

P005 How to interchange decarboxylase and oxidase activities with a single amino acid substitution

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We had previously discovered that oxalate decarboxylases and oxalate oxidases have many common features: a manganese ion with a common ligand set, the substrate oxalate and dioxygen as either a unique cofactor or a substrate. This led to the hypothesis that these enzymes share common catalytic steps that diverge when a carboxylate radical intermediate becomes protonated or not. The *Bacillus subtilis* decarboxylase has two manganese binding sites and we proposed that Glu162 on a flexible lid is the crucial site 1 general acid. We now demonstrate that a decarboxylase can be interchanged with an oxidase by mutating one or more amino acids of the lid that must include Glu162. This work provides compelling evidence for the crucial role of Glu162 in the decarboxylase reaction consistent with it being the general acid, for the role of the lid in controlling the K_m for dioxygen and for site 1 being the sole catalytically active site. This is a rare example of how one enzyme activity could evolve into another with as little as a single nucleotide base-change, bypassing the need for a promiscuous evolutionary intermediate enzyme.