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Understanding a Mechanism of Organic Cosolvent Inactivation in Heme Monooxygenase P450 BM-3

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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 cosolvents, for instance dimethylsulfoxide (DMSO), can be supplemented to increase substrate solubility to achieve higher catalytic efficiencies. Using BM3 as a catalyst for these industrially important biotransformations requires improved tolerance toward organic cosolvents and a better understanding of organic cosolvent effect on the activity of BM3. Purified BM3 retains ~70% of its activity at 14% (v/v) DMSO. Activity drops drastically to ~10% when DMSO concentration reaches 28% (v/v).[3] Previous theoretical studies with molecular dynamics (MD) simulations[4,5] and results from UV−vis spectroscopy indicate the possibility of DMSO perturbing the heme coordination, thus resulting in activity loss. We report here two crystal structures of BM3 heme domain (BMP, T1-L455) cocrystallized with 14% (v/v) and 28% (v/v) DMSO, which shed the light on a cosolvent inactivation mechanism.

Comparisons of the BMP crystal structures in low and high DMSO concentrations {(14% (v/v); Lo-DMSO)//(28% (v/v); Hi-DMSO)} to a previous BM3 structure (1BU7)[6] show similar overall folds without a sign of partial or global unfolding, as predicted by MD[4,5] and confirmed by UV−vis measurements. However, the root-mean-square deviation (rmsd) of monomer B from monomer A in Lo-DMSO (0.376 Å) and in Hi-DMSO (0.468 Å) are significantly lower than that in 1BU7 (1.425 Å). In contrast to Lo-/Hi-DMSO, 1BU7 was crystallized in buffer without DMSO.[6] The resultant lower rmsd's of Lo-/Hi-DMSO suggest a reduction of the flexibility of BMP owing to DMSO addition, which is further supported by the average B-factors for all three structures that lie between 24 and 30 Å^2, resolutions range from 1.7 Å (1BU7 and Hi-DMSO) to 2.1 Å (Lo-DMSO) and low mean coordinate errors of 0.13 Å and 0.07 Å for Lo- and Hi-DMSO, respectively. Interestingly, theoretical studies using MD showed that the flexibility of BMP was slightly lower in water/DMSO mixture compared to water.[4] A tendency of DMSO molecules to cluster on the BMP surface was also found in our previous MD simulations.[4] Hi-DMSO has, in contrast to Lo-DMSO, a DMSO molecule coordinated to S450 by forming hydrogen bonding via its oxygen atom to the backbone amide of S450.

The two monomers in the asymmetric unit of 1BU7 showed large structural differences in the substrate channel entry.[6] Compared to monomer A, monomer B has a more opened substrate binding channel[6] which contributes to the high rmsd between both monomers in 1BU7.[6] In Lo- and Hi-DMSO, we observe high B-factors in the amino acids flanking the substrate access channel: R47 (involved in substrate orientation by interacting with the carbonyl group of fatty acid[7]), F helix (H171-R190), G helix (A197-S226) and F/G loop (A191-P196). Our crystallographic findings are in agreement with previous MD studies in solutions[4,5] which support high flexibility of BMP's substrate binding pocket. Interestingly, the previously specified regions show large conformational changes upon substrate binding in the substrate-bound and substrate-free structures solved by Poulos and co-workers.[8] Crystallographic data from Lo-/Hi-DMSO also suggest that the substrate channel of BMP is highly dynamic and previous MD studies[5] indicated even a DMSO-induced substrate channel opening (defined by distances P45-A191 and R47-Y51).

The most striking difference between Lo-DMSO and Hi-DMSO lies in the heme coordination (Figure 1). In Lo-DMSO, a slight nonplanar distortion of the heme is observed compared to 1BU7 in which the iron is displaced distally by ~0.16 Å on average. Furthermore, the water molecule coordinated to the heme iron is displaced aside (toward A328). Distances between irons and coordinated water molecules ($Fe-O_{water}$) are 3.56 Å for H_2O239 (monomer A) and 3.97 Å for H_2O207 (monomer B). In 1BU7, a water molecule is heme-coordinated at its sixth position with an average distance of 2.63 Å. In Hi-DMSO, a DMSO molecule is found to coordinate surprisingly the heme iron at its sixth position via a sulfur atom ($Fe-S_{DMSO}$ distances are 2.35 and 2.39 Å for monomers A and B, respectively). These covalent $Fe-S_{DMSO}$ bonds have almost identical distances of fifth C400 ligand in the heme center (average $Fe-S_{Cys400}$ distance = 2.33 Å). One methyl group of DMSO tethers to F87 (distance = 3.7 Å) while the other one points to T268. The position of DMSO sulfur was confirmed by anomalous scattering data collected for the sulfur and iron atoms (Figure 1). According to the hard−soft acid−base theory, we would expect an ambident ligand like sulfoxide to coordinate the Fe(III) via oxygen. Furthermore, a Cambridge Structural Database search revealed that all sulfoxide complexes with Fe(III) involved coordination via an oxygen atom.[9] In Hi-DMSO, the coordination via sulfur atom could partially be stabilized by hydrophobic interactions between DMSO's methyl group and the phenyl ring of F87. Interaction between the terminal methyl group of fatty acids with the phenyl side chain of F87 orients fatty acids for subterminal and not terminal hydroxylation by an oxyferryl radical cation intermediate (compound I).[10] Direct coordination of DMSO to the heme iron corresponds well with UV−vis measurements (Figure 2). In phosphate buffer lacking DMSO, a typical low-spin spectrum (peak 420 nm) is obtained which indicates a water molecule coordinated to the heme iron as sixth ligand. In 14% (v/v) DMSO, a mix-spin spectrum (extra shoulder at 390 nm) is observed. When DMSO concentration is raised further to 30% (v/v), a low-spin

