Structural enzymology of bacterial thiolase: the importance of the Asn316 - His 348 pair for catalysis

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Introduction

Bacterial thiolase of Zoogloea ramigera is the most active thiolase characterized so far. Its ability to catalyze the formation of acetoacetyl-CoA (AcAc-CoA) from two acetyl-CoAs (Ac-CoA) or the degradation of AcAc-CoA is a common step in many biosynthetic pathways, for example the formation of polyhydroxybutyrate (PHB) and degradative pathways (for example fatty acid oxidation), respectively.

Reaction cycle for condensation reaction is illustrated in Figure 1.

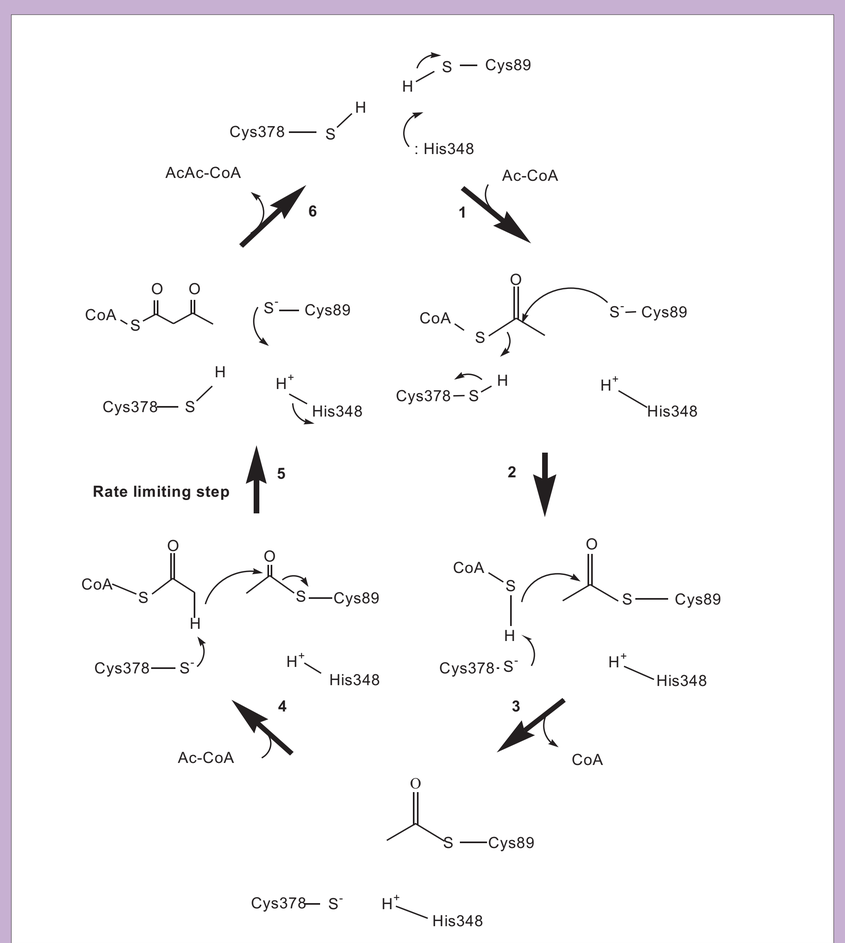

Figure 1. Thiolase reaction cycle. Reaction follows so called ping-pong-mechanism, where one of the reaction products is released before the other is bound. Cysteine 89 gets acetylated and deacetylated during the reaction.

Thiolase active site has conserved water structure, which is thought to play an active role in catalysis. Especially water 82 is important, since it forms the oxyanion hole 1 together with histidine 348. This water molecule is then further hydrogen bonded to asparagine 316 and water 49.

Oxyanion hole 2 is more traditional one, formed by backbone hydrogens of cysteine 89 and glycine 380. It stabilizes the charge in the oxygen of the acetyl group bound to cysteine 89.

Figure 2 shows the trapped enzyme active site geometry suitable for catalyzing the reaction.

