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Pathways of electron transfer and proton translocation in the action of superoxide dismutase dimer

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ABSTRACT

Superoxide dismutase, known to gain large rate enhancement on dimerization, forms a homodimer stabilized by hydrogen bonding between a number of internal water molecules and a few amino acid residues at the interface. Within each subunit the β-sheets provide a sequence of delocalized π-electron units of peptide bonds alternating with hydrogen bonds referred as π-H pathway. These pathways in the two subunits in the dimer are interlinked through a chain of four water molecules bridged by hydrogen bonds at the interface. Connecting the two Cu-centers this π-H pathway can enable rapid electron transfer from one superoxide molecule to the other, crucial for the catalytic reaction and the high rate in the dimer. A proton relay of hydrogen-bonded water molecules in the dimer translocates protons to form the product, hydrogen peroxide.

1. Introduction

The first catalytic activity of erythrocuprein, the copper protein isolated from erythrocytes in 1938 by Mann and Keilin [1] was identified as dismutation of superoxide in 1969 by McCord and Fridovich [2]. The basic action of the enzyme is understood as the donor superoxide (O—O $^{\bullet}$) donates its electron to Cu^{++}, itself becoming O=O (dioxygen), and the reduced Cu^{+} then transfers the electron to the acceptor O—O $^{\bullet}$ forming O—O that yields H_2O_2 on protonation. Renamed superoxide dismutase (SOD), it had a great impact in the area of oxygen radicals in biology ever since.

Early studies had discovered other functions of this protein such as inhibition of autoxidation of catechol compounds, norepinephrine [3], 6-hydroxydopamine [4] and pyrogallol [5]. Similarly reversal of autoxidation of some ortho-quinols (benzo- and naphtho-) [6] indicated that the acceptors are the corresponding semiquinones [$^{\bullet}$O—R—O^{-}]. This effect was recognized and interpreted later as the action of SOD of reversing the first step of electron transfer between dioxygen and a phenolate producing two oxygen radical species, superoxide and phenolate radical [7]. Then the decrease in the rate of autoxidation represents the rate of SOD-catalyzed back reaction, implicitly saving the bioactive catechol substrate. Crane and co-workers discovered the SOD-concentration-dependent inhibition of vanadate-mediated H_2O_2-generating oxidation of NADH by O_2 catalyzed by the plasma membrane enzyme system [8]. This NADH oxidation system involves the first electron transfer reaction between NADH and V^{V} (a diperoxovanadate complex) producing NAD$^{\bullet}$ and a reduced form of vanadium (V^{IV}), both radical species, as in the case of pyrogallol autoxidation [7]. It then became evident that SOD can dismutate other radical species besides superoxide, essentially nullifying autoxidation reactions [9]. The (Cu—Zn)-SOD protein is also capable of oxidizing nitroxyl anions (NO^{-}) to nitric oxide ($^{\bullet}$NO) [10] and cysteine-SH to cysteine-S$^{\bullet}$ followed by dimerization to cystine (-S-S-) [11]. A new perspective emerged from these findings that this ubiquitous copper, zinc-protein, possesses functions besides dismutation of superoxide [12]. These functions essentially depend on electron transfer at the catalytic site, typically by two half reactions, initial reduction of Cu^{++} by donors followed by reoxidation of Cu^{+} by acceptors, subsumed to be in sequence at the same active site Cu center.

Superoxide dismutase occurs as monomeric 16 kDa protein with one atom each of Cu and Zn, and a characteristic protein fold

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described as Greek-key β-barrel tertiary structure [13–15]. Two subunits of the SOD protein form a stable homodimer that includes a number of internal water molecules "associated through unusually strong noncovalent interactions" [14]. Dimerization of the protein enhances its catalytic activity by several-fold. The two Cu sites situated near the surface in each subunit are separated in the dimer by over 30 Å, interspersed by "a flattened eight-stranded antiparallel" β-sheets [16]. This feature provides a stretch of peptide units alternating with hydrogen bonds, known as π-H pathway [17], suitable for electron transfer as the bridging element between delocalized electrons is the hydrogen bond capable of transferring electrons [18,19]. Found in electron transfer proteins, these π-H pathways are employed thus in between cytochrome *c* - heme-Fe and cytochrome oxidase - CuA center [20] and between CuA and metal centers in cytochrome oxidase [21]. Extensive hydrogen-bonded water channel, known to support a proton relay and translocation [22], purportedly includes a rate-contributing step [23] in the formation or removal of the product H_2O_2.

