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Structural Basis for Reductive Radical Formation and Electron Recycling in (*R*)-2-Hydroxyisocaproyl-CoA Dehydratase

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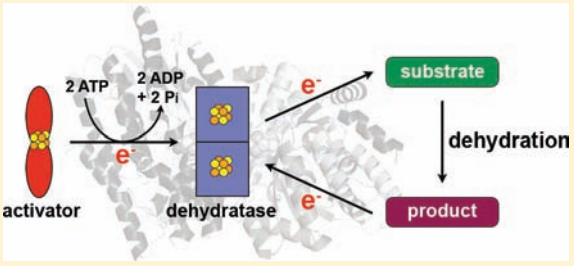
Supporting Information

ABSTRACT: The radical enzyme (*R*)-2-hydroxyisocaproyl-CoA dehydratase catalyzes the dehydration of (*R*)-2-hydroxyisocaproyl-CoA in the fermentation of L-leucine by the human pathogenic bacterium *Clostridium difficile*. In contrast to other radical enzymes, such as bacterial class II ribonucleotide reductase or biotin synthase, the Fe/S cluster containing (*R*)-2-hydroxyisocaproyl-CoA dehydratase requires no special cofactors such as coenzyme B_{12} or *S*-adenosylmethionine for radical generation. Instead it uses a single high-energy electron that is recycled after each turnover. The catalyzed reaction, an atypical α/*β*-dehydration, depends on the reductive formation of ketyl radicals on the substrate generated by injection of a single electron from the ATP-dependent activator protein. So far, it is unknown how the active electron is recycled and how unwanted side reactions are prevented, allowing for up to 10 000 turnovers. The crystal structure reveals that the heterodimeric protein contains two [4Fe-4S] clusters at a distance of 12 Å, each coordinated by three cysteines and one terminal ligand. The cluster in the α-subunit is part of the active site. In the absence of substrate, a water/hydroxide ion acts as the fourth ligand. The substrate replaces this ligand and coordinates the cluster via the carbonyl-oxygen of the thioester group. The cluster in the *β*-subunit has a terminal sulfhydryl/sulfido ligand and can act as a reservoir to protect the electron from unwanted side reactions via a recycling mechanism. The crystal structure of (*R*)-2-hydroxyisocaproyl-CoA dehydratase serves as a model for the reductively radical-generating metalloenzymes of the (*R*)-2-hydroxyacyl-CoA dehydratase and benzoyl-CoA reductase families.



INTRODUCTION

Many chemically challenging reactions in biology are mediated by radical enzymes.[1] Well-known radical enzymes are the human methylmalonyl-CoA mutase[2] and bacterial class II ribonucleotide reductase,[3] which use coenzyme B_{12} as the generator of the 5′-deoxyadenosyl radical. Recently a steadily increasing number of radical enzymes have been discovered, in which *S*-adenosylmethionine (SAM) replaces coenzyme B_{12} to generate the same radical.[4] Prominent examples are the widely distributed biotin synthase,[5] and the activators of glycyl radical enzymes such as pyruvate formate lyase from *Escherichia coli*.[6] In contrast, the (*R*)-2-hydroxyacyl-CoA dehydratases form a distinct family of radical enzymes that only use one high-energy electron for radical generation,[7] which is recycled after each turnover. These enzymes are involved in the fermentation of 12 of the 20 proteinogenic amino acids by anaerobic bacteria, mainly belonging to the order *Clostridiales*.[8]

One representative of the (*R*)-2-hydroxyacyl-CoA dehydratase family is (*R*)-2-hydroxyisocaproyl-CoA dehydratase from the human pathogenic bacterium *Clostridium difficile*. The enzyme catalyzes the key step in the reductive branch of leucine fermentation, the almost irreversible *β*/α-elimination of water from (*R*)-2-hydroxyisocaproyl-CoA to yield isocaprenoyl-CoA,[9] most likely the *E*-isomer (K'_{eq} = 1600).[10] The required elimination of the *β*-hydrogen as proton (p$K \approx 40$) and the removal of the hydroxy group adjacent to the thioester carbonyl are chemically demanding steps. This reaction depends on the reductive formation of ketyl radicals on the substrate[11] generated by injection of a single electron from the ATP-dependent activator protein. The most stable radical, an allylic ketyl radical, involved in the proposed mechanism[12] could be identified by EPR spectroscopy.[11] In the last step, the electron recycles on the dehydratase for up to 10 000 turnovers until another activation is required.[9]

