ISSN 0006-2979, Biochemistry (Moscow), 2012, Vol. 77, No. 5, pp. 456-464. © Pleiades Publishing, Ltd., 2012.
Original Russian Text © O. N. Rogacheva, B. F. Shchegolev, V. E. Stefanov, G. A. Zakharov, E. V. Savvateeva-Popova, 2012, published in Biokhimiya, 2012, Vol. 77, No. 5, pp. 568-577.
Originally published in Biochemistry (Moscow) On-Line Papers in Press, as Manuscript BM12-010, March 18, 2012.

Initiation of the 3′:5′-AMP-Induced Protein Kinase A Iα Regulatory Subunit Conformational Transition. Part I. A202 and A326 Are Critical Residues

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Received January 12, 2012

Abstract—Protein–ligand docking and molecular dynamics studies have shown that the key event initiated by 3′:5′-AMP binding to the A- and B-domains of protein kinase A Iα regulatory subunit is formation of a hydrogen bond between 3′:5′-AMP and A202(A326) (the residue in parentheses being from the B-domain). The A202(A326) amide group movement associated with the bond formation leads to reorganization of the phosphate binding cassette (PBC) (the short 3_{10}-helix becomes the long α-helix). This process results in L203(L327) displacement and finally causes hinge (B-helix) rotation. The L203(L327) displacement and packing into the hydrophobic pocket formed by the PBC and β2β3-loop also depends on the β2β3-loop conformation. The correct conformation is maintained by R, I, E, but not K at position 209(333) of the A- and B-domains. So, the R209K and R333K mutants have problems with reaching B-conformation. The apo-form of the 3′:5′-AMP-binding domain also undergoes transition from H- to B-conformation. In this case, the movement of A202(A326) amide group seems to be a result of reorganization of the PBC into a more stable α-helix.

DOI: 10.1134/S0006297912050057

Key words: A- and B-domains of PKA Iα R-subunit, 3′:5′-AMP, A202(A326), molecular dynamics, β2β3-loop, phosphate binding cassette

Protein kinase A (PKA) is a key protein that is the basis of many of signal cascades. It is known to be involved in cell differentiation, development of immune response, control of metabolic pathways, and many other processes [1, 2]. Only PKA catalytic subunits (C-subunits) possess protein kinase activity *per se*, while the whole enzyme necessarily comprises regulatory subunits (R-subunits) or other peptides and proteins. The function of all noncatalytic subunits is to maintain the inactive state of PKA in the absence of external signal [1-3].

The best-studied and probably the most widespread event is inhibition of the catalytic activity of C-subunits through R-subunits. In this case, inactive PKA complexes consist of a dimer of R-subunits, each bound with one C-subunit. These complexes are divided into four types by the number of R-subunit isomers: PKA Iα, PKA IIα, PKA Iβ, and PKA IIβ. However, in spite of different biological functions, all of them are characterized by a common property: activation in response to the effect of intracellular messenger cyclic adenosine-3′,5′-monophosphate (3′:5′-AMP) [1-3]. The activating effect of 3′:5′-AMP on PKA is caused by the structure of R-subunits. Each R-subunit contains two 3′:5′-AMP-binding domains (*N*-terminal A-domain and *C*-terminal B-domain) forming most of the bonds with the C-subunit [3, 4]. Each of these domains can exist in two final conformations; hence, R-subunits also have two final conformations. The H-conformation of the R-subunit has a higher affinity for the C-subunit and blocks its catalytic activity. The B-conformation is a 3′:5′-AMP-bound form of the R-subunit and cannot interact with the C-subunit [3-6]. Thus, the role of 3′:5′-AMP is reduced to induction of conformational transition of the R-subunit from H- to B-form and, as a result, release of active C-subunit.

The structure of the 3′:5′-AMP-binding domain in the H- and B-conformations is shown in the Fig. 1 (a and

Abbreviations: 3′:5′-AMP, cyclic adenosine-3′,5′-monophosphate; C-subunit, catalytic subunit; PBC, phosphate binding cassette; PKA Iα, protein kinase A Iα; R-subunit, regulatory subunit.

