Molecular simulations of biochemical processes in presence of a MW signal

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Introduction

The influence of microwaves (MW) electromagnetic (EM) fields on metals, metal oxides and ceramics is of relevance in industrial nanotechnology applications regarding material processing, and further insights in these processes can be obtained by performing an analysis at molecular level [1].
This becomes of extreme importance when considering the processes by which an EM field acts on a biological system. Here the MW field action on biological molecules or structures, altering their charge, chemical state, or energy, can be considered as the first "transduction" step for the occurring of a biological effect [2]. Previous authors results on MW EM interaction at molecular level regarded a basic process such as the ligand-binding to a free moving protein [3]-[4]. In this work another equally important process involved in several biochemical reactions is considered: the *electron transfer*. The enzyme specifically chosen is Superoxide Dismutase (SOD), a metalloenzyme containing copper (Cu) and Zinc (Zn) metal ions.
The main function of CuZnSOD is to prevent the accumulation of O_2^-, which is a free radical produced, together with other reactive oxygen species (ROSs), during normal metabolic processes. ROSs are used by many types of cells as part of their ability to defend themselves and can have important roles in cell signaling. Nevertheless, they are potentially dangerous; being unstable and highly reactive, they can participate in unwanted side processes. An uncontrolled free oxygen radical release, named oxidative stress, may indeed cause cellular structural and functional anomalies, as well as DNA damage. Therefore studying MW field action on such enzyme seems to be particularly significative [5].

Models and Methodologies

A brief description of the used molecular model, and of the theoretical computational methods adopted in the simulations, is given in the following.

Models

CuZnSOD is a dimer of identical subunits, composed by a total of 310 residues. The X-ray crystal structure at 3 Å resolution of the bovine CuZnSOD is known [6]. Each subunit contains one Cu and one Zn atom. In Fig. 1a the whole structure is displayed. The Cu is

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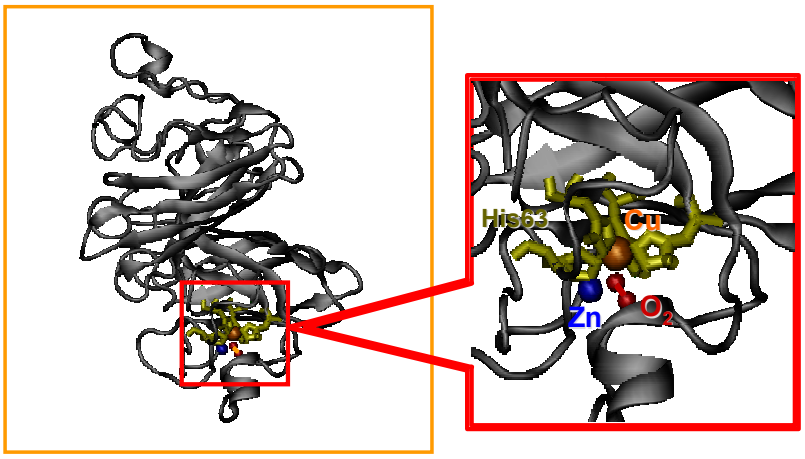
coordinated to four histidines forming a distorted square planar geometry; one of these Histidines (His63) acts as a bridge between Cu and Zn (see Fig. 1b).



(a) (b)

Fig. 1 Molecular view of the CuZnSOD. (a) whole protein; (b) enlargement of the reaction centre, with the four histidines (yellow bonds) surrounding copper, the Cu (orange sphere), the Zn (blue sphere), and the O_2 (red spheres) atoms.

CuZnSOD catalyzes a two-step reaction where hydrogen peroxide (H_2O_2) is produced as a final product, by means of a process where an electron is transferred from the O_2^- to Cu (electron transfer process). The reaction mechanism is defined by two chemical steps: first the O_2^- binding to Cu occurs, (states 1 and 2 of Fig. 2) and then the actual electron transfer process takes place, via the concerted Cu-O_2^- and the His63-Cu bond ruptures (state 3 of Fig. 2).

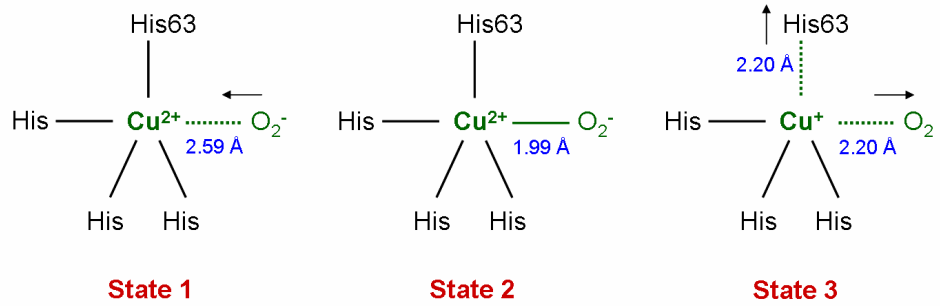


Fig. 2 Schematic view of the analyzed three chemical states. State 1: the Cu-O_2^- bond is heavily stretched at 2.59 Å; State 2: the bond is formed at a distance of 1.99 Å; State 3: electron transfer process and rupture of both O_2 and His63 at a distance of 2.20 Å