Radical chemistry is challenging for enzymes because the generation, storage, and reaction of the single-electron species need to be tightly controlled. Nature has found ways to overcome these problems as shown by the crystal structures of coenzyme B_{12}-dependent glutamate mutase[13] and the SAM-dependent radical enzyme lysine 2,3-aminomutase.[14] In contrast, it is

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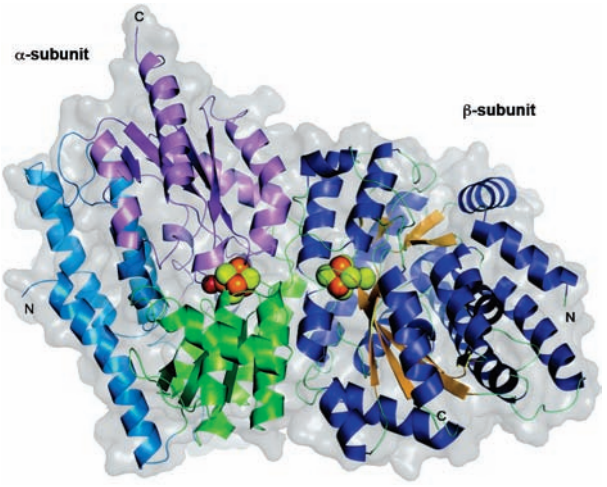

Figure 1. Overall structure of the (*R*)-2-hydroxyisocaproyl-CoA dehydratase. The α-subunit is colored according to its three domains. The β-subunit is displayed according to secondary structure with α-helices in blue, β-sheets in orange, and loops in green. The [4Fe-4S] clusters with terminal ligands are shown as spheres with sulfur in yellow-green, iron in orange, and oxygen in red.

unknown how the highly reactive electron (E_0' ca. −900 mV) is stored in (*R*)-2-hydroxyacyl-CoA dehydratases and how unspecific side reactions are prevented so that the electron can be recycled many times. Here we report the crystal structure of (*R*)-2-hydroxyisocaproyl-CoA dehydratase in complex with its substrate and propose a structure-based mechanism for catalysis.

RESULTS AND DISCUSSION

Overall Structure of the Dehydratase. We crystallized recombinant dehydratase under anoxic conditions and solved the structure by the multiwavelength anomalous dispersion (MAD) phasing method using the iron atoms of the Fe/S clusters as anomalous scatterers (see Table S1, Supporting Information, for statistics). Two copies of the dehydratase heterodimer[9] were found in the asymmetric unit. The 1.8 Å resolution crystal structure (Figure 1) shows that the α-subunit (HadB, 408 residues) consists of three domains and harbors one cubane-type [4Fe-4S] cluster (α-cluster). The α-cluster is located at the interface of two domains, which exhibit similar mixed α/β-topologies each with a central parallel β-sheet pointing to the cluster. The third domain is a α-helical bundle connecting the other two domains. An approximately 17 Å long channel leads from the surface into the interior of the subunit ending with a cavity at the α-cluster (Figure S1, Supporting Information).

The β-subunit (HadC, 375 residues) also contains one [4Fe-4S] cluster (β-cluster), and the superposition of the two subunits (Figure S2, Supporting Information) shows that their overall topology is very similar with a root-mean-square deviation of 1.99 Å of their Cα-atoms despite the modest sequence identity of 19.7%. A survey of the protein data bank using DaliLite v.3[15] reveals no structures with clear similarity to the dehydratase. However, other metalloenzymes with complex Fe/S clusters such as carbon monoxide dehydrogenase and nitrogenase exhibit similar structural motifs, as they also comprise two domains with mixed α/β fold in which parallel β-sheets with the order 2−1−3−4 point to a metal cluster.[16] A superposition of the β-subunit of the dehydratase and one monomer of the carbon monoxide dehydrogenase II from *Carboxydothermus hydrogenoformans* (Figure S3, Supporting Information) clearly shows that the similarity of the structures is limited to the domains containing the β-sheets.

