

## THE BUZZ ABOUT A REMARKABLE ENZYME

*Structure of the most proficient enzyme known stirs a flurry of new proposals about how it works*

A. Maureen Rouhi

C&EN Washington

If enzymes could be superstars, orotidine 5'-monophosphate (OMP) decarboxylase certainly would be one.

OMP decarboxylase is the most proficient enzyme known, accelerating the reaction it catalyzes by a factor of  $10^{17}$ . That acceleration means that the reaction occurs about 39 times per second instead of taking 78 million years to be half complete in the absence of the enzyme. The rate of the catalyzed reaction is not unusually fast, but the slowness of the uncatalyzed reaction was recognized only in 1995 by biochemists Anna Radzicka and Richard V. Wolfenden at the University of North Carolina, Chapel Hill.

The enzyme catalyzes the last step in the biosynthesis of uridine monophosphate (UMP), one of the letters in the alphabet of the genetic code. The substrate, OMP, comprises orotate linked to a ribose that is in turn linked to a phosphate. The chemical transformation involves removal of carbon dioxide from the orotate, which has no obvious means of stabilizing the negative charge formed by decarboxylation. And the enzyme works without the aid of cofactors.

For these reasons, OMP decarboxylase has fascinated structural and mechanistic enzymologists alike, as well as computational and organic chemists. Four groups recently have determined the structures of OMP decarboxylases from various organisms. The structures seem to indicate that electrostatic interactions are key to the enzyme's proficiency. And the structures have given rise to new proposals about how the enzyme works.

Chemistry professors [Tadhg P. Begley](#) and [Steven E. Ealick](#) and graduate students Todd C. Appleby and Cynthia L. Kinsland at Cornell University determined the structure of OMP decarboxylase from *Bacillus subtilis* bound to UMP, the product.

Wolfenden and North Carolina graduate student Brian G. Miller, in collaboration with structural chemists Anne M. Hassell, Michael V. Milburn, and Steven A. Short at GlaxoWellcome, Research Triangle Park, N.C., worked on OMP decarboxylase from *Saccharomyces cerevisiae* bound to 6-hydroxyuridine 5'-monophosphate (BMP), a transition-state analog.

## Anatomy of a proficient enzyme



At left, two barrel monomers of orotidine 5'-monophosphate decarboxylase from yeast join at the top of the barrels to form a dimer with two active sites opposite each other, occupied here by the inhibitor 6-hydroxyuridine 5'-monophosphate (BMP). At right, superposition of monomers of free enzyme (green) and enzyme bound to BMP (blue) shows how the protein loops move when binding occurs. Loops for the free enzyme binds, these loops, shown in red, closed around the ligand. [Images courtesy of Steven A. Short]

At the University of Toronto, biochemistry professor [Emil F. Pai](#) and graduate student Ning Wu worked with OMP decarboxylase from *Methanobacterium thermoautotrophicum* bound to 6-azauridine 5'-monophosphate, an analog of the product. They collaborated with Yirong Mo, a postdoctoral researcher, and [Jiali Gao](#), a chemistry professor, at the State University of New York, Buffalo. Gao is now with the University of Minnesota, Minneapolis.

These groups reported their work in the Feb. 29 issue of the *Proceedings of the National Academy of Sciences (USA)* [97, 2005, 2011, and 2017 (2000)].

A fourth group—consisting of [Pernille Harris](#), a postdoctoral researcher; Jens-Christian N. Poulsen, a Ph.D. student; Kaj Frank Jensen, an associate professor of molecular biology; and [Sine Larsen](#), a professor of structural chemistry, at the University of Copenhagen, Denmark—solved the structure of OMP decarboxylase from *Escherichia coli*, also bound to BMP. Their paper has been accepted for publication in *Biochemistry*.

The four groups provide a consistent picture of the enzyme's architecture and active site.



**The Cornell group: Kinsland, Ealick, Appleby, and Begley. [Photo by Matthew Fondeur]**

The basic fold is a so-called TIM (triose phosphate isomerase) barrel, which typically consists of eight

parallel <sup>3</sup>β-strands linked by eight or more α-helices. The active site is near the top of the barrel. Two such barrels form a dimer, with the top of one barrel opposite that of the other. Some residues from each barrel penetrate into the other to form part of the opposite barrel's active site.

The active site contains a unique alignment of charged residues, including two aspartate and two lysine residues near where the orotate's carboxyl group is expected to be. And it has three mobile portions that close around the substrate.

