Dioxygen Activation by Putidamonooxin - The Influence of Substrate Analogues on the Fate of the Active Oxygen Species

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The 4-methoxybenzoate monooxygenase from P. putida investigated here is an enzyme system consisting of two conjugated iron-sulfur proteins i.e. the oligomeric oxygenase (EPR at g = 1.88) named putidamonooxin (PMO) which needs iron ions as cofactor (EPR at g = 4.29) for its enzymatic activity and the NADH-PMO oxidoreductase (EPR at $g \geq 1.95$) with FMN as an additional prosthetic group (1,2). From kinetic, Mössbauer and EPR studies it was concluded that the reduced cofactor-iron of PMO functions as the dioxygen binding site. Dioxygen activation then is achieved by the uptake of one electron from a reduced 2Fe-2S centre of PMO by the enzyme-bound iron-oxycomplex $(FeO_2)^{2+}$ yielding the iron-peroxocomplex $(FeO_2)^{+}$. It has been suggested from former findings that $(FeO_2)^{+}$ initiates the oxygenation of the substrate or forms H_2O_2 when inactivated by protonation under uncoupling conditions (3). To confirm this conclusion we investigated the influence of uncoupling, partial uncoupling and tight coupling substrates on the fate of the active oxygen species. Measuring the stoichiometry for NADH-oxidation, O_2-uptake, H_2O_2-formation and the formation of hydroxylated products at pH 8.0 using 4-hydroxybenzoate (4-HB), 4-aminobenzoate (4-AB) and 4-methoxybenzoate (4-MOB) as substrates the following ratios of activities were determined:

	4-HB	4-AB	4-MOB
NADH-oxidation	1	2.44	2.79
O_2-uptake	1	2.31	2.54
H_2O_2-formation	1	1.76	0
hydroxylated product/NADH-oxidation	0.48	0.055	1

From these findings in conjuction with the solvent isotope effect on the electron flow which is found only with 4-trifluoromethylbenzoate as uncoupling substrate (3) the following conclusions are drawn: (i) The rate limiting step in the overall hydroxylation reaction catalyzed by the 4-methoxybenzoate monooxygenase is the attack of a CH-bond by the active oxygen species or its inactivation by protonation under formation of H_2O_2. (ii) By uncoupling or partial uncoupling substrate analogues the life time of the ternary complex "enzyme•iron-peroxocomplex•substrate" is normally prolonged except that with these substrate analogues (i.e. 4-AB) the inactivation of the activeoxygen species by protonation is mediated.

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