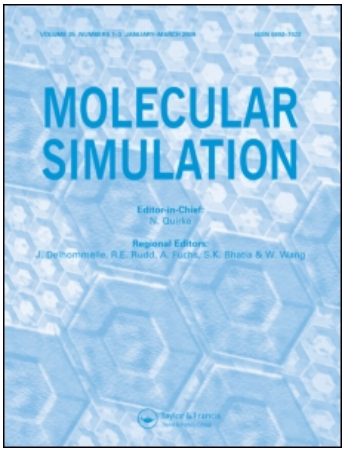

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Structural investigation of human wild-type Cu, Zn superoxide dismutase - a biomolecular case study using DL_POLY_3

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Structural investigation of human wild-type Cu, Zn superoxide dismutase – a biomolecular case study using DL_POLY_3

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This article demonstrates the capability of DL_POLY_3 to study protein molecules at long time scales. The example shown here is the study of early stages of structural destabilisation of human superoxide dismutase (SOD1) as a result of metal depletions. Simulations of up to 4 ns have been carried out on wild-type SOD1. It is shown that the removal of metal ions from the active site essentially destabilises loop structures and provides a potential precursor for unintended protein–protein interactions to take place, as observed experimentally. The results reveal early stages of the destabilisation of structures that may enhance subsequent protein aggregations. It is found that the removal of Zn ion at the active sites of the protein enzyme causes a disruption to the Zn-loop and subsequently the electrostatic-loop (e-loops) with an increase in flexibility and mobility of the structures. The Zn ion is important to maintain structural integrity of the channel loops, whereas the Cu ions apparently play a lesser role in this aspect.

Keywords: Superoxide dismutase; Molecular dynamics; Protein unfolding; Amyotrophic lateral sclerosis; DL_POLY_3

1. Introduction

The implementation of the domain decomposition parallelization technique in DL_POLY_3 enables molecular dynamics (MD) simulations of system sizes larger than 30,000 particles to be efficiently modelled on massively parallel supercomputers [1]. This general-purpose MD program suite has been used to simulate a variety of molecular systems in the areas of material science and condense matter. However, at the time of writing, it is still not common to simulate large and complex biomolecular systems, even though the DL_POLY_3 contains a wide-range of simulation features that are ideally suited to model large and complex biomolecules. The purpose of this article is to demonstrate the capabilities of DL_POLY_3, which can be harnessed in the area of biomolecular modelling, by making reference to our studies of Cu, Zn superoxide dismutase.

The Cu, Zn superoxide dismutase (SOD1) is a bimetallic protein enzyme that catalyses the dismutation reaction of toxic superoxide radical anion to hydrogen peroxide via the cyclic reduction and reoxidation of copper [2]. The enzyme is functionally active as a homodimer orientated by an approximate 2-fold axis along the dimeric interface, containing one Cu and one Zn per subunit monomer. The overall SOD1 structure in ribbon form is shown in figure 1. Each subunit folds into an eight-stranded antiparallel β barrel connected by three external-loops. Two of these loops, the Zn-loop and the electrostatic-loop (e-loop), constitute the channel that guides peroxide substrates from the protein surface to the active sites. The Zn-loop contains a Zn cation which maintains the structural integrity of the loop, whereas, the e-loop contains several charge residues that are involved in the electronic guidance of the peroxide substrate to the active site and thus contribute to the high specificity of the SOD1 catalytic behaviour. Collectively, these two loops are called the channel loops. At the active site, the Cu(II) is coordinated by four histidine residues: H46, H48, H63 and H120. The Zn(II) is coordinated by three histidine residues and an aspartate—H63, H71, H80 and D83—forming an approximate tetrahedral geometry. Both metal ions share the same (bridged) imidazole ring of H63. However, the Cu–H63 bridge is broken when it is reduced to Cu(I), leaving an approximate trigonal planar Cu-coordination [3,4].