The Fe/S Clusters. The β-cluster is completely buried and shielded from bulk solvent within the β-subunit and is coordinated by three cysteines (Figure 2B). The fourth iron ion of the β-cluster is coordinated by an electron-rich ligand with an anomalous scattering contribution at a wavelength of 1.76 Å similar to that of the sulfur atoms of cysteines, methionines, and the [4Fe-4S] clusters (see Table S2, Supporting Information, for statistics). Its location in a hydrophobic pocket with no hydrogen bonding interactions with the protein matrix and an average ligand−iron bond length of 2.30 Å indicate that the terminal ligand of the unique, noncysteine coordinated iron site is a SH group (see Table S2 for statistics). The shortest distance between the edges of the α- and β-cluster is approximately 12 Å, which is within the range allowing facile electron transfer reactions.[17] The α-cluster is coordinated by three cysteines and one terminal ligand at the unique iron site (Figure 2A). Modeling of the terminal ligand as OH_x satisfies the electron density and is in agreement with the refined bond length of 2.03 Å and the lack of anomalous scattering. The coordination of the cluster by three cysteines and a water/hydroxy ligand is similar to the one found in aconitase.[18] The two [4Fe-4S] clusters of the dehydratase have resisted the reduction by conventional reducing agents,[9] indicating that for both clusters the $[4Fe\text{-}4S]^{2+/+}$ redox couple is at low potentials (<−600 mV). The crystal structure reveals for both clusters only one potential hydrogen bond donor, a water molecule near the α- and β-clusters opposite to the unique iron. Hydrogen bonds to the bridging sulfido-ligands and cysteine thiolate ligands have been shown to increase the redox potential of [4Fe-4S] clusters,[19] and consequently a small number of hydrogen bond interactions is anticipated for iron−sulfur clusters with low redox potential.

Substrate Binding and the Active Site. To assess how substrate is activated in the active site, a dehydratase crystal was soaked with (*R*)-2-hydroxyisocaproyl-CoA and a data set was collected to a resolution of 2.3 Å (see Table S3, Supporting Information, for statistics). Electron density maps reveal that (*R*)-2-hydroxyisocaproyl-CoA is located in a channel of the α-subunit leading from the surface to the α-cluster. The substrate coordinates the unique iron atom of the α-cluster via the carbonyl oxygen of the thioester, resulting in a distorted tetrahedral coordination (Figure 3) and positioning the carbonyl carbon of the substrate at a distance of 3.0 Å from the unique iron. The 2-hydroxy group is positioned 4.0 Å away from the unique iron site. The only direct interaction of the (*R*)-2-hydroxyisocaproyl moiety with the surrounding amino acids is provided by the carboxylate group of Glu55, which is at a distance of 2.9 Å to the carbonyl oxygen of the substrate. Glu55 is highly conserved in the family of (*R*)-2-hydroxyacyl-CoA dehydratases (Figure S8, Supporting Information), and its carboxylate group is oriented toward the substrate by Ser37 (Figure 3), which is also conserved (Figure S8). The CoA moiety makes direct contacts with the protein as well as indirect interactions via water molecules. In the absence of substrate, three water molecules can be found in close proximity of the α-cluster in addition to the water ligand providing a hydrophilic environment. These molecules are displaced when substrate enters the active site and coordinates the cluster. Except for the 2-hydroxy group, the isocaproyl moiety is apolar. Additionally, the dehydratase was

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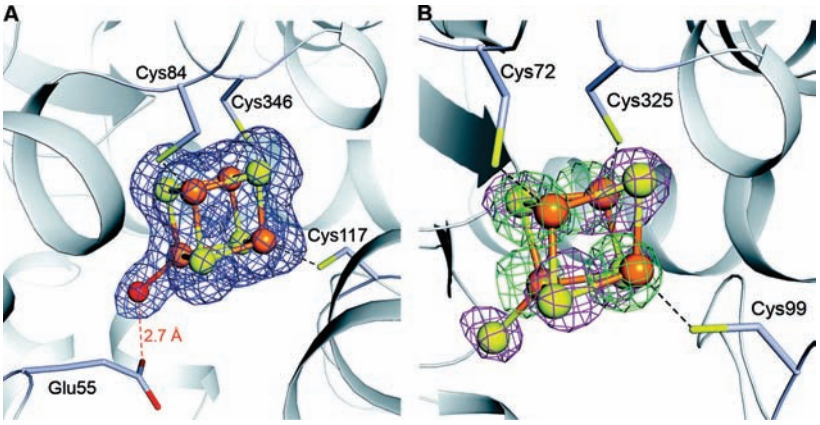

