MS5-P5 Structure function studies of a catechol oxidase from *Aspergillus oryzae*

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Catechol oxidase (EC. 1.10.3.1) belongs to a family of coupled binuclear copper oxidoreductases together with tyrosinase (1.14.18.1) and *o*-aminophenol oxidase (1.10.3.4). Catechol oxidase oxidizes *p*-substituted *o*-diphenols to corresponding *o*-quinones. Tyrosinase has an additional monophenolase activity *i.e.* it can catalyze the *o*-hydroxylation of p-substituted monophenols to *o*-diphenols, which then subsequently oxidizes to corresponding *o*-quinones. *o*-Aminophenol oxidases catalyze the mono-oxygenation of p-substituted o-aminophenols into the corresponding o-nitrosophenols. Several crystal structures of tyrosinases have been solved, but catechol oxidases are much less studied.

The first crystal structure of a fungal catechol oxidase from *Aspergillus oryzae* (AoCO4) was recently solved [1]. In addition, a crystal structure of a plant catechol oxidase from *Ipomoea batatas* is known. Now we want to determine the complex structures of AoCO4 to understand the structural determinants for different substrate specificities among coupled binuclear copper enzymes and to elucidate the reaction mechanisms. At the moment, we are optimizing the recombinant overexpression of AoCO4.

[1] Hakulinen, N., Gasparetti, C., Kaljunen, H., Kruus, K., Rouvinen, J., 2013 *J.Biol.Inorg.Chem.*, 18, 917-929

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MS5-P6 Structural insight into the phytoene desaturase of rice

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Vitamin A deficiency is a major health problem in developing countries resulting in blindness in children, increased risk of infection or death that has to be tackled via drugs or dietary supplementation. The biosynthesis of β-carotene, one of its precursors, has been extensively researched. So far a structural insight into involved proteins lacked however. In plants and cyanobacteria two desaturases, two *cis-trans* isomerases and a cyclase are involved in the biosynthesis of β-carotene starting with the isoprenoid 15-*cis*-phytoene.[1] The first enzyme in this biochemical pathway is the FAD containing phytoene desaturase (PDS) that is located at the stromal side of plastid membranes and catalyzes the insertion of two *trans* double bonds in 15-*cis*-phytoene. We have successfully crystallized PDS of *Oryza sativa* in complex with its herbicidal inhibitor norflurazon. The structure was solved using the weak anomalous signal of the soaked mercuric compound thiomersal and refined to a resolution of 2.77 Å. PDS shows a kinked arrangement of two globular domains linked by a single FAD moiety. A highly hydrophobic channel leads the substrate to the putative quinone binding site that is blocked by norflurazon in our model thus showing competitive binding of substrate and electron acceptor.

[1] Beyer P, Al-Babili S, Ye X, Lucca P, Schaub P, Welsch R, et al. Golden Rice: introducing the beta-carotene biosynthesis pathway into rice endosperm by genetic engineering to defeat vitamin A deficiency. J Nutr. 2002;132:506S-10S.

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