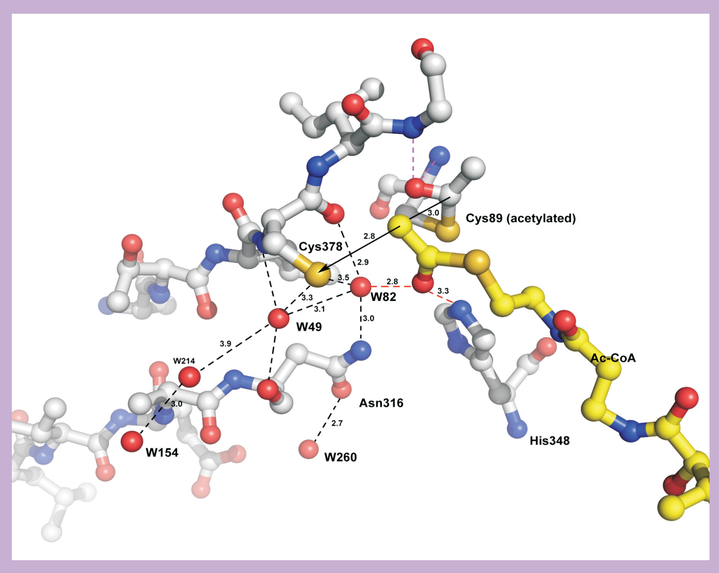

Figure 2. Active site of the bacterial thiolase with acetyl-CoA bound. Cysteine 89 is acetylated. Oxyanion holes 1 and 2 shown in red and magenta dashed line, respectively. Conserved water molecules (W) and hydrogen bonding distances (Å) are drawn in the figure.

Aims

Aims of the project were to characterize the importance of active site waters and the amino acids histidine 348 and asparagine 316 to the successful catalysis and to determine the X-ray crystal structures of mutated variants from bacterial thiolase.

Results & Discussion

We have shown that mutations in the active site have much stronger effect for the synthesizing than for thiolytic reaction (Table I) and the binding is not effected by mutations (data not shown).

The X-ray crystal structures reveal small changes in the active site architecture, but the backbone stays unchanged. The catalytic histidine 348 has rotated somewhat in every structure of asparagine 316 variants compared to the wild type (Figure 3). Such big changes are not seen in histidine 348 variants (Figure 4).

Both active site waters are absent in the H348A variant and water 82 is absent in N316H. It is notable that the totally inactive N316D has smallest structural changes compared to the wild type. This implies that the charges in the active site are more important than waters for catalysis.

Table I: Kinetic parameters for the variants of bacterial thiolase.

	Thiolytic reaction			Condensing reaction
	K_M (uM)	k_{cat} (sec^{-1})	k_{cat}/K_M ($uM^{-1}s^{-1}$)	k_{cat}/K_M ($M^{-1}s^{-1}$)
Wild type	24	813	34	60
N316D	-	-	inactive	inactive
N316A	14	43	3.1	0.1
N316H	12	181	15	0.2
H348A	9	0.6	0.07	0.05
H348N	15	6	0.4	0.2
H348N-N316H	14	20	1.4	2.0