The two superoxide molecules needed for the dismutation reaction interact with the enzyme, albeit differently. It was reported that superoxide, cannot bind to the enzyme protein in the absence of Arg141, donates its electron to Cu^{2+} and exits as dioxygen. It can form a stable complex with the enzyme and Arg141 at the Cu-center [24]. Simultaneously, the bridge of Cu-His61-Zn breaks [25,26]. The Cu^{+} atom moves 1.7 Å [25] towards Arg141 and the zinc center. His61 residue, now free, acquires a proton (His61-$N^{\varepsilon}H$) used later for protonation of the substrate. Known as the two half reactions, the reduction of Cu and its reoxidation might occur in the two Cu-centers in the dimer with the electron rapidly transferring between them.

In this communication, we describe a π-H pathway assembled across the β-sheets from both subunits in the SOD-dimer protein connecting the two Cu-centers and thus the two half-reactions. In addition, water channel/proton relay is also tracked between the histidine residues/Cu-centers in the dimer protein.

2. Methods

Crystal structure of bovine superoxide dismutase (PDB ID: 1Q0E) was analyzed using pymol software for 'Hydrogen Bonds' starting from Cu and Zn centers of both the subunits by locating residues below 4 Å distance and marking the polar atoms, including the water molecules, which are within the distance of 2.6–3.3 Å. Whenever more than one hydrogen bond is encountered the short and/or the strong (in terms of distance and the most favored angle between the donor and acceptor) one is selected.

A chain of alternate peptide units and hydrogen bonds across β-sheets, as in α-helix, is found between the two Cu-centers in the dimer of SOD. Referred as 'π-H pathway', it can rapidly mobilize electrons from one delocalized peptide unit (π-electron cloud) to another of the β-sheets enabled by bridged hydrogen bonds, now found experimentally to transfer electrons [18,19]. Internal water molecules do invariably form part of these pathways, and the pathway using smaller number is selected.

Based on clues from the D-path of cytochrome oxidase [21], Asp-carboxyl groups and a peptide flip of glycine residue (lacking side chain) are included in a chain of large number of water molecules to form the proton relay in translocating protons to the substrate [23].

3. Results

The subunits, designated A and B, are bridged by hydrogen bonds of several water molecules and a few peptide bonds at the interface (Fig. 1). It is noteworthy that the whole cross-section of the interface is studded, across and along, with multiple hydrogen-bonded water molecules. These apparently give unusual stability to the homodimer. Out of sixteen peptide units found in the interface area, the pairs Ile149 (HN–C=O) Gly112 and Ile149 (O=C–NH) Gly49 from each subunit contribute four inter-subunit hydrogen bonds. Other peptide units form hydrogen bonds with either interface water molecules or with polar side chains of the subunits. Remarkably, the flattened eight-stranded antiparallel β-sheets [16] of each subunit provides a string of peptide units bridged by hydrogen bonds. Six strings of these arising from water molecules in each subunit from opposite direction merge in the interface water pool. Combined, all these interactions contribute to the extraordinary stability of the dimer.

In addition, β-sheets are found to provide two additional pathways of alternating delocalized electron units (peptide bonds) bridged by hydrogen bonds as in cytochrome *c* and cytochrome oxidase [20,21]. Bridged by four hydrogen-bonded water molecules at the interface, one such π-H pathway (131 Å-long) links the two Cu centers (33.5 Å) in the dimer of SOD (Fig. 2) and enables rapid electron transfer between them.

Using the structures and metal centers described above, the likely events after the substrate molecules reach the dimer are given below. One molecule of superoxide transfers its electron to reduce Cu^{2+} to Cu^{+} at a Cu-center (subunit A) and dissociates as dioxygen (first half of the reaction). The connecting π-H pathway rapidly transfers the electron to the other Cu-center (subunit B). In the reduced state, Cu^{+} can only donate its electron to the second molecule of superoxide (or any of the acceptor substrates). Because of the "increased proton affinity" in the reduced protein [24], superoxide is protonated to form HO–$O^{\bullet}$ and is retained in the stable complex in subunit B. Thus prepared, the substrate molecule can receive the crucial second electron from Cu^{+} followed by another protonation to form H_2O_2 (second half of the reaction). Thus the two subunits in the dimer perform the two half-reactions with the connecting π-H pathway relocating the electron from one molecule of the substrate to the other, the core reaction of the enzyme. These electron paths start or end with a metal center (Cu). By linking the two Cu-centers, this π-H pathway ensures rapid electron transfer in the dimer. This explains the rate enhancement. It is self-evident that the architecture of interior β-sheets of the dimer protein makes this possible.

