A Two-domain Structure of One Subunit Explains Unique Features of Eukaryotic Hydratase 2*

Received for publication, January 12, 2004, and in revised form, March 29, 2004
Published, JBC Papers in Press, March 29, 2004, DOI 10.1074/jbc.M400293200

M. Kristian Koski‡, Antti M. Haapalainen‡, J. Kalevero Hiltunen‡, and Tuomo Glumoff‡§

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

2-Enoyl-CoA hydratase 2, a part from multifunctional enzyme type 2, hydrates trans-2-enoyl-CoA to 3-hydroxyacyl-CoA in the (3R)-hydroxy-dependent route of peroxisomal β-oxidation of fatty acids. Unliganded and (3R)-hydroxydecanoyl coenzyme A-complexed crystal structures of 2-enoyl-CoA hydratase 2 from Candida tropicalis multifunctional enzyme type 2 were solved to 1.95- and 2.35-Å resolution, respectively. 2-Enoyl-CoA hydratase 2 is a dimeric, α-β protein with a novel quaternary structure. The overall structure of the two-domain subunit of eukaryotic 2-enoyl-CoA hydratase 2 resembles the homodimeric, hot dog fold structures of prokaryotic (R)-specific 2-enoyl-CoA hydratase and β-hydroxydecanoyl thiol ester dehydrase. Importantly, though, the eukaryotic hydratase 2 has a complete hot dog fold only in its C-domain, whereas the N-domain lacks a long central α-helix, thus creating space for bulkier substrates in the binding pocket and explaining the observed difference in substrate preference between eukaryotic and prokaryotic enzymes. Although the N- and C-domains have an identity of <10% at the amino acid level, they share a 50% identity at the nucleotide level and fold similarly. We suggest that a subunit of 2-enoyl-CoA hydratase 2 has evolved via a gene duplication with the concomitant loss of one catalytic site. The hydrogen bonding network of the active site of 2-enoyl-CoA hydratase 2 resembles the active site geometry of mitochondrial (S)-specific 2-enoyl-CoA hydratase 1, although in a mirror image fashion. This arrangement allows the reaction to occur by similar mechanism, supported by mutagenesis and mechanistic studies, although via reciprocal stereochemistry.

Hydratase 2 (2-enoyl-CoA hydratase 2; (R)-specific 2-enoyl-CoA hydratase) catalyzes reversibly the addition of water to the β-carbon of trans-2-enoyl-CoAs with the opposite chiral specificity to classic, (S)-specific, 2-enoyl-CoA hydratase 1 (hydratase 1; crotonase). In eukaryotic hydratase 2 is a ~31-kDa integral part of multifunctional enzyme type 2 (abbreviated MFE-2 in mammals and Mfe2p in fungi) (1, 2). In mammals, hydratase 2 together with the 2-enoyl-CoA hydratase/α-sorbose part of multifunctional enzyme type I (MFE-1) (3, 4), are responsible for catalyzing the second reaction of the peroxisomal β-oxidation spiral. It has been shown by patient mutations as well as in vitro studies that hydratase 2 is a crucial enzyme in lipid metabolism of α-methyl-branched fatty acids (e.g. pristanic acid), bile acid intermediates, and very-long-chain fatty acids (5–8).

Multiple sequence alignment of eukaryotic hydratase 2 has revealed a conserved region showing a motif, [YF]-X$_2$-[LVI]-[STGC]-GDXNP-[LIV]-HX$_2$-[AS], called the hydratase 2 motif (9) (see Fig. 1C). The motif contains an aspartate residue (Asp-510 in human MFE-2), whose function as a catalytic residue has been suggested by site-directed mutagenesis studies (9). The closest prokaryotic homologs known that contain the hydratase 2 motif are from the polyhydroxylkanoate (PHA)producing bacteria, such as Aeromonas caviae (10), Pseudomonas aeruginosa (11), Rhodopseudomonas aerogenosa (12), and Methylenebacterium rhodesianum (13). These enzymes, referred to as (R)-specific 2-enoyl-CoA hydratases or (R)-hydratases, catalyze a similar hydration reaction as eukaryotic hydratase 2, but in prokaryotes the product, (3R)-hydroxyacyl-CoAs, are polymerized to PHA by PHA synthase (14). In general, the size of (R)-hydratases are around 15 kDa, and they show the highest catalytic efficiency with short-chain enoyl-CoAs (C$_9$-C$_13$). However, at least one (R)-hydratase makes an exception. A PhaF$_2$p$_{pa}$ gene product, PhaF$_2$p$_{pa}$ characterized from P. aeruginosa has approximately the same subunit size as the eukaryotic hydratase 2 and shows a preference to enoyl-CoAs of C$_9$-C$_12$ (11).

