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Sequence analysis and structure prediction of enoyl-CoA hydratase from *Avicennia marina*: Implication of various amino acid residues on substrate–enzyme interactions

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ABSTRACT

Enoyl-CoA hydratase catalyzes the hydration of 2-trans-enoyl-CoA into 3-hydroxyacyl-CoA. The present study focuses on the correlation between the functional and structural aspects of enoyl-CoA hydratase from *Avicennia marina*. We have used bioinformatics tools to construct and analyze 3D homology models of *A. marina* enoyl-CoA hydratase (AMECH) bound to different substrates and inhibitors and studied the residues involved in the ligand–enzyme interaction. Structural information obtained from the models was compared with those of the reported crystal structures. We observed that the overall folds were similar; however, AMECH showed few distinct structural changes which include structural variation in the mobile loop, formation and loss of certain interactions between the active site residues and substrates. Some changes were also observed within specific regions of the enzyme. Glu106 is almost completely conserved in sequences of the isomerases/hydratases including AMECH while Glu86 which is the other catalytic residue in most of the isomerases/hydratases is replaced by Gly and shows no interaction with the substrate. Asp114 is located within 4 Å distance of the catalytic water which makes it a probable candidate for the second catalytic residue in AMECH. Another prominent feature of AMECH is the presence of structurally distinct mobile loop having a completely different coordination with the hydrophobic binding pocket of acyl portion of the substrate.

1. Introduction

In higher plants, fatty acids are stored in large amounts as triacylglycerols that are degraded by β-oxidation and sequential removal of carbon units resulting in the formation of acetyl-CoA (Parani et al., 1999). The peroxisome is the site of numerous important biochemical reactions in plants, including β-oxidation cycle (Reumann et al., 2004). β-Oxidation also plays a significant role during the vegetative and reproductive growth phases, and is involved in plant responses to stress mainly in the synthesis of jasmonic acid (Goepfert and Poirier, 2007). The complete β-oxidation process consists of the cyclic repetition of four basic reactions catalyzed by a dehydrogenase, a hydratase, a second dehydrogenase and a thiolase (Modis et al., 1998). Enoyl-CoA hydratase (ECH) (EC 4.2.1.17) catalyzes one of the reactions in fatty acid metabolism i.e. the hydration of 2-trans-enoyl-CoA into 3-hydroxyacyl-CoA (Engel et al., 1996). This reaction involves addition of a hydroxyl group from an activated water molecule to the β-carbon (C3) and protonation of the α-carbon (Müller-Newen and Stoffel, 1993).

Enoyl-CoA hydratase has also been identified as one of the proteins related to various salt stress responses (Goepfert and Poirier, 2007). Being the first member of crotonase superfamily, it consists of repeated ββα units that assemble into two approximately perpendicular β-sheets surrounded by α-helices (Hamed et al., 2008). This enzyme has broad substrate specificity. It is active with substrates of varying chain lengths (C4–C20) (Kim and Battaile, 2002). Crotonyl-CoA, hexadienoyl-CoA, and β-hydroxyacyl-CoA derivatives containing, 4, 6, 8, 9, and 12 carbon atoms are all potent substrates (Engel et al., 1996).

The structural studies done so far have revealed that two glutamic acid residues act as catalytic acid for providing the α-proton during the hydratase reaction and as the catalytic base for the activation of a water molecule (Muller et al., 1995; Gerlt and Gassman, 1993; Bahnson et al., 2002). However, one of the glutamic acids is not conserved in all isomerases/hydratases suggesting that some other residue may be involved in water activation reaction. The

Abbreviations: ECH, enoyl-CoA hydratase; AMECH, *Avicennia marina* enoyl-CoA hydratase; DAC-CoA, dimethyl amino cinnamoyl-CoA; SPDV, swiss PDB viewer.

