Heterodimeric Structure of Superoxide Dismutase in Complex with Its Metallochaperone

A. L. Lamb,[1] A. S. Torres,[2] T. V. O'Halloran,[2,1] A. C. Rosenzweig[1,2]
[1]Department of Biochemistry, Molecular Biology, and Cell Biology and [2]Department of Chemistry,
Northwestern University, Evanston, IL, U.S.A.

Introduction

Transition metal ions such as copper and iron are crucial components of all living organisms, serving as cofactors for numerous proteins with diverse functions, including electron transfer, dioxygen binding, and catalysis [1]. Cellular systems for controlling uptake and distribution of metal ions prevent toxic accumulation and subsequent oxidative damage of macromolecules [2]. For copper, trafficking factors called metallochaperones deliver the required metal ion to target active sites by direct protein-protein interactions [3]. Copper,zinc superoxide dismutase (SOD1) — a homodimeric, antioxidant enzyme [4] implicated in some forms of familial amyotrophic lateral sclerosis (FALS) [5] — obtains its copper cofactor *in vivo* from the copper chaperone for superoxide dismutase (CCS) [6]. Although crystal structures of both SOD1 [7] and CCS [8, 9] are available, the details of chaperone-target protein recognition and docking for metal ion transfer are not understood. The structure of yeast CCS (yCCS) [8] revealed a homodimer, in which each monomer comprises two domains. The N-terminal domain (domain I) contains an MHCXXC metal binding motif and resembles the Atx1 metallochaperone [10]. The second domain (domain II) is similar to SOD1 but lacks all the structural elements important for catalysis. The C-terminal 27's residues (domain III), which are essential for yCCS function [11] and contain a highly conserved CXC sequence motif, were disordered. We have determined the structure of a mutant yeast SOD1 complexed with yCCS to 2.9-Å resolution.

Methods and Materials

The complex between yCCS and H48F-SOD1 was prepared as described previously [12]. Crystallization of the complex was carried out by the hanging drop method at 25°C. Drops containing 1.5 µL of ~40 mg/mL protein (determined by the Bradford assay) in 50 mM MES at pH 6.0, 150 mM NaCl, 20 mM DTT, and 20 µM $ZnSO_4$ were mixed with equal volumes of a reservoir solution composed of 100 mM MES at pH 6.5, 1.8 M ammonium sulfate, and 5% dioxane. The crystals belong to the space group $P3_221$ with unit cell dimensions of $a = b = 104.1$ Å and $c = 233.7$ Å. For data collection, crystals were transferred to the reservoir solution supplemented with 20% ethylene glycol as a cryosolvent and flash-cooled by immersion in liquid nitrogen. The data were processed with DENZO and SCALEPACK [13]. The structure was solved by molecular replacement with AmoRe [14] by using data in the 8.0- to 4.0-Å resolution range. After model building with the programs O [15] and XtalView [16], refinement was conducted by simulated annealing and individual B-value refinement with CNS [17].

Results and Discussion

The asymmetric unit contains two heterodimers related by a noncrystallographic twofold axis. Each heterodimer consists of a single SOD1 monomer and a single yCCS monomer. Thus, contrary to some predictions, the very stable SOD1 homodimer [18] can dissociate to interact with its chaperone. Conserved dimer interface residues are the key element in chaperone-target protein recognition and docking.

Drastic conformational changes occur in both the yCCS and SOD1 monomers upon reorganizing from homodimers into the heterodimeric complex. These changes are particularly striking for the yCCS monomer. Domain I, although quite similar in overall structure to domain I in the yCCS homodimer, occupies a completely different position. The second domain of yCCS is very similar in structure to that in the yCCS homodimer, but there are several notable differences. The most significant difference is the ordering of 23 of the 27 C-terminal amino acid residues that were disordered in the yCCS homodimer structure. The domain III region is a long, random coil that terminates in a 10-residue α helix.

The presence of yCCS domain III also causes a completely unexpected conformational change in the SOD1 monomer. A comparison of the SOD1 monomer in the heterodimer with that in the previously determined SOD1 homodimer structure [19] indicates that the two monomers are quite similar except for one loop, the S-S subloop. This loop, conserved in all SOD1s [20], is characterized by a disulfide bond between Cys 57 and Cys 146, linking the loop to the β barrel structure. In the heterodimeric complex with yCCS, this disulfide is not present. Instead, Cys 57 forms a disulfide bond with Cys 229 from yCCS domain III. This new disulfide alters the conformation of the S-S subloop and opens up the SOD1 active site.

The conformational changes observed in the complex between yCCS and SOD1 have important implications for

the mechanism of metal ion transfer. Since both yCCS domains I and III contain potential metal binding motifs, either domain or both domains could insert the metal ion into the SOD1 active site. Although the conformation of domain I has changed significantly in the heterodimer as compared with the yCCS homodimer, its MHCXXC motif remains ~35 Å from the SOD1 copper site, suggesting that domain I does not directly deliver the metal ion. By contrast, domain III is absolutely required for CCS function [11], and its two cysteines are adjacent to the SOD1 active site in the complex, poised to deliver metal ions.

The presence of a disulfide bond between SOD1 and yCCS is perhaps the most surprising aspect of the structure. This disulfide is not a requirement for stable heterodimer formation. Although crystallization of the complex was performed in the presence of excess reductants, crystals grew aerobically after 1 month, providing ample time for oxidation to occur. Both pairs of cysteines in domain I remain reduced, however, suggesting that the disulfide between SOD1 and yCCS may not be an artifact of crystallization. It is possible that transient intermonomer disulfide formation plays a role in yCCS function.

