34th Annual International Conference of the IEEE EMBS
San Diego, California USA, 28 August - 1 September, 2012

Signal Transduction on Enzymes: the Effect of Electromagnetic Field stimuli on Superoxide Dismutase (SOD)

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Abstract— **Protein functions and characteristics can highly differ from physiological conditions in presence of chemical, mechanical or electromagnetic stimuli. In this work we provide a rigorous picture of electric field effects on proteins behavior investigating, at atomistic details, the possible ways in which an external signal can be transduced into biochemical effects. Results from molecular dynamics (MD) simulations of a single superoxidismutase (SOD) enzyme in presence of high exogenous alternate electric fields will be discussed.**

I. Introduction

Under physiological conditions, proteins, enzymes and peptides spend most of their life-time in the native conformation, facilitating their biological functions. Exogenous perturbations may alter this equilibrium starting a transition toward denaturated states. Several authors have demonstrated, through molecular dynamic simulations, that microwave electromagnetic (EM) fields may excite macromolecular vibrational modes and alter protein conformation, even including denaturation and stability [1], [2]. A strong coupling with such fields is argued at least for high intensities of the fields.

Similarly from experimental side, it is demonstrated that non-ionizing radiation in the form of nanosecond Pulsed Electric Fields (nsPEFs) with intensities up to several MV/m, unipolar or bipolar, can interact both with cellular membranes and cellular substructures, including proteins and genetic materials [3], [4]. Furthermore, given the low energy content of nsPEFs (millijoule/cc), no thermal effects are involved [5]. It is known that nsPEFs are signals whose frequency spectrum extends well in the microwave region with a quite large bandwidth, similarly to what happens to ultra-wideband technology signals. Their pattern is usually a train of electric field pulses with duration going from 1 to 60 ns, with specific pulse rise and fall times from hundreds of ps to tens of ns [6]. Due to their particular frequency content, nsPEF, when acting on macromolecules such as proteins and enzymes, may solicit a coupling on different frequencies in the microwave region.

Therefore possible relevant outcomes of a detailed theoretical investigation of microwave electromagnetic field interaction with biomolecular systems might be important for a deeper comprehension of the effects induced by nsPEF on proteins. Moreover, the possibility to control the protein effects due to EM fields with well defined frequency and intensity may open up a wide range of possibilities in protein engineering and medicine [7]-[8].

In this context it seems appropriate to identify and apply powerful tools and models able to describe interaction phenomena occurring at atomistic level: molecular simulations approach is able to properly investigate the dynamic behavior and functioning of molecules in their own realistic environment, both in physiological condition and under exposure to EM fields. Through the simulation of a "virtual" experiment, such an approach allows the observation of the EM field action on microscopic structures in an accurate and rigorous way. Given the characteristic time associated with a typical nsPEF signal, molecular dynamic simulations seem to be a perfect tool to dynamically study conformational and thermodynamic changes induced on a protein.

As a case study we have selected SOD enzyme, an important defense in nearly all cells exposed to oxygen since it catalyzes the dismutation of superoxide into oxygen and hydrogen peroxide [9], [10]. Given its fundamental antioxidant role, SOD enzyme has been widely investigated both experimentally and theoretically [11], [12]. Moreover, many efforts have been devoted to the study of possible effects on SOD reactivity in presence of exogenous electromagnetic perturbation, in a wide range of frequencies [13], [14].

Our idea is to theoretically study by means of molecular dynamics simulations the sensitivity of SOD enzyme to different EM stimuli, in particular 1 and 2.45 GHz continuous wave (CW) and a Gaussian pulse centered at 2.45 GHz with a bandwidth of 2 GHz, resulting in a pulse duration in time of less than 8 ns, with the perspective to possibly act on SOD activity in a controlled and efficient way.

II. Materials and Methods

A. Simulation Details

We carried out molecular dynamic simulations of a single SOD enzyme in water using GROMACS package [15]. The simulated system consisted of a rectangular box (around 7-nm side), in which we placed a single SOD enzyme, 10480 Single Point Charge (SPC) [16] water molecules and 9 sodium counterions resulting in a typical density of 1000 kg/m^3. Note that, to properly describe SOD physiological behavior, it was necessary to simulate a box of water

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molecules large enough to reproduce both the first hydration shells and the remaining bulk water. Following an energy minimization and subsequent solvent relaxation, the system was gradually heated from 50 K to 300 K. Trajectories were propagated up to 80 ns in a NVT ensemble using an integration step of 2 fs, fixing the SOD centre of mass, preventing translations but with no constraints on its related rotation. The temperature was kept constant at 300 K by proper coupling with the same time-step used for the integration algorithm using the Berendsen thermostat [17]. Long range electrostatics were computed by the Particle Mesh Ewald method [18]. The ffG43a1 force field [19] parameters were adopted.

