A fast conformational sampling approach to investigate enzymatic cold-adaptation mechanisms

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Motivation

The huge amount of sequence and structural data provided by genomic projects, opened new intriguing possibilities to understand at the molecular level the plasticity of life in adaptation to the so-called "extreme" environments, such as low temperatures at a molecular level. Comparisons among protein sequences or entire genomes from extremophilic and mesophilic organisms suggested possible adaptative strategies, confirming that there is no general solution to the achievement of structural and functional stability in extreme environments. In particular, the number of reports and the structural data on enzymes from psychrophilic organisms has increased significantly only recently, revealing that adaptative strategies are specific inside an enzymatic family and related to an increased molecular flexibility that in turn leads to increased catalytic efficiency and reduced stability. Computational methods which allow a good sampling of the protein conformational space, such as classical molecular dynamics simulation (MD), are a suitable tool to investigate structural mechanisms at the basis of enzymatic cold-adaptation, by comparing psychrophilic enzymes with the mesophilic homologues. However, in order to get reliable information, long simulation times are required, allowing the investigation of only few cold-adapted enzymes, generally in comparison with one mesophilic counterpart. The number of threedimensional structures of cold-adapted enzymes is increasing as well as the number of mesophilic homologues that could be studied in order to carry out comparative analysis. Therefore, the employment of computational methods which are not time-consuming but which, at the same time, allow a conformational sampling comparable to MD, can be useful to clarify the details of the different molecular strategies used by different enzymatic families in order to face low temperature conditions.

Methods

In order to extend the previously performed analysis to a larger set of psychrophilic enzymes, CONCOORD was employed to sample the conformational space of whole families of cold-adapted proteins. CONCOORD is a fast and reliable method to generate an ensemble of conformations from a list of distance constraints derived from the starting structure. Each structure is generated by a random algorithm and optimised to satisfy the constraint list. A collection of 500-2000 structure is a good representative of the ensemble of accessible conformations in the neighbourhood of the starting structure. To this extent, the CONCOORD ensembles can be analysed with traditional Molecular Dynamics tools. Essential Dynamics analysis is particularly effective in defining and extracting a meaningful subspace of informative motions and it was employed in this study to highlight evolutionary adapted flexibilities in the structure of psychrophilic enzymes. Additionally a comparison of CONCOORD and MD results was exploited for some representatives with the aim of further validate the fast sampling in the specific case of psychrophilic proteins. MD simulations were carried out with GROMACS and encompassed a timescale of tens of nanoseconds. Independent runs with different initial distributions of velocities were performed in the NPT ensemble and explicit solvent.

Results

Preliminary results on some enzymatic families support the hypothesis that psychrophilic enzymes carried out different evolutionary strategies in order to cope with the detrimental effects of low temperature environments, among which structural flexibility emerges as the main adaptative character. Moreover, the employment of a fast sampling method appears to be suitable to perform

large screening of whole families before the application of more time-consuming simulation methods.

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