"What's nice about these four structures is that the enzymes are all from different organisms," comments [Dagmar Ringe](#), a professor of biochemistry and chemistry at Brandeis University, Waltham, Mass., and an expert in protein crystallography. "One of the things we always worry about is whether one organism does it differently from another." In this case, the crystal structures are of the enzyme from gram-positive and gram-negative prokaryotes, an archaeobacterium, and a eukaryote. "It's clear that they use the same architecture and the same interactions in the active site. They work the same way," she says.

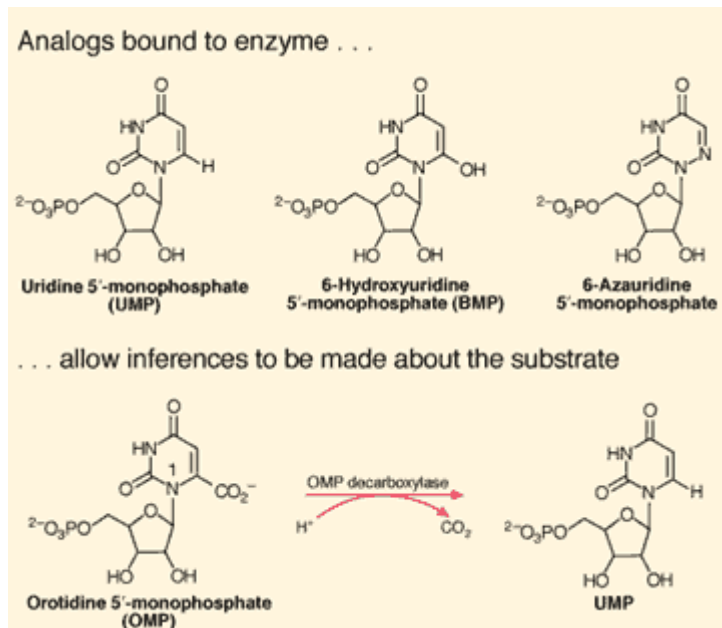
When the enzyme is free by itself in solution, the active site appears to be open, Wolfenden says. But when it binds to a ligand that looks like the substrate, the ligand is "completely engulfed by the protein."



**The North Carolina group: Hassell and Miller. [Glaxo Wellcome Creative Services]**

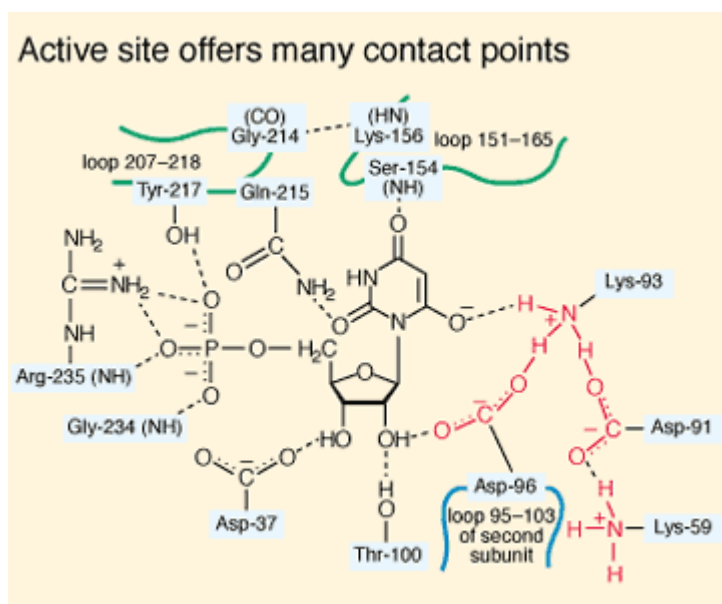
That behavior might seem paradoxical, Wolfenden notes. If the enzyme is stable in an open conformation in solution, then it has to expend energy to distort itself to form the enzyme-substrate complex. But by wrapping around its substrate, the enzyme maximizes its contacts with the substrate, producing a tight binding that, it seems, releases more than enough energy to compensate for the distortion.

In the active site, all four teams conclude, the substrate is anchored by the phosphoribosyl group through extensive hydrogen bonding, and the orotate lies in an awkward position that would be relieved by decarboxylation. A lysine residue previously identified as essential for catalysis is in a position that could allow easy donation of a proton to the pyrimidine ring.



Commenting on the four structures, [Daniel Herschlag](#), an associate professor of biochemistry at Stanford University and an expert on the energetics of enzyme-catalyzed reactions, says that they "present a remarkable testament to the fundamental principles of enzymatic catalysis laid out over the past 50 years—that binding interactions are integral to enzymatic catalysis, with enzymes using these binding interactions to position substrates and catalytic groups and to manipulate their environment to facilitate catalysis."

