Heating a Lipase: Theoretical and Experimental Circular Dichroism Comparison.

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Abstract: Classical molecular dynamics have been developed since the 1950s and have since been applied to many fields, from solid state modeling to the development of drugs and biocatalysts. In the biological context, molecular dynamics has been used to gain understanding about processes of interest such as transport of ions through the cell barrier, action of antibodies and inhibition of viruses. Nowadays, such processes can be studied at an atomic level since the available software can be distributed in a cluster of CPU’s / GPU’s making the calculations involved in the classical molecular dynamics be realized in a finite computational time. However, despite the advances, the simulation times still large enough to avoid the study of the complete unfolding pathway of a protein (~10^{-6}s), or the study of systems containing a very large number of atoms (>10^6).

Since the beginning of the last century, researchers have discovered that proteins from biological systems could be used in other types of processes and understanding these processes would enable improvement and even optimization of the intended effect. Regarding biocatalysts, molecular dynamics has been used in their development as well as in the study of the mechanisms that lead to proteins to act. These biocatalysts, in which the lipases are included, are of industrial interest since they can be used in the environmental and food industries because they do not generate toxic residues. In the specific case of the enzyme studied in this work, it was found in contaminated soil near a treatment lagoon in the metropolitan region of Curitiba, an environment with high fat concentration and its potential application has been investigated since then.

In this work, classical molecular dynamics techniques were used to study LipC12 lipase with the objective of obtaining the qualitative evolution of the circular dichroism spectrum during its unfolding caused by heat and comparing it to the evolution of the experimental circular dichroism spectrum.

The simulations were performed using the GROMACS software, using the CHARMM36 force field, using water as explicit solvent and the SPC/E model for the description of the water molecules. The volume of the simulation box used was 85x85x85 Å. After the energy minimization, the system was balanced in the ensemble NVE for 1 ns, and in the NPT by 1 ns at 500 K and 1 bar, respectively, and in both equilibrations the positions of the protein atoms were kept fixed. After equilibration, 2 production simulations of 25 ns were performed and their trajectories were analyzed. The theoretical circular dichroism spectrum was calculated using the PDB2CD tool available for free online.
Once in possession of the trajectories, they were divided into 5 intervals of 5 ns. At each interval, the clusters formed were calculated using the mean value of the RMSD of that interval as cutoff and the structure with the smallest difference between its RMSD and that of all the others was chosen for the calculation of the CD. Figure 1 shows the comparison between the experimental spectrum and that obtained by the method described.

As can be seen in the comparison of Figure 1 (a) with 1 (b), the spectrum shows that in both cases the circular dichroism curve is reaching a zero plateau indicating that the protein is in fact unfolding. This development can be further confirmed by the direct observation of the form the protein acquires during the simulation. This comparison presents a validation of the parameters used in the simulation, especially with respect to the force field, and that it can be used to collect other information about the system and a possible prediction of a structure for the protein more thermally stable.

Key-words: Lipase, Molecular Dynamics, Circular Dichroism.

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References: