Cold-adapted enzymes: structure, stability and function investigated by computational methods

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Motivation

Extremophiles are organisms living at temperatures considered "extreme" for the human life. The study of the molecular mechanisms that rule the relationship between stability, flexibility and activity in their proteins is fundamental to shed light on the protein folding, unfolding and misfolding processes that are present in cell life cycle. We are presenting the molecular characterization of an esterase isolated from the antarctic organism Pseudoalteromonas haloplanktis TAC125 (PhEST). PhEST presents a dimeric structure and exhibits an optimum activity at 20°C. We used computational methods to model the putative structure of this enzyme, and molecular dynamics simulations at different temperatures to probe the variation of stability and flexibility of this cold-adapted enzyme.

Methods

The structure of PhEST was predicted by homology modeling with the program MODELLER, using S. cerevisiae S-formylglutathione hydrolase (PDB code: 1PV1) as suitable template. The sequences of target and templates were aligned to those of other 30 carboxylesterases and formylglutathione hydrolases, to exploit information of common features of their family. Also information about type and position of secondary structures were used before submitting the alignment to the modelling steps. Two monomers were assembled to create the model of the dimeric structure of the enzyme, then, a mild optimization was applied to reduce steric clashes. The stereochemical and energetic parameters of the best model obtained for PhEST assess its good quality. Molecular dynamics simulations were carried out using the program GROMACS 3.3.1. The dimeric protein was included in a triclinic box filled with water molecules and Na+ ions to neutralize the net negative charge of the system. Periodic boundary conditions were used to exclude surface effects. A short MD simulation (20 psec) with position restraints was applied to soak the solvent into the macromolecule. Then, three 10 ns-long simulations at 4°C, 20°C and 45°C and at a pressure of 1 atm were performed with a time step of 2 fs and without any position restraints. The systems were coupled to a temperature and pressure bath using Berendsen's method. Long-range electrostatics were handled using the PME method. Cut-off was set at 0.9 nm for Coulomb interactions, and at 1.4 nm for van der Waals interactions. Several analyses were conducted using programs built within GROMACS package. Moreover, for each simulation, an "average" structure representative of the trajectory was calculated. These "average" structures were saved in .pdb format, and were subsequently minimized with the Steepest Descent method. Visualization and analysis of model features was carried out using InsightII facilities. The percentage of residues embedded in secondary structure elements, and their variation during the simulations has been evaluated using the program DSSP. H-bonds were calculated with HBPLUS. Identification of salt bridges was made on "average" minimized structures.

Results

The analysis of the three average minimized structures obtained at three different temperatures shows that there are no major changes of the secondary structure of PhEST between 4°C and 45°C. The root mean square fluctuations (RMSF) of the atomic position of PhEST identify several zones of the enzyme characterized by different mobility at different temperatures, in particular in loops joining segments of secondary structures and in segments surrounding the active site. This observation suggests a molecular explanation for the known activation of the enzyme at 20°C. The gain in flexibility of these segments may favor the entry of substrates and/or the catalysis, whereas at low temperature the active site of the protein shows a more rigid conformation and the catalysis is slowed down. We have also analyzed the variation of the distance between D225 (D505) and H258 (H538) belonging to the catalytic triad, and found that at 4°C and 20°C the distance appears to be quite stable, whereas at 45°C there are major

fluctuations. Thus, it is possible to infer that the excessive flexibility of the protein caused by high temperatures impairs the correct activity of the enzyme. We have used also the program HBPLUS to detect the presence of the H-bond between the side chains of D225 and H258 on the average minimized structures, and we found it only at 20°C. Therefore, from these simulations it seems that the reason for the low activity of PhEST at 4°C is the lack of the proper flexibility of the structures around the active site, whereas at 45°C is the lack of the proper interactions among the residues of the catalytic triad. We have also identified several ion pairs, eight of which are conserved also at high temperatures, suggesting a crucial role in stabilizing this enzyme, bringing together the two subunits of the protein.

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