Section: Molecular Biophysics

Mössbauer and Kinetic Studies on Putidamonooxin (PMO)

W. Adrian[+], E. Bill[++], F.H. Bernhardt[+++] and A. Trautwein[++]

[+]Universität des Saarlandes, Physiologische Chemie, D-665o Homburg/Saar
[++]Universität des Saarlandes, Fachbereich 12.1, Angewandte Physik, D-66oo Saarbrücken and Medizinische Hochschule Lübeck, Institut für Physik, D-24oo Lübeck
[+++]RWTH Aachen, Physiologische Chemie, Melatener Straße 213, D-51oo Aachen

The oligomeric PMO functions as the oxygenase in a 4-methoxybenzoate monooxygenase system (O-demethylating) from Pseudomonas putida. By different methods (ERR[1], Mössbauer[2] and kinetic[3]studies) PMO was proved to be a conjugated iron-sulfur protein with non-chromophore bound iron (NC-iron) as cofactor. Our Mössbauer investigations on reduced and oxidized enzyme show that the chromophores of PMO are 2Fe-2S centers and that the NC-iron is the dioxygen binding site and mediates the electron flow from the 2Fe-2S centers to the dioxygen[4].
Binding of different substrates to PMO yields characteristic influence on the Mössbauer spectra of the 2Fe-2S clusters. The substrate binding spectra of oxidized PMO could be fitted only under the assumption of two or three slightly different 2Fe-2S centers per enzyme molecule. The spectra also show that both iron within the oxidized 2Fe-2S clusters are influenced differently by substrate binding. Adding 4-methoxybenzoate to oxidized PMO seems to optimize the asymmetry within 2Fe-2S centers with respect to the reduction of PMO by its native reductase. The NC-iron could be characterized by Mössbauer measurements as five- or sixfold coordinated. From Mössbauer spectra of oxidized, reduced and aerob reoxidized PMO samples we conclude that dioxygen activation occurs by the uptake of one electron from an NC-iron and one from a 2Fe-2S chromophore. (Fig. 1)
We performed measurements of substrate isotope effects for hydroxylation reactions catalized by the enzyme system. By comparing the overall reaction velocities in the oxidative O-demethylation reaction, using as substrate deuterated and non-deuterated 3-phenyl-4-methoxybenzoate, the ratio $k_{1H}/k_{2H} = 1.47$ was found for the apparent (or intermolecular) isotope effect[5]. The true (or intramolecular) isotope effect was determined by measuring the O-demethylation of the symmetrical substrate 3-$^{1}H_3$ methoxy-5-$^{2}H_3$ methoxybenzoate. The ratio of the relative rates of reaction at the labelled and unlabelled sites of this substrate was determined to be $k_{1H}/k_{2H} = 1.36$.
Since for P45o dependent hydroxylation reactions a big difference between intermolecular ($k_{1H}/k_{2H} = 1.o - 2.o$) and intramolecular ($k_{1H}/k_{2H} \simeq 1o$) was reported[6], we concluded from comparison with our findings that different hydroxylation mechanisms exist for both enzyme systems.
The reduction kinetics of PMO reveal that the reduction of the

2Fe-2S chromophores by the native reductase is strongly enhanced in presence of substrate[7], while the reduction using dithionite as reductant is significantly lowered in presence of substrate. This indicates that the reduction of PMO by the two reductants occurs from different sites which are both influenced by substrate binding.

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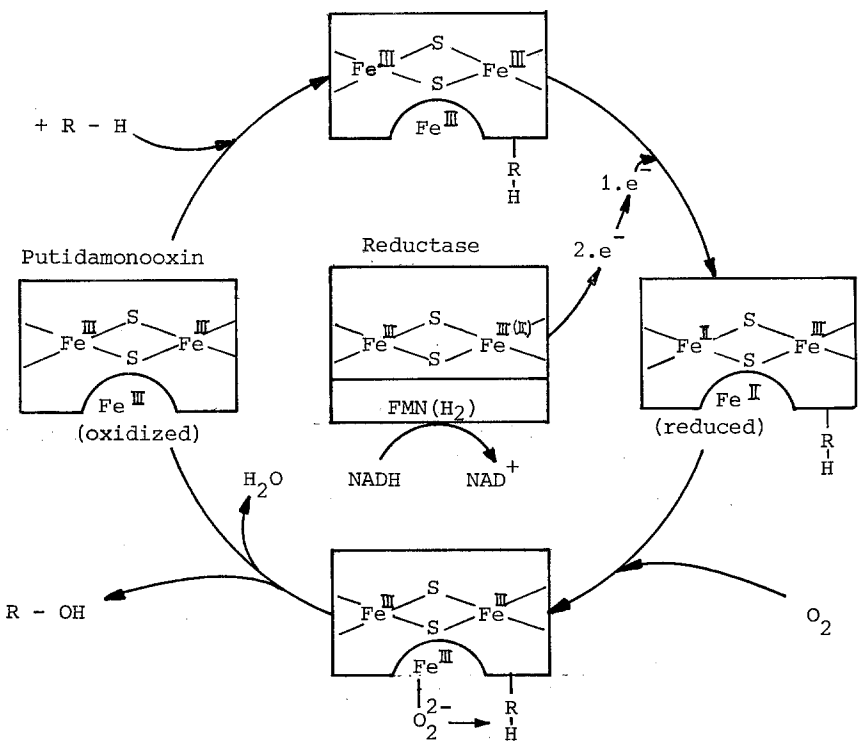

Fig. 1: Proposed reaction cycle of PMO for the hydroxylation of 4-methoxybenzoate, as derived from our Mössbauer measurements of oxidized reduced, and aerobically reoxidized samples. R - H = substrate, R - OH = hydroxylated reaction product.