Monomeric sarcosine oxidase: structure of a covalently flavinylated amine oxidizing enzyme

Peter Trickey[a], Mary Ann Wagner[b], Marilyn Schuman Jorns[b], and F. Scott Mathews[a]
[a] *Department of Biochemistry and Molecular Biophysics, Washington University School of Medicine, 660 S. Euclid Ave, St. Louis, MO 63110 USA*
[b] *Department of Biochemistry, MCP Hahnemann School of Medicine, 2900 Queen Lane, Philadelphia, PA 19129 USA*

Introduction

Monomeric sarcosine oxidases (MSOX) are among the simplest members of a recently recognized family of prokaryotic and eukaryotic enzymes that contain covalently bound flavin and catalyze similar oxidation reactions with various secondary or tertiary amino acids [1–3]. MSOX from *Bacillus sp*. B-0618 has 386 residues, a molecular mass of 43.8 kDa, and an NH_2-terminal ADP-binding motif [4]. The enzyme contains 1 mol of FAD, which is bound covalently through an 8-α-S-cysteinyl linkage to Cys315. MSOX catalyze the oxidation of sarcosine (N-methylglycine) by molecular oxygen and water to form glycine, formaldehyde, and hydrogen peroxide [5].

Methods and Materials

Multiwavelength anomalous diffraction (MAD) data were collected at beamline 19-ID at the Advanced Photon Source, Argonne, Illinois. Data processing was carried out using the HKL package [6]. All MAD data were collected from a single selenomethionine MSOX crystal, which was frozen at 100 K. Data sets were collected at four wavelengths at or near the K absorption edge of selenium. Wavelengths were selected on the basis of an x-ray fluorescence spectrum collected directly from the crystal: edge (0.9794 Å) was optimized for S-'; peak (0.9792 Å) was at maximal S-", low (1.0679 Å), and high (0.9392 Å) were recorded to obtain the dispersive differences. Due to the alignment of the crystal in the cryoloop, an inverse beam experiment was performed in which at each energy data were recorded over a range of Ω at X = 0, K = 0, and fixed Φ and again at Φ + 180.

The MAD data were combined with MADMRG [7] and analyzed using HEAVY and SOLVE [8, 9]. From Patterson maps calculated with the anomalous differences and with the dispersive differences, 14 selenium sites were found in the asymmetric unit, indicating the presence of two molecules per asymmetric unit. Visual analysis of the heavy atom coordinates revealed the presence of a local two-fold axis approximately parallel to the crystallographic b axis relating the two molecules in the asymmetric unit. This allowed us to establish the noncrystallographic symmetry (NCS) operator relating the two molecules using LSQKAB [10].

The selenium parameters were refined with the maximum-likelihood program SHARP [11] and solvent flattened using a solvent content of 42%. The resulting electron density map was averaged about the NCS two-fold axis with further solvent flattening using DM [10]. Masks were calculated with NCSMASK [10]. The final figure of merit was 0.92 to 2.0 Å. The quality of the experimental map was exceptionally good as expected from the phasing statistics. All main chain and side chains, except for a few terminal residues, could readily be modeled in the density using the program TURBO [12].

The selenomethionine MSOX model was subjected to simulated annealing refinement with X-PLOR [13] using tightly restrained NCS. This was followed by several rounds of positional and temperature factor refinement with loose NCS restraints combined with manual rebuilding. About 200 water molecules were added. The final model consists of residues 1–385 for both molecules as there was no density for the remaining four residues. The refined selenomethionine structure without water was subjected to simulated annealing refinement against the native data set, followed by a refinement procedure similar to that employed with the selenomethionine structure. Two fo-fc difference density maps were calculated for the seleno-methionine inhibitor-soaked data sets, followed by modeling of the inhibitor into the resulting density.

