

A Structural View into the Complexity of Carbon Dioxide Fixation

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Enzyme catalysis beyond active sites: novel structures of an enoyl-CoA carboxylase/reductase reveal complex intersubunit coupling that is crucial for CO₂ fixation.

In this issue of *ACS Central Science*, DeMirici, Erb, Vöhringer-Martinez, Wakatsuki, and colleagues¹ dive deep into the inter- and intrasubunit communication of the tetrameric enoyl-CoA carboxylase/reductase (ECR) from *Kitasatospora setae*. ECR is one of the most efficient CO₂-fixing enzymes described to date.² Through a combination of structure elucidation (providing not one but *four* high-resolution structures of this challenging system) and molecular simulations, they provide unprecedented insight into how this enzyme can use conformational coupling of its catalytic domains to achieve CO₂ fixation with elusive catalytic proficiency.

CO₂ fixation is arguably the most important reaction of life. It requires both a metabolic energy module and enzymes that activate the thermodynamically stable and thus highly unreactive CO₂ molecule. The Calvin–Benson–Bassham cycle from plants and algae uses ribulose-1,5-bisphosphate carboxylase-oxygenase (Rubisco) as the central carbon fixing unit. This and five microbial pathways are the known natural metabolic routes that account for the entirety of the biomass on this planet. The tremendous progress in the elucidation of the structures of large protein complexes and membrane proteins has fueled improvements in our mechanistic and structural understanding of these pathways and of biological carbon capture mechanisms (CCMs).³ Yet, the success of the biotechnological utilization of natural CO₂ fixation in photoautotrophs and chemoautotrophs lags remarkably behind. This is due to very diverse reasons including the overall very low

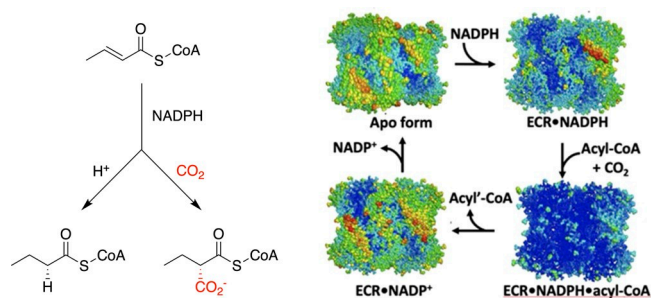
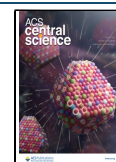


Figure 1. Overview of the reaction catalyzed by the ECR from *K. setae*, as well as the key structural rearrangement during the catalytic cycle. The different ECR complexes along the cycle are color coded according to B-factor (blue = high, red = low). Adapted with permission from ref 1. Copyright 2022 The Authors. Published by American Chemical Society.

growth rates of autotrophs and technical challenges such as difficulties supplying light or gaseous substrates in aqueous systems or the challenge of providing strictly oxygen-free cultivation conditions at a large scale for anaerobic microorganisms. The optimization of CO₂-fixing organisms not only has to cope with the structural complexity of the multiprotein complexes responsible for photosynthesis and carbon capturing systems but also with their highly sophisticated regulation and the involvement of protection systems.⁴ Despite tremendous research effort, it has not been possible to improve the catalytic efficiency of Rubisco itself.

The limitations of natural CO₂-fixation systems and their resilience against optimization has spurred interest in CO₂ enzymes from different metabolic pathways. Some of them are distinguished by much higher reaction rates than

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Kitasatospora setae ECR generates a reactive enolate species by the reduction of an enoyl-CoA substrate with NADPH, which then conducts a nucleophilic attack on a bound CO₂ molecule, challenging the notion that the enolate intermediate must be protected against protonation by the solvent.

Rubisco and could find applications in artificial metabolic pathways.^{2,5} In this context, Wakatsuki and co-workers have achieved remarkable insight into the complex mechanism of an enoyl-CoA carboxylase/reductase (ECR) from *Streptomyces* (Figure 1). *Kitasatospora setae* ECR generates a reactive enolate species by the reduction of an enoyl-CoA substrate with NADPH, which then conducts a nucleophilic attack on a bound CO₂ molecule, challenging the notion that the enolate intermediate must be protected against protonation by the solvent.

The structure of the homotetrameric *K. setae* ECR is highly complex. It is composed of a pair of dimers that take open and closed configurations, undergoing significant conformational rearrangement to achieve a catalytically active state.¹ Insight into the mode of action of ECRs, as well as the chemistry involved, has been limited by a dearth of high-resolution structures of ECRs in complex with catalytic intermediates and carboxylated products. The authors present four high-resolution structures of the *K. setae* ECR, one in the unliganded apo form, and three liganded forms of the enzyme: two binary complexes with reduced NADPH and oxidized NADP⁺, respectively, as well as a ternary complex with NADPH and butyryl-CoA.