The crystal structure of A. caviae (R)-hydratase has been solved recently (15). The core structure is a hot dog fold, which is built up of a long and hydrophobic α-helix (“sausage”) packed against anti-parallel β-sheet (“hub”). In a functional dimer, two subunits associate side by side to form an extended 10-stranded anti-parallel β-sheet layer. A similar hot dog fold and subunit organization is also found in Escherichia coli β-hydroxydecanoyl thiol ester dehydrase (FabA; dehydrase) of fatty acid synthesis type II (16). The major difference between these two enzymes is the additional loop structure in (R)-hydratase, referred to as overhanging segment (15). Despite the FabA lacking the overhanging segment housing the conserved hydratase CtMfe2p(dha$b_{H9251}$), 2-enoyl-CoA hydratase 2 part of C. tropicalis Mfe2p; SeMet, selenomethionine; 3RHDc, (3R)-hydroxydecanoyl-CoA; CtMfe2p(dha$b_{H9251}$H813Q), CtMfe2p(dha$b_{H9251}$) containing mutation H813Q; H-bond, hydrogen bond.

* This work was supported by European Community, Access to Research Infrastructure Action of the Improving Human Potential Programme to the EMBL Hamburg Outstation, Contract HPRN-CT-200017, and by grants from the Academy of Finland and Sigrid Juselius Foundation. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The atomic coordinates and structure factors (code 1PN2 and 1PN4) have been deposited in the Protein Data Bank, Research Collaboratory for Structural Bioinformatics, Rutgers University, New Brunswick, NJ (http://www.rcsb.org/).

‡ To whom correspondence should be addressed. Tel.: 358-8-553-1200; Fax: 358-8-553-1141; E-mail: tuomo.glumoff@oulu.fi.

§ This work was supported by European Community, Access to Research Infrastructure Action of the Improving Human Potential Programme to the EMBL Hamburg Outstation, Contract HPRN-CT-200017, and by grants from the Academy of Finland and Sigrid Juselius Foundation. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The abbreviations used are: hydratase 2, (R)-specific 2-enoyl-CoA hydratase; MFE-2, mammalian peroxisomal multifunctional enzyme type 2; Mfe2p, fungal peroxisomal multifunctional enzyme type 2; MFE-1, peroxisomal multifunctional enzyme type 1; PHA, polyhydroxylkanoate; FabA, β-hydroxydecanoyl thiol ester dehydrase from E. coli;
2 motif, the proposed catalytic residues, Asp-84 and His-70′ of the neighboring subunit, are arranged identically with the proposed catalytic residues, Asp-31 and His-36 (from the same subunit), of (R)-hydratase proposing an equal enzyme mechanism in addition/elimination of water molecule to/from 2-enoyl-(3R)-hydroxacyl thioesters. Incongruously, the catalytic dyad of human hydratase 2 is proposed to form by an aspartate (Asp-510) and a glutamate (Glu-366) residues (9). Interestingly, the suggested catalytic glutamate locates in the N-terminal half of eukaryotic protein, which is absent in the prokaryotic (R)-hydratase.

Even though the prokaryotic (R)-hydratase from *A. caudae* and eukaryotic hydratase 2 are related enzymes, they have major differences concerning the size of the enzymes, acyl chain substrate specificities, as well as biological functions in the cell. In the present work we show for the first time the three-dimensional structures for an eukaryotic hydratase 2 (*Candida tropicalis* MFE-2) as a free (apoenzyme) and enzyme-product complex (holoenzyme). The crystal structures locate the catalytic residues and suggest a novel catalytic mechanism for (R)-specific hydratases/dehydratases. Moreover, the structures introduce novel features for quaternary structure, evolution, substrate binding mode, and stereospecificity of hydratase 2 from the fatty acid β-oxidation pathway.