The obtained trajectories represent a time dependent behavior of the simulated system, i.e. molecular dynamic simulations generate information at the microscopic level, including atomic positions and velocities. The conversion of this microscopic information to macroscopic observables such as energy, electric profiles, geometric properties, etc., has required statistical mechanics calculations.

B. Exogenous Signals

Once obtained an exhaustive unexposed trajectory we decided to evaluate possible effects induced by high exogenous EM field on the system, hence introducing different signals, two continuous waves (1 GHz and 2.45 GHz) and a complete pulse (see Fig. 1), each with two different intensities of 10^9 and 10^8 V/m acting on all atoms in the simulation box as explained in [12]. The time-signal of Fig. 1 is obtained considering a gaussian pulse with a bandwidth of 2 GHz modulating a 2.45 GHz cosine, for a time duration of about 8 ns.

In classical simulations the magnetic field force is several order of magnitude smaller than that of the electric component [20], so hereinafter only electric field will be considered. Moreover, since the simulation tool does not provide the insertion of an electric field perturbation except the constant electric field, we modified and compiled the source code to let the user decide which kind of signal to adopt, in terms of intensity, frequency and shape. To note that the application of the electric field takes place starting from the last frame of the unexposed simulation, thus allowing a direct evaluation of the characteristic response in time of the system to the exogenous perturbation starting from an equilibrium (unexposed) condition.

C. Numerical Observables

Exogenous signal transduction can be evaluated by monitoring possible conformational and electric changes on SOD enzyme before and after the electric field application.

In order to evaluate structural properties of a molecular system it is often convenient to approximate its geometry to known 3D regions. For this reason, we adopted the Covariance Matrix Method, which provides an immediate path for evaluating dynamical fluctuations of a macromolecular target such as a SOD enzyme in a classical three-dimensional space. The focus is seeking those collective degrees of freedom that best approximate the total

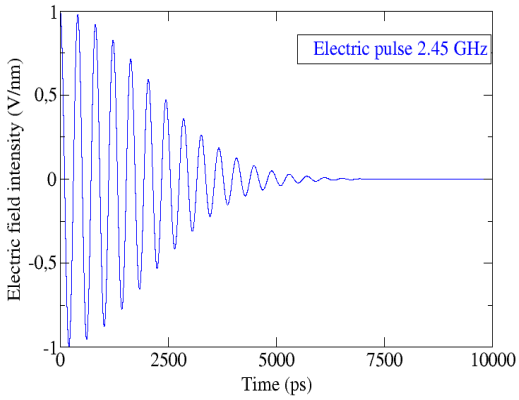

Figure 1. Time trend of the exogenous electric pulse (first 10 ns). The time-signal is obtained considering a gaussian pulse in the frequency domain with f_0=2.45 GHz, BW= 2 GHz and amplitude of 10^9 V/m.

amount of fluctuations of a dynamical system [21]. Diagonalization of the 3x3 Covariance Matrix provides the eigenvectors, which represent the directions along which the overall system fluctuates with a positional mean square fluctuation given by the corresponding eigenvalues. To note that once obtained the three eigenvectors we can express data both in external (Cartesian) and internal (ellipsoidal) coordinate systems (see Fig. 2).

Since polarization effects are expected in exposure conditions, as second observable we have taken into account the dipole moment of the whole enzyme, as obtained by the atomic charge distribution around SOD center of mass. For convenience dipole components have been projected along the internal coordinate system.