It is known that mutation of the SOD1 gene encoding the enzyme causes about 20% of the familial cases of the

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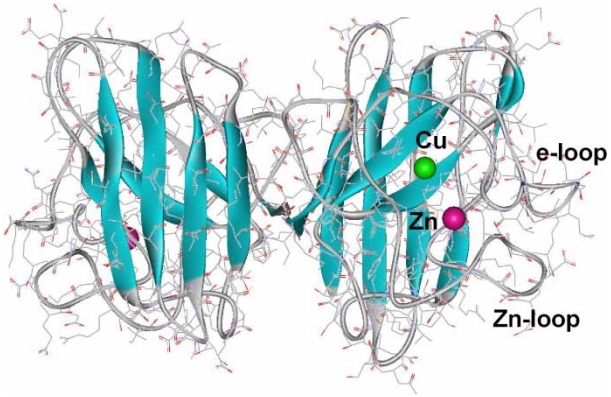

Figure 1. Atomic configuration of a wild-type holo-SOD1. The molecule consists of an approximate 2-fold symmetry dimer and each monomer folds into eight-stranded antiparallel beta barrel connected by three external loops. Only two loops are indicated, the Zn-loop and the e-loop, which together form the channel loop for that guides peroxide substrates from the protein surface to the active sites.

progressive amyotrophic lateral sclerosis (ALS), a neurodegenerative disease [5] that may lead to death within 5 years. The pathogenic mutations often involve only a single amino acid residue, of which more than a 100 distinct single-site mutations have been identified. Unfortunately, the aberrant biological roles of these mutations and their precise pathological contributions to the disease are still not known, let alone an effective treatment to cure the disease. An obvious explanation of the disease is dysfunctional enzymatic activity. However, there is a growing body of evidence suggesting that it is the change in molecular structure in the absence of metals which leads to the gain of toxic behaviour via misfolding, unfolding and aggregation. More remarkably, it was found that wild-type SOD1 devoid of metals can form aggregates which have a similarity to the structures seen for other pathogenic mutants [6]. This important finding leads to a possible conclusion that these mutations may induce a more labile metal binding and a less stable protein than the wild-type metallated protein, resulting a protein that is more prone to aggregation.

The protein X-ray crystallographic technique [7] has been used successfully to investigate, down to atomistic levels, the structure of mutants and aggregates over many years. However, the underlying atomic mechanisms leading to protein destabilisation have to be inferred from these static studies in crystalline forms and extrapolated to aqueous, physiological conditions. To this end, molecular simulation techniques offer a crucial complement to experimental studies to aid the understanding of the molecular basis of protein destabilisation as a result of loss of metals. Answer to these questions are penultimate to the construction of a clearer picture of the abnormal structural behaviour of mutant SOD1 and the provision of a better understanding of pathogenesis in familial ALS.

Our investigation involves the study of protein structural destabilisation and at least the early stages of unfolding and misfolding. This poses difficult challenges to molecular simulation, since these processes often involve long time scales. The DL_POLY_3 program was chosen to take on such challenges to identify the sign of early misfolding resulting from metal depletion. The program code is designed for a variety of stand-alone and parallel platforms. On the latter platforms, the communication bottle-necks between the CPUs are greatly reduced by using the domain decomposition technique, through which up to a 1000 CPU nodes can be used efficiently [1]. This is in contrast to DL_POLY_2 which uses an entirely different parallelization technique, namely, the replicated data [8], which is more efficient for smaller model systems and using processor node numbers of perhaps up to 64.

In this work we consider two fully solvated wild-type SOD1 models: the fully-metallated (holo model) and SOD1 that is completely devoid of metals (apo model). The MD simulations were carried out on the HPCx, a national computing facility based on IBM Power5 processors, located at Daresbury Laboratory and maintained by the HPCx Consortium [9].

2. Simulation model

Unlike many material simulations where the models often consist of a crystalline structure, which can be easily generated from computer programs, in the case of complex biomolecules such as SOD1, the starting structures are often derived from experimental sources such as X-ray protein crystallography. These structures are usually available from the Protein Data Bank (PDB) [10]. High resolution structures will give more precise positions of each individual atom in the molecule and thus keeping speculation to a minimum. In our case we used the crystal structure of copper-reduced holo wild-type SOD1 at atomic resolution of 1.15 Å [11] as our starting configuration.

