Crystal Structure of *m*-hydroxybenzoate Hydroxylase from *Comamonas Testosteroni*

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m-Hydroxybenzoate hydroxylase (MHBH) from *C. testosteroni* is a flavin-containing monooxygenase of the glutathione reductase family. It catalyzes the conversion of *m*-hydroxybenzoate derived from lignin to 3,4-dihydroxybenzoate with requirements of NADPH and molecular oxygen. To establish a structural basis for characterizing its substrate specificity, the crystal structure of MHBH in complex with its substrate was determined to 1.8 Å resolution. The active-site architecture, including the positions of FAD- and substrate-binding sites, is similar to those of other members of the family, suggesting that flavoprotein aromatic hydroxylases share a similar catalytic mechanism for the hydroxylation of their respective phenolic substrates. Structural comparison of MHBH with the homologous enzymes, however, shows some structural differences in the substrate-transport and the recognition mechanisms. In particular, the presence of a large channel between the catalytic domains indicates a unique pathway for substrate transport. The size of the entrance and the characteristic stratified environment of the channel interior would enable the enzyme to select *m*-hydroxybenzoate on the basis of its molecular size and charge distribution. In addition, the Xe-derivative structure at 2.5 Å resolution led to identification of a putative oxygen-binding site adjacent to the substrate-binding pocket.

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