Mössbauer studies on the cofactor of Putidamonooxin (PMO)

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

[+]Universität des Saarlandes, FB 12.1, D-66 Saarbrücken, and Medizinische Hochschule Lübeck, Physik, D-24 Lübeck

[++]RWTH Aachen, Physiologische Chemie, D-51 Aachen

Putidamonooxin (PMO) - the oxygenase of a 4-methoxybenzoate monooxygenase - catalyzes in presence of the NADH-PMO oxidoreductase, NADH and O_2 the O-demethylation of 4-methoxybenzoate. By different methods (EPR[1],Mössbauer[2] and kinetic studies[3]) PMO was proofed to contain 2Fe-2S and, in addition, mononuclear non-heme iron as cofactor, with 1:1 being the ratio of both. The mono-nuclear non-heme iron serves as the dioxygen binding site and mediates the electron flow from the 2Fe-2S cluster to dioxygen[2]. PMO can be depleted from the cofactor iron by gel filtration in absence of substrate. The cofactor iron can be reincorporated and fixed in presence of substrate[1,2,3]. After selective enrichment with ^{57}Fe we were able to characterize the cofactor by Mössbauer spectroscopy.

The reduced cofactor iron yields a quadrupole doublett typical for Fe^{2+} high spin (quadrupole splitting: 3.19 mm s^{-1}, isomer shift relative to α-iron at 3oo K: 1.2 mms^{-1},line width: o.4 mm s^{-1} at 77 K). The relatively large isomer shift indicates a five or six fold coordination. The spectrum of the oxidized cofactor taken at 2.7 K in a field of o.1 T was simulated using a spin hamiltonian pertinent to Fe^{3+} high spin. This analysis yields two iron sites with different rhombicities E/D. This is in accordance with recent EPR results[1]. In addition we derived hyperfine fields (>5o T) for both sites which support our finding from above that the cofactor iron is five or six fold coordinated.

To study the influence of the cofactor iron on the electronic structure of the 2Fe-2S clusters we investigated the effect of substrate binding on the oxidized 2Fe-2S centers (in vivo enriched with ^{57}Fe) when the cofactor was depleted and incorporated. Binding of different substrates to depleted and oxidized PMO showed characteristic influence on the spectra of the $2^{57}Fe$-2S clusters. From reconstituted and oxidized PMO, we derived substrate independent spectra which were practically identical to the pattern obtained from the depleted enzyme in presence of 4-methoxybenzoate, the physiological substrate. From these findings we conclude, that either the binding of the cofactor iron to PMO in presence of different substrates or the binding of the physiological substrate to the depleted enzyme leads to an electronic structure of the oxidized 2Fe-2S clusters, which is the physiological structure.

Recently, a large difference between the Michaelis-Menten constants (K_M) and the dissociation constants (K_S) for PMO and its substrates was reported[4]. The measurements of K_S in contrast to those of K_M were performed with depleted enzyme. These kinetic results together with our Mössbauer results imply that the affinity of PMO to its substrates is enhanced in presence of cofactor iron. - Supported by Deutsche Forschungsgemeinschaft.

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