The crystal structure of dienoyl-CoA isomerase at 1.5 Å resolution reveals the importance of aspartate and glutamate sidechains for catalysis

Y. Modis, S.A. Filppula[1], D.K Novikov[1], B. Norledge[1], J.K. Hiltunen[1] and R.K. Wierenga[1]

European Molecular Biology Laboratory, Postfach 10.2201, D-69012, Heidelberg, Germany
[1]Department of Biochemistry, University of Oulu, FIN-90570, Oulu, Finland

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The degradation of unsaturated fatty acids is vital to all living organisms. Certain unsaturated fatty acids must be catabolized *via* a pathway auxiliary to the β-oxidation pathway. Dienoyl-coenzyme A (CoA) isomerase cloned from rat liver [1] catalyzes one step of this auxiliary pathway, the isomerization of 3-*trans*,5-*cis*-dienoyl-CoA to 2-*trans*,4-*trans*-dienoyl-CoA [2]. The enzyme is imported into both mitochondria and peroxisomes. Dienoyl-CoA isomerase belongs to a family of over thirty CoA-binding proteins that share the enoyl-CoA hydratase/isomerase sequence motif.

The crystal structure of rat dienoyl-CoA isomerase has been determined by isomorphous replacement at 1.5 Å resolution [3]. The fold closely resembles that of the two other enzymes of this family whose structures have been solved: enoyl-CoA hydratase and 4-chlorobenzoyl-CoA dehalogenase, suggesting a common evolutionary origin for these enzymes. Dienoyl-CoA isomerase forms hexamers. The structure contains a well ordered peroxisomal targeting signal type-1 (PTS1) which is mostly buried in the inter-trimer space. A key feature of these enzymes is the conserved anion hole for binding of the thioester oxygen of the substrate to enhance catalysis. The active site pocket is deeply buried and entirely hydrophobic, with the exception of Asp176, Glu196 and Asp204. Site-directed mutagenesis of Asp204 revealed that this residue is essential for catalysis. A molecule of 3-*trans*,5-*cis*-octadienoyl-CoA was docked into the active site in a molecular modeling simulation.

The structural data, supported by the mutagenesis data, suggest a reaction mechanism where Glu196 acts as a proton acceptor and Asp204 acts as a proton donor (Figure 1). Asp176 is paired with Glu196 and is important for optimizing the catalytic proton transfer properties of Glu196. An oxyanion hole stabilizes the transition state by binding the thioester oxygen, in the predicted mode of substrate binding. The presence of a buried peroxisomal targeting signal suggests that dienoyl-CoA isomerase is prevented from reaching its hexameric quaternary structure in the cytosol [3].

The coordinates and structure factors have been deposited at the Protein Data Bank (PDB), with the accession code 1dci.

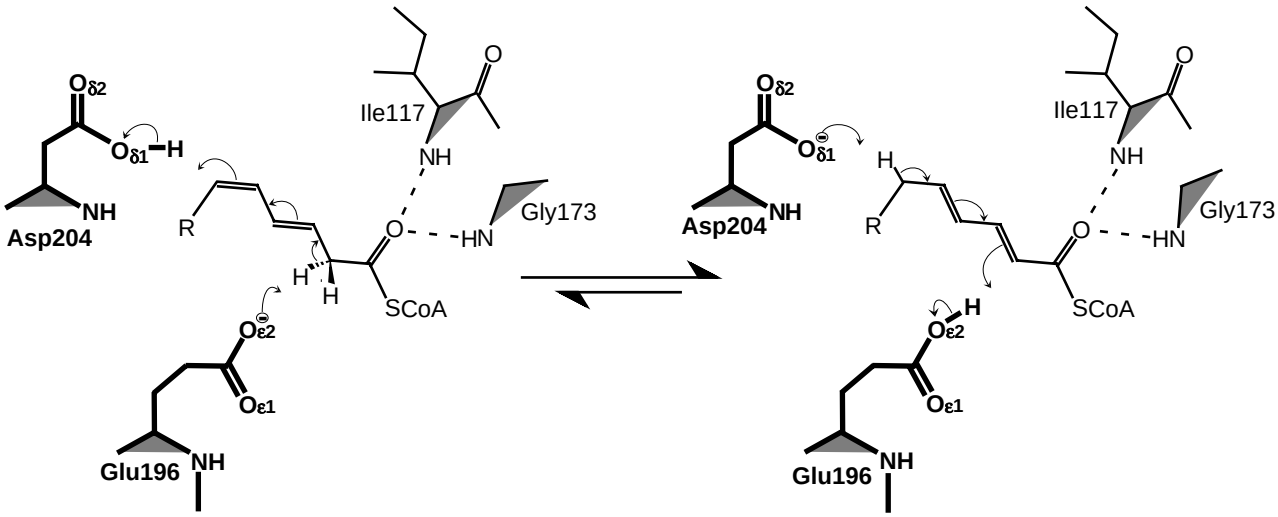

Figure 1 The proposed reaction mechanism for dienoyl-CoA isomerase. The dashed lines indicate hydrogen-bonding interactions of the thioester oxygen atom with the residues forming the oxyanion hole. These interactions are observed in the crystal structure of both enoyl-CoA hydratase and 4-chlorobenzoyl-CoA dehalogenase in complex with their respective substrate analogs. This reaction mechanism implies that Glu196 is unprotonated and Asp204 is protonated in the presence of 3-*trans*,5-*cis*-dienoyl-CoA, and *vice versa* for the reverse reaction. Some small rearrangements in the conformation of the sidchain of Glu196 (with respect to the unliganded structure) may be necessary during catalysis to allow for its proposed protonation states.

References

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