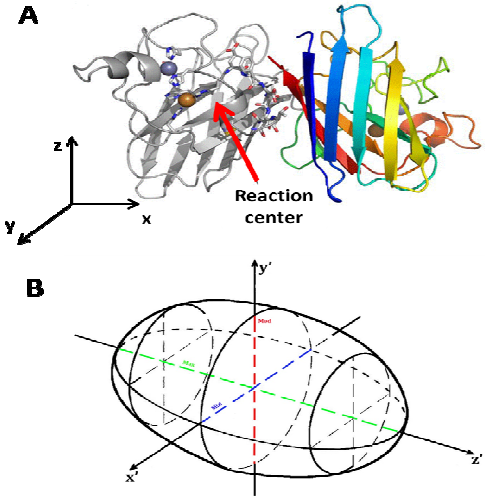

Figure 2. Panel A, molecular representation of SOD enzyme as obtained by VMD software (http://www.ks.uiuc.edu/Research/vmd/) in the external coordinate system {x,y,z}. Panel B, ellipsoidal region approximating SOD enzyme, as stated by covariance matrix method; to note the internal coordinate system {x',y',z'} determined by SOD eigenvectors.

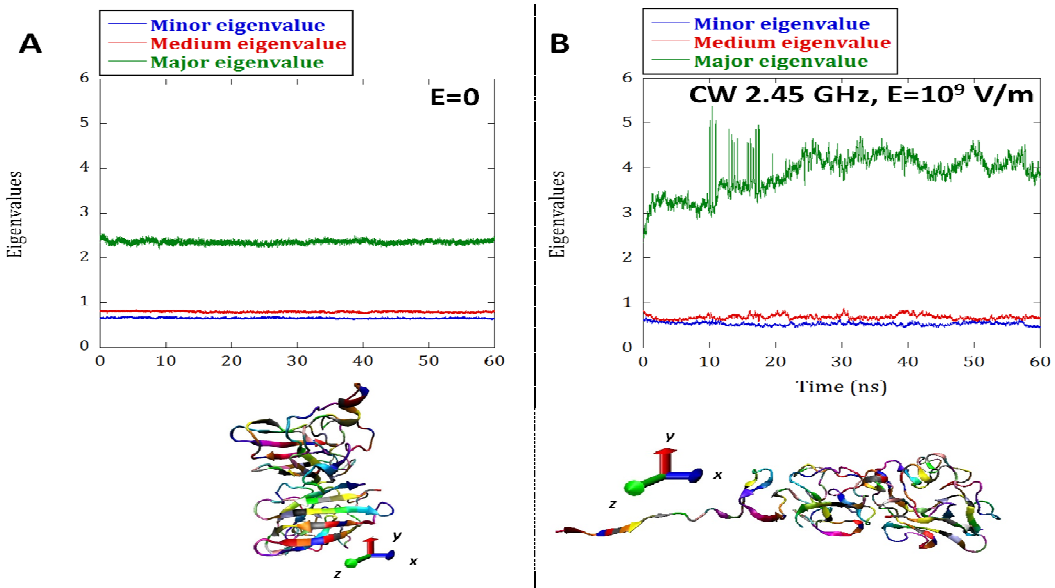

Figure 3. Panel A, time course of the three eigenvalues in unexposed conditions. To note the equilibrium condition during the simulation and the compact globular shape of SOD enzyme. During the application of the CW {2.45 GHz, 10^9 V/m} signal SOD major eigenvalue is stretched up to 200% of the physiological value (panel B).

D. Post Elaboration Analysis

To better evaluate the possible transduction process of the exogenous electric signals to the molecular system, we performed a post processing of the trajectories that simulations provided in the time domain, converting them into the frequency domain. Time courses of the observables were first numerically filtered through an Hamming pass-band filter, with the low cut-off frequency of 500 MHz and the high one of 5 GHz. Then the average periodogram was calculated over 10 segments of the trace.

Regarding the gaussian pulse, spectrograms were adopted to represent how the spectral density of a signal varies with time. Spectrograms have been obtained as the square modulus of the short time Fourier transform algorithm. This algorithm implies the windowing of the temporal signal under analysis and the fast Fourier transform of each time sequence. Even in this case the output traces were filtered in order to leave components in the given frequency range and eventually spectrograms are produced.

III. RESULTS AND DISCUSSSION

As explained in section II.C, we can directly evaluate electric field effect on SOD enzyme conformation by means of the covariance matrix method, applied to different exposure conditions. In Fig. 3 we present a comparison between the unexposed simulation (panel A) and the {2.45 GHz, 10^9 V/m} CW exposed simulation (panel B). It appears quite clear that high exogenous electric field, even with duration of few nanoseconds, can alter SOD native conformation, inducing a sharp stretching along the electric field direction (in our case the x-axis in the external coordinate system).