**EXPERIMENTAL PROCEDURES**

**Primary Phasing and Structure Refinement of SeMet-labeled CtMfe2p(dha*/H813Q*)—**The recombinant and selenomethionine (SeMet)-labeled 2-enoyl-CoA hydratase 2 part (residues 628–906) of *C. tropicalis* Mfe2p (CtMfe2p(dh*/*)) was overexpressed, purified, and crystallized as described previously (17). Primary phases for a SeMet-crystal were determined by the multilength wavelength anomalous dispersion method using data measured at three wavelengths (17). Data were processed using HKL2000 (25) and scaled using CNS (20), leading to two additional sites. The figure of merit at the beginning of the structure determination was 0.56, and the electron density calculated with the help of these 10 sites already revealed clear secondary structure elements. The initial maps were improved by solvent flattening calculations using a water content of 56%, after which the maps were interpretable. The building of the initial model was performed manually using O (21). Phases were extended to 1.95 Å using CNS (20), followed by refinement cycles with REFMAC (23). The non-crystallographic statistics of both crystal forms are presented in Table I. The more detailed structural statistics of both crystal forms are summarized in Table I.

**The Overall Structure of CtMfe2p(dh*/*Δ)—**The CtMfe2p(dh*/*Δ) subunit is comprised of 5 well defined α-helices (α1 and α4–α7) and 11 β-strands (β1–β11), which form a compact molecule with dimensions of 35 × 40 × 45 Å (Figs. 1A and 2A). The subunit structure can be further divided into an N-terminal domain (N-domain, residues 631–770), a C-terminal domain (C-domain, residues 789–900), and an intervening bridge (residues 771–788), which connects the two domains and includes a short α-helix, α4 (Fig. 2A). The core structure of the C-domain of CtMfe2p(dh*/*Δ) consists of a 16-residue α-helix, α7, and covering β-strands, β7–β11, forming a typical hot dog fold first identified in FabA (16). Moreover, the C-domain of the CtMfe2p(dh*/*Δ) contains a solvent-exposed loop structure (residues 806–830), included by an amphipathic α-helix, α6, and an α-helix, α5, which sandwiches the α7 with the β-sheet layer. The folding of the C-domain of CtMfe2p(dh*/*Δ) is strikingly similar with the subunit of the A. caudae (R)-hydratase (the root mean square deviation = 1.54 Å) as atoms can be...
proposed by a 15% overall sequence identity. The solvent-exposed loop structure, mainly composed of residues belonging to the hydratase 2 motif and referred to as the overhanging segment in *A. caviae* (R)-hydratase, is a unique feature for (R)-specific 2-enoyl-CoA hydratases and distinguishes the C-domain of eukaryotic hydratase 2 and (R)-hydratase from the crystal structure of FabA (16). However, the similar overall fold indicates that all these enzymes/domains, utilizing trans-2-enoyl(3R)-hydroxyacyl metabolites, most probably share a common ancestor.

Interestingly, the N-domain of Ctmfe2p(dha<sub>a</sub>) resembles significantly the C-domain, although no clear sequence similarity can be found between the domains. If the N-domain is rotated 180° around the vertical axis perpendicular to the β-sheet layer, the β-strands, β1–β5, superimpose with the β-strands, β′-β11, of the C-domain, as do α1 and α5. Moreover the N-domain contains a solvent-exposed loop structure (residues 647–668) resembling the overhanging segment of the C-domain; although it lacks the well defined α-helix, a PRO-MOTIF analysis (30) revealed α-helical structure (α1/2) also in this loop (Fig. 1C). The major difference between the two domains is, however, that the core helix, the “sausage” of the hot dog fold is replaced in the N-domain by a region consisting of short stretches of α-helices, α2 and α3, connected by a random coil structure (Figs. 1A and 2A). Therefore the features of the hot dog fold are only partially fulfilled in the N-domain of Ctmfe2p(dha<sub>a</sub>).

