Mechanistic Studies of Novel Biosynthetic Enzymes

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Terpenoids are a family of secondary metabolites that are widely distributed in nature and are rich in biological activities. The terpenoid building block is a 5-carbon unit known as isoprene, which has long been established to be derived from acetate via the mevalonate pathway. However, a new isoprene biosynthetic pathway has recently been discovered where the isoprenoid unit is formed from pyruvate and glyceraldehyde-3-phosphate. It has been shown that 2-*C*-1-deoxy-D-xylulose-5-phosphate (DXP) is the immediate product resulting from condensation of these two precursors, and conversion of DXP to methyl-D-erythritol 4-phosphate (MEP), catalyzed by the NADPH-dependent DXP reductoisomerase (DXR), is the first pathway-specific transformation en route to the basic isoprenoid unit. Two possible mechanisms involving either an α-ketol rearrangement or a retroaldolization/aldolization rearrangement have been proposed for DXR-catalyzed reaction. Experiments to distinguish these mechanistic possibilities will be presented.

The O_2-activating, non-heme iron enzymes catalyze a wide range of oxygenation and oxidation reactions with important biological implications, such as DNA repair, hypoxic response, collagen biosynthesis, and histone demethylation. Most of these enzymes contain a single iron center coordinated by two His and one Asp/Glu residues in a tridentate binding motif referred to as "2-His-1-carboxylate facial triad". Understanding the O_2-activation process for these enzymes may provide key insights into the source of their divergent substrate specificity despite similarly coordinated active site metal centers. To study the mechanism of (*S*)-2-hydroxypropylphosphonic acid (HPP) epoxidase, which catalyzes the final step in the biosynthesis of fosfomycin, we have determined the competitive $^{18}O/^{16}O$ kinetic isotope effects (^{18}O KIEs) on $k_{cat}/K_m(O_2)$ of this enzyme in order to probe the early steps of O_2 activation and to reveal the nature of the metal/O_2 intermediate formed in the rate determining step (RDS) of $k_{cat}/K_m(O_2)$. The results of these experiments along with studies using radical probes to gain insight into the mechanism of this enzyme will be presented.