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The dimeric assembly of *Photobacterium leiognathi* and *Salmonella typhimurium* SodC1 Cu,Zn superoxide dismutases is affected differently by active site demetallation and pH: An analytical ultracentrifuge study

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Abstract

To establish whether the species-specific variations at the subunit interface of bacterial Cu,Zn superoxide dismutases affect dimer assembly, the association state of the *Photobacterium leiognathi* (PlSOD) and *Salmonella typhimurium* (StSOD) enzymes, which differ in 11 out of 19 interface residues, was investigated by analytical ultracentrifugation.

The same linkage pattern correlates quaternary assembly, active site metallation, and pH in the two enzymes albeit with quantitative differences. Both holo-enzymes are stable dimers at pH 6.8 and 8.0, although their shape is altered at alkaline pH. In contrast, dimer stability is affected differently by metal removal. Thus, apo-StSOD is a stable dimer at pH 6.8 whereas apo-PlSOD is in reversible monomer–dimer equilibrium. In both apoproteins a pH increase to 8.0 favors monomerization. These effects prove the existence of long-range communication between the active site and the subunit interface and provide a structural explanation for the known functional differences between the two enzymes.

Keywords: Bacterial Cu,Zn superoxide dismutase; Quaternary structure; Metal occupancy; Long-range communication

The recent characterization of an increasing number of prokaryotic Cu,Zn superoxide dismutases (Cu,Zn SODs)[1] has revealed a considerable structural variability between proteins from different bacteria and distinctive structural features with respect to the eukaryotic enzymes. All bacterial Cu,Zn SODs conserve the characteristic fold based on a flattened Greek-key eight-stranded β-barrel, but the organization of their active site channel and the quaternary structure are distinct. In particular, in prokaryotic Cu,Zn SODs the catalytically relevant residues involved in the electrostatic recognition and steering of the superoxide anion have a different location in the folded monomer than in eukaryotes [1] such that the active site is surrounded by a rather flexible protein environment and is more accessible to solvent [2,3]. Moreover, whereas eukaryotic Cu,Zn SODs are always homodimers, highly active and stable monomeric enzymes have been identified in *Escherichia coli* [4], *Brucella abortus* [5] and *Salmonella enterica* [6]. Other bacterial Cu,Zn SODs are dimeric, although their state of association in solution has not been characterized rigorously. The crystallographic characterization of the enzymes from *Photobacterium leiognathi* [2,3], *Actinobacillus pleuropneumoniae* [7] and *Salmonella enterica* serovar Typhimurium (here after referred to as *S. typhimurium*) SodC1 [8] has brought out that the subunit interface is built from different structural elements and is characterized by looser intermolecular contacts relative to eukaryotic Cu,Zn SODs. Moreover, the bacterial interface defines a wide intermolecular

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[1] *Abbreviations used*: Cu,Zn SODs, Cu,Zn superoxide dismutases; PlSOD, *Photobacterium leiognathi* Cu, Zn superoxide dismutase; StSOD, *Salmonella typhimurium* Cu, Zn superoxide dismutase.

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cavity containing several ordered water molecules, at variance with the eukaryotic one where the tightly complementary surfaces exclude solvent. The interface of the *P. leiognathi* enzyme (PlSOD) can be taken as reference, since PlSOD was the first Cu,Zn SOD to be isolated from a prokaryote [9] and to be crystallized [3]. The residues stabilizing the interface form three clusters. The first is centered around the side-chains of Trp83 and Phe81 which form a wide hydrophobic patch involving several residues from the facing subunit, the second is built in proximity of the 2-fold axis by the side-chains of Met41 and Phe96 from both subunits, and the third comprises Lys25, Tyr26 from one subunit and Asp85 from the symmetry-related one. Additional stability is provided by other residues, like Ala94, Asp129 and His131, whose surface is only marginally involved in the interaction with the partner subunit (Fig. 1).

The numerous and radical mutations at these clusters occurring in the *E. coli* enzyme (e.g., Lys25→Asp, Tyr26→Lys, Phe81→Gly, Trp83→Glu, Met41→Glu) account for its monomeric state, but it is not known, for example, whether the substantial number of substitutions characterizing *S. typhimurium* SodC1 (StSOD) results in a different dimerization capacity in solution relative to PlSOD. In StSOD, the most efficient superoxide dismutase known to date, the mutations concern 11 out of 19 interacting residues and are located mainly in the first and second cluster (Fig. 1A). It is hard to establish *a priori* whether the loss and/or the replacement of such a large number of interactions, the slightly larger molecular surface buried at the interface (805 Å^2 vs. 754 Å^2 in PlSOD) and the smaller number of ordered interface water molecules (5–6 per dimer vs. 8–10 in PlSOD) result in an increased or decreased interface stability with respect to PlSOD.

