Insights into the enantiospecificities of (*R*)- and (*S*)-dichlorprop/α-ketoglutarate dioxygenases

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(*R*)- and (*S*)-dichlorprop dioxygenases (RdpA and SdpA) from *Sphingomonas herbicidovorans* MH are mononuclear Fe(II)-containing α-ketoglutarate (αKG)-dependent dioxygenases that hydroxylate the side chains of their chiral substrates mecoprop [(R,S)-2-methyl-4-chlorophenoxypropanoic acid] and dichlorprop [(R,S)-2,4-dichlorophenoxypropanoic acid] to form the achiral 2-methyl-4-chlorophenol and 2,4-dichlorophenol, respectively. The substrate hydroxylation is coupled to the oxidative decarboxylation of αKG to form succinate. RdpA and SdpA share 30% amino acid identity with each other and both enzymes are strictly enantiospecific. In order to study the basis of enantiospecificity, the enzyme structures were modeled using the crystal structure of taurine/αKG dioxygenase as a template. According to the models and sequence alignments with other αKG dependent dioxygenases, the Fe(II)-binding and possible αKG-binding residues were predicted. Furthermore, several residues possibly involved in substrate binding were identified: V80, Q93, I106, G107, Y221, and R285 in RdpA, and E69, G97, N98, H208, H272, and R274 in SdpA. Site-directed mutagenesis was carried out and the kinetic parameters of the mutants were determined to assess the roles of these residues in substrate binding and enantiospecificity. Activity was abolished in the Y221A and I106G/G107N mutants of RdpA, whereas all other RdpA and SdpA mutants showed activity in the range of 0.1-65% with the corresponding mecoprop enantiomer compared to the wild type enzymes. For the V80A, Q93A, and R285A mutants of RdpA and the E69A, H272A, and G97N/N98G mutants of SdpA, the apparent K_m-values were at least ten times higher than the value of the wild type enzyme. Two SdpA mutants, E69A and G97I/N98G, showed slight activity with the (*R*)-mecoprop. These results indicate that enantiospecificity of RdpA and SdpA is due to several amino acid residues which are located at different positions of the active site cavity.