Since the discovery of the phenomenal rate of acceleration caused by OMP decarboxylase [*Science*, **267**, 90 (1995)], various mechanisms have been proposed for the enzyme-catalyzed reaction, focusing on how the negative charge developed by decarboxylation could be stabilized.



The active site of orotidine 5'-monophosphate decarboxylase from yeast allows many hydrogen bonds to be formed between the enzyme and the inhibitor 6-hydroxyuridine 5'-monophosphate. The array Asp-96

**Lys-59 Asp-91 Lys-93 is close to where the carboxyl group of orotate is expected to be. [Modified from PNAS © 2000]**

"Most enzymatic decarboxylations are of molecules where the electrons can be delocalized, and you can see how that can happen," comments W. [Wallace Cleland](#), a professor of biochemistry at the University of Wisconsin, Madison, and an expert on the use of enzyme kinetics to determine enzyme mechanisms. With the pyrimidine ring, there's no obvious way to stabilize the charge.

Two of the suggested ways to accommodate the charge are formation of a carbene (C&EN, May 12, 1997, page 12) and formation of a zwitterion involving nitrogen in position 1. Both require protonation of orotate at a carbonyl group.

"What we were looking for was to verify one of those mechanisms by finding out which carbonyl oxygen was being protonated," Ealick tells C&EN. "But there was nothing around either oxygen that could serve as a proton donor." Furthermore, recent results of nitrogen-15 isotope effect experiments in Cleland's lab indicate that mechanisms involving changes in the bond order of nitrogen in position 1 also can be ruled out.

What the Cornell, Toronto-New York, and Copenhagen teams found—by modeling the substrate in their crystal structures and predicting where the carboxylate of orotate would be situated—was a carboxyl from an aspartate residue facing the orotate carboxyl. Juxtaposition of two negative charges would create electrostatic repulsion, which would force decarboxylation of orotate, the three teams suggest.

"It is an interesting way to catalyze the reaction," Pai says. "No other enzyme easily comes to mind that works like this. A completely different chemistry is going on."

What seems to be going on, according to the Cornell and Toronto-New York teams, is ground-state destabilization in an enzymatic reaction, a much-discussed idea for which direct experimental evidence has been lacking.

Enzymes are believed to accelerate reactions by stabilizing, or lowering the energy of, the transition state. This stabilization reduces the energy hump—the difference in the ground- and transition-state energies—that must be overcome for the reaction to proceed. But that difference also can be reduced by raising the energy of, or destabilizing, the ground state.

"People have argued that ground-state destabilization couldn't contribute to enzymatic reactions," Cleland says. "Here's a classic case."



### Gao [Photo by Lisa Wilson]

Calculations by Gao show that ground-state destabilization raises the energy of the substrate by 18 kcal per mole, whereas transition-state stabilization lowers the energy barrier by 5 kcal per mole. "There is still transition-state stabilization, but it is a much smaller than ground-state destabilization," he says.

"I accept this charge-repulsion theory," Cleland says. "The calculations by Gao show that there's enough rate acceleration from this destabilization to drive the reaction."

The Toronto-New York team's interpretation of their structure and calculations also points to electrostatic repulsion as a mechanism for removing the product. As the decarboxylation and proton transfer proceed, Gao explains, the hydrogen-bonding relationships shift, leaving two aspartates without ion-pair stabilization. "Another electrostatic repulsion occurs between these two aspartates. And that will induce conformational changes in the enzyme that will unhinge the product," which is still tightly bound through the phosphoribosyl group, he says.



### The Toronto-New York group: Wu (left) and Pal. [Photo by Allan Connor]

The Cornell researchers propose that the reaction proceeds through bimolecular electrophilic substitution ( $S_E2$ ), in which decarboxylation and protonation of the pyrimidine ring by the catalytic lysine residue are concerted. "In the end, you still have to stabilize the carbanion or avoid its formation," Ealick says. Although decarboxylation in such a manner is unprecedented, he says evidence for a similar mechanism exists for certain alkyl metal lyases, which catalyze the cleavage of a bond between an alkyl group and a metal by direct protonation of the bond.

However, data from multiple solvent deuterium and carbon-13 kinetic isotope effects suggest that a protonation occurs before decarboxylation [ [J. Am. Chem. Soc., 121, 6966 \(1999\)](#) ]. "In an enzyme, conformational changes take place; solvation and desolvation of substrate occurs," Ealick says. "There are all sorts of ways that the solvent can play a role that is really complicated and difficult to measure and could result in those effects."

