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REVIEW

The accidental assignment of function in the tautomerase superfamily ☆

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Abstract
Cg10062 from *Corynebacterium glutamicum* is a tautomerase superfamily member with the characteristic $\beta-\alpha-\beta$ fold and catalytic Pro-1. It is a *cis*-3-chloroacrylic acid dehalogenase (*cis*-CaaD) homologue with high sequence similarity (53%) that includes the six critical active site residues (Pro-1, His-28, Arg-70, Arg-73, Tyr-103, and Glu-114). However, Cg10062 is a poor *cis*-CaaD: it has much lower catalytic efficiency and lacks isomer specificity. Two acetylene compounds (propiolate and 2-butynoate) and an allene (2,3-butadienote) were investigated as potential substrates for Cg10062. Cg10062 is a hydratase/decarboxylase using propiolate and *cis*-3-chloro- and 3-bromoacrylates, where malonate semialdehyde is the product of hydration and acetaldehyde is the product of decarboxylation. The two activities occur consecutively using the initial substrate. In contrast, 2-butynoate and 2,3-butadienote only undergo a hydration reaction with Cg10062 to afford acetoacetate. *cis*-CaaD does not function as a hydratase/decarboxylase using any of these substrates, yielding only the products of hydration. Cg10062 proceeds by direct hydration or covalent catalysis (using Pro-1) depending on the substrate. Direct hydration yields the hydration products and covalent catalysis yields the hydration and decarboxylation products. Cg10062 mutants shift the reaction toward one or the other mechanism. The observation that propiolate is the best substrate suggests that Cg10062 could be a hydratase/decarboxylase in a pathway that transforms an unknown acetylene compound to acetaldehyde via propiolate. The bifunctional activity of Cg10062 might also have implications for the evolution of the dehalogenase and decarboxylase activities in the tautomerase superfamily.

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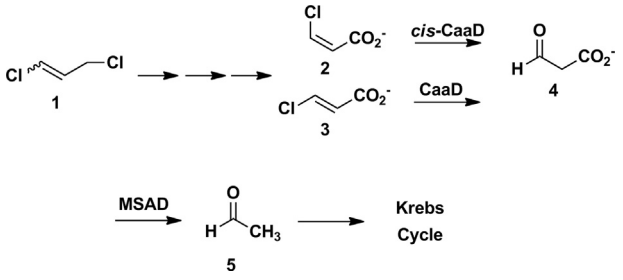
Introduction

cis-3-Chloroacrylic acid dehalogenase (*cis*-CaaD) and Cg10062, a *cis*-CaaD homologue in *Corynebacterium glutamicum*, are two closely related enzymes in the tautomerase superfamily (Poelarends et al., 2004a, 2008a, 2008b). The first one has a known function in a well-established catabolic pathway (Poelarends et al., 2004a). The second one does not have a known function or a genomic context that provides clues about possible functions (Poelarends et al., 2008a). However, our recent studies of *cis*-CaaD and Cg10062 with acetylene and allene substrates suggest a possible function and biological role for Cg10062. Cg10062 might also be representative of a common ancestral enzyme that diverged to give the separate dehalogenase and decarboxylase activities in the tautomerase superfamily (Poelarends et al., 2005; Almrud et al., 2005).

cis-CaaD catalyses the conversion of *cis*-3-chloroacrylic acid (**2**, Scheme 1) to malonate semialdehyde (**4**). The enzyme is a trimer where each monomer consists of 149 amino acids. It does not use coenzymes or metal ions to assist in the reaction. The enzyme is part of a degradative pathway for the nematicide 1,3-dichloropropene (**1**). In three enzyme-catalysed steps, the isomeric mixture of **1** is converted to the *cis*- and *trans*-isomers of 3-chloroacrylate (**2** and **3**, respectively). Isomer-specific dehalogenases (*cis*-CaaD and *trans*-3-chloroacrylic acid dehalogenase or CaaD) convert the respective isomers to **4**. Subsequently, malonate semialdehyde decarboxylase (MSAD) processes **4** to acetaldehyde (**5**), which is then channelled to the Krebs cycle (Poelarends et al., 1998).



Scheme 1

The tautomerase superfamily

One interesting feature of the 1,3-dichloropropene catabolic pathway is that three of the enzymes (*cis*-CaaD, CaaD, and MSAD) are tautomerase superfamily members. The tautomerase superfamily is a group of structurally homologous proteins characterized by a $\beta-\alpha-\beta$ building block and a catalytic amino-terminal proline (Poelarends et al., 2008b). Nature has used this building block to produce diverse structures and activities. Characterization of the individual members provides insight into how nature creates these activities and how divergent evolution proceeds.

The amino-terminal proline in the tautomerase superfamily can function as a general base catalyst or a general acid catalyst, depending on its pK_a value (Poelarends et al., 2008b). In several members, Pro-1 has a low pK_a value ($\sim$6.4) so that it functions as a general base catalyst (Stivers et al., 1996). In other members, the pK_a value is higher ($\sim$9.2) so that Pro-1 exists in the cationic form and functions as a general acid catalyst. In *cis*-CaaD, the pK_a value of Pro-1 is estimated to be 9.2, based on a pH rate profile (Poelarends et al., 2004b). More generally, the pK_a values for Pro-1 in tautomerase superfamily members are determined by direct titration using ^{15}N NMR spectroscopy and uniformly ^{15}N-labelled enzyme (Poelarends et al., 2008a). These experiments have not been carried out with *cis*-CaaD or Cg10062.

Characterization of *cis*-CaaD

Sequence analysis, mutagenesis and kinetic experiments, inhibition studies, and several crystal structures (with and without ligands) identified six key active site residues for *cis*-CaaD activity (Pro-1, His-28, Arg-70, Arg-73, Tyr-103, and Glu-114) (de Jong et al., 2007; Guo et al., 2011). Based on the positions of these residues in crystal structures, a working hypothesis was formulated for the mechanism (Scheme 2). The substrate (*cis*-3-bromo or 3-chloroacrylate) is bound in the active site where three residues (His-28, Arg-70, and Arg-73) interact with the C-1 carboxylate group. These interactions bind and polarize the substrate to create a partial positive charge at C-3. The combination of Glu-114 and Tyr-103 activates a water molecule, which attacks at C-