The N-domain is paired with the C-domain via β-strands β2 and β8 such that an extended 11-stranded anti-parallel β-sheet layer is formed in a Ctmfe2p(dha<sub>a</sub>) subunit (Fig. 2A). This arrangement of the two domains in Ctmfe2p(dha<sub>a</sub>) resembles strikingly the pairing of the two subunits of FabA and especially to that of *A. caviae* (R)-hydratase (Fig. 2). The origin of the N-domain of the Ctmfe2p(dha<sub>a</sub>), which replaces the neighboring subunit when compared with prokaryotic homologs, is an intriguing question. Although the N- and C-domains of the Ctmfe2p(dha<sub>a</sub>) share only <10% amino acid sequence identity, both domains show in addition to the similar folds a 50% identity at the level of DNA. This suggests that they have arisen via gene duplication rather than via gene fusion of two non-related genes with subsequent structural convergence.

The dimer of Ctmfe2p(dha<sub>a</sub>) is elongated, with dimensions of 70 × 40 × 45 Å, and the dimerization is accomplished by a four-helix bundle structure where the pairwise arranged α-helices, α1 from the N-domain and α5 form the C-domain, from one subunit are packed against their counterparts from the other one in an anti-parallel fashion (Fig. 1B). In addition to the major contacts via four-helix bundle, the N-domain solvent-exposed loop and the overhanging segment are participating in the dimeric interactions (Fig. 1B). One of the strengthening contacts formed between the subunits is the salt bridge Glu-659 to Arg-804. Importantly, Glu-659 corresponds to the proposed catalytic Glu-366 in the human enzyme suggesting that the role of the N-domain glutamate is rather to stabilize the folding of the hydratase 2 motif and to strengthen the dimeric interactions than to participate directly in the catalytic reaction. Despite the resemblance of the subunit structure of Ctmfe2p(dha<sub>a</sub>) with the homodimeric bacterial counterparts, the dimerization of the two subunits of Ctmfe2p(dha<sub>a</sub>) makes the quaternary fold of the eukaryotic hydratase 2 a novel feature.

**Substrate Binding Mode**—The bound 3RHDC molecule locates between β-strands β2 and β8 of the extended β-sheet at the interface of the N- and C-domains (Figs. 1A and 2A). The CoA molecule is in a bent conformation, a state often found in the CoA molecules bound to proteins (33). The funnel formed in the domain interface engulfs part of 3RHDC but not the 3′-phosphate ADP and half of the pantetheine moiety. The adenine ring of the bound substrate lies in a pocket surrounded

---

**Table I**

| Data set | Apoenzyme | Holoenzyme |
|----------|-----------|------------|
| Space group | C2 | P2<sub>1</sub> |
| a (Å) | 178.80 | 48.64 |
| b (Å) | 60.65 | 151.29 |
| c (Å) | 131.12 | 81.62 |
| β (°) | 94.57 | 90.50 |
| Resolution range (Å)<sup>a</sup> | 3.0–1.95 (2.92–1.95 Å) | 35.0–2.35 (2.50–2.35 Å) |
| Wavelength (Å) | 0.97985 | 0.8019 |
| Hkl (observed/unique)<sup>b</sup> | 442,494/97,595 (15,282/8,811) | 140,876/47,255 (21,501/7,461) |
| Completeness (%) | 95.2 (85.9) | 96.4 (90.1) |
| Overall I/σ(I) | 17.3 (3.05) | 12.10 (4.53) |
| R<sub>merge</sub>(%) | 4.8 (24.3) | 6.4 (23.2) |
| Refinement |
| R<sub>free</sub>(%) | 21.4 (10.0) | 22.5 (5.5) |
| Residues included in the model | 630–901 (A); 621–678, 683–901 (C); 631–902 (D) | 630–902 (A); 631–902 (B); 630–677, 698–728, 733–900 (C); 630–902 (D) |
| Number of atoms<sup>d</sup> | 9,157 | 8,963 |
| Protein<sup>d</sup> | 8,347 | 8,307 |
| SHDHC | 175 | |
| Water/ethylene glycol<sup>e</sup> | 79020 | 46516 |
| Geometry statistics |
| Root mean square deviations | |
| Lengths (Å/angles (°)) | 0.015/1.442 | 0.014/1.550 |
| Average B-factor (Å<sup>2</sup>) | 10.64 | 15.55 |
| Ramachandran plot |
| Most-favored region (%) | 91.1 (A, 92.2; B, 90.3; C, 91.4; D, 90.7) | 92.2 (A, 92.4; B, 92.4; C, 92.5; D, 91.6) |
| Additionally allowed regions (%) | 8.3 (A, 7.4; B, 9.3; C, 7.8; D, 8.9) | 7.7 (A, 7.6; B, 7.6; C, 7.1; D, 8.4) |
| Generously allowed regions (%) | 0.5 (A, 0.4; B, 0.4; C, 0.9; D, 0.4) | 0.1 (A, 0.0; B, 0.0; C, 0.5; D, 0.0) |

<sup>a</sup> Values in parentheses refer to the highest resolution shell.

