Computational and experimental approaches to characterize the molecular organization and structural stability of an Arginine-binding protein from Thermotoga maritima

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Motivation

ABC transport systems provide selective passage of metabolites across cell membranes and typically require the presence of a soluble binding protein with high specificity to a specific ligand. In addition to their primary role in nutrient gathering, the binding proteins associated with bacterial transport systems have been studied for their potential to serve as design scaffolds for the development of fluorescent protein biosensors. For these applications, proteins isolated from thermophilic organisms possess added intrinsic value in the design of new biosensing technology that features enhanced stability. Before utilizing a protein as the basis for a sensing device, however, it is of high importance to fully characterize the biomolecule with respect to its stability in the potential operating conditions. Here we present a combined computational and experimental approach to investigate the physicochemical properties of an arginine binding protein (ArgBP) from the hyperthermophilic eubacterium Thermotoga maritima. Levels of available arginine are very important for NO synthesis in patients with hypercholesterolemia or atherosclerosis. Thus, the development of biosensors to detect and titrate arginine levels in bodily fluids could be of interest for diagnosis and treatment of these diseases.

Methods

The structure of ArgBP is not yet available by experimental methods, therefore we used a comparative modelling approach to model both the ligand-free and the ligand-bound forms of the protein. For ArgBP/Arg, the structure of Arg-, Lys-, His-binding protein ArtJ from Geobacillus stearothermophilus bound to Arg available in PDB database (PDB code: 2Q2A) was selected as suitable template after a BLAST search. In addition to the ArtJ template, information was taken from the structure of the open unliganded form of the Gln-binding protein from E. coli (PDB code: 1GGG) to model the ligand-free ArgBP. The software MODELLER 9v7 was used to create the final models of the protein, that were validated using both

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stereochemical and energetic criteria. Investigations were performed on topology, secondary structures, solvent accessibility and role of H-bonds, salt bridges, cavities and hydrophobic clusters in enhancing protein stability, in order to define a “molecular portrait” of the protein itself and of its interactions with the ligand. Then, molecular dynamics simulations at different temperatures and pH were performed in order to investigate the effects of these two physical perturbations on protein structure. The program GROMACS v. 4.0.5 was used to perform simulations of 10 ns at pH 7.5 and 9.5 at increasing temperatures (from 27 to 100°C) and to analyze results, that were compared and integrated with experimental results obtained from Fourier transform infrared spectroscopy experiments in the same conditions of pH and temperature.

Results

The “molecular portrait” of ArgBP obtained with the computational approach is that of an extremely stable protein, whose structure is composed by two domains, each formed by a central beta sheet surrounded by helices, connected by a hinge region. A tightly packed and structured hydrophobic core in each domain is surrounded by protic residues potentially interacting with each other and with the medium. The non-N-, non-Cterminal domain shows a central beta sheet formed by hydrophobic residues almost completely shielded from solvent; in the N+C-terminal domain Ile and Leu residues are inserted in the strands and around them, creating a large cluster of bulky hydrophobic residues. The ligand is inserted in the cleft between the two domains and is bound to ArgBP via interactions with residues belonging to both domains: polar residue that promote H-bond formation between the charged moiety of Arg, and hydrophobic residues that contact the long apolar side chain of the ligand. The effect of temperature and pH was also studied to provide a more complete picture of the protein dynamics. At high temperatures, the first elements of secondary structures that seem to be destabilized are helices, whereas beta strands seem more resistant. In particular, it is possible to identify a group of beta structures in the non-N-, non-C-terminal domain of ArgBP, hydrophobic and deeply buried in the protein structure, that are not disrupted either by high temperature and high pH. Finally, the presence of the ligand is able to increase substantially the thermostability of the protein. These results are in agreement with experimental data and depict a portrait of an ideal candidate as a biological component for a biosensor to detect arginine concentration in bodily fluids. Our data on dynamics and stability will contribute to our understanding of bacterial binding protein family members and their potential biotechnological applications.

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Supplementary information

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