loupatty, F.J., Ruiter, J.P., Duran, M., Lehnert, W. and Wanders, R.J. (2002) Am. J. Hum. Genet. 71, 1463–1466.
[10] Ly, T.B., Peters, V., Gibson, K.M., Liesert, M., Buckel, W., Wilcken, B., Carpenter, K., Ensenauer, R., Hoffmann, G.F., Mack, M. and Zschocke, J. (2003) Hum. Mutat. 21, 401–407.
[11] Gurvitz, A., Mursula, A.M., Firzinger, A., Hamilton, B., Kilpelainen, S.H., Hartig, A., Ruis, H., Hiltunen, J.K. and Rottensteiner, H. (1998) J. Biol. Chem. 273, 31366–31374.
[12] Mursula, A.M., van Aalten, D.M.F., Hiltunen, J.K. and Wierenga, R.K. (2001) J. Mol. Biol. 309, 845–853.
[13] Mursula, A.M., van Aalten, D.M.F., Modis, Y., Hiltunen, J.K. and Wierenga, R.K. (2000) Acta Crystallogr. D56, 1020–1023.
[14] Palosaari, P.M. and Hiltunen, J.K. (1990) J. Biol. Chem. 265, 2446–2449.
[15] Zeelen, J.Ph., Hiltunen, J.K., Ceska, T.A. and Wierenga, R.K. (1994) Acta Crystallogr. D50, 443–447.
[16] Kabsch, W. (1993) J. Appl. Crystallogr. 26, 795–800.
[17] Navaza, J. (1994) Acta Crystallogr. A50, 157–163.
[18] Murshudov, G.N., Vagin, A.A. and Dodson, E.J. (1997) Acta Crystallogr. D55, 247–255.
[19] Jones, T.A., Zou, J.Y., Cowan, S.W. and Kjeldgaard, M. (1991) Acta Crystallogr. A47, 110–119.
[20] Yang, X., Purdue, P.E. and Lazarow, P.B. (2001) Eur. J. Cell Biol. 80, 126–138.
[21] Gurvitz, A., Mursula, A.M., Yagi, A.I., Hartig, A., Ruis, H.,

Rottensteiner, H. and Hiltunen, J.K. (1999) J. Biol. Chem. 274, 24514–24521.
[22] Hiltunen, J.K., Mursula, A.M., Rottensteiner, H., Wierenga, R.K., Kastaniotis, A.J. and Gurvitz, A. (2003) FEMS Microbiol. Rev. 27, 35–64.
[23] Laskowski, R.A., MacArthur, M.W., Moss, D.S. and Thornton, J.M. (1993) J. Appl. Crystallogr. 26, 283–291.
[24] Kabsch, W. and Sander, C. (1983) Biopolymers 22, 2577–2637.
[25] DeLano, W.L. (2002) The PyMOL Manual, DeLano Scientific, San Carlos, CA.