Results and Discussion

Monomeric sarcosine oxidase is a two-domain protein of the flavoenzyme structural class first observed in *p*-hydroxybenzoate hydroxylase (PHBH) [14]. One domain contains a "classic" FAD-binding motif common to the PHBH class and to the glutathione reductase (GR) [15] class of flavoproteins. In MSOX, this motif consists of a five-stranded parallel β-sheet flanked on one side by two α-helices (αF1 and αF2) preceding strands βF2 and βF3 of the sheet, respectively, and by a three-stranded antiparallel β-sheet (a "β-meander", strands βF4–βF6) on the other side of the sheet preceding strand βF7. A third and fourth helix (αF3 and αF4) are packed on either side of the sheet near strands βF8 and βF9. The FAD-binding motif is interrupted twice by excursions into domain 2, the "catalytic" domain, a two-part, eight-stranded mostly antiparallel β-sheet. One part interrupts the flavin-binding motif`between strands βF2 and βF3 and comprises three antiparallel β-strands (βC1–βC3) interspersed with three α-helices. The other part interrupts the motif between strands βF7 and βF9 and comprises five antiparallel β-strands (βC4–βC8) plus one α-helix. The two-part nature of domain 2 is characteristic of the PHBH flavoprotein class. In the GR flavoprotein class, the second domain, a dinucleotide-binding domain closely

resembling the FAD-binding domain, occurs as a single excursion interrupting the FAD-binding domain between β-strands 4 and 5.

FAD is bound to MSOX in a manner similar to that of other members of the PHBH class of flavoenzymes, in particular to DAAO. It is in an extended conformation and is almost totally isolated from the bulk solvent; this includes the adenosine portion of the molecule, which is partially exposed to the bulk solvent in many FAD-containing flavoenzymes. The covalent, hydrogen-bonding and electrostatic interactions made by FAD in the enzyme include one covalent bond, 28 hydrogen bonds (of which eight are to solvent molecules), and two helix dipoles. One of the helix dipoles, at the N-terminus of αF4, points toward the N(1)/O(2) portion of the flavin ring; the other dipole, at the N-terminus of αF1, points toward the pyrophosphate group of FAD. Both of these helix dipole interactions are found in all members of the PHBH and GR classes of FAD-binding proteins, since both classes share the same FAD dinucleotide-binding motif. The αF1 helix serves to partially neutralize the double negative charge on the pyrophosphate group, while the αF4 helix serves to help stabilize the electrophilic character of the flavin ring and the anionic forms of the hydroquinone and semiquinone. The latter may also help promote covalent flavinylation of certain members of these two flavoprotein classes.

Acknowledgments

This work was supported by U.S. Public Health Service grants No. GM20530 (FSM) and GM31704 (MSJ). We thank Dr. Masanori Sugiyama for his generous gift of plasmid pOXI103.

Use of the Advanced Photon Source was supported by the U.S. Department of Energy, Basic Energy Sciences, Office of Science, under Contract No. W-31-109-Eng-38.

References

[1] L.J. Chlumsky, L. Zhang, and M.S. Jorns, *J. Biol. Chem.* **270,** 18252–18259 (1995).
[2] B.E. Reuber, C. Karl, S.A. Reimann, S.J. Mihalik, and G. Dodt, *J. Biol. Chem.* **272,** 6766–6776 (1997).
[3] Y. Koyama and H. Ohmori, *Gene* **181,** 179–183 (1996).
[4] K. Suzuki, H. Sagai, S. Imamura, and M. Sugiyama, *J. Ferm. Bioe* **77,** 231–234 (1994).
[5] K. Kvalnes-Krick and M.S. Jorns, *Chemistry and biochemistry of flavoenzymes* (CRC Press, Inc., Boca Raton, FL, 1991) 425–435.
[6] Z. Otwinowski and W. Minor, *Methods Enzymol*. **276**, 307–326 (1997).
[7] T.C. Terwilliger, *Acta Cryst*. **D50**, 17–23 (1994).
[8] T.C. Terwilliger, S.-H. Kim, and D. Eisenberg, *Acta Cryst*. **A43**, 1–5 (1987).
[9] T.C. Terwilliger, *Acta Cryst.* **D50**, 11–16 (1994).
[10] *Acta Cryst*. **D50**, 760–763 (1994).
[11] E. de La Fortelle, J. J. Irwin, and G. Bricogne, *Crystallographic Computing 7*, (1997).
[12] A. Roussel and C. Cambillau, *Silicon Graphics Geometry Partners Directory 86*, (Silicon Graphics, Moutain View, CA, 1991).
[13] A.T. Brunger, X-PLOR (version 3.1) *Manual of a system for crystallography and NMR*, (Yale University, New Haven, CT, 1993).
[14] H.A. Schreuder, P.A. Prick, R.K. Wierenga, G. Vriend, K.S. Wilson, W.G. Hol, and J. Drenth, *J. Mol. Biol*. **208**, 679–696 (1989).
[15] P.A.Karplus and G.E. Schulz, *J. Mol. Biol*. **195**, 701–729 (1987).