<sup>b</sup> Values in parentheses refer to the percentage of reflections taken randomly for the test set calculations.

<sup>c</sup> The non-hydrogen atoms used in refinement.
The structure of 2-enoyl-CoA hydratase 2 part of C. tropicalis multifunctional enzyme type 2. A, stereo view of the ribbon diagram of the 3R HDC-complexed CtMfe2p(dha b) subunit. α-Helices and β-strands are colored in magenta and green, respectively. The 3R HDC molecule is shown as sticks and is colored as follows: carbon, grey; nitrogen, blue; oxygen, red; sulphur, yellow; phosphorus, green. B, the ribbon diagram showing an overview of the quaternary structure of the 3R HDC-complexed CtMfe2p(dha b) dimer. The view of the subunit A (on the top) is obtained by a counter clockwise rotation of that in panel A by 90° around the vertical axis. The α-helices participating in dimerization, as well as the hot dog core helices, are labeled. The 3R HDC molecules are shown as black sticks. C, alignment of the amino acid sequence of 2-enoyl-CoA hydratase 2 part of C. tropicalis Mfe2p (Ct_H2) with the corresponding parts of Saccharomyces cerevisiae (Sc_H2), human (Hs_H2), and rat (Rn_H2) multifunctional enzyme type 2s, as well as with monofunctional PhaJ2 from P. aeruginosa (Pa_H2) and A. caviae (Rc_H2). The corresponding SwissProt identifiers are: P22414, Q02207, P51659, P97852, Q9LBK1, and O32472. The secondary structure elements of CtMfe2p(dha b), α-helices (red lines) and β-strands (green arrows), are marked above the sequence alignment. Black vertical arrows indicate some functionally important residues in CtMfe2p(dha b). The black line below the sequence alignment indicates the hydratase 2 motif, and the yellow lines above the sequence alignment show the regions of flexible loops 1, II, and III. The Glu-627 (shown by the asterisk) of full-length C. tropicalis Mfe2p corresponds to the initial methionine of CtMfe2p(dha b). The remaining ten-carbon acyl chain of the substrate is buried in the hydrophobic cleft formed by the N-terminal β-strands β2 and β5 and by the N-terminal loop structure (residues 678–697). The loop contains hydrophobic residues (Phe-676, Phe-685, Phe-692, and Leu-697), which interact with the ω-end of the acyl group. In apoenzyme, this loop had either high temperature factors or poorly visible electron densities indicating high flexibility (therefore referred to as flexible loop I, Figs. 1C and 2A). In addition, the N-domain of CtMfe2p(dha b) contains two other flexible loops (flexible loop II, residues 726–736; flexible loop III, residues 756–769; Figs. 1C and 2A), which were difficult to interpret in the apoenzyme but well defined in the holoenzyme. Upon ligand binding the flexible loops move toward the overhanging segment of the C-domain excluding the hydrophobic parts of the ligand out of the solvent and completing the CoA binding pocket. The substrate binding mode of CtMfe2p(dha b), where the ligand is bound to the domain interface, is similar to that found in FabA (16). However, the crystal structure of FabA complexed with an inhibitor molecule, 3-decynoyl-N-acetylcysteamine, shows two equal binding sites in the subunit interface in contrast to only one found in CtMfe2p(dha b). Docking experiments based on the FabA-inhibitor crystal structure also prompted the suggestion of two binding sites in the subunit interface of the A. caviae (Rc)-hydratase (15). The available space in the suggested substrate binding tunnel of (R)-hydratase is restricted partly by the rigid hot dog helix (α4) of an adjacent subunit (Fig. 2B), and the depth of the pocket allows only the entrance of fatty enoyl-CoAs up to Ca 6 in length. In
contrast in the N-domain of CtMfe2p(dha\_b/H\_b/H\_a) the region corresponding to the rigid hot dog helix is replaced by the short \(\beta\)-helix, \(\beta\)-mercaptoethylenamine moiety of 3RHDCA are shown. Water molecules W6, W7, and W8 give way to the substrate as it enters the binding site, whereas the first five waters remain in position. The black arrow shows the \(\beta\)-carbon of the acyl moiety, and the red arrow indicates the H-bond crucial for oxyanion hole formation. The \(\beta\)-hydroxyl of the 3RHDCA molecule is H-bonded to the carboxylate oxygen of Asp-808 as well as to the N\_2 atoms of Gln-813 and Asn-810. The point mutation, H813Q, does not affect the folding of the active site. The atom colors correspond to the coloring in panel A, in addition to two sulfur atoms, which are shown in yellow. c, the final 3\(_F^\prime\) – 3\(_F^\prime\) map around 3RHDCA molecule at 2.35-Å resolution (contoured at 1.0 \(\sigma\)). The map is drawn also around the catalytic residue, Asp-808, as well as Gln813, which replaces the catalytically active His-813 in the mutant protein. A black arrow points to the electron density for the \(\beta\)-hydroxyl group.