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primary conserved feature of the enzyme is a common protein fold that produces an oxyanion hole formed by alanine and glycine as well as side chain atoms of the two glutamic acid residues that stabilize carbanion transition states of the enzyme (Holden et al., 2001; Bell et al., 2002).

In the present study, we have analyzed the primary sequence of *Avicennia marina* enoyl-CoA hydratase (AMECH) and constructed 3D homology models of AMECH bound to different ligands using the known structural coordinates of enoyl-CoA hydratases. *A. marina* (grey mangrove) is a salt tolerant plant. As ECH has been shown to have some role in stress related conditions (Goepfert and Poirier, 2007), we wanted to observe whether there is any significant correlation with the structural aspects of the enzyme as well. The structural information obtained from the models is compared with those of the reported crystal structures. This includes the study of the active **site residues with substrate and inhibitors which play important roles in** catalysis, and mutation predictions at the crucial sites to see if the change in the amino acids resulted in different interactions with the substrates as well as with the neighboring residues.

2. Results and discussion

2.1. Multiple sequence alignment and phylogenetic analysis

Multiple sequence alignment of *A. marina* enoyl-CoA hydratase (AMECH) was performed with the members of enoyl-CoA hydratase/isomerase family whose structures have been solved and deposited in the protein data bank (Supplemental material; Table S1). These sequences are part of family HD2 (abbreviated for hyhydroxyacyl dehydratases that catalyze the forward reaction) as classified in the ThYme (Thioester-active EnzYmes) database at http://www.enzyme.cbirc.iastate.edu (Cantu et al., 2011, 2012). Of the 22 amino acid residues involved in the interaction with ligand atoms, none of them is entirely conserved. Two glutamatic acid residues are conserved in most of the enoyl-CoA hydratases and are involved in the catalytic reaction of hydration (Bell et al., 2002; Bahnson et al., 2002). In AMECH, one of the two catalytic residues is Glu106 which is better conserved in ECH members while the other glutamic acid is replaced by Gly at position 86 (AMECH numbering). The probable candidate for the second catalytic residue, Asp114 is only weakly conserved. Analysis of the multiple sequence alignment shows that the second glutamic acid residue is not conserved in 9 species; out of which 5 possess Asp at their respective equivalent positions. Multiple sequence alignment shows that Gly34, Asp36, Gly78 and Gly84 are completely conserved in ECH sequences while 22 other residues are conserved in more than 70% sequences. Aligned residues were analyzed in the homology models and crystal structures of the templates. It was observed that Glu106 hydrogen bonds with the substrate while Asp114 is located within the hydrogen bonding distance with the catalytic water. Other amino acid residues that show interaction with the ligands are Ala32 and Val36 that form H-bonds with the carbonyl oxygen of hexadienoyl-CoA and Gly34 and Gly83 that form H-bonds with the thiocarbonyl group of DAC-CoA, hexadienoyl-CoA and acetoacetyl-CoA. One of the noticeable differences between AMECH sequence and other members of the ECH family is the presence of Arg28 which is involved in the formation of two ionic interactions with the phosphates of the substrate, hexadienoyl-CoA. Arg28 is very weakly conserved and is replaced by Lys in most of the members of this family. Unrelated amino acids are also present in other sequences at this position.

Multiple sequence alignment of *A. marina* enoyl-CoA hydratase with that of two salt sensitive (*Zea mays* and *Glycine max*) and two

