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Structural basis for carbon dioxide binding by 2-ketopropyl coenzyme M oxidoreductase/carboxylase ☆

Arti S. Pandey [a], David W. Mulder [a], Scott A. Ensign [b], John W. Peters [a,*]

[a] Department of Chemistry and Biochemistry and Astrobiology Biogeocatalysis Research Center, Montana State University, Bozeman, MT 59717, United States
[b] Department of Chemistry and Biochemistry, Utah State University, Logan, UT 84322, United States

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ABSTRACT

The structure of 2-ketopropyl coenzyme M oxidoreductase/carboxylase (2-KPCC) has been determined in a state in which CO_2 is observed providing insights into the mechanism of carboxylation. In the substrate encapsulated state of the enzyme, CO_2 is bound at the base of a narrow hydrophobic substrate access channel. The base of the channel is demarcated by a transition from a hydrophobic to hydrophilic environment where CO_2 is located in position for attack on the carbanion of the ketopropyl group of the substrate to ultimately produce acetoacetate. This binding mode effectively discriminates against H_2O and prevents protonation of the ketopropyl leaving group.

Structured summary:
2-KPCC binds to **2-KPCC** by x-ray crystallography (View interaction)

1. Introduction

Xanthobacter Py2 is one of several microorganisms that can grow on ethylene, propylene or butylene as sole carbon and energy source. During aerobic metabolism of short chain alkenes by *Xanthobacter autotrophicus* Py2, coenzyme M is conjugated to highly reactive, short chain epoxides for subsequent reductive carboxylation to β-keto acids [1–3]. The last step of the pathway is facilitated by 2-ketopropyl coenzyme M oxidoreductase/carboxylase (2-KPCC), a flavin adenine dinucleotide (FAD)-containing nicotinamide adenine dinucleotide phosphate (NADP)-dependent oxidoreductase. 2-KPCC catalyzes reversible carboxylation of ketopropyl-CoM thioether (2-K-S-CoM), yielding acetoacetate and regenerating coenzyme M (2-mercaptoethanesulfonate) (CoM-SH) (Scheme 1) [4].

In contrast to the role of CoM-SH as a C1 carrier in methanogenesis, CoM-SH acts as a C3 carrier throughout multiple steps in the epoxide carboxylation pathway. 2-KPCC is an FAD-containing $NADP^+$-dependent carboxylase that reductively cleaves and carboxylates a thioether substrate.

2-KPCC is a member of the disulfide oxidoreductases (DSOR), a family of enzymes which catalyze reductive cleavage of a disulfide-bound substrate followed by subsequent protonation of leaving groups (for a review, see [5]). At the core of DSOR enzymes there is a highly redox active cysteine pair, separated by 4 residues, at which substrate reduction occurs [6]. The reaction catalyzed by 2-KPCC is similar to that of other DSORs, but is unique in two ways: (i) 2-KPCC cleaves a thioether rather than a disulfide bond, and (ii) 2-KPCC catalyzes reversible carboxylation. The catalytic mechanism of 2-KPCC was originally proposed to involve a base-stabilized enolate intermediate of acetone (Scheme 2) [4].

Subsequent structural characterization of 2-K-S-CoM bound enzyme suggested that the catalytic acid for stabilization of the acetone enolate is a histidine bound H_2O molecule [7]. In contrast with other DSOR mechanisms, which require protonation of the leaving group for productive catalysis, protonation would result in dead-end, irreversible production of acetone, which occurs only when CO_2 is not present (Scheme 3) [4].

In the presence of CO_2, protonation to form acetone is negligible, and nearly all 2-K-S-CoM is converted into acetoacetate and CoM [4].

The reverse of the physiological reaction, decarboxylation of acetoacetate in the presence of CoM-SH and $NADP^+$, also presumably occurs through the formation of an enolate intermediate of acetone and yields 2-K-S-CoM (Scheme 4) [4].

Abbreviations: 2-KPCC, 2-ketopropyl coenzyme M oxidoreductase/carboxylase; FAD, flavin adenine dinucleotide; NADP, nicotinamide adenine dinucleotide phosphate; 2-K-S-CoM, 2-ketopropyl coenzyme M; CoM-SH, coenzyme M (2-mercaptoethanesulfonate); DSOR, disulfide oxidoreductase; RMSD, root mean square deviation; Rubisco, 1,5-bisphosphate carboxylase/oxidase

☆ Coordinates and structure factors have been deposited in the Protein Data Bank under the accession code 3Q6J.

* Corresponding author. Fax: +1 406 994 7212.
E-mail address: john.peters@chemistry.montana.edu (J.W. Peters).