Figure 2. Coordination of the Fe/S clusters. The [4Fe-4S] clusters and the terminal ligands are represented as balls and sticks with sulfur in yellow-green, iron in orange, and oxygen in red. Coordinating bonds are displayed as black dashed lines. (A) [4Fe-4S] cluster in the α-subunit (α-cluster). The simulated annealing omit map shown for the α-cluster and its terminal ligand is contoured at 2.5σ. The occupancies of the coordinating water/hydroxide ion and the unique iron have been estimated to 0.7. The coordinating water/hydroxide ion is in hydrogen-bonding distance to Glu55, which is oriented with its anti electron lone pair toward the hydroxy group. (B) [4Fe-4S] cluster in the β-subunit (β-cluster). The anomalous difference Fourier maps for iron (data set collected at λ = 1.73980 Å) and sulfur (data set collected at λ = 1.76 Å) are shown in green and purple, contoured at 15.5σ and 4.5σ, respectively.

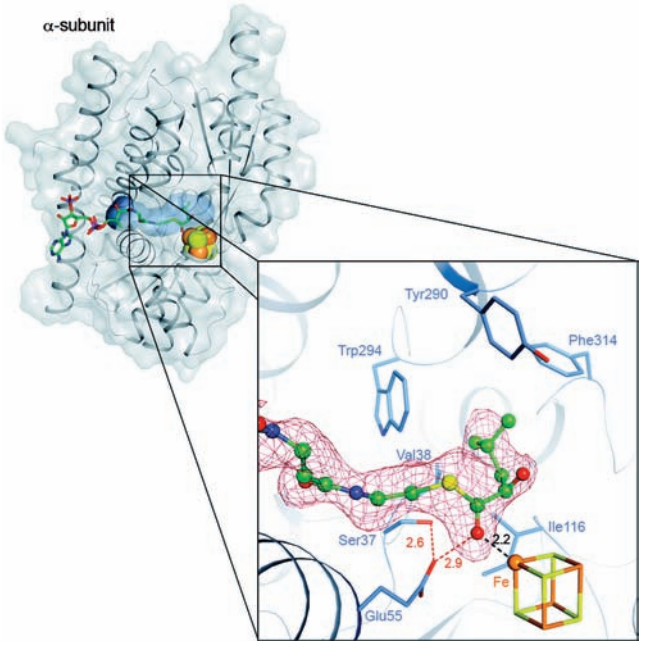

Figure 3. Substrate binding mode. The α-subunit with bound (*R*)-2-hydroxyisocaproyl-CoA is shown. The substrate channel is displayed in blue. The panel shows an enlargement of the active site with substrate bound to the α-cluster. Key residues are shown as sticks, and the unique iron ion of the α-cluster is displayed as a sphere. Carbon atoms are colored in green, oxygen atoms in red, nitrogen atoms in blue, sulfur atoms in yellow-green, phosphorus atoms in purple, and iron atoms in orange. The simulated annealing omit map for the substrate contoured at 3σ is shown in dark red. All distances are given in angstroms. The end of the binding pocket contains primarily hydrophobic and aromatic amino acids. As the substrate channel has an approximate length of 17 Å, the 3′-phosphorylated adenosine moiety of CoA is outside the protein and is not defined in the electron density map. The occupancy of the substrate has been estimated to be approximately 70%.

cocrystallized with (*R*)-2-hydroxyisocaproate, and the structure was refined to 2.0 Å resolution (see Table S3 for statistics). Like in the substrate bound structure, (*R*)-2-hydroxyisocaproate coordinates the unique iron of the α-cluster by one oxygen of the carboxyl group, but the 2-hydroxy group points in another direction compared to the substrate-bound state (see Figures S4 and S5, Supporting Information). This different orientation of the hydroxyl group in the (*R*)-2-hydroxyisocaproate complex is probably due to the lack of conformational restraints imposed by CoA in the substrate complex. The binding of the free acid does not lead to conformational changes of the dehydratase.

