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Structural Basis for Cofactor-Independent Dioxygenation in Vancomycin Biosynthesis

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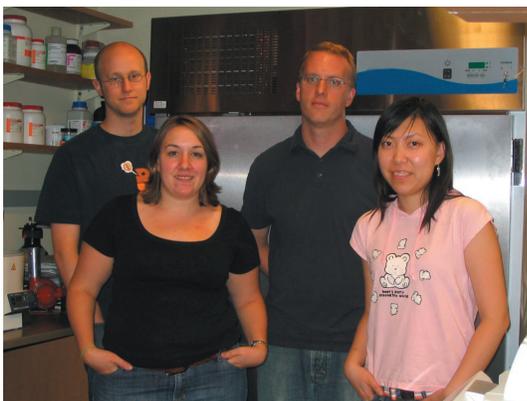
The enzyme DpgC performs a key step in the biosynthesis of the important antibiotic vancomycin. The catalyst performs unique oxidation chemistry without the assistance of any metal or cofactor. Typically, a cofactor is needed to activate molecular oxygen for downstream reactions. We have solved the first structure of DpgC using atomic resolution x-ray diffraction crystallography. The structure provides an exceptional amount of detail regarding the novel DpgC reaction pathway and the general mechanism of enzymatic oxygen activation. In a rare example, molecular oxygen is observed bound to the enzyme in the reactive conformation.

All living organisms exploit the reactivity of molecular oxygen for critical processes such as energy production and the synthesis of cellular components. The direct reaction of molecular oxygen with organic molecules is formally a disallowed, spin-forbidden transformation. The vast majority of characterized oxygenases utilize bound transition metals or flavin cofactors to activate triplet molecular oxygen in order to carry out diverse oxidation chemistry. The vancomycin biosynthetic enzyme DpgC is a unique catalyst that performs a dioxygenation reaction independent of metals or cofactors. The enzyme DpgC catalyzes a key step in the biosynthesis of 3,5-dihydroxyphenylglycine (DPG), a nonproteinogenic amino acid found in the vancomycin family of antibiotics (**Figure 1A**). DpgC is a dioxygenase, incorporating two oxygen atoms from the same molecule of molecular oxygen into the substrate resulting in the four-electron oxidation and the cleavage of the thioester bond (**Figure 1B**). This combination of transformations has no precedent in characterized enzymes.

The x-ray structure of DpgC was solved through single-wavelength anomalous diffraction (SAD) phasing of a selenomethionine derivative to 2.75 Å resolution. The structure of the apo-enzyme contains numerous disordered regions, especially in proximity to the predicted active site. To resolve the problems of disorder in the crystal, a substrate analog was designed and synthesized for use in co-crystallization studies. The co-complex crystallized in the space group P2₁2₁2 to 2.40 Å resolution (**Figure 2A**). The substrate analog is clearly evident in the electron density maps (**Figure**

2B). The substrate analog is positioned properly in an "oxyanion hole" similar in architecture to that previously described for other homologous members of the crotonase superfamily.

The orientation of the substrate suggests molecular oxygen will react with the α -carbon of the substrate. At this predicted position, unassigned electron density consistent in size and shape with diatomic molecular oxygen is evident in the electron density maps. Oxygen is bound in a well-defined, solvent-accessible hydrophobic pocket. Soaking of our co-complex crystals under high pressures of xenon gas failed to displace the observed bound oxygen, suggesting the oxygen is bound with unique specificity. An ordered dioxygen molecule at the observed position is consistent with the unusual chemistry of DpgC. The proposed mechanism first involves formation of a thioester enolate. Molecular oxygen, bound in a hydrophobic pocket, is properly positioned to react with the substrate through a two-step process. The first step is the transfer of an electron



Authors (from left) Paul Widboom, Elisha Fielding, Steven Bruner and Ye Liu

from the substrate to triplet oxygen, forming a conjugated radical cation/superoxide pair. The pair can then collapse in a spin-allowed, bond-forming process to give a peroxide intermediate. This peroxide can proceed to the ob-

served product, DPGX, through a 1,2-dioxetanone.

In summary, the structure of DpgC provides detailed information regarding the specific interaction with an oxygenase and a bound,

isolated oxygen molecule. In addition, the structure reveals novel insights that can be applied to the general mechanism of enzymatic oxygen activation.

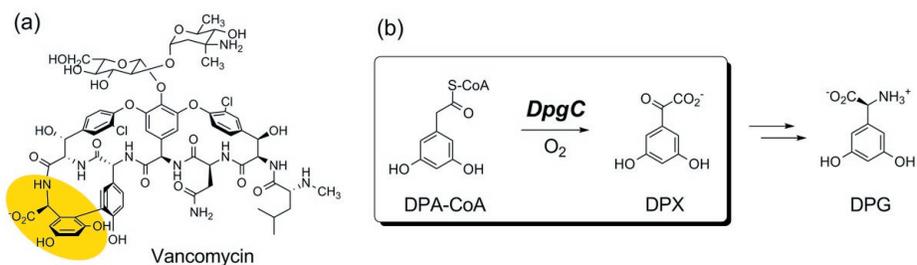


Figure 1. (a) Chemical structure of the antibiotic vancomycin with the amino acid building block DPG (3,5-dihydroxyphenylglycine) highlighted in yellow. (b) The conversion of DPA-CoA to DPGX and coenzyme A (CoA) catalyzed by DpgC.

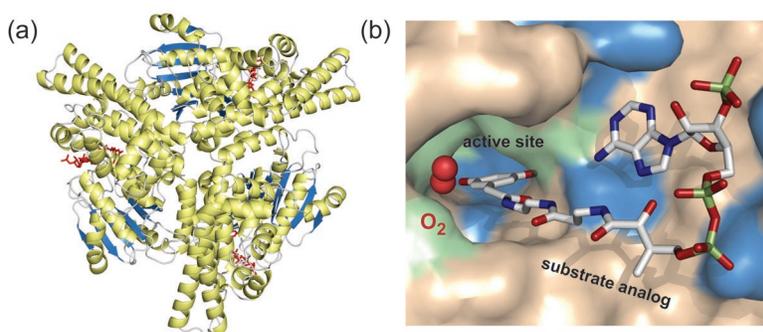


Figure 2. (a) Ribbon diagram of DpgC looking down the 3-fold axis of the protein hexamer, α -helices are colored yellow and β -sheets blue. The bound substrate analog is shown in red. (b) Surface representation of the hydrophobic oxygen-binding pocket of DpgC. The hydrophobic surfaces are colored green and areas directly involved in substrate recognition blue. Molecular oxygen is shown in van der Waals representation (red).