Comparative molecular dynamics simulations of homologous enzymes to investigate enzymatic cold-adaptation: a family-centred point of view

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Motivation

In recent years, there has been increasing interest in the origin of enzymatic adaptation to low temperatures to understand both the protein folding and structure-function relationships, and for biotechnological and industrial applications. The number of reports on enzymes from cold adapted organisms has increased significantly over the past years, revealing that adaptative strategies varies among enzymes, which use different small selections of structural features in order to gain increased molecular flexibility that in turn leads to increased catalytic efficiency and reduced stability. Molecular flexibility and the characteristics related to cold-adaptation are often difficult to estimate using experimental methods, whereas molecular dynamics (MD) provides a suitable tool to evaluate flexibility and molecular properties of proteins and correlate them to their structural and functional features. In light of the above scenario, the systematic investigation of different enzyme families becomes crucial to unravel the cold-adaptation strategies discovered by specific families. In the present contribution we report an approach based on several long MD simulations of representative structures for mesophilic and psychrophilic homologous at different temperatures, to explore the molecular basis inside different enzymatic classes. The MD trajectories were compared and analyzed considering the time-evolution of different properties: secondary structure content, molecular flexibility indexes, intramolecular and protein-solvent interactions, solvent accessibility, molecular surroundings of selected residues, to electrostatic interactions and properties of the protein surfaces. This analysis lead to unravel putative structural and molecular determinants of thermolability, flexibility and activity at low temperatures for psychrophilic enzymes.

Methods

Several MD simulations were performed in the NPT ensemble, using the GROMACS simulation software package and allowing the collection of 36-48 ns trajectories for each system. The solvent was explicitly treated by periodic boundary conditions and the ionization state of charged residues was set to mimic a neutral pH environment. In order to sample efficiently the conformational space, independent MD simulations were carried out starting from the same atomic coordinates and using different initial velocities from a Maxwellian distribution. Multiple trajectories help to identify recurring features and to avoid artifacts arising from the simulation procedure. The trajectories were checked to assess the quality of the simulation using GROMACS routines. In particular, the root mean square deviation (rmsd) calculated with respect to the initial structure and detection of the structural clusters sampled during MD simulation by the Linkage algorithm allowed the determination of the stable portions of the trajectories, which were further analyzed using GROMACS tools, and the NACCESS, DSSP, DELPHI, PYMOL, VMD programs as well as suitable tools developed in our laboratory.

Results

The comparative MD approach reveals that modulation of the number of protein-solvent interactions is not the evolutionary strategy followed by the analyzed enzymatic families to enhance catalytic activity al low temperature. In addition, flexibility and solvent accessibility of the residues forming the catalytic sites are generally comparable in the cold- and warm- adapted enzymes. In some test cases, it turns out a localized flexibility and peculiar electrostatic surface properties or salt-bridge pattern, related to a particular amino acid composition, which clustered around the

functional sites of the psychrophilic enzymes. In contrast, the mesophilic counterparts show a scattered flexibility in non-functional regions and additional stability in the surroundings of the active site as well as specificity pocket due to the presence of unique stabilizing ion-pairs. The results support the hypothesis that flexibility is the main adaptative character of psychrophilic enzymes, being responsible for the decrease of activation enthalpy that leads to increased kcat values at low temperature. On the contrary, other cold-adapted enzymes have evolved weakening intramolecular interactions, increasing structural disorder, showing a less stable binding of ion cofactors, and therefore enhancing the structural flexibility of the main protein domains, indicating a strategy of increased overall flexibility. In conclusion, the present investigation contributes, by means of a suitable computational and comparative approach to the clarification and enforcement of the picture that, among the several putative mechanisms of molecular and structural coldadaptation, different enzymatic families can pursue different strategies according to their function, cellular localization and specific substrate.

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