From these findings we conclude that the active site is harbored in the α-subunit at the α-cluster. In the absence of substrate, a water molecule or hydroxide ion acts as the fourth ligand of the cluster. Binding of the substrate or a cofactor to the open coordination site of a [4Fe-4S] cluster is similar to that found for aconitase[18] and SAM radical enzymes.[5] But unlike the substrate/cofactor coordination found in these enzymes, (*R*)-2-hydroxyisocaproyl-CoA coordinates the [4Fe-4S] cluster only in a monodentate manner via the carbonyl oxygen of the thioester (Figure S6, Supporting Information). In SAM radical enzymes, the radical-generating cofactor *S*-adenosylmethionine coordinates the unique iron bidentately via its COO^- and NH_2 group, and it is partially wrapped around the cluster. Similarly, in aconitase the unique iron is coordinated by two functional groups of citrate and a solvent molecule. The arrangement found in the (*R*)-2-hydroxyisocaproyl-CoA dehydratase allows for the closest possible proximity between the unique iron of the α-cluster and the carbonyl group to be reduced to the ketyl-radical anion. The use of a [4Fe-4S] cluster coordinated by three Cys residues to reductively generate a radical is reminiscent of the SAM-dependent radical generation,[4] and similar to that found in these enzymes, the unique iron is expected to mediate an inner-sphere electron transfer to an appropriate molecular orbital in close proximity.[20] The unusual interaction of 2-hydroxyisocaproyl-CoA with the α-cluster positions the electron-accepting antibonding π^*-orbital of its thioester carbonyl close to the unique Fe ion and may thus be rationalized through the mechanistic demands of a controlled inner-sphere electron transfer to generate a ketyl radical anion. As the β-cluster is in electron transfer distance to the α-cluster, the radical-generating electron may be transferred between the two clusters. While no substrate is

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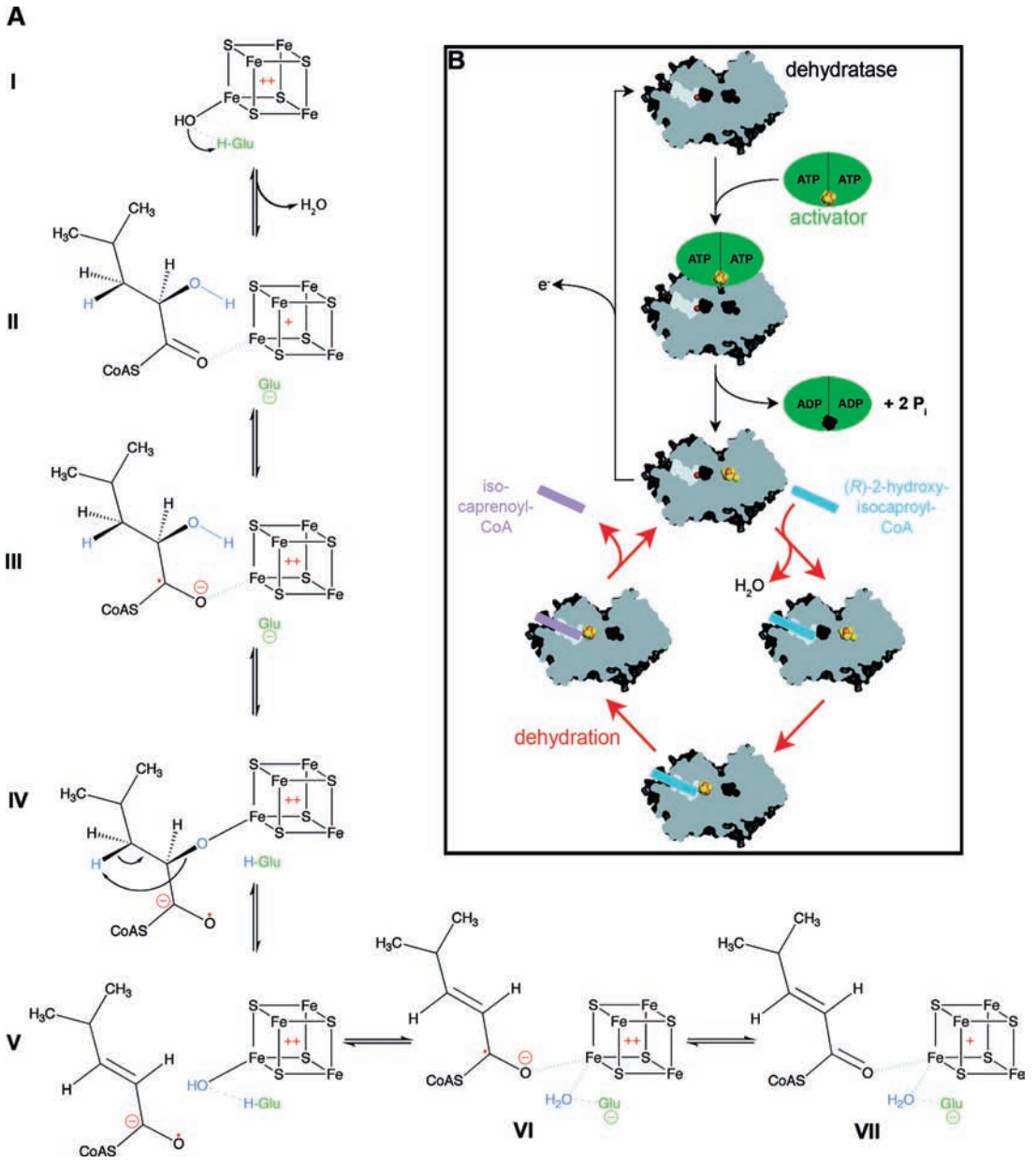