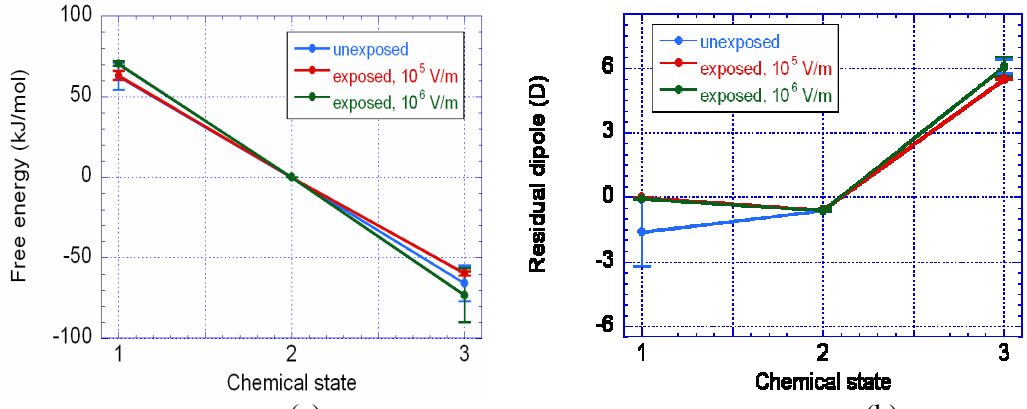
Methods

When dealing with a theoretical-computational description of a biochemical reaction, it is necessary to take into account the electronic state transitions of the reaction center, together with its interactions to the molecular environment (i.e. protein and water). The use of quantum-mechanical (QM) methods, which explicitly take into account electronic behavior, should be strictly required, although pure QM methods are unfeasible for complex molecular systems (more than 10^4 atoms). Classical molecular dynamics (MD) could easily follow the atomic and molecular motions of the complex systems, but

without any evaluation on the electronic properties. Modern techniques based on mixed QM and MD methods offer the possibility to overcome these limitations of pure quantum calculations. In particular the Perturbed Matrix Method (PMM) proposes a computational approach recently applied to the study of biochemical reactions [7]-[8]. PMM is based on the perturbation theory and states that the electric properties of the atoms of the reaction center can be perturbed by the electric field due to the protein and solvent surrounding them. By this way, a pure quantum approach is preserved and limited to the few atoms of the reaction center, while the electric perturbation due to the whole molecular environment is evaluated by means of MD simulations. PMM reduces the calculation of the electronic properties of the whole biochemical system, to a simple diagonalization of the Hamiltonian matrix associated to the perturbed Hamiltonian (*H*) operator, including the electric field perturbation. Such diagonalization is performed on a basis set represented by the wavefunctions of the unperturbed *H* operator. The EM field, in the MW frequency range, is introduced in PMM as an exogenous electric field signal. In particular, since it is known that quasi-diffusive motions, occurring between a few ns down to 100 ps, are typical of large macromolecules as enzymes [9], a possible coupling of the MW field within the protein and its influence on the reaction can be investigated, introducing the electrical signal in the MD simulations. Such analysis seems to be feasible, since typical duration lengths of such simulations (tens of ns) are consistent with some periods of the applied MW signal.

Results

Results were obtained from simulations ran in parallel on two nodes of a cluster composed of 20 Intel Xeon (Pentium 4) equipped with 1 GB RAM memory each; a computational effort of 672 hours (i.e. 30 days) was required, in order to obtain a single MD trajectory of 10 ns. Three MD trajectories were simulated both for the unexposed case (no exogenous field applied) and for each exposure condition, i.e. in the presence of continuous wave (CW) fields, with f=1 GHz at amplitudes of 10^3, 10^4, and 10^5 V/m. Two further MD runs were obtained under exposure to a CW 1 GHz field at 10^6 V/m. The duration of each MD run simulating the CuZnSOD in the presence of 10^5 and 10^6 V/m CW fields was of 19 ns; in all the other cases, the temporal length was equal to 30 ns. Results are given in Fig. 3 in terms of free energy and residual dipole for the three chemical states previously introduced. They are relative to the highest intensity values of electric field applied (10^5 and 10^6 V/m).



(a) (b)

Fig. 3 Mean value ± standard error of three states free energy (a), residual dipole along the $Cu\text{-}O_2^-$ (b), both for unexposed and the exposed MD trajectories.

The free energy furnishes a complete thermodynamic and kinetic description of the chemical reaction, the residual dipole provides a direct measure of the charge density modification involved in the electron transfer, obtained with respect to the unperturbed reference charge distribution.
By assuming a Gaussian distributed noise for all the MD trajectories, unpaired student t-tests were performed to compare all unexposed and exposed data. Differences were considered significant for $p<0.003$. For all observables the differences were not significant but remained within the intrinsic variability of the unexposed simulations. Similar results were obtained for the lower electric field values (data not shown).

Conclusions

Molecular simulations on a metalloenzyme involved in electron transfer process have been performed in presence of a CW (1 GHz) electric signals with growing intensities up to 10^6 V/m. The overall indication emerging from this preliminary analysis is that no effect is observable for exogenous MW fields intensities quite lower than the molecular endogenous ones (which are in the order of 10^9 V/m) at least within some tens of nanoseconds of exposure.

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