The next stage is to convert the PDB structure into a configuration that is recognizable by the DL_POLY_3 program (the CONFIG file), and the corresponding force field to describe the atomic interactions (the FIELD file). In our simulation we used standard all-atom CHARMM22 force field [12]. Unfortunately, unlike most other molecular simulation models, manual preparations of FIELD files for large biomolecular systems are very complex and can be impractical. Furthermore, there is yet a user-friendly interface program available for DL_POLY to achieve this. However, conversions can be carried out rather straightforwardly in two stages. Firstly, an input script was run in the CHARMM program environment [13] to read the PDB file and produce the CHARMM's protein structure file and system coordinate file. Secondly, using these output files and CHARMM22's protein parameter and topology files, the DL_POLY's FIELD and CONFIG files were produced using the ChemShell script [14] developed at Daresbury Laboratory. ChemShell is a computational chemistry script driven system based on the Tcl interpreter. It is modular-based software that

integrates various modeling software packages including DL_POLY and CHARMM.

Two fully solvated molecular models have been considered: the fully-metallated (holo model) and SOD1 that is completely devoid of metals (apo model). A dimer molecule (consisting of monomer A and B) was selected and placed at the center of the simulation box of size $70 \times 70 \times 89$ Å with all the original water of crystallisation retained. Additional water molecules were added into the box to give the bulk density of the water at approximately 1.0 g/cm^3. This gave a total of about 45,000 atoms in a system. Long-range interactions were calculated using smoothed particle mesh Ewald method [15] and the water molecules were represented by the TIP3P model [16]. All atomic bonds of SOD1 were represented by flexible harmonic springs. Both the electrostatic real space cut-off and short-range interaction cut-off was set to 8.0 Å.

In the case of the holo model, the geometries of the active sites were maintained by having the metal bonded covalently to the ligating amino acid residues. Additional three body and four body bond angle and dihedral interactions were also introduced to maintain the correct geometrical orientations of the active sites. Charges on metal ions were estimated from *ab initio* gas phase cluster calculations involving all ligating residues at Hartree-Fock level using 6-31G* basis set. This gave charge values of +1.4e and +0.6e for Zn and Cu ions, respectively. Of note is that, in our model, the copper was in an activated form with oxidation state of +1, as derived from the crystal structure, and bonded to three ligating histidine residues, instead of four in normal oxidised state of Cu(II).

A fixed timestep of 1.4 fs was used to update the tracjectories. Initially, the system was equilibrated in the NVE ensemble for 160 ps. It was then switched to the NPT ensemble, with the temperature and pressure coupling constants set at 0.4 and 10.0 ps, respectively, using the Nosé-Hoover formalism [17]. A low temperature of 150 K was set and this was gradually increased to 300 K over 300 ps at 1 atmospheric pressure. A further 300 ps simulation was carried out for equilibration before the configurational structures were extracted every 0.2 ps for analysis over 4 ns.

The apo model was derived from the holo model by simply removing the metal ions and configurations were extracted without further equilibration. In this case, the MD simulation essentially tracked atomistically the structural changes the moment metal ions were removed. Sodium counter cations were added in both models to maintain the neutrality of the system. These ions were added such that they were far apart from one another and from the protein molecule.

According to the DL_POLY_3, the domain decomposition parallelization scheme [1] works by dividing simulation box into geometric domains, each of which is further divided into subcells (link cells), of which the width must not be smaller than the radius of the cut-off applied in the potential energy and force calculations. Hence, combinations of system model size and the cut-off value are crucial factors that determine the maximum number of CPU nodes that can be used in a simulation. In this case, we set the cut-off to 8.0 Å for simulation box size of $70 \times 70 \times 89$ Å and this allows up to a maximum of 32 processor nodes for a simulation. Generally speaking, a larger system size allows more processors to be used and DL_POLY_3 has been demonstrated to work efficiently for as many as 1024 processors on the HPCx system [9]. However, the easier way to increase the number of maximum processors' utilization is to reduce the cut-off value for the long-range real space calculation. For example, for our model, reducing the cut-off value from 10 to 8 Å allows maximum CPU nodes to increase from 16 to 32, although this will also increase slightly the computational overheads to calculate the reciprocal space calculations. Obviously, care must be exercised to ensure the accuracy of the Ewald sum does not suffer.

The current simulation models therefore indicate the lower limit for the parallelization scheme to work efficiently in DL_POLY_3. This scheme is capable to run up to 14,000 time steps in one hour (wall clock time) on the HPCx system. Even then, we found that there is still almost four times reduction in computational time when compared with DL_POLY_2 using the same number of processor nodes.