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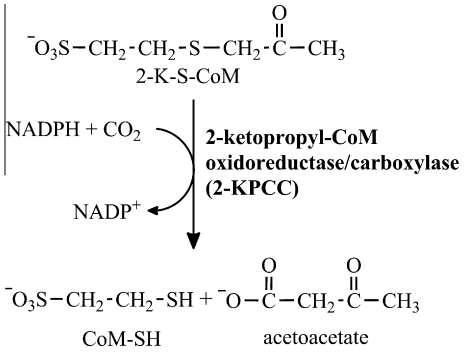
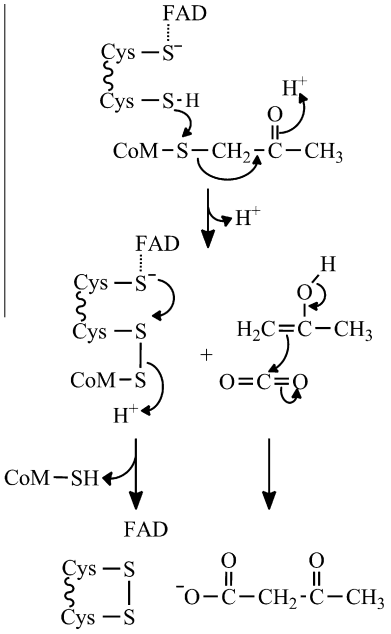
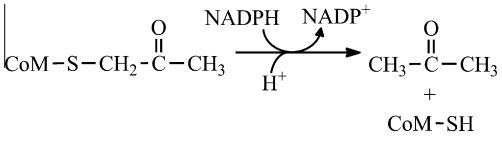


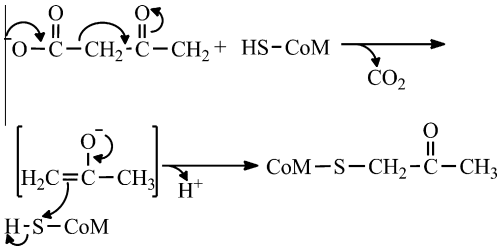
Scheme 1.



Scheme 2.



Scheme 3.



Scheme 4.

Stabilization of a carbanion through charge delocalization with an adjacent carbonyl or imine that has been polarized by coordination to a Lewis acid is widely used in enzyme-catalyzed decarboxylation, isomerization and aldol reactions [8,9]. Other enzymatic carboxylation mechanisms include Schiff-base formation between a lysine and pyridoxal phosphate to provide an electron sink [10,11]. This strategy is not used by 2-KPCC, which has an active site devoid of lysine residues.

By soaking crystals of FAD-containing 2-KPCC with $NADP^+$ and products CoM-SH and acetoacetate, we have enabled the reverse of the physiological reaction resulting in catalytic decarboxylation of acetoacetate, producing the substrates 2-K-S-CoM and CO_2. The structure of this complex represents the first direct visualization of the mode of CO_2 recognition used by 2-KPCC. Comparison of the CO_2 binding site of 2-KPCC with other known enzyme/CO_2 complexes reveals a hydrophobic channel with unique structural features for CO_2 insertion and a mode of CO_2 recognition that aids in discrimination against H_2O at the active site.

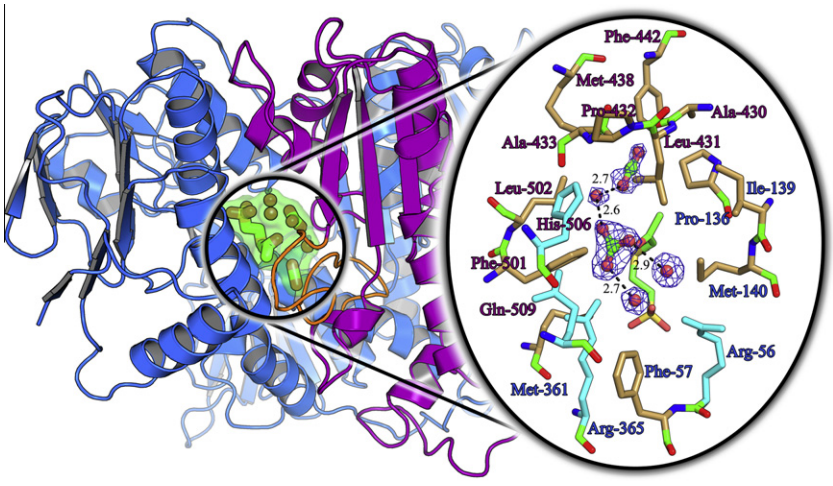


Fig. 1. (A) Cartoon representation of the structure of 2-KPCC observed in a state with bound CO_2. The overall structure is colored according to subunit (subunit 1, blue; subunit 2, purple) and a unique loop region is colored orange. The transparent surface rendering (green) depicts the active site formed at the subunit interface. The zoom region displays the active site area as sticks and depicts CO_2 bound along with bicarbonate, 2-K-S-CoM and selected neighboring residues with side chains colored according to hydrophobicity/hydrophilicity (brown/cyan). The blue mesh represents the $2F_o - F_c$ map for CO_2, bicarbonate and H_2O molecules contoured at 1.3σ. Hydrogen bonds at the active site are shown as dashes with distance in angstroms. The residue label color corresponds to the residues contributed by the two subunits. Atomic coloring scheme for CO_2, bicarbonate, and 2-K-S-CoM: C, green; O, red; S, orange. All figures were generated with PyMOL [28].