In silico Phosphorylation of the SuperSH3 domain of p47phox

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Motivation

Computer simulations can be a valid tool to study the properties of biomolecular systems that are not easily accessible to experimental means [1]. One of these mechanisms is phosphorylation, amongst the most important regulatory events in signal transduction processes. NADPH oxidase is a multi-protein enzyme that catalyzes the reduction of oxygen to a superoxide anion (O2-) in response to microbial infection. The enzyme complex comprises a heterodimeric membrane-bound flavocytochrome b558 (gp91phox and p22phox), and four cytosolic regulatory subunits [2]. A crucial step for the assembly and activation of this enzyme is the phosphorylation of one of the cytosolic subunits, p47phox, on multiple serine residues [3]. The p47phox subunit contains a PX domain, a tandem SH3 domain (SuperSH3 domain), a polybasic region (PBR/AIR) and a proline-rich C-terminus. In the inactive state the SH3 domains interact through intramolecular interactions with the PBR/AIR region, resulting in an autoinhibited, inactive protein. Phosphorylation of p47phox induces conformational changes that lead to intramolecular rearrangements that allow the SH3 domains to interact with the p22phox membrane subunit of the complex. The changes, at the molecular level, produced by phosphorylation are difficult to analyse using experimental techniques, therefore we focus our attention on this region of NADPH oxidase by the use of computational techniques. Phosphorylation of Ser303, Ser304 and Ser328 was shown to be absolutely required for the NADPH oxidase activation [4]. Starting from the crystal structure of the auto-inhibited form [5] we investigated by Molecular Dynamics (MD) simulations and Essential Dynamics (ED) sampling the effect of these phosphorylated sites on the dynamical behaviour of the superSH3 domain.

Methods

Results

Molecular Dynamics simulations of the phosphorylated form compared to the auto-inhibited form have demonstrated that phosphorylation-induced conformational changes lead to the loss of about 70% of the interactions between SH3 domains and the PBR/AIR region. Principal components analysis of the simulation reveals that the first 2 eigenvectors account for 56% of the global motion. The projection of the trajectories of the first 2 eigenvectors has shown that the protein explores five conformational states and remains trapped until the end of the simulation in the last state. The opposite direction of motion shown by these two eigenvectors highlights that the superSH3 tends to open in the hinge region connecting the two SH3 domains.

Nevertheless the simulation didnt explore any new conformational area after 50 ns, therefore effective sampling techniques were required to explore the phosphorylation-induced opening mode.

Essential Dynamics was employed to encourage the protein to move in direction of the first and the second eigenvectors to explore a larger region of space than it would in free MD. After this sampling, most of the residues interacting with p22phox become exposed, suggesting that phosphorylation induces a conformational change leading to the accessibility of these residues for a competitive binding. In particular we observe a more significant exposure of residues belonging to SH3A. It has been experimentally demonstrated that isolated SH3A is able to bind p22phox while SH3B alone is not. The results obtained with using both these simulation (MD and ED) are in excellent agreement with these experimental finding.

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