Understanding the inactivation of cytochrome P450 BM3 by organic co-solvents

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Cytochrome P450 BM-3 (BM3)[1] isolated from *Bacillus megaterium* catalyzes the hydroxylation and/or epoxidation of a broad range of substrates, including alkanes, alcohols, fatty acids, amides, polyaromatic hydrocarbons and heterocycles.[2] The K_m values of BM3 are in the millimolar range for many of these notoriously water-insoluble compounds. Polar organic co-solvents, for instance dimethylsulfoxide (DMSO), can be supplemented to increase substrate solubility in order to achieve higher catalytic efficiency. Using BM3 as a catalyst for these industrially important biotransformations requires improved tolerance towards organic co-solvents and an understanding how organic co-solvents reduce BM3 activity. The activity of BM3 is retained fully up to ~15 % (v/v) DMSO and drops drastically when concentration reaches ~30 % (v/v).[3]

Data sets of different crystals grown in increasing amounts of DMSO were collected at beamlines X13 and X12. Structures were solved by MR with the pdb entry 1BU7 as target. Comparisons of BM3 crystal structures in low (14% (v/v); resolution 2.1 Å; Lo-DMSO), high (28 % (v/v); resolution 1.7 Å; Hi-DMSO) DMSO concentration with a previously published native structure (1BU7)[6] show similar overall structures with no sign of partial or global unfolding, as predicted by MD[4, 5] and UV-vis. The striking difference between Lo-DMSO and Hi-DMSO lies in the heme coordination (Figure 1). In Lo-DMSO, a slight nonplanar distortion of heme is observed as compared to 1BU7 in which iron is displaced by ~0.16 Å on average. Furthermore, water coordinating heme is displaced aside; closest water molecules to the heme irons found are H_2O239 (Monomer A; distance Fe-O_{Water} 3.56 Å) and H_2O207 (Monomer B; distance Fe-O_{Water} 3.97 Å). On the contrary, water molecule is coordinating to heme iron directly at its 6th position in 1BU7 with an average distance of 2.63 Å. In Hi-DMSO, a DMSO molecule is found to coordinate heme iron at its 6th position via a sulfur atom (distances Fe-S_{DMSO} are 2.35 Å and 2.39 Å for monomer A and B respectively). Heme iron is covalently bonded to both sulfurs of DMSO and the 5th ligand C400 with almost identical distances (average distance Fe-S_{Cys400} (2.33 Å). One methyl group of DMSO tethers to F87 (distance 3.7 Å) while the other one points to T268. This DMSO orientation is confirmed by anomalous scattering data. Hi-DMSO represents the first monooxygenase crystal structure reported with a direct DMSO coordination to heme iron. This coordination modulates very likely BM3 activity at high DMSO concentrations. DMSO is, compared to water, more difficult to be displaced by a substrate molecule; substrate binding triggers channel closure and is a pre-requisite for catalysis in BM3.

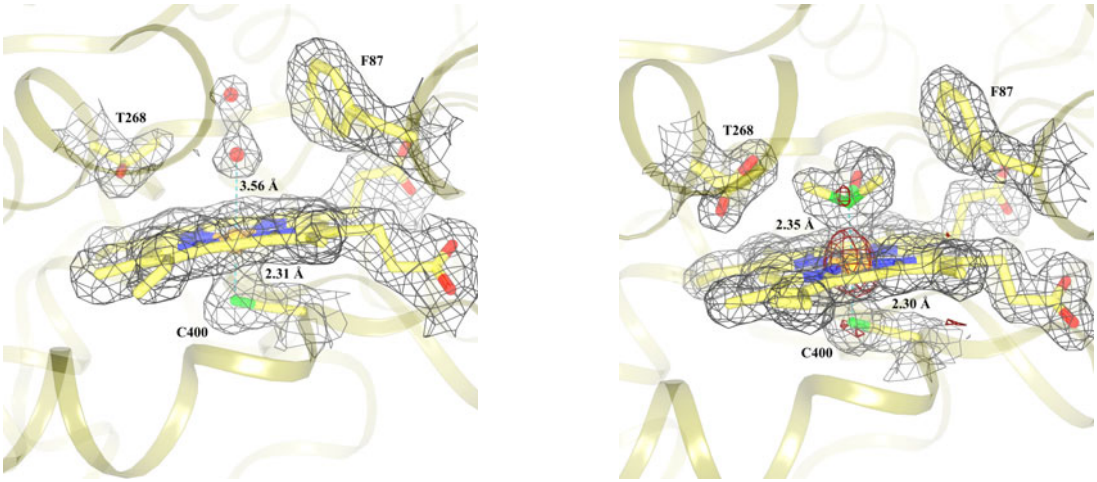

Figure 1. Active site of Lo-DMSO (left) and Hi-DMSO (right). In Lo-DMSO, H_2O239 is displaced aside from the heme iron. In Hi-DMSO, a DMSO is coordinating to heme iron via sulfur atom. The orientation of DMSO is confirmed by anomalous scattering data (shown as red density). Prepared with DINO (http://www.dino3d.org) and rendered with POVRAY (http://www.povray.org).

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