The proton relay of the water chain is also shown to be present in the dimer protein (Fig. 3). It starts from His61-N^{ε}H-N^{ε}H of subunit A and reaches His-coordinated Cu in subunit B (96.5 Å) passing through 26 water molecules, Asp96 and Asp122 residues (carboxyl groups), and a peptide unit Leu82-Gly83. The residues that form proton path are present in the both subunits but only one set forms the path but not the other (hydrogen bond network absent) (Fig. 3). The transfer of an electron and a proton from the subunit A to the substrate bound in the subunit B completes the dismutase reaction.

4. Discussion

Dimerization of the SOD protein confers enormous rate enhancement. It occurred to us that the dimer protein might assemble a link π-H pathway for directional, rapid electron transfer between the two Cu-centers in the two subunits, one acting as the acceptor of an electron from the first superoxide anion, which becomes dioxygen, and the other as the donor of the electron to the second superoxide anion, converting it to hydrogen peroxide on protonation – the two recognized half-reactions. Such a link π-H pathway, made possible by dimerization, is indeed found to be present in this study. An advantage of such a defined pathway is that an electron that enters one Cu-center rapidly reaches other Cu-center. The calculated high electron transfer frequency (ν) of 1.5×10^{11} per sec for a dipeptide and enhancing further on

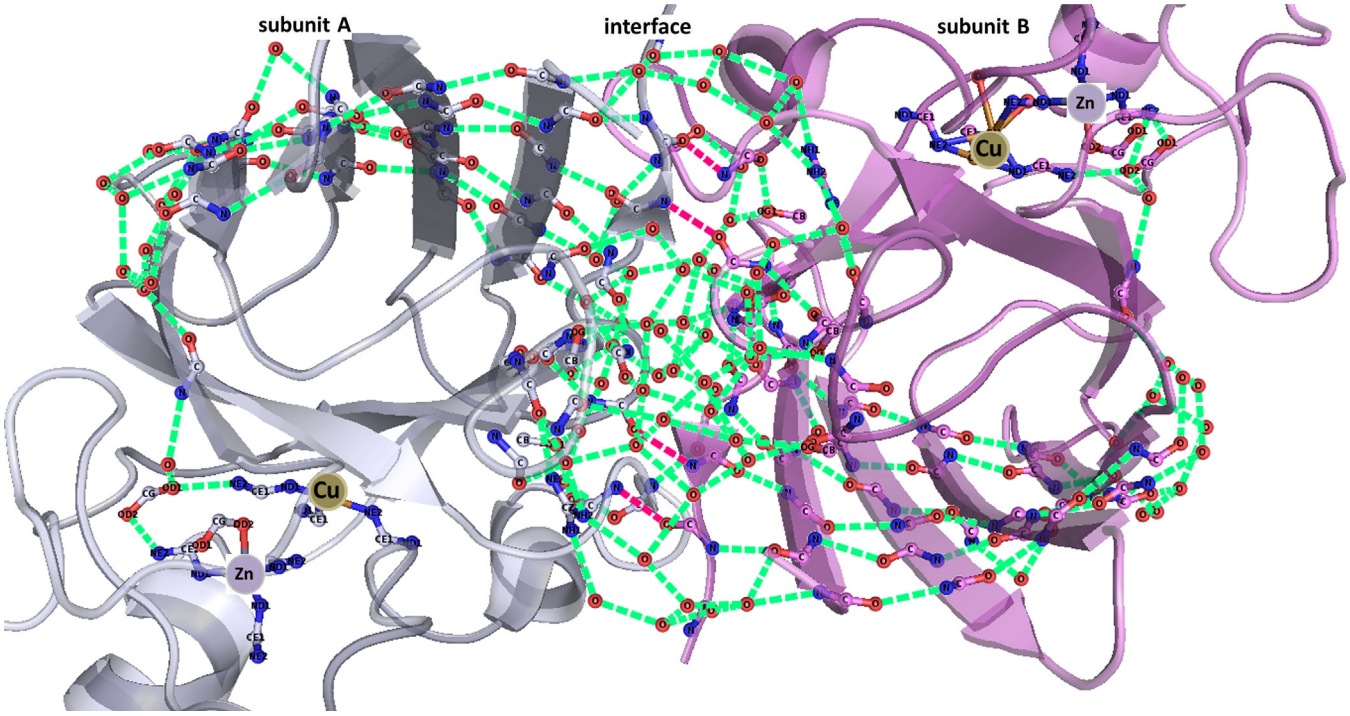

Fig. 1. The dimer of superoxide dismutase. Two subunits of superoxide dismutase protein are bridged by hydrogen bonds of multiple water molecules, peptide units and polar amino acid residues at the interface forming a stable homodimer. Six strings of peptide units bridged by hydrogen bonds formed across the β-sheets in each subunit merge in the water pool at the interface and give additional stability to the dimer. [Subunits A (light blue) and B (pink) are shown only as folded backbone. The atoms are identified (same in all figures) as colored spheres: oxygen (red), nitrogen (blue), carbon (light gray), copper (light green sphere) and zinc (white sphere). Hydrogen bonds are shown as green broken lines; only those between peptide units in the water pool at the interface are shown in purple]. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

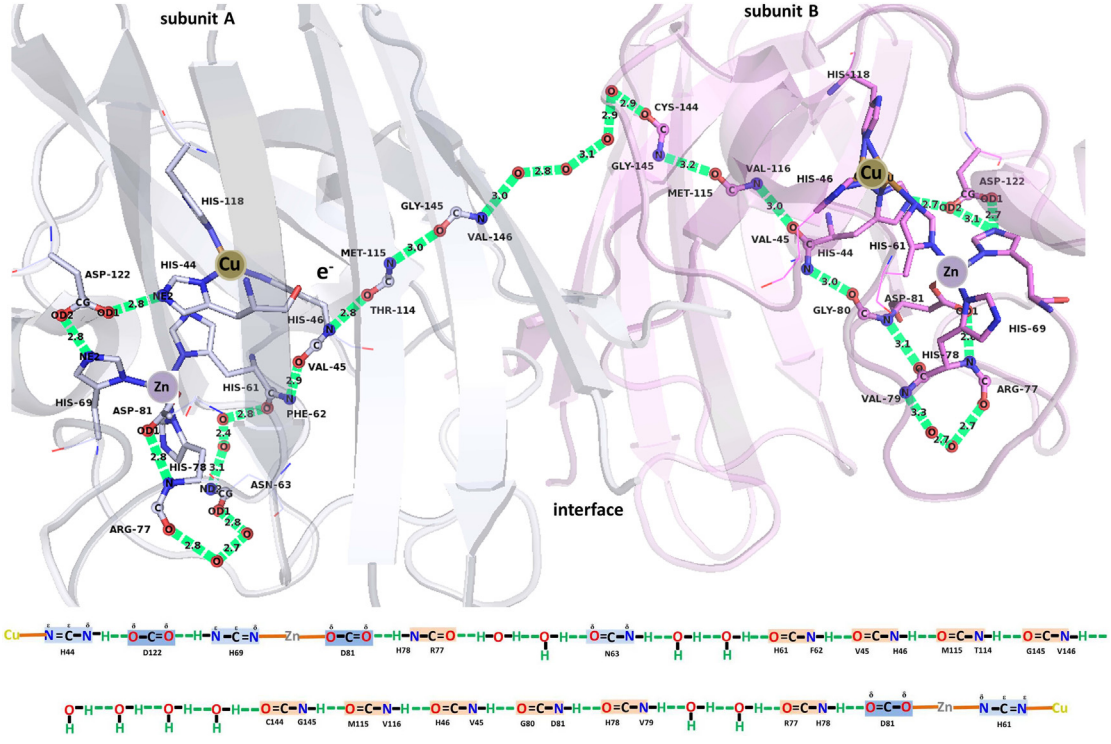

Fig. 2. The π-H pathway between the Cu-centers in superoxide dismutase dimer. The pathway is assembled by the alternating peptide bond and hydrogen bond sequence available over the β-sheets between the Cu-centers. The oxygen atoms over the Cu atom on subunit B, shown in the crystal structure, represent two water-oxygen molecules in the place of the substrate, superoxide. The same pathway is represented below on atom to atom basis.