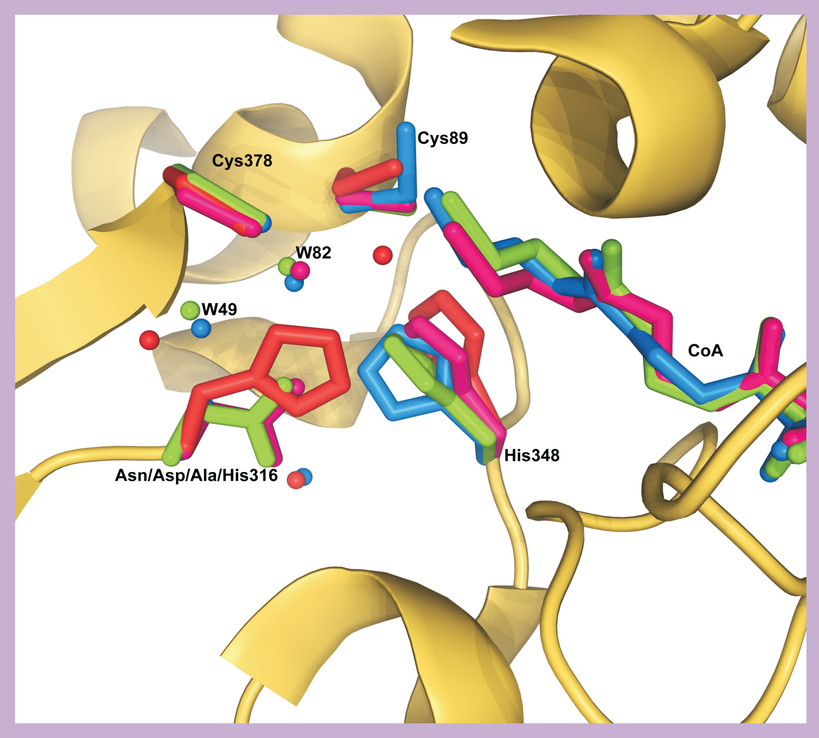

Figure 3. Active sites of wild type (green), N316A (blue), N316D (pink) and N316H (orange) with CoA (except in N316H). Waters and CoA share the same color code. In N316H water 82 is absent, but there are two extra waters. One of them is seen also in N316A in the place of the Asn side chain, the other would probably be pushed away by CoA if bound to the molecule. In the N316A structure cysteine 89 is oxidized.

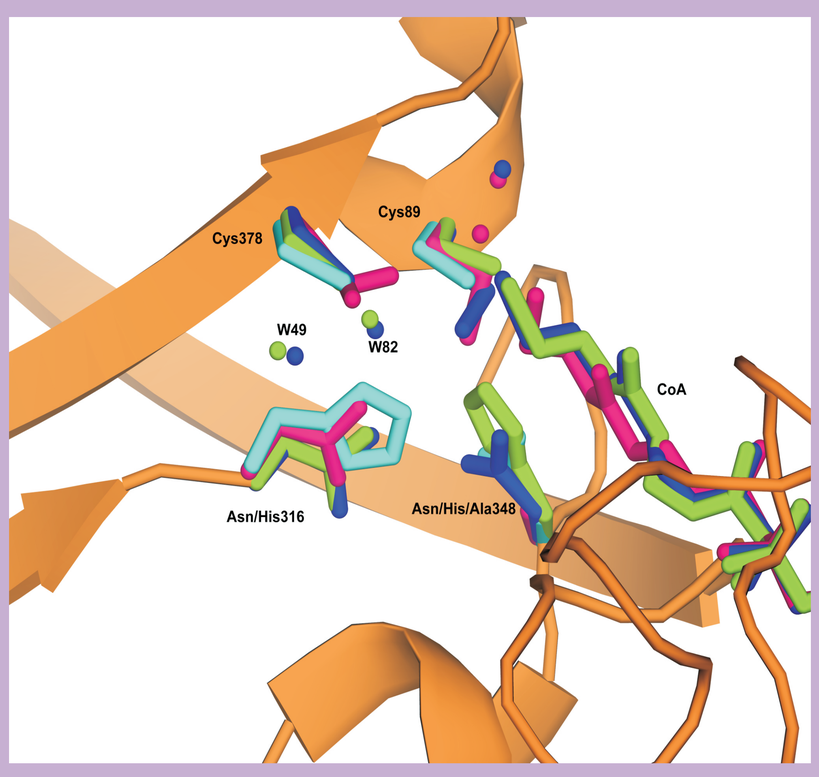

Figure 4. Active sites of wild type (green), H348A (pink), H348N (violet) and H348N-N316H (cyan) with CoA (except in H348N-N316H). Color codes are the same for waters and CoA. Both active site waters are absent in H348A and the mode of binding of CoA is changed in this variant. Resolution of double mutant was too low to see the waters. Cysteine 89 is oxidized in the H348A and H348N structures and cysteine 378 is oxidized in H348A structure.