Synopsis of Original Research Paper

Structual Aproach to Molecular Mechanism of Tyrosinase

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Tyrosinase is a copper enzyme widely distributed in nature and has anti-ferromagnetically coupled binuclear copper ions in the active site, where the dioxygen molecule can be reductively activated into [(μ-η^2:η^2-peroxide)dicopper(II)] species. This active oxygen species play a central role in catalyzing the hydroxylation of phenols to catechols and the subsequent oxidation of catechols to o-quinones. However, the details of the binding mode of substrate tyrosine have been still a long-standing mystery to be resolved. We have reported the high-resolution crystal structures of recombinant pro-form tyrosinase from yellow mold. In the pro-form tyrosinase, the active site is covered by *C*-terminal shielding domain, which functions as copper chaperone and can be proteolytically cleaved off to induce the catalytic activity. In this work, we have determined the crystal structure of the active-form tyrosinase in order to compare the structures between the pro- and active-form tyrosinase. First, the active-form tyrosinase was prepared by trypsin treatment of pro-form tyrosinase and purified by gel filtration chromatography. The purified protein was crystallized by hanging-drop vapor diffusion method using the precipitant solution containing polyethylene glycol. X-ray diffraction data was collected at Spring-8 and determined the crystal structure of the active-form tyrosinase. There was little difference in overall structures of pro- and active-form tyrosinase. We have succeeded in determining the crystal structures of complex with inhibiters. Kojic acid was stacked on histidine coordinated to copper ion. Based on the details of their binding modes, the reaction mechanism will be updated and discussed.