While the application of the The CW signal at {1 GHz, 10^9 V/m} produces the same result, conversely, neither CW signals at lower intensities ($\leq 10^8$ V/m) nor the pulse, produced evident modifications in SOD geometry during the 60 ns trajectories (data not shown).

In its native state SOD enzyme possesses a dipole moment of about 80-120 Debye. As explained in section II-C SOD dipole moment is a good observable to study polarization effect induced by the exogenous field, even at those intensities not affecting other observables. At the same time the spectral representation of dipole moment can, in principle, clarify the entity of signal transduction, as confirmed by the density spectra profiles shown in Fig. 4 for 10^8 V/m and pulsed signals. We can observe a sharp effect at 1 and 2.45 GHz even for such a lower intensity. Surprisingly

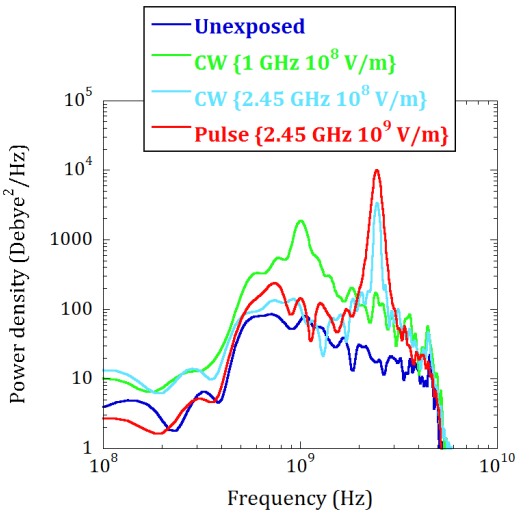

Figure 4. Power density spectrum of dipole component along SOD major axis.

the 10^9 V/m Gaussian pulse seems to exhibit a similar behavior to those of the 10^8 V/m, with a peak at 2.45 GHz about 20 dB below the one of almost 10^4 Debye^2/Hz, due to the {2.45 GHz, 10^9 V/m} signal. This mismatch can be explained with some further comments: from Fig. 1 it is evident that only for a small fraction of the total simulation time (60 ns) the signal is on, hence providing a duty cycle of about 14%. Therefore the representation via the density power spectrum may be misleading.

A possible alternative analisys is the signal representation via a spectrogram. In Fig. 5 we show two spectrograms for unexposed and exposed (to the pulse) conditions: in panel A we can appreciate the native spectra components possessed by SOD enzyme, while in panel B the high components around 2.45 GHz are pronounced mainly within the first ten ns. Moreover it is quite evident that the signal pulse is able to increase the frequency content of the SOD enzyme spectra even below the 2.45 GHz, since as it was expected, due to its wide frequency band, it is able to solicit the protein vibrational modes which are in the frequency range of [0.5-1.5] GHz.

IV. Conclusion

From these results it is possible to make some first evaluations. Molecular dynamics simulations are a useful tool to investigate the interaction of EM fields with enzyme molecules. Classical observables like covariance matrix and dipole moment are able to provide information on major effects as denaturation or large conformational changes, even if further studies on the nsPFEs on-off transitions could better clarify how SOD activity can be controlled. Nonetheless signal transduction of the fields into the molecular system is still detectable for field intensities which do not induce relevant molecular variations, when using a proper spectral analysis. Finally more sophisticated time-frequency techniques seem to be the best approach for investigating such a transduction for short nanosecond pulses.

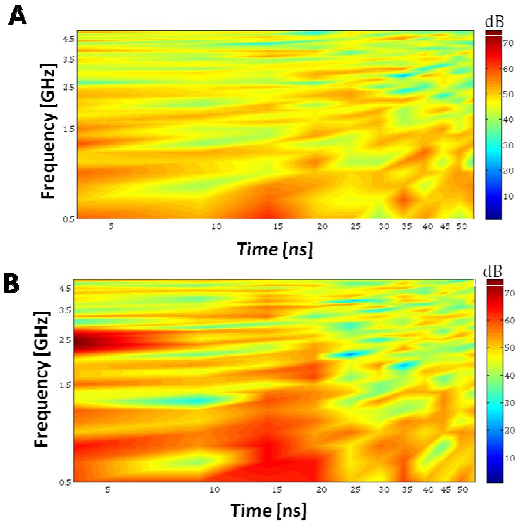

Figure 5. Spectrograms of unexposed (panel A) and exposed (panel B) simulations

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