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Most Proficient Enzyme Works Traditionally

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The most proficient enzyme known, orotidine 5'-monophosphate (OMP) decarboxylase, owes its effect to transition-state stabilization rather than ground-state destabilization, according to chemistry professor [Arieh Warshel](#) and others at the University of Southern California, Los Angeles.

This enzyme catalyzes the last step in the biosynthesis of uridine monophosphate, one of the letters in the alphabet of the genetic code. The enzyme has gotten much attention since it was recognized that it accelerates that decarboxylation by 10^{17} . One explanation offered for this remarkable effect was the involvement of a carbene intermediate generated in a nonpolar pocket of the enzyme (C&EN, May 12, 1997, page 12). That proposal was refuted earlier this year when the enzyme's structure was solved, revealing an active site full of charges and dipoles ([C&EN, March 13, page 42](#)).

The crystal structure shows two carboxylates juxtaposed: one from the substrate and another from the active site. This juxtaposition led to the proposal that the stress from the repulsion of like charges destabilizes the ground state and is responsible for OMP decarboxylase's action. Calculations of the difference between the energies of the transition state and the ground state in the enzyme and in solution have been especially supportive of this idea [[Proc. Natl. Acad. Sci. \(USA\), 97, 2017 \(2000\)](#)].

Enzymes accelerate reactions by stabilizing the transition state, reducing the energy barrier that must be overcome for the reaction to proceed. This barrier could also be lowered by destabilizing the ground state. Although much discussed, enzyme catalysis by ground-state destabilization has lacked experimental evidence to support it. OMP decarboxylase has seemed a perfect example for this untraditional mode of enzyme action.

But now, Warshel and collaborators make the case that the rate enhancement must be due to transition-state stabilization [[Biochemistry, 39, 14728 \(2000\)](#)].

From the enzyme's crystal structure and computer simulations, the Warshel group concludes that electrostatic stresses indeed account for the enzyme's action. However, the group says, these electrostatic effects are due to protein-protein interactions--that is, repulsions between two carboxylates from two aspartate residues in the enzyme, rather than a protein carboxylate repelling a substrate carboxylate.

In the transition state, the group finds, these repulsive carboxylates on the enzyme stabilize the positive charge on a lysine residue that serves as the proton donor in the reaction. "When the same reaction occurs in water only, the water molecules that stabilize the proton donor in the transition state also repel each other," **Warshel** says. "In solution, this water-water repulsion is part of the activation barrier. In the enzyme, the carboxylates are preorganized to repel each other in the ground state so

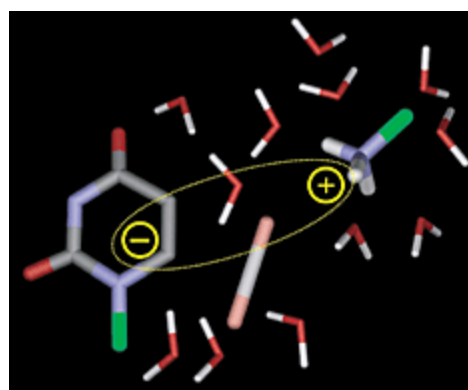
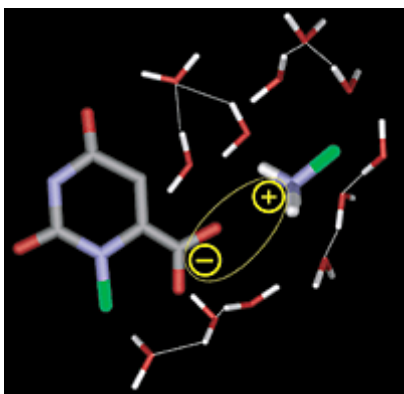
that their repulsion is not part of the reaction barrier. The net result is a very large transition-state stabilization relative to the reaction in water."

Warshel and coworkers suggest that experiments with mutants could help distinguish between the two modes of stabilization. If ground-state destabilization is involved, then some mutants--those lacking the residues with the mutually repulsive carboxylates--should increase the stability of the ground state. If transition-state stabilization is involved, then the same sort of mutants should increase the energy of the transition state. And if both mechanisms are involved, then no net effect will be observed.

Experiments of this type are under way in the lab of chemistry professor Richard V. Wolfenden at the University of North Carolina, Chapel Hill. Wolfenden's group is one of four that solved the crystal structure of OMP decarboxylase earlier this year. It was also in his lab that the amazing proficiency of OMP decarboxylase was discovered in 1995.

Reaction costs much more in water than in enzyme

In water, the ground state of the orotidine monophosphate-lysine system (left) is characterized by extensive hydrogen bonding among water molecules. The negative charge on orotate is stabilized by the positive charge on lysine. In the transition state (right)--when orotate has lost carbon dioxide (pink-white bar) but lysine has not yet transferred a proton--the distance between the opposite charges increases, and water molecules have to stabilize the ion pair. So water molecules that have been happily hydrogen-bonding to each other then have to break those hydrogen bonds, reorient around the charged species, and end up repelling each other. This reorientation costs energy that is not expended when the reaction occurs in the enzyme.



[Courtesy of Jordi Villà]