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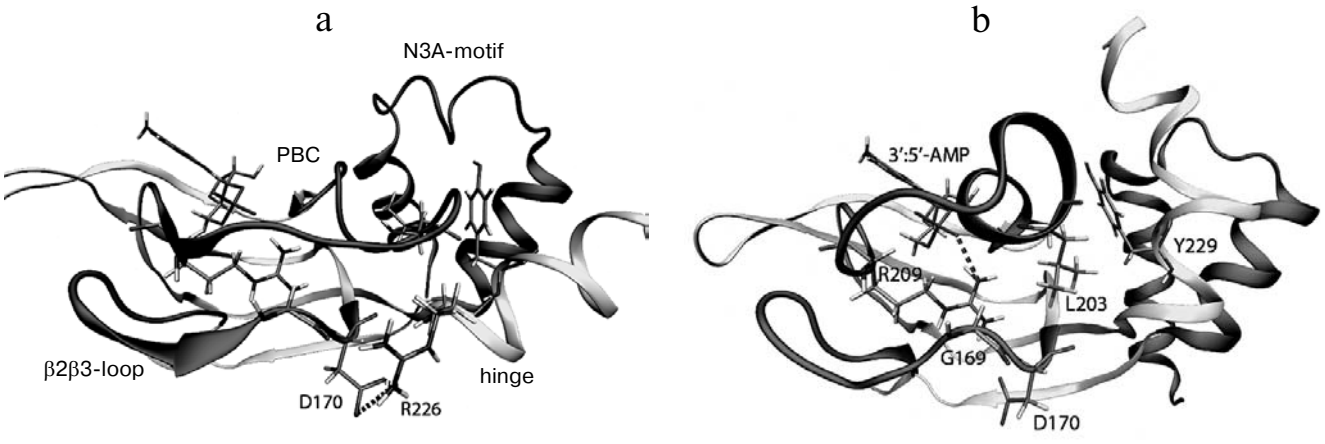

Fig. 1. Structure of the 3′:5′-AMP-binding A-domain of PKA Iα. a) H-conformation; b) B-conformation. Hydrogen bonds are shown by dotted lines.

b) by the example of PKA Iα A-domain. The figure is based on the data of X-ray structure analysis [7, 8]. The 3′:5′-AMP-binding site of the domain is located within a conformationally stable structure called a β-barrel and represented by a conservative region: a phosphate binding cassette (PBC) [9]. Most of the amino acid residues of the PBC in the H-conformation form a 3_{10}-helix (G199-I204 and E324-L328 for the A- and B-domains, respectively) [4, 7]; in B-conformation they form a longer α-helix (G199-G206 and G323-N330 for the A- and B-domains, respectively) [6, 8].

It has been shown [6, 8] that in the B-conformation the hydrogen bonds with 3′:5′-AMP are formed by five amino acid residues of the PBC: G199(G323), E200(E324), A202(A326), R209(R333), and A210(A334)[1], and all of them are invariant. However, in the literature due consideration has been given only to R209(R333) [10-12]; the degree of involvement of other mentioned residues in the 3′:5′-AMP-induced activation of all PKA isoforms is still rather unstudied.

Under the PBC (Fig. 1, a and b) there is the second conservative region of the 3′:5′-AMP-binding domain: β2β3-loop (the loop between β-strands numbered 2 and 3 [9]), which is represented by the I163-N171 and V281-E289 sequences in the A- and B-domains of PKA Iα, respectively [4, 6]. According to available data, G169(G287), being a component of this loop, forms CH–π interaction with the guanidinium group of R209(R333) [13], which is essential for the conformational transition of 3′:5′-AMP-binding domains [13]. The second significant residue of the β2β3-loop, D170, forms in the B-conformation an ionic bond with R209 and thereby stabilizes the 3′:5′-AMP-bound form of the domain [14]. This bond is not formed in the B-domain in spite of the presence of homologous residue D288 [6, 8, 14].

The third conservative region of the 3′:5′-AMP-binding domain called a "hinge" is located at the *C*-terminus from the β-barrel [9] and represented by the B- and C-helices (Fig. 1, a and b). The term "hinge" was proposed in the work of Rehmann et al. [15, 16] and was completely explained by their hypothesis suggesting the principle of conformational transition of 3′:5′-AMP-binding domains. As noted in the cited paper, the key moment of conformational transition is the turn of the B-helix of the domain (Fig. 1, a and b). However, this turn is impossible in the H-conformation because a conservative residue of the PBC, L203(L327), sterically prevents the movement of the B-helix conservative residue Y229(F353) (Fig. 1). In the B-conformation, L203(L327) is in a different position, and the turn of the B-helix becomes possible. The reasons why the binding of 3′:5′-AMP results in L203(L327) displacement are still unknown.

The fourth conservative region of the 3′:5′-AMP-binding domain, the N3A-motif [9], is also shown in the Fig. 1.

Comprehensive study of the PKA Iα A-domain resulted in development of the following concepts of mechanisms of its conformational transformations. According to the suggested hypothesis [5], there are two allosteric switches through which the A-domain responds to 3′:5′-AMP binding by varying its conformation.

The first switch is hydrophobic. The final stage of its functioning is based on the above-mentioned mechanism stated in the papers of Rehmann et al. [15, 16], while the initial stage is obviously initiated by 3′:5′-AMP binding and completed with displacement of the L203 residue; the association between these events has not yet been

[1] Here and below the homologous residues of the A- and B-3′:5′-AMP-binding domains of PKA Iα are given with the B-domain residue in parentheses.

established. As mentioned in the discussion of Badireddy's paper [5], A202 may be involved in the operation of the switch because it is a part of the PBC hydrophobic core and forms a hydrogen bond with 3′:5′-AMP; however, the involvement of other amino acid residues in this switch is also possible.