**Fig. 3. The active site architecture of CtMfe2p(dha\_b/H\_a).** A, stereo view of the H-bonding network in the active site of apo form of CtMfe2p(dha\_b/H\_a). The H-bond network is shown as black dashed lines. A black arrow shows the catalytic water molecule, which is H-bonded to the carboxylate oxygen of Asp-808, the N\_2 atom of His-813, and the N\_2 atom of Asn-810. The carbons, nitrogens, oxygens, and selenium have been colored in gray, blue, red, and brown, respectively, and the water molecules are colored in magenta. B, stereo view of the active site of CtMfe2p(dha\_b/H\_a/H813Q) complexed with 3R-HDC. Only the acyl part and \(\beta\)-mercaptoethylenamine moiety of 3RHDCA are shown. Water molecules W6, W7, and W8 give way to the substrate as it enters the binding site, whereas the first five waters remain in position. The black arrow shows the (3R)-hydroxyl group, which is added to the \(\beta\)-carbon of the acyl moiety, and the red arrow indicates the H-bond crucial for oxyanion hole formation. The (3R)-hydroxyl of the 3RHDCA molecule is H-bonded to the carboxylate oxygen of Asp-808 as well as to the N\_2 atoms of Gln-813 and Asn-810. The point mutation, H813Q, does not affect the folding of the active site. The atom colors correspond to the coloring in panel A, in addition to two sulfur atoms, which are shown in yellow. C, the final 3\(_F^\prime\) – 3\(_F^\prime\) map around 3RHDCA molecule at 2.35-Å resolution (contoured at 1.0 \(\sigma\)). The map is drawn also around the catalytic residue, Asp-808, as well as Gln813, which replaces the catalytically active His-813 in the mutant protein. A black arrow points to the electron density for the (3R)-hydroxyl group.

**Reaction Mechanism**—The role of the conserved aspartate in...
the hydratase 2 motif (510 and 31, in human MFE-2 and A. caviae (R)-hydratase, respectively) has been shown to be crucial for hydratase 2 activity (9, 15). On the contrary, the present structure of the fungal enzyme challenges the potential catalytic role of the N-domain glutamate for eukaryotic hydratase 2 as discussed above. Instead, the location of the side chain of His-813 with respect of Asp-808 is optimal for catalysis in the apoenzyme of CtMfe2p(dha/H11001b/H9004). Furthermore, the mutagenesis studies showed that the mutation H813Q reduced the enzyme activity of CtMfe2p(dha/H11001b/H9004) around 15,000 times (kcat value was 0.026 s-1 with 60 μM trans-2-decenoyl-CoA) compared with the wild type protein (17) without affecting the folding of the enzyme. These facts indicate that His-813 may have an important role in catalysis analogous to the corresponding histidine, His-36, in A. caviae (R)-hydratase (15).

