cAMP-induced conformational changes of Protein Kinase A Iα A-domain

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A-domain of protein kinase A (PKA) regulatory subunit RIα is an example of cAMP-binding domains, which are widely spread among different proteins and 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. In order to explain conformational changes of these domains (particularly, A-domain of RIα), two allosteric switches have been proposed. The first one is the electrostatic switch R209–D170–R226 that links the B/C helix (R226) to the phosphate binding cassette (PBC) in the cAMP-binding site (R209) through the β2β3-loop (D170) [1]. The second one is the hydrophobic switch or hinge L203–Y229 that links the B/C helix (Y229) directly to PBC (L203) [2]. Both switches can assume two distinctive ‘‘on’’ and ‘‘off’’ positions so that switching between them causes the domain to undergo transition between H- and B-conformations. The hydrophobic switch model is confirmed experimentally, but the mechanism of the cAMP-dependent activation of this switch remains unknown. In contrast, the electrostatic switch model, explains how information about cAMP binding is transferred outside the cAMP-binding site, however, this model cannot account for activation of PKA with mutational substitutions for R209 and D170 [3,4].

We combined MD (molecular dynamics) and aMD (accelerated MD) methods to find the average path (the first approximation to the minimum free energy path) of A-domain RIα (a. a 118 – 242) transition from H- to B-conformation. We got thirteen productive trajectories, which we processed sequentially by factor and cross-correlation analyses. In the end, we presented the conformational transition of the A-domain in the form of a partly deterministic sequence of six events: 1) B 2) C1, D1, 3) E, 4) C2[D2], F, 5) G.

The sequence of the items of this scheme corresponds to the process of the conformational transition in time. Events listed in one item can occur in any sequence, including the situation when one occurs on the background of another one. On the contrary, the events described in the different items proceed in a certain order: each of the events of the next item may occur simultaneously with the events of the preceding item, but not earlier.

The event B represents the transition of the PBC from H- to B-conformation, including the packing of the L203 side chain into the hydrophobic pocket, which is formed by the β2β3-loop. We named the main participants of this event (the amide group A202, carbonyl group G199 and the equatorial oxygen atom of the ligand) electrostatic switch cAMP(O6)–A202(N-H)–G199(C=O). Through this switch cAMP transmits the fact of its binding to the hydrophobic switch L203–Y229 and triggers thus conformational transition of A-domain.

The events C and D consist, respectively, in the N3A-motif displacement to the side of the PBC and the rotation of the B/C-helix. The event E involves an increase in the interaction energy between Y229 and β-subdomain. The events C and D, due to long duration, tend to occur in two stages, which we labeled with "1" and "2". At the second stage the event C affects the progress of the event D. We designated this fact as D2[C2].

Taken together, the events B, D1 and E correspond to the switching of the hydrophobic switch L203–Y229 to the state, which is characteristic of the B-conformation. These data confirm the key role of the hydrophobic switch in the conformational transition of A-domain.

The events F and G are specific for A-domains of protein kinases A and B-domains of EPACs. Transition of the B/C-helix turn (a. a. 229 – 234) from α- to π-form, accompanied by rotation of L233 and M234 side chains to the N3A-motif and the PBC, respectively, accounts for the event F. This event leads to an increase in the interaction energy between B/C-helix and N3A-motif, on the one hand, and B/C-helix and PBC on the other hand. In the course of the event G a kink replaces the π-helical turn. Electrostatic interaction R241–E200 and to a lesser extent van der Waals interaction L238–I204 become possible in resulting conformation and facilitate the kink formation.

The results give insight into possible evolution and functioning of other cAMP-binding domains.

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