It is also not known whether and how far the metal cofactor influences the association state in solution. The existence of a sort of allosteric link between the interface and the active site has been suggested by previous studies on site-specific PlSOD mutants bearing substitutions at the dimer interface. These mutants have an altered copper accessibility and reactivity and in addition are characterized by the onset of a metal-dependent subunit association–dissociation process [10–13]. Intriguingly, mutations at the subunit interface (i.e., Ala4→Val) have been reported recently to affect metal affinity also in a human Cu,Zn SOD mutant. The affinity for

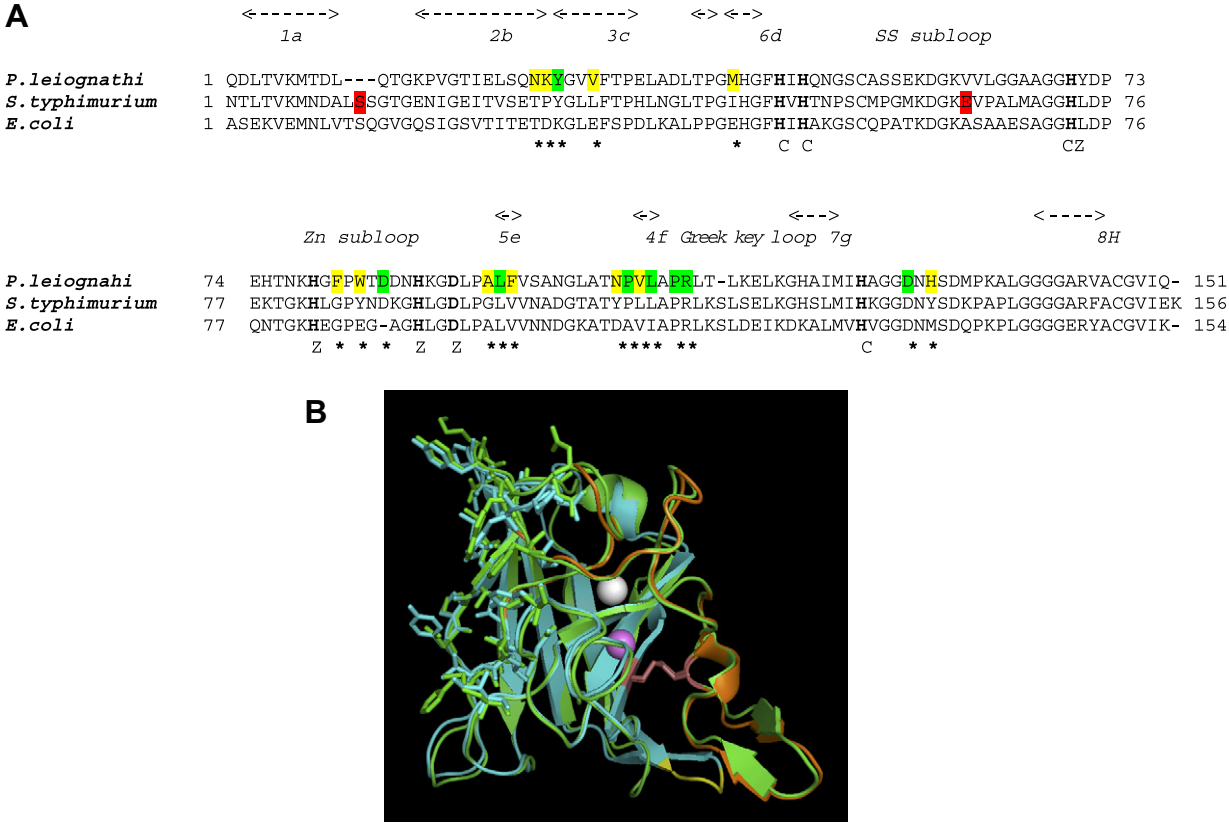

Fig. 1. (A) Structure-based sequence alignment of bacterial Cu,Zn SODs. The β-strand elements are indicated above amino acid sequences with the < –> symbol. The numbering is based on PlSOD. The histidine residues coordinating copper and zinc ions in the active site of the enzyme are shown in bold and are indicated by C and Z, respectively. The residues involved in the contacts between the two subunits are marked by asterisks and highlighted in yellow when they differ between PlSOD and StSOD and in green whey they are conserved in the two enzymes. The two residues highlighted in red in the StSOD sequence may form an hydrogen bond in StSOD. (B) Superimposition of PlSOD and StSOD. One subunit of PlSOD (green) was superimposed to one subunit of StSOD (blue). The three amino acids insertion in loop 1,2 of StSOD (yellow) is close to the long and flexible loop 6,5 (green/orange). Zinc and copper are represented as grey and purple spheres, respectively. The conserved disulphide bond is shown in salmon. The residues participating in the dimer contacts are shown as sticks. The figure was prepared with PyMOL (DeLano, W. L. (2002) *PyMOL*, DeLano Scientific, San Carlos, CA).