The presence of a natural product in the crystal structure of CtMfe2p(dha/H11001b/H813Q) indicates the active site as well as the catalytic residues for hydratase 2. In the holoenzyme the (3R)-hydroxyl group of the bound 3RHDC is H-bonded to Oδ2 of Asp-808 and Nε2 of His-813 and can be proposed to be the catalytic water activated by Asp-808 and His-813 in hydration reaction (Fig. 3A). The role of His-813 in water activation is enhanced by Nδ1 of the imidazole ring donating proton via H-bond to the backbone oxygen of Ile-828 (Fig. 3A), thus creating a basic lone pair of electrons on Nε2. In the optimal configuration for the hydration reaction, the electron pair of the fourth tetrahedral position of the catalytic water, W6, would be directed toward the β-carbon of the substrate. Because W6 is also H-bonded to the Nε2 atom of Asn-810 (Fig. 3A), this requirement is fulfilled only if the carboxylate oxygen of Asp-808 and Nε2 of His-813 are both unprotonated. This being the case, activated W6 is the source of both the proton added to the β-carbon and the (3R)-hydroxyl group added to the β-carbon, as suggested for hydratase 1 reaction (35). Previously, the reaction mechanism of (R)-specific hydration/dehydration is proposed to occur via acid-base catalysis (15, 16), which is now challenged by the current observations with the crystal structures of apoenzyme and enzyme-product complex of eukaryotic hydratase 2. The proposed reaction

The Structure of Eukaryotic Hydratase 2

Fig. 4. The proposed reaction mechanism of hydratase 2. The essential active site interactions for the hydration/dehydration reactions are shown. The reaction is suggested to proceed via concerted transition state where the atoms across the double bond of trans-2-enoyl-CoA thioester are derived from the single water molecule, defined as catalytic water. The numbering of the residues is based on the sequence of C. tropicalis Mfe2p.
mechanism of hydratase 2 is presented in Fig. 4. The carboxyl oxygen of the substrate is H-bonded to the backbone amide of Gly-831 and water molecule, W1 (Figs. 3B and 5B), which together form an oxyanion hole, found upon substrate binding in many CoA-ester metabolizing enzymes (36). The role of the oxyanion hole is to stabilize the kinetically very unfavorable intermediate state of a thiol ester substrate, enabling catalysis of the hydration reaction to occur (Fig. 4). The polarizing effect of Gly-831 is enhanced due to the position of this residue at the N terminus of the long hot dog α-helix (α7) giving Gly-831 a positive dipole. These findings underline the importance of the long central helix of the hot dog fold. Because the core helix is replaced by the discontinuous helical region in E. coli b-Enoyl-CoA hydratase 2 is the key enzyme of peroxisomal convergence during evolution.

REFERENCES

1. Qin, Y.-M., Poutanen, M. H., Helander, H. M., Kvist, A.-P., Siivari, K. M., Schmitz, W., Conzelmann, E., Hellman, U., and Hiltunen, J. K. (1997) J. Biol. Chem. 272, 21–28

2. Hiltunen, J. K., Wenzel, B., Beyer, A., Erdmann, R., Fossa, A., and Kunau, W. H. (1992) J. Biol. Chem. 267, 6646–6653

3. Kiema, T.-R., Engel, C. K., Schmitz, W., Filippula, S. A., Wierenga, R. K., and Hiltunen, J. K. (1999) Biochemistry 38, 2991–2999

4. Osumi, T., and Hashimoto, T. (1979) Biochem. Biophys. Res. Commun. 89, 560–564

5. Möller, G., van Grunsven, E. G., Wanders, R. J. A., and Adamaki, J. (2001) Mol. Cell. Endocrinol. 171, 61–70

6. van Grunsven, E. G., Mosjier, P. A. W., Aubourg, P., and Wanders, R. J. A. (1999) Hum. Mol. Genet. 8, 1539–1546

7. Dieuaide-Noubhani, M., Novikov, D., Baumgart, E., Vanhooren, J. C., Fransen, M., Goethals, M., Vandenkervochte, J., Van Veldhoven, P. P., and Maes, G. P. (1996) Eur. J. Biochem. 240, 660–668

8. Qin, Y.-M., Haapalainen, A. M., Conry, D., Cuebas, D. A., Hiltunen, J. K., and Novikov, D. K. (1997) Biochem. J. 328, 377–382

