The structural basis of orotidine-5'-phosphate decarboxylase catalysis: Ground-state destabilisation by electrostatic repulsion is not a driving force

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Enzymes are nature's catalysts as they speed up the rate of virtually every biochemical reaction in the cell by up to 20 orders of magnitude. These enormous rate enhancements have been attributed to both tighter binding of the transition state compared to the substrate ground-state as well as ground-state destabilisation of the substrate. For the latter paradigm, it is assumed that favourable binding of non-reacting parts of the substrate is used to steer the reactive substrate portion into an environment where it experiences "stress" through e.g. electrostatic repulsion. The enzyme orotidine-5'-monophosphate decarboxylase (OMPDCase) serves as the textbook example in this context by virtue of a negatively charged amino acid at the active site that is thought to facilitate catalysis by exerting "electrostatic stress" on the substrate carboxylate leaving group. Despite decades of research, structural snapshots of how the substrate binds to the active site and interacts with the negative charge have remained elusive. I will present crystallographic snapshots of human OMPDCase in complex with the genuine substrate, substrate analogs, transition state analogs and product - all at true atomic resolution - that defy the proposed ground-state destabilisation by revealing that the substrate carboxylate is protonated and forms a favourable low-barrier hydrogen bond with the negatively charged amino acid. The catalytic prowess of OMPDCase seems to almost entirely result from transition-state stabilisation by favourable electrostatic interactions of the enzyme with charges spread over the substrate and "enforced catalysis" in a pre-association mechanism, in which the evolving carbanion becomes protonated in the transition-state of decarboxylation avoiding the internal return of CO2 of an otherwise reversible decarboxylation. These findings not only shed light on fundamental principles of enzyme catalysis but also necessitate a revised strategy for the design of enzymatic and synthetic carboxylase catalysts that act on green house gas carbon dioxide in the quest to transform it into non-gaseous states. Rather than excluding negatively charged moieties to avoid destabilisation of the formed carboxylate products, these are required for differential binding of reactant and transition state and should be vital elements of carbon dioxide fixating catalysts with OMPDCase serving as one of nature's blueprints.