Most impressively from a conformational perspective, the all-atom molecular dynamic simulations indicate highly complex allosteric regulation, involving catalytically essential swing and twist motions of peripheral catalytic domains that are coupled both within and across pairs of dimers.

While the elucidation of the structures is itself impressive, the authors then use these structures as a basis for detailed molecular dynamics and quantum mechanics/molecular mechanics (QM/MM) simulations to reveal substantial

conformational transitions that occur during the catalytic cycle. In brief, (1) the unliganded apo form of the enzyme is a symmetric homotetramer, composed of a dimer of dimers, with each subunit being comprised of two domains: a larger catalytic domain and a smaller oligomerization domain. The catalytic domains are at the periphery of the tetramer, and the associated active sites are open and accessible for cofactors and substrates in the apoenzyme. (2) Once NADPH binds, this symmetry is broken, and instead the ECR converts to a pair of asymmetric dimers. In all four subunits, NADPH spans both the catalytic and oligomerization domains, with its adenine moiety bound to the oligomerization domain and its nicotinamide moiety bound to the catalytic domain. (3) The cofactor–substrate complex indicates an additional layer of complexity: while NADPH appears to be bound to all four subunits, only two closed-form subunits show density for the complete thioester, whereas the two open-form subunits represent catalytically incompetent ternary complexes. QM/MM simulations indicate that the substrate is conformationally constrained in the closed-form subunits, and highly dynamic in the open-form subunits, suggesting half-site reactivity. (4) Most impressively from a conformational perspective, the all-atom molecular dynamic simulations indicate highly complex allosteric regulation, involving catalytically essential swing and twist motions of peripheral catalytic domains that are coupled both within and across pairs of dimers. Removing the substrate from the closed-form subunits resulted in rapid conformational changes in just a few tens of nanoseconds of simulation time, causing the closed-form subunits to transition to an open-form conformation, coupled with a twist motion of one subunit to the other in each dimer. In the A/C dimer, which opens its closed subunit first, this twisting motion was shown to rotate the catalytic domain of the now-empty open-form active site in subunit A, which would then be expected to push a bound substrate toward the NADPH cofactor in the C-subunit. Further simulations indicated coupling between the dynamics of each dimer pair in the tetrameric complex (Figure 2), that is regulated by the absence or release of substrate.

Overall, this is a tremendous effort that provides unprecedented structural and biochemical detail into the most proficient known enzyme for CO fixation.

ECR needs to protect the enolate intermediate from protonation by the solvent. From a biochemical perspective, it is

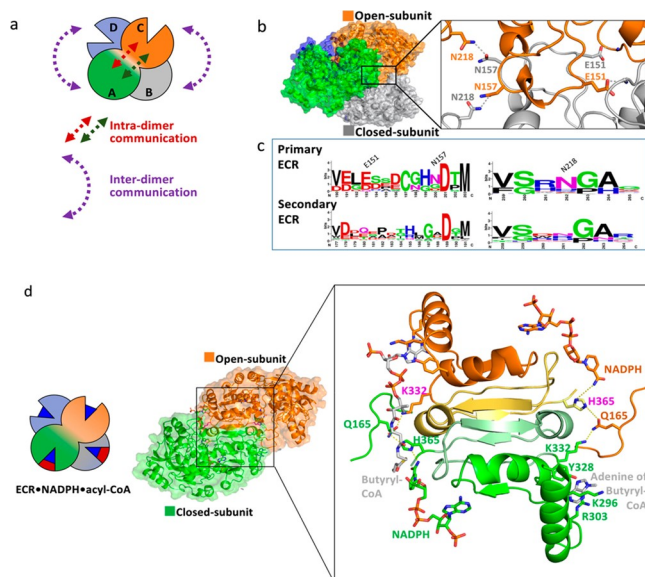


Figure 2. Overview of the communication networks that drive fast CO₂ fixation by this ECR. These are a combination of both (a) inter- and (b) intradimer interactions, facilitated in particular by (b, c) key highly conserved residues in ECRs. Also important are (d) communications between the closed and open states of the ECR. For further details, see ref 1. Reproduced with permission from ref 1. Copyright 2022 the Authors. Published by American Chemical Society.

fascinating that conformational changes leading to a Michaelis complex deeply buried within the protein can be represented by molecular modeling. It is intriguing to see how the modeling also indicates a possible mode of communication of the allosteric changes between the monomers, which is confirmed by the pronounced effect of the substitution of several peripheral residues on the kinetic parameters of the enzyme. Overall, this is a tremendous effort that provides unprecedented structural and biochemical detail into the most proficient known enzyme for CO₂ fixation.¹ A recent computational study captured the time evolution of the allosteric activation of a different enzyme, imidazole glycerol phosphate synthase, on the millisecond time scale.⁶ Wakatsuki and colleagues¹ provide fine details of large-scale conformational changes in a system that only recently would have been considered computationally intractable due to its system size, showcasing just how far (and how fast) the scope of the challenges that simulation can address is progressing.

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