[John A. Gerlt](#), a professor of biochemistry at the University of Illinois, Urbana-Champaign, and an expert on mechanisms of enzyme-catalyzed reactions, finds the case for ground-state destabilization and the  $S_E2$  mechanism persuasive. "I'm convinced enough that I'm already teaching it in class," he tells C&EN. "While no mechanism can be determined by structures alone," he adds, "the mechanism of this reaction will surely be the textbook example of how enzymes use simple structural principles to catalyze reactions whose mechanisms have proven elusive."

Other people think differently.

**Arieh Warshel, a professor of chemistry and biochemistry at the University of Southern California, Los Angeles**, and an expert on modeling of chemical reactions in enzymes and solutions, is not persuaded that ground-state destabilization is taking place in this reaction. "In principle, you can take a charge and put it in a place where it is unhappy, and this will be ground-state destabilization," he tells C&EN. "But that doesn't happen in biology, because biological systems use charged groups like carboxylates and amines that have no problem paying a small price to give up or pick up protons when they are unhappy."

According to Gao's calculations, the electrostatic stress of the carboxylate in the ground state—or the extent of its unhappiness, as **Warshel** puts it—is about 18 kcal per mole. That's the cost of moving the carboxylate group from water to the enzyme. "You know what will happen when you do that?" **Warshel** asks. "It will instantly pick up a proton and become happy. It will become uncharged."

However, Gao points out that the unhappiness in part of the substrate should not be confused with the happiness of the whole. "OMP is quite happy," he explains, releasing 8 kcal per mole upon binding. And he notes that the reaction is fastest at pH 7, at which conditions the carboxyl group is not protonated.



**The Copenhagen group: Jensen, Harris, Larsen, and Poulsen. [Photo by Johann Gotthardt Olsen]**

The Toronto-New York team's analysis also suggests that the energy used to destabilize the ground state comes from the binding of the phosphoribosyl group. That means that the energy released by the binding (binding energy) of that group is about 26 kcal per mole: 18 kcal per mole goes to destabilization, and 8 kcal per mole is manifested as total binding energy.

"There's no precedent for such a large binding energy in biology," Warshel says. He notes that even for the tightest complex known so far, between avidin and biotin, the total binding energy is only about 20 kcal per mole.

Warshel also points out that the paper from the North Carolina team indicates that the binding energy due to the phosphoribosyl group is only approximately 8 kcal per mole for the potent inhibitor BMP. "That hardly leaves anything for destabilization," he says.

But Gao says, "One should not simply expect to observe a binding energy of 26 kcal per mole for the phosphoribosyl group" when it is not connected to the orotate. The nature of its binding when connected to orotate will be different from that when it is disconnected, he argues.

The importance of this connectivity is supported by recent results obtained by North Carolina's Miller.

When the substrate is orotate only, no decarboxylation can be detected, even with radioactive orotic acid, he comments. And when the ligand consists only of the phosphoribosyl group, he adds, the total binding energy is only about 5 kcal per mole, compared with 8 kcal per mole for the whole substrate.

The Copenhagen team interprets the juxtaposition of substrate and enzyme carboxylates differently. "Catalysis should be based on a gain of energy," Larsen says. Putting two charged groups about 2.4 Å apart—which is the team's estimate of the distance between the substrate and enzyme carboxylates—will cost about 36 kcal per mole, she says.

"If you put two carboxylates that close, they are going to catch a proton, because protons are everywhere," Larsen explains. Her team suggests that a proton bridges the two carboxylates through a very short hydrogen bond in the transition state. The energy gained by forming such a hydrogen bond, she notes, is equivalent to the reduction in reaction free energy due to the enzyme, about 24 kcal per mole. The proposal, she adds, is consistent with kinetic isotope effects showing that protonation occurs before decarboxylation.

But it seems inconsistent with the reaction being fastest at pH 7.

Larsen agrees that a free carboxylate group will not tend to accept a proton at pH 7. But being near a negative charge will increase its tendency to do so by several orders of magnitude, she says.

"Given the complexity of the active site, with the two lysines and the two aspartates, there are a lot of possibilities of how these could work," Ringe says. She notes that the structures are based on different ligands, not one of which is the substrate. "Making inferences from the ligand to what the actual substrate is doing is what we do all the time. And very often, these inferences are at least partially correct."

Unraveling this mechanism is not over, Herschlag says. The structure is a milestone, but it should just be "a starting point for understanding a fascinating enzyme in depth, providing a fantastic opportunity to move forward with structure, mechanistic investigations, and calculational approaches hand-in-hand."

"In a general sense," Wolfenden says, the new mechanisms proposed "are similar enough to provide some confidence that their common features are correct in a qualitative sense. Further experiments . . . will be needed to place these speculations on a quantitative footing." ◀

[\[Previous Story\]](#)[\[Next Story\]](#)