Understanding of the cAMP-induced conformational transition of Protein Kinase A Iα A-domain. Role of the invariant R209.

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A-domain of Protein Kinase A Iα is an example of cAMP-binding domains, which are widely spread among different proteins and so thoroughly studied using various methods. Nevertheless, in spite of this fact, the average paths of cAMP-induced transition from H- (cAMP-free) to B- (cAMP-binding) conformation are not defined for any members of this domain family, presenting the main challenge for further investigation. The second challenge arises from contradictory data regarding the role of invariant arginine which binds cAMP (R209 in case of A-domain). Some papers say for irreplaceability of this arginine due to its implication for the discovered electrostatic switch [1]. Others [2] demonstrate that low-polar residues isoleucine or threonine at position 209 are relatively benign in their effects on transition mechanism, whereas basic lysine, which seems to be a good candidate for maintaining switch function, behaves almost as well as glutamic acid and makes A-domain about 20-times less sensitive to cAMP than isoleucine. This study presents the molecular dynamics (MD) solution to the both abovementioned challenges.

Two elements (the N3A-motif and the B/C-helix) were removed from the A-domain structure (PDB ID 3PVB) in order to prepare the systems for the first part of the study. The resulting stable domain fragments (150 - 225 a.a.) with one of the five residues (R, I, G, E or K) at position 209 were equilibrated in presence or absence of docked cAMP for 80-150 ns under NVE ensemble with mean kinetic temperature close to 310 K, aqueous environment and periodic boundary conditions. The chosen time proved to be enough for all systems to scan the energetic landscapes. Different conformations resulted from undertaken simulations were distinguished by Cluster Analysis. The second part of the study was carried out on the whole A-domain (118-242 a.a.) with R or K at position 209 and is presented by MD and

activated MD simulations in NVE and NVT ensembles. Five trajectories for wild-type protein and one trajectory for R209K mutant, each of about 25 ns in length, described A-domain transition from H- to B- conformation. Resulting data were processed by Principle Component and Time Series Analyses. All simulations were performed with NAMD2.8 software.

The data didn't confirm the crucial role of electrostatic switch in A-domain conformational transition, but demonstrated that correct twist of β2β3-loop is necessary for B-conformation realization. According to our results, only R209I and R209E mutants can maintain correct β2β3-loop conformation and only R209I and R209K mutants can keep cAMP in binding site. So, only isoleucine has negligeable effect on A-domain cAMP-induced conformational transition if substitutes for lysine at position 209. However, if external forces were applied to β2β3-loop all the mutants except for R209K underwent transition to B-conformation. Lysine at position 209 not only distorted β2β3-loop twist, but also sterically interfered with A202 movement, which is crucial for domain transition. If both β2β3-loop and K209 were fixed by external forces in suitable position, transition to B-conformation was observed.

The results obtained on whole A-domain were used to highlight the main stages of its transition from H- to B-conformation. These stages involve Phosphate Binding Cassette conformational changes, C-helix rotation (for the first time demonstrated by H.Rehmann), formation of π-helix turn within C-helix, and finally substitution of this turn by kink. R209K mutant goes through the same stages, provided that K209 and β2β3-loop are fixed by external forces as shown for truncated systems.

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