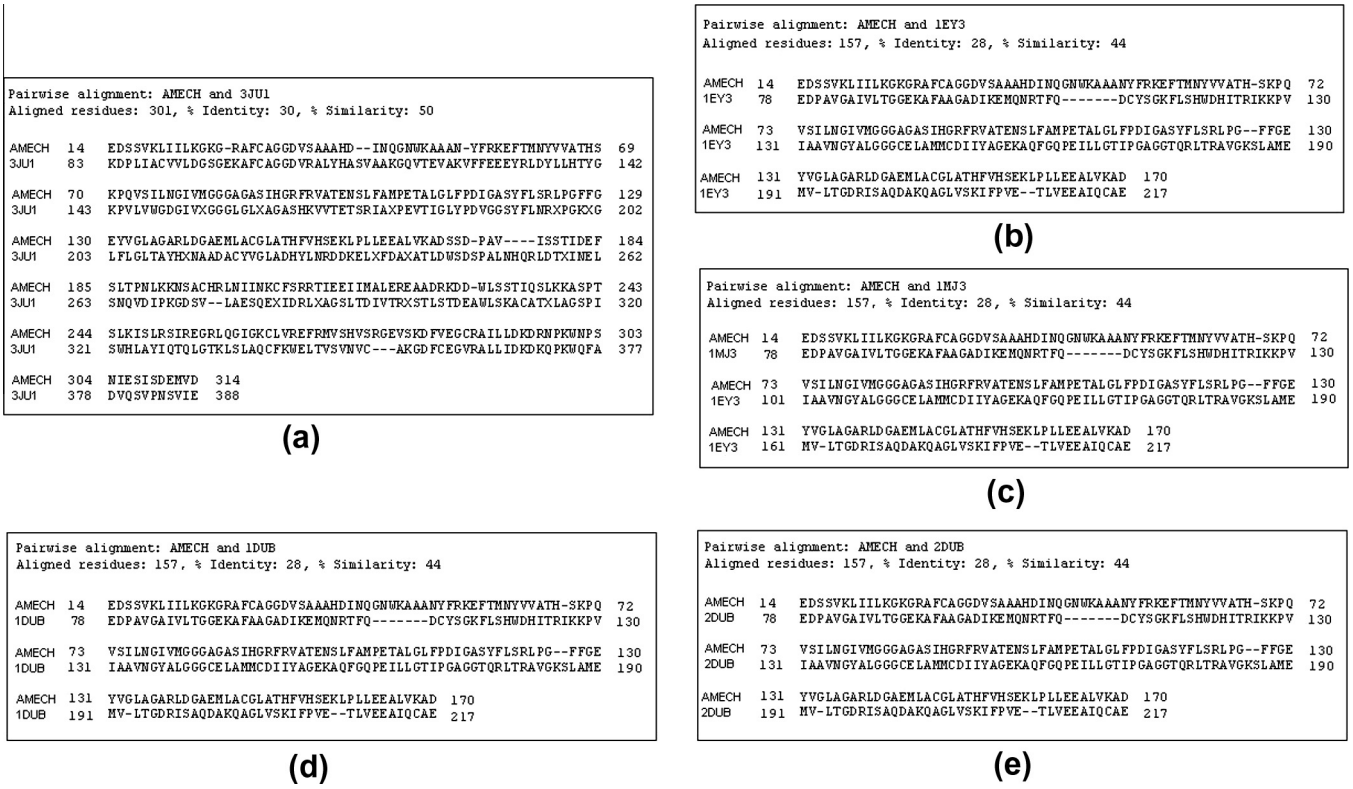

Fig. 1. Pairwise sequence alignment of *Avicennia marina* enoyl-CoA hydratase (AMECH) against protein data bank entries using BLAST server: (a) *Shewanella oneidensis* enoyl-CoA hydratase/isomerase (PDB id: 3JU1) showing 30% identity, while rat enoyl-CoA hydratases bound with (b) dimethylamino cinnamoyl-CoA (DAC-CoA) (PDB id: 1EY3), (c) hexadienoyl-CoA (PDB id: 1MJ3), (d) acetoacetyl-CoA (PDB id: 1DUB) and (e) octanoyl-CoA (PDB id: 2DUB) each showing 28% identities with the target sequence. In case of 3JU1, 301 out of 321 residues of AMECH were aligned while in case of 1EY3, 1MJ3, 1DUB and 2DUB, 157 residues were aligned. The alignments were manually modified where necessary. These sequences and corresponding crystal structures were used for model building of AMECH.