Identification of Novel Catalytic Strategies in the Enolase Superfamily

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The mechanistically diverse enolase superfamily has evolved a conserved strategy for the stabilization of enolate anions during catalyzed reactions. All enolase superfamily members initiate catalysis by the base-assisted abstraction of an α-proton adjacent to a carboxylate to form an enolate anion which is stabilized by a divalent metal ion, hydrogen bonding and electrostatic redistribution. From this intermediate, β-elimination of hydroxide or ammonia, 1,1-proton transfer (racemization), and cycloisomerization reactions have been observed in this superfamily. Enolase superfamily members proceed by general acid/base catalysis with the catalytic residues located in a TIM-barrel catalytic domain. In our attempts to identify catalytic diversity within the enolase superfamily, we have identified two enzymes which take unique routes to catalyze similar reactions. D-mannonate dehydratase (ManD) and L-rhamnonate dehydratase (RhamD) both dehydrate acid-sugars using a general acid-base mechanism but each have evolved unique catalytic residues to perform this reaction. ManD is a highly specific enzyme, dehydrating only its substrate D-mannonate whereas RhamD is a promiscuous enzyme with the ability to dehydrate substrates with stereochemical similarity to L-rhamnonate. These enzymes demonstrate that even within a mechanistically diverse superfamily with shared structural and mechanistic features, multiple avenues can evolve to afford a similar end result.