3. Results

The radius of gyration, R_g, was calculated in order to measure the overall size of a chosen group of atoms within a molecule. The quantity is defined as

$$R_g = \sqrt{\frac{\sum m_i c_i^2}{M}} \quad c_i = r_i - \bar{m} \quad \bar{m} = \frac{1}{M}\sum_i m_i r_i \qquad (1)$$

where m_i is the mass of atom i, r_i is the position vector of atom i, M is the total mass of the molecule group and $\bar{m}$ is the centre of mass.

In order to quantify the shape of a molecular group, the asphericity, A, was calculated, defined as

$$A_S = \frac{\sum_{i>j}^{3} (\lambda_i^2 - \lambda_j^2)^2}{2(\sum_{i=1}^{3} \lambda_i^2)^2} \qquad (2)$$

where λ is the eigenvalue of the radius of gyration tensor. The value of A_S falls between 1 and 0. When $A_S = 0$ (where all three eigenvalues are equal), the shape is a perfect sphere and when $A_S = 1$ the object is a rod. All other values give a measure of the shape tending towards one of these extremes.

The shapes and sizes of the main monomer bodies for holo-SOD1, excluding the channel loops, are shown in figure 2(a) as functions of time. The radius of gyration of each subunit of the holo-enzyme dimer remains steady during the simulation, at about 13.8 Å, which is comparable to the theoretical value based on the crystal

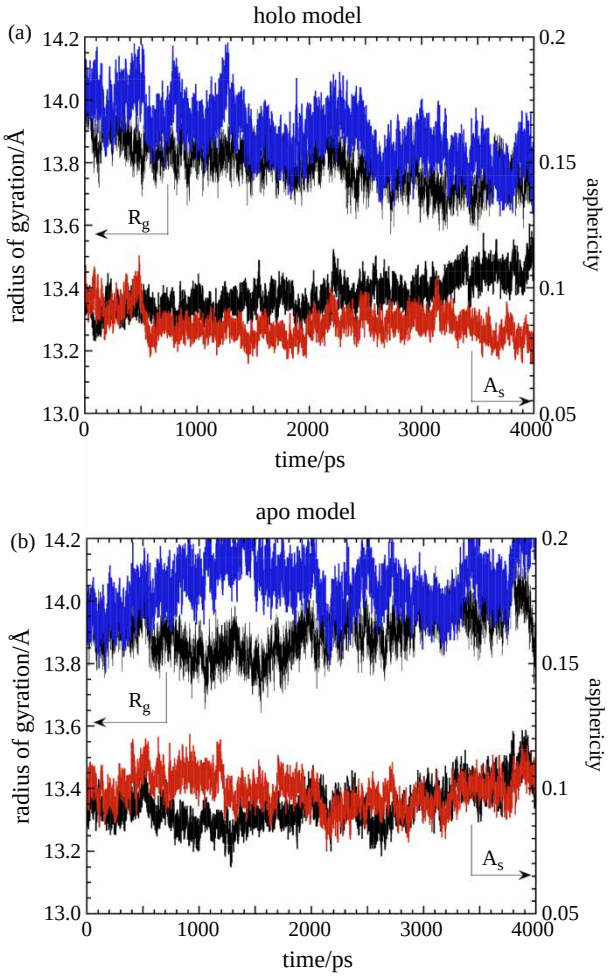

Figure 2. The variation of size and shape of the two subunits of the SOD1 dimer excluding the channel loops. The time profile for subunit A is shown by a black line while that for subunit B is shown by dark grey (indicating R_g) and light grey (indicating A_s); blue and red respectively in online version. (a) Results for the holo-SOD1, (b) Results for the apo-SOD1.

