

## Mechanistic Characterization of the RuBisCO Superfamily: Stereochemical Studies of the Methionine Salvage Pathway “Enolase”

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DK-MTP-1-P “enolase” carries out the enolization of an intermediate, 2,3-diketo-5-methylthio-pentose-1-P (DK-MTP-1-P), in the methionine salvage pathway of *Bacilli* species. To date, this is the only function elucidated in the newly identified form IV group of the RuBisCO superfamily. We have characterized the reaction mechanism to better understand the RuBisCO active site architecture in the context of non-RuBisCO reactions. Stereochemistry of enolization has been analyzed with stereospecifically deuterated substrates and substrate analogs. From these experiments, along with crystallographic and mutagenic studies, we have determined that the pro-*S* proton is abstracted by lysine 98. In authentic RuBisCOs this position is occupied by a conserved asparagine that interacts with the reaction intermediate. From this we conclude that while the fundamental RuBisCO active site architecture is utilized in other superfamily members, there is no strict conservation in position or identity of catalytic residues.