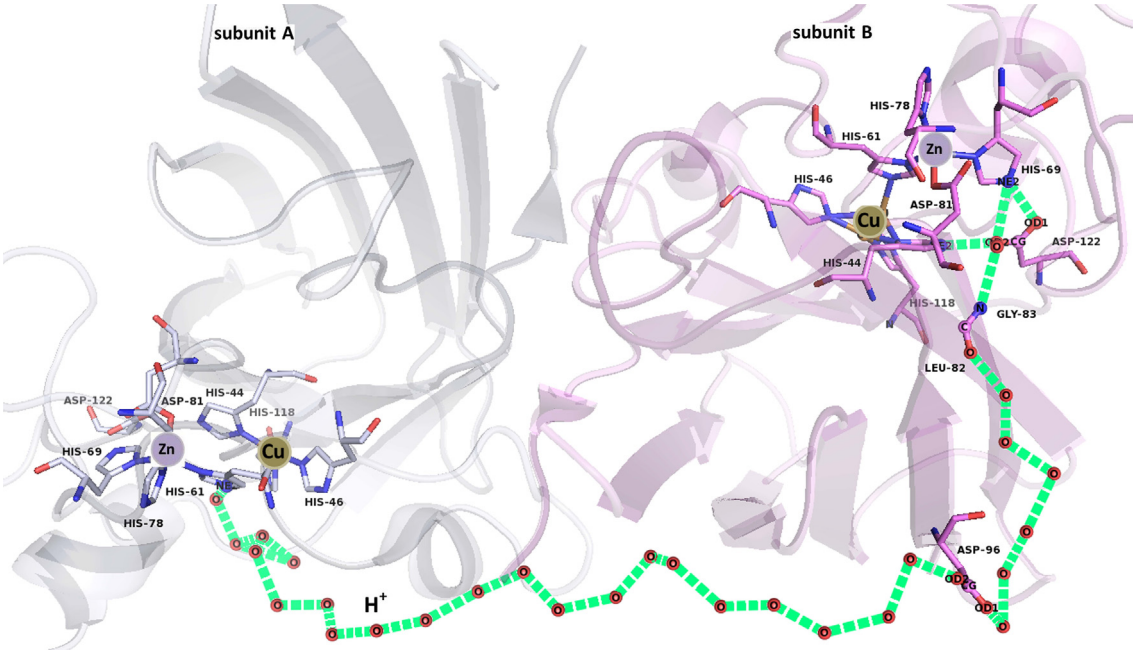

Fig. 3. The water chain and proton relay in superoxide dismutase dimer. A chain of water molecules bridged by hydrogen bonds connect the histidine residues at the Cu–Zn centers in the dimer. Note the inclusion of two aspartate residues and a peptide bond of glycine-leucine needed for completing the path.

displacement of the connecting hydrogen atom in the bond [27] is likely to suffice for the observed rate enhancements.

SOD protein folded in the absence of zinc is without activity [28]. Catalytic activity needs both the constituent metals, Cu and Zn. It is noted that the π-H pathway linking the two Cu-centers, essential for the electron transfer activity, passes through Zn-center.

Protons needed for forming the product, H_2O_2, seem to derive from Arg141 and His61 within the active site of the protein and not directly from the medium. The first one is added to superoxide from an arginine residue preparing it to bind at the electron donor Cu-center, and the second one is derived from distant His61-N$^{\varepsilon}$H (released from the Cu–Zn complex on reduction by superoxide) through a long proton relay aided by hydrogen-bridged water-chain. Importance of the proton translocation is underscored by its rate-control [23] possibly in releasing the product.

It is worthy of note that another Cu–Zn protein, cytochrome *c* oxidase, also employed similar pathways between Cu-centers for transfer of electrons as well as translocation of protons to form the basis of reduction of dioxygen to water molecules [20,21]. Electron transfer through a π-H pathway across the bulk of the protein using the four protein structural features is the core reaction in the vignette that emerged from these studies. It dawned upon us that these enzymes may gain large rate enhancement by using rapid electron transfer through the inbuilt π-H pathways generated by the secondary structure, also explaining the need for a large protein.

The authors declare

"No conflict of interest".

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