9. Qin, Y.-M., Haapalainen, A. M., Kilpelainen, S. H., Marttila, M. S., Koski, M. K., Glumoff, T., Novikov, D. K., and Hiltunen, J. K. (2000) J. Biol. Chem. 275, 4965–4972

10. Fukui, T., and Doi, Y. (1997) J. Bacteriol. 179, 4821–4830

11. Tasaka, T., Fukui, T., Matsuakashi, H., Taguchi, S., Kobayashi, G., Ishizaki, A., and Doi, Y. (2000) FEBS Microbiol. Lett. 184, 193–198

12. Reiser, S. E., Mitsky, T. A., and Grays, K. J. (2000) Appl. Microbiol. Biotechnol. 53, 209–218

13. Mothes, G., and Babel, W. (1995) Can. J. Microbiol. 41, 68–72

14. Madison, L. L., and Huisman, G. W. (1999) Hum. Mol. Genet. 8, 1539–1546

15. Engel, C. K., and Wierenga, R. K. (1998) J. Mol. Biol. 275, 617–624

16. Leesong, M., Henderson, B. S., Gillig, J. R., Schwab, J. M., and Smith, J. L. (1998) Acta Crystallogr. D Biol. Crystallogr. 54, 849–861

17. Mothes, G., and Babel, W. (1995) Can. J. Microbiol. 41, 68–72

18. Leesong, M., Henderson, B. S., Gillig, J. R., Schwab, J. M., and Smith, J. L. (1998) J. Mol. Biol. 275, 617–624

19. Mothes, G., and Babel, W. (1995) Can. J. Microbiol. 41, 68–72

20. Brunger, A. T., Adams, P. D., Clore, G. M., DeLano, W. L., Gros, P., Grosse-Kunstleve, R. W., Jiang, J. S., Kuszewski, J., Nilges, M., Pannu, N. S., Read, R. J., Rice, L. M., Simonson, T., and Warren, G. L. (1998) Acta Crystallogr. D Biol. Crystallogr. 54, 905–921

21. Jones, T. A., Zou, J. Y., Cowan, S. W., and Kjeldgaard, M. (1991) Acta Crystallogr. Sect. A 47, 110–115

22. Kabsch, W. (1993) J. Appl. Crystallogr. 26, 795–800

23. Murshudov, G. N., Vagin, A. A., and Dodson, E. J. (1997) Acta Crystallogr. Sect. D Biol. Crystallogr. 53, 240–255

24. Perrakis, A., Morris, R., and Lamzin, V. S. (1999) Nat. Struct. Biol. 6, 458–463

25. Hiltunen, J. K., Palosaari, P. M., and Kunau, W. H. (1999) J. Biol. Chem. 274, 13536–13540

26. Brzozowski, A. M., and Walton, J. (2001) J. Appl. Crystallogr. 34, 97–101

27. Engel, C. K., Kiema, T.-R., Hiltunen, J. K., and Wierenga, R. K. (1998) J. Mol. Biol. 275, 847–859

28. Laskowski, R. A., MacArthur, M. W., Moss, D. S., and Thornton, J. M. (1993) J. Appl. Crystallogr. 26, 283–291

29. Friend, G. (1990) J. Mol. Graph. 8, 52–56

30. Hutchinson, E. G., and Thornton, J. M. (1996) Protein Sci. 5, 212–220

31. Guex, N., and Peitsch, M. C. (1997) Electrophoresis 18, 2741–2743

32. Gould, S. J., Keller, G. A., Hosten, N., Wilkinson, J., and Subramani, S. (1989) J. Mol. Biol. 198, 1657–1664

33. Engels, C., and Wierenga, R. (1996) Curr. Opin. Struct. Biol. 6, 790–797

34. Tasaka, T., Hirasawa, T., Takami, Z., and Doi, Y. (2003) J. Appl. Crystallogr. 36, 4830–4836

35. Akopyan, R. A., Anderson, V. E., and Petsko, G. A. (2002) Biochemistry 41, 6653–6663

36. Holden, H. M., Benning, M. H., Haller, T., and Gerlt, J. A. (2001) Acc. Chem. Res. 34, 145–157

37. Mohrig, J. R., Moerke, K. A., Cloutier, D. L., Lane, B. D., Person, E. C., and Onasch, T. B. (1995) Science 269, 527–529