How a copper ion coordinated by thiolates can be transferred directly to the histidine coordination environment in SOD1 is a major unresolved question. Given that SOD1 activation by CCS has recently been shown to require dioxygen (A. S. Torres and T. V. O'Halloran, unpublished data), oxidation of the cysteines might be necessary to promote release of the metal ion from domain III into the SOD1 active site. The formation of an intermolecular disulfide would have the added benefit of propping open the SOD1 active site to facilitate metal ion insertion.

Acknowledgments

This work was supported a National Institutes of Health (NIH) grant and a grant from the ALS Association to A. C. Rosenzweig and by an NIH National Research Service Award (NRSA) fellowship to A. L. Lamb. The DuPont-Northwestern-Dow Collaborative Access Team (DND-CAT) sector at APS is supported by E. I. DuPont de Nemours & Co., The Dow Chemical Company, the U.S. National Science Foundation through Grant No. DMR-9304725, and the State of Illinois through the U.S. Department of Commerce and Illinois Board of Higher Education, Higher Education Cooperation Act (IBHE-HECA) Grant No. NWU 96. Use of the APS was supported by the U.S. Department of Energy, Office of Science, Office of Basic Energy Sciences, under Contract No. W-31-109-ENG-38.

References

[1] R. H. Holm, P. Kennepohl, and E. I. Solomon, "Structural and functional aspects of metal sites in biology," Chem. Rev. **96**, 2239-2314 (1996).

[2] D. Radisky and J. Kaplan, "Regulation of transition metal transport across the yeast plasma membrane," J. Biol. Chem. **274**, 4481-4484 (1999).

[3] A. C. Rosenzweig, "Copper delivery by metallochaperone proteins," Acc. Chem. Res. **34**, 119-128 (2001).

[4] J. M. McCord and I. Fridovich, "Superoxide dismutase. A enzyme function for erythrocuprein (hemocuprein)," J. Biol. Chem. **244**, 6049-6055 (1969).

[5] M. Gaudette, M. Hirano, and T. Siddique, "Current status of SOD1 mutations in familial amyotrophic lateral sclerosis," Amyotroph. Lateral Sc. **1**, 83-89 (2000).

[6] V. C. Culotta et al., "The copper chaperone for superoxide dismutase." J. Biol. Chem. **272**, 23469-23472 (1997).

[7] I. Bertini, S. Mangani, and M. S. Viezzoli, "Structure and properties of copper-zinc superoxide dismutases," Adv. Inorg. Chem. **45**, 127-250 (1997).

[8] A. L. Lamb, A. K. Wernimont, R. A. Pufahl, T. V. O'Halloran, and A. C. Rosenzweig, "Crystal structure of the copper chaperone for superoxide dismutase," Nature Struct. Biol. **6**, 724-729 (1999).

[9] A. L. Lamb, A. K. Wernimont, R. A. Pufahl, T. V. O'Halloran, and A. C. Rosenzweig, "Crystal structure of the second domain of the human copper chaperone for superoxide dismutase," Biochem. **39**, 1589-1595 (2000).

[10] A. C. Rosenzweig et al., "Crystal structure of the Atx1 metallochaperone protein at 1.02 Å resolution," Structure **7**, 605-617 (1999).

[11] P. J. Schmidt et al., "Multiple protein domains contribute to the action of the copper chaperone for superoxide dismutase," J. Biol. Chem. **274**, 23719-23725 (1999).

[12] A. L. Lamb, A. S. Torres, T. V. O'Halloran, and A. C. Rosenzweig, "Heterodimer formation between superoxide dismutase and its copper chaperone," Biochem. **39**, 14720-14727 (2000).

[13] Z. Otwinowski and W. Minor, "Processing of x-ray diffraction data collected in oscillation mode," Meth. Enz. **276**, 307-326 (1997).

[14] Collaborative Computational Project N, "The CCP4 suite programs for protein crystallography," Acta Crystallogr. Sec. D **50**, 760-763 (1994).

[15] T. A. Jones, J.-Y. Zou, S. W. Cowan, and M. Kjeldgaard, "Improved methods for building protein models in electron density maps and location of errors in these models," Acta Crystallogr. Sec. A **47**, 110-119 (1991).

[16] D. E. McRee, "XtalView Xfit — A versatile program for manipulating atomic coordinates and electron density," J. Struct. Biol. **125**, 156-165 (1999).

[17] A. T. Brünger et al., “Crystallography & NMR system: A new software suite for macromolecular crystallography,” Acta Crystallogr. Sec. D **54**, 905-921 (1998).
[18] J. S. Valentine and M. W. Pantoliano, “Protein-metal ion interactions in cuprozinc protein (superoxide dismutase),” in *Copper Proteins*, edited by T. G. Spiro (Wiley-Interscience, New York, NY, 1981), pp. 291-358.
[19] K. Djinovic et al., “Crystal structure of yeast Cu,Zn superoxide dismutase: Crystallographic refinement at 2.5 Å resolution,” J. Mol. Biol. **225**, 791-809 (1992).
[20] D. Bordo, K. Djinovic, and M. Bolognesi, “Conserved patterns in the Cu,Zn superoxide dismutase family,” J. Mol. Biol. **238**, 366-386 (1994).