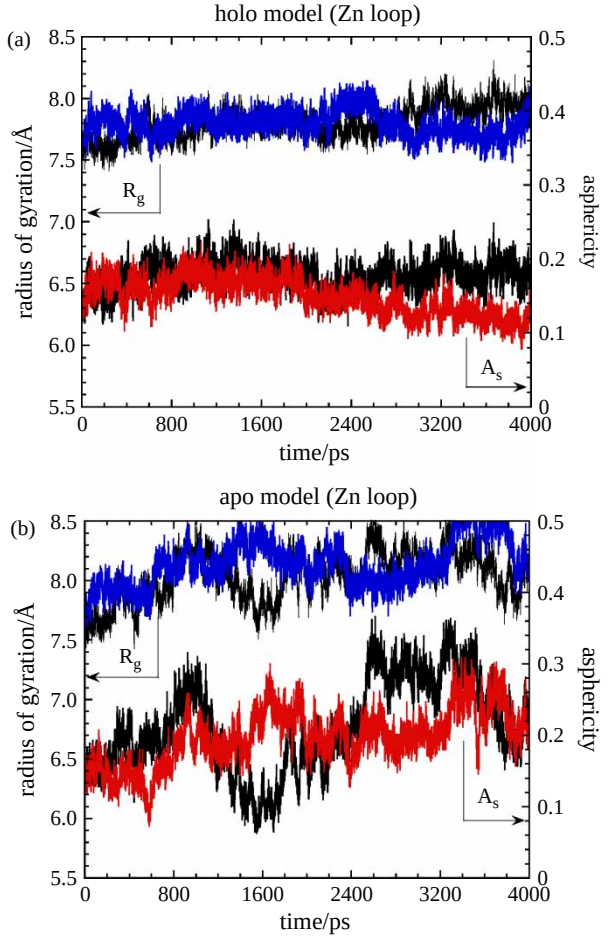

Figure 3. The variation of size and shape of the Zn-loops on two subunits of the SOD1. The time profile for subunit A is shown by a black line while that for subunit B is shown by dark grey (indicating R_g) and light grey (indicating A_s); blue and red respectively in online version. (a) Results for the holo-SOD1, (b) Results for the apo-SOD1.

structure, while the asphericity stays close to 0.095, a near spherical shape, as shown in figure 1. The general constancy of the shape and size of the main monomer body of the holo-dimer over the course of the simulation suggests that the system is sufficiently equilibrated within the simulation time scale of the calculation and that all the β-strands are conserved. However, for the apo-SOD1, figure 2(b), shows more significant change of size but not the shape. There is also evidence for asymmetry of R_g in the behaviour of the two subunits of the apo-SOD1 and the sizes were generally larger (by up to about 0.4 Å) when compared with the holo model. This shows that the effect of metal depletion does not localise around the active sites and has a far-reaching structural perturbation over the whole protein monomer body. In addition, all the β-strands in the apo molecule were also found to be largely conserved.

The Zn- and e-loops of the holo-SOD1 (shown in figures 3(a) and 4(a)) show a similar, stable behaviour. Although these regions of the structure are more flexible than the main monomer body, there are no significant size and shape variations, an indication of the structural stability nature of the loops. On the contrary, an overall increase in A_S is observed for the Zn- (figure 3(b)) and e-loops (figure 4(b)) of both apo subunits, indicating that they are elongated relative to their conformation in the holo-SOD1. The Zn-loops of the apo-SOD1 also show a more asymmetric variation of R_g compared to the holo-SOD1, while the e-loop of one of the apo-subunit is significantly elongated.

Although e-loops do not associate directly with metal ions, the results show that removal of metal ions had a direct consequence on the electrostatic loops (e-loops). The holo case demonstrated stabilisation of the e-loops in the presence of stable Zn-loops. Whereas, the e-loops for the apo case were expanded and elongated over time. This highlights the importance of Zn cations, not only to preserve the structure of Zn-loops, but also indirectly the e-loops. The less constrained e-loops with large increases in freedom of movement thus indicate the potential source for unexpected protein–protein interactions as observed in the experiment studies involving both mutant and