Figure 4. Structure-based reaction mechanism. (A) Seven states of the α-cluster occurring in the dehydration are shown. Coordinative and hydrogen bonds are indicated by hashed and dashed lines, respectively. (I) Oxidized α-cluster with hydroxy ligand at the unique iron ion. (II) Substrate replaces the coordinating water molecule and coordinates the α-cluster, increasing its redox potential, which results in electron transfer from the β-cluster to the α-cluster. (III) Inner-sphere electron transfer from the α-cluster to the carbonyl carbon of the thioester. (IV) The change in the coordination favors the negative charge on the ketyl carbon, allowing the expulsion of the 2-hydroxy group. (V) The 2-hydroxy group is expelled, and the resulting enoxy radical lowers the pK_a of the β-proton, which is abstracted by the negatively charged Fe−O^-. (VI) Product-related allylic ketyl radical and water are bound to the unique iron atom. (VII) The electron is transferred back to the α-cluster, yielding the product isocaprenoyl-CoA. Finally the product leaves the active site, and the water ligand is deprotonated by Glu55, favoring electron transfer to the β-cluster. (B) Electron recycling. Reduced [4Fe-4S] clusters are shown in bright colors and oxidized [4Fe-4S] clusters in dark colors.

bound in the active site the β-cluster could be used to store the catalytic electron.

Structure-Based Reaction Mechanism for Ketyl Radical Formation. For activation, the dehydratase forms a complex with its reduced activator with bound ATP.[9] The homodimeric activator contains a surface-exposed [4Fe-4S] cluster from which an electron is transferred to one of the Fe/S clusters of the dehydratase at low redox potential with ATP hydrolysis as the driving force. The shortest distance of each cluster of the dehydratase to the protein surface is approximately 11 Å, making this electron transfer structurally possible. On the basis of the structures of the dehydratase, we propose the catalytic cycle shown in Figure 4. At the beginning, the dehydratase is in an oxidized, inactive state and a hydroxy ligand is bound to the α-cluster. In the first step, the reduced activator with bound ATP forms a complex with the dehydratase.[21] ATP hydrolysis provides the energy necessary to transfer an electron at low redox potential to the dehydratase, where it is stored at the β-cluster.

