First experimental visualization of the gaseous product CO_2 in the active site of ODCase supports substrate strain as an integral part of the catalytic mechanism

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Orotidine-5'-monophosphate decarboxylase (ODCase) accelerates the last step of *de novo* pyrimidine biosynthesis by 17 orders of magnitude and is therefore one of the most proficient enzymes known. Numerous structural studies have described in detail key role of conserved electrostatic residue network in catalysis as well as binding of substrate, many substrate analogs and product UMP in the active site. Despite this extensive research exact catalytic mechanism of the enzyme is still debated. Besides widely accepted transition state stabilization in enzymatic catalysis, contribution of steric and electrostatic repulsion leading to substrate destabilization has been proposed for ODCase. One missing piece of experimental data that could impact this hypothesis is position of the second product – carbon dioxide – in the active site that has not yet been observed.

We therefore crystallized ODCase in both the apo-enzyme and UMP-ODCase complex form and then pressurized these crystals with CO_2 to facilitate it's binding to the protein. This allowed us to identify exact position of CO_2 in the ternary product complex which shows carbon dioxide twisted and at $\sim 46°$ to the pyrimidine ring of UMP. This structure represents end state of the decarboxylation reaction and is in very good agreement with previously reported structures of substrate analog complexes and molecular dynamics data. Furthermore, it supports substrate strain as an integral part of the catalytic mechanism of ODCase.