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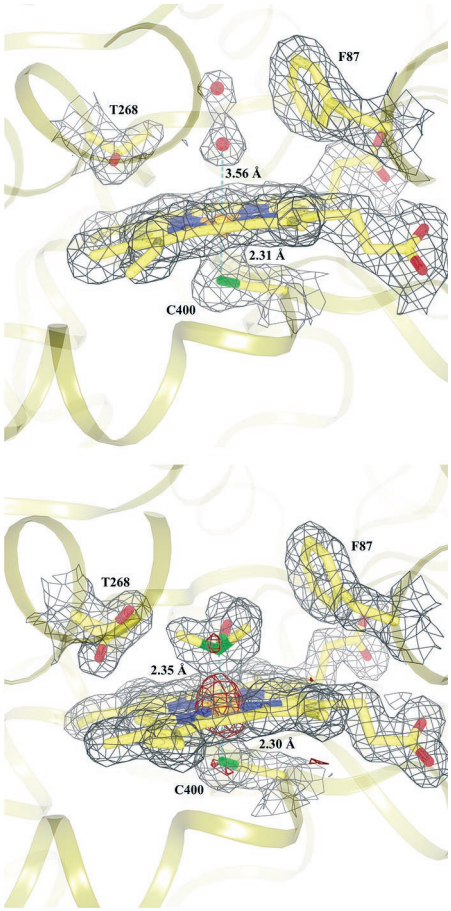

Figure 1. Active sites of Lo-DMSO (top) and Hi-DMSO (bottom). In Lo-DMSO, H_2O239 is displaced aside from the heme iron. In Hi-DMSO, a DMSO is coordinating to the heme iron via sulfur atom. The orientation of DMSO is confirmed by anomalous scattering data (shown as red density). Prepared with DINO (http://www.dino3d.org)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

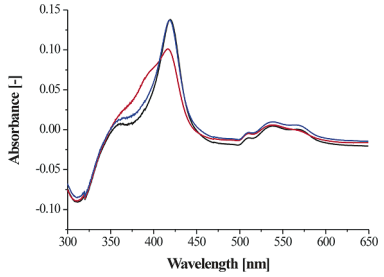

Figure 2. UV−vis difference spectra of BMP in phosphate buffer (black), in 14% (v/v) DMSO (red), and in 30% (v/v) DMSO (blue).

spectrum is regained with a DMSO coordinating the heme iron in Hi-DMSO, causing a reduced activity.

The I-helix (D250−K282) of BMP contains a number of catalytically important residues, including A264[11] and T268.[12] In the P450 reaction cycle, T268 provides proton for oxygen activation.[12] Interestingly in Hi-DMSO monomer B, two alternating conformations can be observed for residues H266−T268. In monomer A, alternating conformations can be found for T268. The I-helix in Hi-DMSO is bent to a larger degree compared to 1BU7 and Lo-DMSO (see Supporting Information Figure S9; rmsd of Hi-DMSO vs 1BU7 is 0.85 Å, rmsd of Lo-DMSO vs 1BU7 is 0.28 Å). Bending of the I-helix in Hi-DMSO could provide additional space to accommodate a DMSO molecule which is bulkier than a water molecule.

To our best knowledge, Hi-DMSO represents the first monooxygenase crystal structure reported with a direct DMSO sulfur coordination to the 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 prerequisite for catalysis in BM3. DMSO diffusion into the substrate channel is likely facilitated by the DMSO-induced widening of substrate channel. Current efforts are devoted to crystallizing W5F5 and F87A. W5F5 is an organic solvent resistant mutant identified by directed evolution.[3] F87 residue modulates BM3 sensitivity for the cosolvent DMSO.[3] These investigations will help us to understand the cosolvent effect and to tailor BM3 for industrially relevant catalysis in nonaqueous medium.

Coordinates of Lo-DMSO and Hi-DMSO were deposited in PDB, with entries 2J4S and 2J1M respectively.