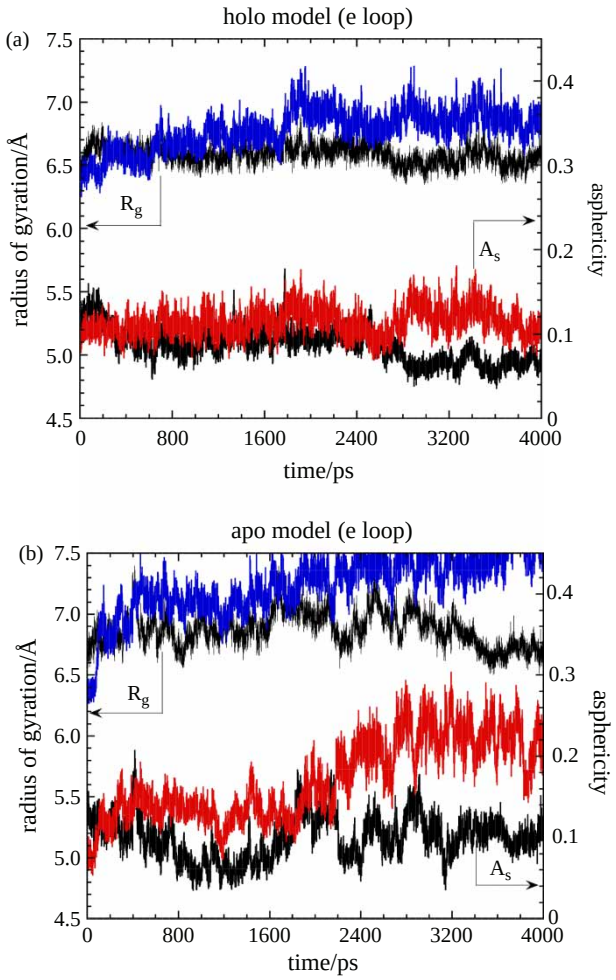

Figure 4. The variation of size and shape of the e-loops on two subunits of the SOD1. The time profile for subunit A is shown by a black line while that for subunit B is shown by dark grey (indicating R_g) and light grey (indicating A_s); blue and red respectively in online version. (a) Results for the holo-SOD1, (b) Results for the apo-SOD1.

wild-type species [6,18]. Furthermore, the aggregation may also further enhanced by the fact that structural disruptions to the channel loops also diminish the protective roles to the β-strands beneath them [11].

4. Discussion

In this work, computer simulation shows that the overall wild-type SOD1 molecular structure is preserved. However, the greatest structural change when the metal ions are removed is due to the channel loops, as confirmed by the experimental studies. Unfortunately, these disordered structures are not resolved in X-ray analysis and are identified as amorphous regions in the apo crystal samples. The MD simulations are able to reveal their atomistic details and track mechanistically the structural evolution of these loops. The MD technique is therefore an important complimentary tool to X-ray crystallography in the study of structural biology.

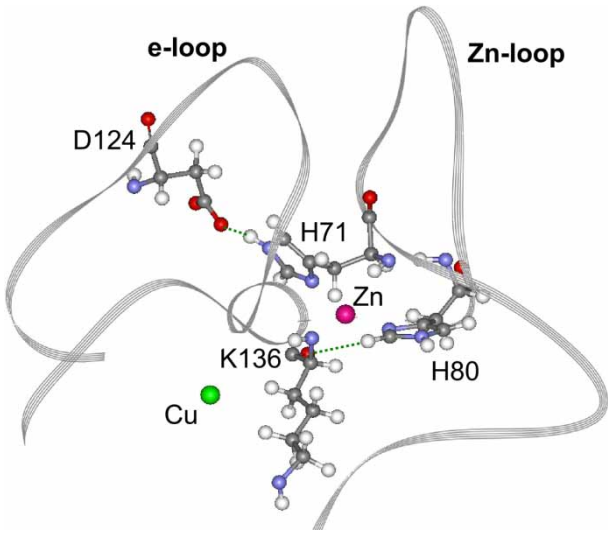

Figure 5. Atomic configuration (at 2.3 ns) of channel loops for the holo molecule in flat ribbon notation. The H71, H80, D124 and K136 have been highlighted showing the hydrogen-bonds (green dotted lines in online version) that stabilised the loops.

The analysis of the holo model showed that stable hydrogen-bonds located along the channels which were formed between residues D124–H71, T135–H71 and K136–H80. The hydrogen-bondings were found to survive throughout the whole simulation time. Figure 5 shows the atomic configuration snapshot of the channel loops for the holo-SOD1 at 2.3 ns. For clarity, only two sets of stable hydrogen-bonds are highlighted, the D124–H71 and K136–H80. It can be seen that the Zn-bound histidine residues are important to maintain a proper structural configuration of the e-loop for the holo molecule—H71 with D124 from one side of the e-loop

