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Comparative structural and conformational studies on H43R and W32F mutants of copper–zinc superoxide dismutase by molecular dynamics simulation

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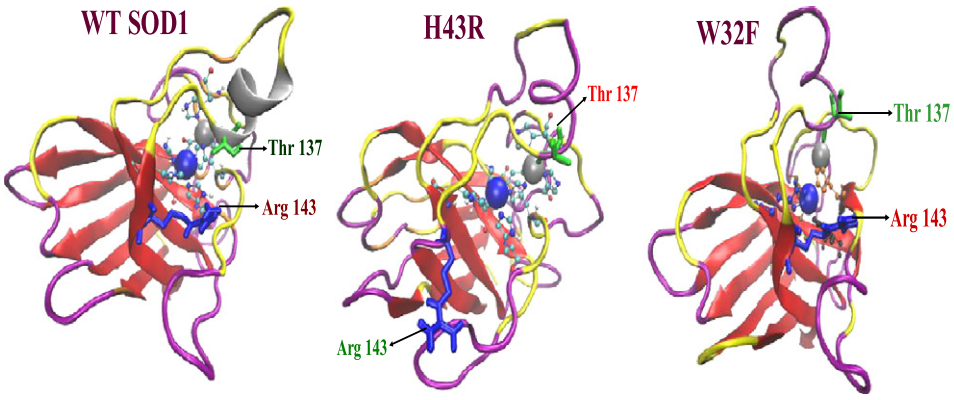
HIGHLIGHTS

- H43R exhibits greater mobility at Zn-binding and electrostatic loops.
- Dimer interface interaction in H43R is weakened compared to that of WT and W32F.
- Distances between the highly conserved amino acids increase for H43R.
- The decreased activity should be attributed to the opening of active channel in H43R.

GRAPHICAL ABSTRACT



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ABSTRACT

Recently, mutations in copper–zinc superoxide dismutase (SOD1) have been linked to familial amyotrophic lateral sclerosis (fALS), a progressive neurodegenerative disease involving motor neuron loss, paralysis and death. It is mainly due to protein misfolding and aggregation resulting from the enhanced peroxidase activity of SOD1 mutants. In this study, we have carried out a 20 ns molecular dynamics simulation for wild type (WT), H43R and W32F mutated SOD1's dimer and compared their structure and conformational properties by extracting several quantitative properties from the trajectory to understand the pathology of fALS disease. Our results show considerable differences in H43R compared to WT and W32F mutated SOD1, such as increasing distances between the critical residues results in open conformation at the active site, strong fluctuations in the important loops (Zinc and electrostatic loops) and weakening of important hydrogen bonds especially between N (His 43/Arg 43) and carbonyl oxygen (His 120) in agreement with the experimental report. The calculated buried surface area of dimer interface for WT, H43R and W32F are 682, 726 and 657 $Å^2$ respectively, representing the loss of dimerization in H43R. Essential dynamics reveal that overall motions of WT and W32F are mainly involved in three to four eigenvectors, but in H43R the overall motions are mainly in the first eigenvector. These data thus provide a unifying description for the structural destabilization, enhanced peroxidase activity, loss of dismutation activity and increase in aggregation propensity in the pathology of fALS diseases.

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1. Introduction

Copper–zinc superoxide dismutase (SOD1) is a metalloenzyme, abundantly found in the intracellular cytoplasmic space of aerobic organisms and is responsible for defending cells from oxidative damage [1]. SOD1 is a functional homodimer, (molecular weight of ca. 32 kDa) with each monomer containing one Cu(II) and Zn(II) ions that are bridged by an anionic histidinato ligand. The Cu(II) ion in SOD1 is further coordinated to three other histidine residues in a distorted square pyramidal geometry, while Zn(II) is bound to two histidine and an aspartate residue in a distorted tetrahedral geometry [2]. Each monomer of SOD1 forms an eight-stranded antiparallel β-barrel. The β-barrel of SOD1 is protected against self-aggregation by having each end of the dimer covered by well ordered electrostatic and zinc binding loops [3]. Further, these two extended loop regions form a wall for the channel formed between the surfaces of enzyme to the active site [4].

SOD1 catalyzes the disproportionation of superoxide anions into molecular oxygen and hydrogen peroxide and the widely accepted catalytic mechanism [5] involves the two following steps,

$$SOD\text{-}Cu^{2+} + O_2^{\cdot -} \text{---------} > SOD\text{-}Cu^{+} + O_2 \quad (1)$$

$$SOD\text{-}Cu^{+} + O_2^{\cdot -} + 2H^{+} \text{--------} > SOD\text{-}Cu^{2+} + H_2O_2. \quad (2)$$

Early biochemical studies, measuring the SOD1 activity in amyotrophic lateral sclerosis (ALS) patients, suggested that the reduction or the loss of enzyme activity and an increase in the peroxidase activity can be suppressed through post-translational modifications (such as the attainment of copper and zinc ions, formation of disulphide bond and dimerization) that help in the regaining of enzymatically active quaternary structure [6,7]. Mutations affecting the post-translational modifications in human SOD1 were identified as an important cause for approximately 20% of fALS [3,8–10]. The fALS-associated mutants do not essentially reduce the activity of SOD1, but gain some new activities such as peroxidase/thiol oxidase activity that might also lead to protein misfolding followed by its aggregation [11–14].