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Supporting Information Available: Details of BMP cloning, protein expression, protein purification, UV−vis measurement, crystallization, data collection, refinement statistics and structural analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Denisov, I. G.; Makris, T. M.; Sligar, S. G.; Schlichting, I. *Chem. Rev.* **2005**, *105*, 2253−77. (b) Munro, A. W.; Leys, D. G.; McLean, K. J.; Marshall, K. R.; Ost, T. W.; Daff, S.; Miles, C. S.; Chapman, S. K.; Lysek, D. A.; Moser, C. C.; Page, C. C.; Dutton, P. L. *Trends Biochem. Sci.* **2002**, *27*, 250−7. (c) Warman, A. J.; Roitel, O.; Neeli, R.; Girvan, H. M.; Seward, H. E.; Murray, S. A.; McLean, K. J.; Joyce, M. G.; Toogood, H.; Holt, R. A.; Leys, D.; Scrutton, N. S.; Munro, A. W. *Biochem. Soc. Trans.* **2005**, *33*, 747−53.

(2) (a) Appel, D.; Lutz-Wahl, S.; Fischer, P.; Schwaneberg, U.; Schmid, R. D. *J. Biotechnol.* **2001**, *88*, 167−71. (b) Boddupalli, S. S.; Estabrook, R. W.; Peterson, J. A. *J. Biol. Chem.* **1990**, *265*, 4233−9. (c) Capdevila, J. H.; Wei, S.; Helvig, C.; Falck, J. R.; Belosludtsev, Y.; Truan, G.; Graham-Lorence, S. E.; Peterson, J. A. *J. Biol. Chem.* **1996**, *271*, 22663−71. (d) Carmichael, A. B.; Wong, L. L. *Eur. J. Biochem.* **2001**, *268*, 3117−25. (e) Glieder, A.; Farinas, E. T.; Arnold, F. H. *Nat. Biotechnol.* **2002**, *20*, 1135−9. (f) Li, Q. S.; Schwaneberg, U.; Fischer, M.; Schmitt, J.; Pleiss, J.; Lutz-Wahl, S.; Schmid, R. D. *Biochim. Biophys. Acta* **2001**, *1545*, 114−21. (g) Li, Q. S.; Schwaneberg, U.; Fischer, P.; Schmid, R. D. *Chemistry* **2000**, *6*, 1531−6. (h) Miura, Y.; Fulco, A. J. *Biochim. Biophys. Acta* **1975**, *388*, 305−17. (i) Ost, T. W.; Miles, C. S.; Murdoch, J.; Cheung, Y.; Reid, G. A.; Chapman, S. K.; Munro, A. W. *FEBS Lett.* **2000**, *486*, 173−7.

(3) Wong, T. S.; Arnold, F. H.; Schwaneberg, U. *Biotechnol. Bioeng.* **2004**, *85*, 351−8.

(4) Roccatano, D.; Wong, T. S.; Schwaneberg, U.; Zacharias, M. *Biopolymers* **2005**, *78*, 259−67.

(5) Roccatano, D.; Wong, T. S.; Schwaneberg, U.; Zacharias, M. *Biopolymers* **2006**, *83*, 467−76.

(6) Sevrioukova, I. F.; Li, H.; Zhang, H.; Peterson, J. A.; Poulos, T. L. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 1863−8.

(7) Noble, M. A.; Miles, C. S.; Chapman, S. K.; Lysek, D. A.; MacKay, A. C.; Reid, G. A.; Hanzlik, R. P.; Munro, A. W. *Biochem. J.* **1999**, *339* (2), 371−9.

(8) Li, H. Y.; Poulos, T. L. *Biochim. Biophys. Acta* **1999**, 141−9.

(9) Lushington, G. H.; Cowley, A. B.; Silchenko, S.; Lukat-Rodgers, G. S.; Rodgers, K. R.; Benson, D. R. *Inorg. Chem.* **2003**, *42*, 7550−9.

(10) Schwaneberg, U.; Schmidt-Dannert, C.; Schmitt, J.; Schmid, R. D. *Anal. Biochem.* **1999**, 269, 359−66.

(11) Joyce, M. G.; Girvan, H. M.; Munro, A. W.; Leys, D. *J. Biol. Chem.* **2004**, *279*, 23287−93.

(12) (a) Clark, J. P.; Miles, C. S.; Mowat, C. G.; Walkinshaw, M. D.; Reid, G. A.; Daff, S. N.; Chapman, S. K. *J. Inorg. Biochem.* **2006**, *100*, 1075−90. (b) Truan, G.; Peterson, J. A. *Arch. Biochem. Biophys.* **1998**, *349*, 53−64. (c) Yeom, H.; Sligar, S. G.; Li, H.; Poulos, T. L.; Fulco, A. J. *Biochemistry* **1995**, *34*, 14733−40.

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