Acetohydroxyacid Synthase and related decarboxylase-carboligases: origins of their substrate specificities

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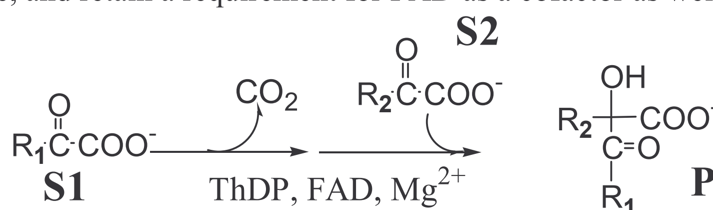
Acetohydroxyacid synthases (AHAS) and glyoxylate carboligase (GCL) are thiamindiphosphate (ThDP) -dependent enzymes which normally catalyze decarboxylation of 2-oxoacids and condensation of the resulting hydroxyalkylThDP anions/enamines with a second oxoacid to form 2-acyl-2-hydroxyacids. These enzymes probably evolved from a pyruvate oxidase, and retain a requirement for FAD as a cofactor as well as for ThDP.



At least three different types of structural and mechanistic factors determine the varied specificities of these enzymes. In AHASs, pyruvate (R_1=methyl) is preferred by >10 fold over any other oxoacid as first substrate (**S_1**) *(2)*. Simple steric hindrance is the major determinant of this specificity, as a mutant AHAS with a strategically-placed Val→Ala mutation (*e.g.*, *E. coli* isozyme II V375A) allows 2-oxobutyrate (R_1=ethyl) to be a good first substrate and can synthesize proprionohydroxybutyrate (**P**, R_1 = R_2 = C_2H_3). An Ile residue in the position in GCL equivalent to the Val375 in AHAS II plays the analogous role in restricting wild-type GCL to glyoxylate as **S_1**.

GCL (R_1 = H) is highly unusual among ThDP-dependent enzymes in that it has no carboxylate residue in H-bonding distance of the aminopyrimidine N1' of ThDP *(3)*. Deprotonation at N2 of the thiazolium is apparently possible because of a very low dielectric constant in the vicinity of the thiazolium, lowering the pK_a for the thiazolium cation ⇒ ylide ionization. Glyoxylate is also more reactive to nucleophilic substitution than a ketoacid. The selective advantage of *loss* of the canonical activating Glu in GCL seems to be related, *inter alia*, to its effect on product release

The specificity of these enzymes for 2-oxoacids as **S_2** is due to an arginine residue which probably interacts with the carboxylate of **S_2** (*e.g.*, Arg276 in AHAS II) *(1)*. Mutants altered at this arginine can utilize aromatic aldehydes as **S_2** and form chiral arylacyl carbinols, of interest as chiral synthons in pharmaceutical syntheses.

NMR measurements of the distribution of ThDP-bound intermediates reveals that faster rates of *release* of **P** when R_2 = ethyl compared to R_2 = methyl plays the major role in product specificity in AHASs *(4)*. The crucial role of a Trp residue (Trp 464 in AHAS II) in determining this specificity may be due to control of a conformational change involved in product release rather than to affinity for 2-ketobutyrate. It is significant that in AHAS I, without the required Trp and with a low specificity for **R_2** = ethyl, the product release step is readily reversible.

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