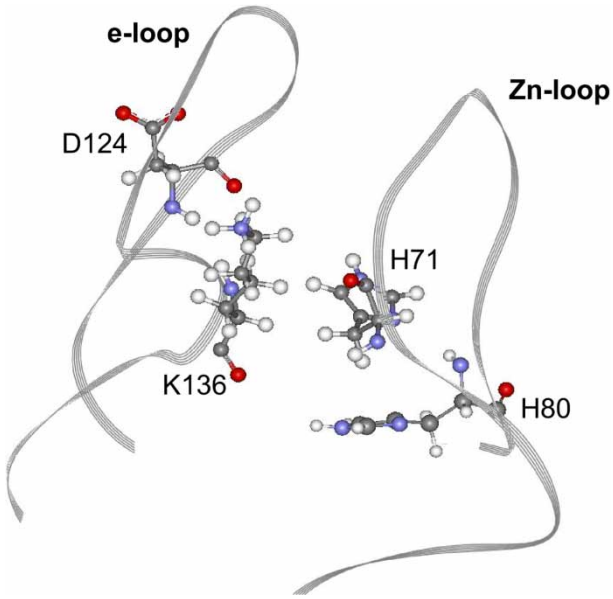

Figure 6. Atomic configuration of channel loops for the apo molecule in flat ribbon notation. The His71, His80, D124 and K136 have been highlighted showing the absence of hydrogen-bond and detachment of the e-loop from the Zn-loop.

and H80 with K136 from the other side of the e-loop. Furthermore, strong interactions between D124 and H71 also reinforce the idea that the former residue is an important element in stabilising the Zn-binding, in addition to the e-loop [6,19].

In the case of the apo model, the analysis showed that the channel loops were highly uncorrelated with each other. None of the above mentioned hydrogen-bond network can be identified, in which case the channel loops at monomer B did not contain any hydrogen-bonding for more than 300 ps. The loops were moved wide apart, before approaching each other again during the course of the simulation. In the case of monomer A, the number of hydrogen-bonds was generally fewer than that for the holo model, with a series of short-lived hydrogen-bonds. The longest hydrogen-bond that survived the consequence of metal removal was the D124–H71, which remained stable for about 1.7 ns from the onset of the metal removal, whereas, the T135–H71 hydrogen-bond was still evident, albeit considerably weakened, with occasional breaking and reforming of the hydrogen-bond. Figure 6 shows the atomic configuration snapshot for the apo-SOD1 at 2.3 ns. It can be seen that when the metals were removed, these hydrogen-bonds were destroyed. For instance, the H80 imidazole ring was flipped to the other side, while the side group of K136 was rotated upward and the carboxylate group from the D124 was swung completely away from the H71 residue. Consequently, the e-loops were partially uncoiled, elongated and moved away from the Zn-loop. This shows that without Zn, these residues would prefer to be in positions that do not favour the formation of hydrogen-bonds between the loops.

We have also recently carried out root-mean-square structure difference and found that the Cu ion apparently does not play any part in maintaining the molecular structure, with no significant change to most of its ligating residues [20]. The only notable exception is the H46, which was significantly displaced when the metal ions were removed. However, it is not clear whether such displacement is due to the absence of solely Cu, Zn or both ions. Nevertheless, H46, H71 and D124 are believed to form a stable hydrogen network that ensures correct orientations at the Cu and Zn sites [6]. In fact, previous studies [21] showed that mutation of the histidine residue (H46R) can severely disrupt the native Cu site and thus lower the affinity for copper metal.

5. Conclusion

Molecular dynamics (MD) simulations of human wild-type SOD1 have been carried out using DL_POLY_3 in order to study the effects of removing the zinc and copper ions from the SOD1 molecules. In general, metal removal only produces small structural differences to the global molecular structure. However, most of the differences can be located at the channel loops, whereby they gained in flexibility and degree of movement and became structurally disordered. From the FALS pathological point of view, this may result in unintended protein–protein interactions. Our simulations suggest how the structures would have to change to accommodate such interactions.

This work also demonstrates the feasibility of using MD simulations to elucidate the underlying atomic mechanisms that may give rise to the cytotoxic nature of the SOD1 that leads to FALS. It was found that "long" simulation time of at least ~4 ns or more was required to effectively map out the initial stage of the structural disorder behaviour of SOD1. For instance, the structural results in figure 4(b) indicate that the differences for the e-loops become apparent only after 2 ns. In fact, the calculations we have carried out are much longer than most other previous molecular simulation studies on SOD1 which, in most cases, only up to the order of 1 ns were achieved [22,23]. However, with improved parallelization techniques (as employed in DL_POLY_3 software) and advances in computational capabilities (such as the HPCx), bio-molecular simulations of up to 10 s of nanoseconds can now be routinely achieved. This opens up the opportunity to provide a better understanding of structural biology of a bio-molecule by providing a more complete picture of structural evolution.

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