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Glu55 can transfer a proton to the hydroxyl ligand at the unique iron of the α-cluster to form water. Upon substrate binding, the water ligand coordinating the α-cluster is replaced by the thioester carbonyl oxygen of the substrate. Additionally, the water molecules in the substrate-binding pocket are displaced by the substrate, leading to a change in the electrostatic environment of the cluster that favors the electron transfer from the β-cluster to the α-cluster. The highly energetic electron reduces the thioester carbonyl bound to the α-cluster to a ketyl radical.[11] We suggest that the formation of the ketyl radical anion drives a ligand substitution, such that the reduced substrate coordinates the cluster via its 2-hydroxy group that is stabilized by hydrogen-bonding interaction with Glu55. This change in coordination can be easily achieved by the rotation of the 2-hydroxyisocaproyl moiety around the σ-bonds of the β-mercaptoethylamine moiety adjacent to the thioester group and small rearrangements (Figure S7, Supporting Information). The substitution is necessary to favor the localization of the negative charge at the ketyl carbon to increase its nucleophilicity and expel the adjacent hydroxy group. A change in the coordination of a substrate at a [4Fe-4S] cluster during the catalytic cycle is already known from aconitase where *cis*-aconitate changes from the citrate to the isocitrate mode which corresponds to a flip of 180°.[22] After the change in coordination and removal of the hydroxy group, the acidity of the β-hydrogen of the resulting enoxy radical is increased by 26 p*K* units[23] and can be removed as a proton. As the β-hydrogen is not near to any amino acid which could act as a base, the only possible base for this elimination appears to be the alkoxide group bound at the [4Fe-4S] cluster, which explains the experimentally determined *syn*-eliminations catalyzed by the related (*R*)-lactyl-,[24] (*R*)-3-phenyllactyl-,[25] and (*R*)-2-hydroxyglutaryl-CoA dehydratases.[26] The formed product-related allylic ketyl radical, which was detected by Kim et al.,[11] coordinates the cluster again via its carbonyl oxygen and transfers the electron back to the α-cluster. The eliminated water molecule remains bound to the cluster. Without the change in coordination of the substrate-related ketyl radical anion, the formation of the enoxy radical as well as the product-related ketyl radical anion would not be possible, as the negative charge would be stabilized at the oxygen atom of the ketyl group. Finally, the product leaves the enzyme and the electron recycles from the α-cluster to the β-cluster, facilitating multiple turnovers. Cycling of the electron between α- and β-cluster is necessary to minimize the risk that the radical-generating electron is wasted in unwanted side reactions with small, oxidized molecules abundant under physiological conditions, which could accidentally enter the active site through the wide substrate channel. Recycling tames the radical and allows the dehydratase to catalyze up to 10 000 turnovers before a new activation is required.[9] Loss of the electron and multiple activations should also be avoided, as 2 mol of ATP is needed for each activation and only −145 kJ (equivalent to approximately 1.75 mol of ATP) can be gained from the consumption of 3 mol of leucine.[27] The likely driving force for the electron recycling between the two [4Fe-4S]-clusters are changes in the redox potential of the α-cluster caused by altered cluster environments upon substrate binding and product egress. In this proposed mechanism, two [4Fe-4S] clusters are used to prevent the loss of the radical during catalysis by side reactions. A different way of how a radical enzyme tames highly reactive intermediates during turnover has been demonstrated for the SAM-dependent lysine 2,3-aminomutase,[28] where hydrogen atom transfer between radicals and their reaction partners is enforced by tight van-der-Waals contacts, thus eliminating side reactions of the reactive partners.

Comparison to Phylogenetically Related Enzymes. Systems phylogenetically related to the (*R*)-2-hydroxyacyl-CoA dehydratases are the benzoyl-CoA reductases, which catalyze the ATP-dependent formation of cyclohexadiene carboxyl-CoA in two one-electron steps.[8,29] Biochemical experiments have shown that benzoyl-CoA reductases are composed of four different subunits, two of which are related to the activator and share one [4Fe-4S] cluster, whereas the other two are homologous with the (*R*)-2-hydroxyacyl-CoA dehydratases and contain one [4Fe-4S] cluster each.[29] A sequence alignment of (*R*)-2-hydroxyacyl-CoA dehydratases and benzoyl-CoA reductases (Figure S8) reveals that all cysteine residues coordinating the iron−sulfur clusters in the dehydratase as well as Glu55 in the active site are conserved in the two enzyme families, and we therefore conclude that the crystal structure of the (*R*)-2-hydroxyisocaproyl-CoA dehydratase is a structural model for these reductively radical-generating metalloenzymes.

