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The active site and substrate binding mode of 1-aminocyclopropane-1-carboxylate oxidase of Fuji apple (*Malus domesticus L.*) determined by site directed mutagenesis and comparative modeling studies

Ahrim Yoo[1], Young Sam Seo[2], Soon-Kee Sung[3], Dae Ryook Yang[1],
Woo TaeK Kim [2] and Weontae Lee[4]

[1]Department of Chemical and Biological Engineering, Korea University, Seoul
[2]Department of Biology, College of Science, Yonsei University, Seoul
[3]Plant Biotechnology Team, Dongbu Advanced Research Institute, Daejeon
[4]Department of Biochemistry, College of Science, Yonsei University, Seoul

Active sites and substrate bindings of 1-aminoxyclopropane-1-carboxylate oxidase (MD-ACO1) catalyzing the oxidative conversion of ACC to ethylene have been determined based on site-directed mutagenesis and comparative modeling methods. Molecular modeling based on the crystal structure of Isopenicillin N synthase (IPNS) provided MD-ACO1 structure. MD-ACO1 protein folds into a compact jelly roll shape, consisting of 9 α-helices, 10 β-strands and several long loops. The MD-ACO1/ACC/Fe(II)/Ascorbate complex conformation was determined from automated docking program, AUTODOCK. The MD-ACO1/FeII complex model was consistent with well known binding motif information (HIS177-ASP179-HIS234). The cosubstrate, ascorbate is placed between iron binding pocket and Arg244 of MD-ACO1 enzyme, supporting the critical role of Arg244 for generating reaction product. These findings are strongly supported by previous biochemical data as well as site-directed mutagenesis data. The structure of enzyme/substrate suggests the structural mechanism for the biochemical role as well as substrate specificity of MD-ACO1 enzyme.