More recently, pulsed EPR and NMR spectroscopic studies were used to establish the oxidation of His 43 residue during peroxidase activity of SOD1 resulting in the loss of enzyme activity [15]. It has been found that the fALS sites are associated not only with the active site His residues, but also with the integrity of the β-barrel fold, the dimer interface, and other structural features [6]. In addition to the metal-bound His residues in the active site, His 43 is only the residue located in the vicinity of the active site, (ca. 11 Å away from the Cu (II) site) [15] positioned at an opening of the β-barrel and stabilizes the ends of the compact structure of β-barrel through its hydrophobic packing interactions with the nearby residues [16,17]. His 43 also assists with the exact orientation of the Arg 143 side chain with respect to the Cu active site by forming a hydrogen bond bridge [16,17]. Therefore, His 43 to Arg mutation in human SOD1 (H43R), enhances the disruption of the Thr39-His43-His120 hydrogen bond bridge that indeed alters the position and orientation of Arg 143 residue, resulting in a reduced stability of SOD1 protein structure leading to protein aggregation [6]. Further, H43R mutation has been identified in patients of fALS [9,10,16].

Similarly, Trp 32 is located on the surface of human SOD1 and the oxidation of Trp 32 occurs in vivo as a natural modification to SOD1 leading to peroxide-induced aggregation [18]. The enhanced peroxidase activity of human SOD1, resulting in the formation of a tryptophanyl radical by the reaction with carbonate anion radical ($CO_3^{\bullet -}$), has been found to cause protein aggregation [13,14]. Further, mutating Trp 32 with Phe (W32F) slows down the rate of oxidative modification [13,14,18] that has been found to decrease the cytotoxicity in motor neuronal cell culture model and the rate of aggregation of SOD1[14].

Information about the dynamical properties of these mutants of SOD1 is crucial in understanding their role in protein activities/aggregation on a molecular level. Since, crystal structures of SOD1 mutants give only the static behavior, molecular dynamics (MD) simulations were used as an alternative tool for probing the structural as well as conformational changes due to mutations [3]. MD simulations reveal atomic-level insights which remain inaccessible by present high resolution experimental methods, and that are expected to shed light on the unresolved issues connecting the protein's dynamics and structure to its biological activities [19]. Studies describing the structure and dynamics of WT and selectively mutated SOD1's are available [3,20–26]. More recently, Schmidlin et al. [27] performed MD simulations on A4V mutated SOD1 and demonstrated the structural changes to monomeric SOD1 but have not focused on describing the dynamical behavior of these two mutations (H43R and W32F) and their relation to protein aggregation and its stability. Indeed, this is essential in understanding the pathology of fALS diseases and therefore by using classical molecular dynamics, we address here the dynamical properties of these mutated systems and explain several properties and their relation to the protein activities/aggregation on a molecular level.

2. Computational methods

The starting co-ordinates of the WT SOD1 dimer (pdb entry 1spd) at 2.4 Å resolution were obtained from the protein data bank [28]. We have constructed H43R and W32F mutants using the interactive computer graphics package Swiss pdb viewer v 3.6 [29] by substituting His 43 residue with Arg and Trp 32 residue with Phe in the WT crystal structure. Molecular dynamics (MD) simulations were carried out using the GROMACS 4.5.3 [30] package using GROMOS96 [31] 43a1 force field for all residues and metal ions. The protonation state was treated with the help of optimal H-bonding conformation. The standard GROMOS96 charges were used for all the atoms except for the residues of the active site, for which the partial atomic charges calculated from ab initio methods reported in Ref. [32] were employed. Each structure was fully solvated with SPC cubic water in a box with side's 6.939 nm × 4.719 nm × 4.190 nm with box angles 90° for each side. The whole dimeric enzyme of WT, H43R and W32F mutated SOD1's was embedded in 22639, 22642 and 22647 water molecules respectively.

The steepest descent algorithm was used for energy minimization and the maximum step size for energy minimization considered was 0.01 nm. The tolerance used in this simulation was 6000 kJ/mol/nm. The WT protein and W32F mutant both have a net charge of −5 e, while the H43R mutant has a net charge of −4 e. Precise simulation of protein dynamics needs the inclusion in the model of, at least, a neutralizing counter ion's atmosphere, therefore in this work, five and four Na^+ ions were added by replacing solvent molecules with the highest electrostatic potential for WT, W32F and H43R mutants respectively, thus the investigated proteins have a net charge of zero. Initially, position-restrained MD was carried out for 200 ps which restrains the position of atoms in the protein and allows the distribution of solvent in the system. The LINCS algorithm was used to constrain all bonds [33] and SETTLE algorithm [34] for solvent distribution.

In our simulations, the temperature was maintained at 300 K by weak coupling to an external temperature bath and the simulation is continued for 20 ns (including the equilibration steps) for each WT, H43R and W32F mutated SOD1's. A time step of 2 fs was used and the data were collected every 500 fs. A twin range cut-off was used for the calculation of the non-bonded interactions. The short range cut-off radius was set to 0.9 nm and the long range cut-off radius was set to 1.4 nm for both columbic and Lennard–Jones interactions. The electrostatic interaction was handled by the particle-mesh Ewald (PME) method [35]. The distances measured at the active site metals after 20 ns of MD simulations are shown in Supplemental Table S1. It reveals that the geometry of the active site is maintained, and shows that the set of parameters employed for the metal ions and their ligands are reliable for studying the dynamical behavior of this molecule.