CONCLUSION

The crystal structure of the radical enzyme (*R*)-2-hydroxyisocaproyl-CoA dehydratase from *C. difficile* reveals a heterodimeric enzyme with two [4Fe-4S] clusters at a distance of 12 Å, allowing facile electron transfer. The α-cluster is located at the active site and has a OH_x group as the fourth ligand in the absence of substrate. Substrate binding replaces the water/hydroxide ion and coordinates the cluster via its carbonyl oxygen of the thioester, positioning the carbonyl group to be reduced to the ketyl-radical anion in close proximity to the unique iron of the cluster. The terminal ligand of the β-cluster is a sulfhydryl/sulfido group, and the cluster probably stores the highly reactive electron in the absence of substrate. The structure-based mechanism explains how the generation and storage of the energetic electron is controlled. Cycling of the electron between the clusters in the presence of substrate prevents loss of the electron by unwanted side reactions and allows high turnover numbers.

EXPERIMENTAL SECTION

Protein Production, Purification, and Crystallization of the Dehydratase (HadBC). All manipulations were performed under strictly anaerobic conditions in anaerobic glove boxes (Coy Laboratories). 2-Hydroxyisocaproyl-CoA dehydratase was obtained by overexpression of its genes in *E. coli* BL21 CodonPlus(DE3)-RIL and purified as previously described.[9] Crystals of the dehydratase were grown at 16 °C by the vapor diffusion technique from a reservoir solution containing 18−29% (w/v) PEG 3350 and 100 mM BisTris (pH 6.5). The dehydratase was cocrystallized by incubation with 5 mM (*R*)-2-hydroxyisocaproate 10 min prior to crystallization. Soaking was accomplished by incubating dehydratase crystals for 30 min in a solution containing 100 mM BisTris (pH 6.5), 20% (w/v) PEG 3350, 25% (v/v) PEG 400, and 3.8 mM (*R*)-2-hydroxyisocaproyl-CoA. (*R*)-2-Hydroxyisocaproyl-CoA was synthesized as previously described.[11]

Data Collection, Structure Determination, and Refinement. Diffraction data were collected at the synchrotron beamline BL14.2 (BESSY, Berlin, Germany). The data were processed and scaled with XDS.[30] In the MAD data set, four [4Fe-4S] clusters were localized by SHELX,[31] and phases were calculated using SHARP. The 16 individual Fe atoms were manually positioned, and phases were recalculated with SHARP.[32] The obtained electron density was of good quality so that a first model could be built using ARP/Warp.[33] Subsequent rounds of model

building and refinement were performed using the programs COOT,[34] CNS,[35] REFMAC5,[36] and PHENIX.[37] Initial phases of the dehydratase with (*R*)-2-hydroxyisocaproyl-CoA or (*R*)-2-hydroxyisocaproate were obtained by Patterson search techniques with a homologous search model using PHASER.[38] The refined model of the dehydratase was used as the search model. Model building and refinement of those structures were performed with COOT,[34] CNS,[35] and PHENIX.[37]

The structure of the dehydratase has been refined to 1.8 Å resolution with *R* and free *R* values of 17.3% and 22.0%, respectively. The bond lengths of the terminal ligands to the unique iron sites have been refined with weak restraints. The occupancies of the water-derived ligand and the unique iron of the α-cluster have been estimated on the basis of the *B*-factors of the other atoms of the α-cluster. The final model of the dehydratase in complex with (*R*)-2-hydroxyisocaproyl-CoA was refined to 2.3 Å resolution and has *R* and free *R* values of 21.1% and 27.3%, respectively. The occupancy of the substrate has been approximated with the help of its own *B*-factors and the ones of the α-cluster. The bond length between the carbonyl oxygen of the thioester and the unique iron of the α-cluster has been refined with weak restraints. The structure of the dehydratase in complex with (*R*)-2-hydroxyisocaproate has been refined to 2.0 Å resolution with *R* and free *R* values of 20.4% and 25.7%, respectively. The proposed change of the coordination of the substrate has been modeled using COOT,[34] and the resulting structure has been energy-minimized by CNS.[35] Superimpositions have been generated by LSQMAN.[39] HOLLOW[40] was used to calculate cavities. All figures showing protein structures have been generated with PyMol.[41] Sequence alignments were calculated with ClustalW[42] and edited with GeneDoc.[43]

■ ASSOCIATED CONTENT

Supporting Information. Complete reference 37, crystallographic statistics, and supporting tables and figures. This material is available free of charge via the Internet at http://pubs.acs.org.

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