The second switch is electrostatic [5, 17]. In the A-domain, it is represented by three amino acid residues: R209, D170, and R226, the latter being a part of the B-helix (Fig. 1). The essence of the "electrostatic switch" is as follows: as a result of 3′:5′-AMP binding to the A-domain, the D170 side chain switches over from R226 to R209 (Fig. 1), thereby stabilizing the 3′:5′-AMP-bound conformation of the domain. At the same time, the loss of connection between the B-helix and the β2β3-loop (R226-D170) releases the B-helix for its subsequent turn [17].

While the presence of the electrostatic switch in the A-domain of PKA Iα is an experimentally confirmed fact, which is not doubted, its importance for conformational transitions of other 3′:5′-AMP-binding domains seems to be somewhat exaggerated. A number of experimental facts do not confirm the presence of the described "switch" in the B-domains of PKA [6, 8, 14]. The EPAC proteins, which also contain 3′:5′-AMP-binding domains, lack even the amino acid residues it is formed of [16]. In addition, the paper of Steinberg et al. published in 1996 [12] is a serious counterargument against the key role of an electrostatic switch in conformational transitions of the A-domain of PKA Iα. The research conducted by the authors has shown that mutational substitutions of residues I, T, K, S, W, N, and E for R209 worsen the activation of PKA Iα to varying degrees but do not make it impossible. Thus, the processes triggered by 3′:5′-AMP in the PBC and β2β3-loop are still quite unstudied. In this work, we attempted to eliminate this gap in understanding the PKA Iα activation.

MATERIALS AND METHODS

Preparation of the A- and B-domains of PKA Iα. Spatial structures of the PKA Iα B-domain (residues 235-376) in the H- and B-conformations were taken from the PDB bank. The B-conformation was obtained from the ligand-bound form of the R-subunit with the number 1NE6 [8], and the H-conformation was obtained from the only existing spatial structure of the 2QCS complex of R- and C-subunits [4]. However, the B-domain within the 2QCS structure could not bind ligands due to the point mutation of invariant arginine, R333K [17]. In order to use this structure in our research, the arginine residue was substituted for the K333 residue. The substitution was made in DeepView/Swiss-PdbViewer software (http://www.expasy.org/spdbv/) [18], followed by manual selection of the R333 conformer completely coinciding with the R209 conformer realized in the H-conformation of the A-domain. The described selection of the arginine conformer was more preferable under these conditions than determination of equilibrium position of its side chain, because equilibrium modeling in the absence of the C-subunit could result in escape of the protein from the H-conformation.

Spatial structures of the PKA Iα A-domain (residues 118-242) in the H- and B-conformations were taken from the PDB bank with the numbers 3PVB [7] and 1NE6 [8], respectively. In addition to the 3PVB structure, the A-domain is represented by the H-conformation also in the 2QCS [4] and 3FHI [19] structures. However, the hydrogen bond between the carbonyl group of A202 and the amide group of G206 is realized only in the 3PVB structure. This bond is typical of the PBC α-helix but obviously absent in the 3_{10}-helix. Therefore, the 3PVB structure can be considered as being at the earliest stages of A-domain transition from the H- to B-conformation. Based on preliminary data, the use of such structure considerably reduces the time of modeling; hence, it has been taken as the reference for all calculations performed during this work on the basis of the H-conformation of the A-domain.

All of the tested protein complexes of PKA Iα were visualized using the VMD software [20].

Docking of ligands in the 3′:5′-AMP-binding sites of the A- and B-domains of PKA Iα. The docking of 3′:5′-AMP into 3′:5′-AMP-binding sites of the A- and B-domains was performed using Quantum 3.3.0 software (2008-2009)[1] [21]. This software performs the docking of a flexible ligand into a rigid protein and then minimizes the energy of the resulting complex.

Modeling of 3′:5′-AMP-induced conformational transitions of the A- and B-domains using molecular dynamics. Conformational changes in the A- and B-domains resulting from 3′:5′-AMP binding were modeled by of molecular dynamics in the NVE ensemble. The complexes obtained in the course of docking, being A- and B-domains in the H-conformations with 3′:5′-AMP in the binding sites, were taken as initial structures for modeling. The modeling was performed with the NAMD 2.7 b3 and NAMD 2.8 software [22] with a step of 2 fsec using aqueous environment under the periodic boundary conditions and the CHARMM27 force field [23, 24]. The electrostatic component of interactions was taken into consideration by the PME method [25]. During the first 600 psec of modeling, the systems were heated in 20 K increments from 0 to 300 K and then equilibrated for 20 nsec.

[1] The docking into the 3′:5′-AMP-binding site of the A-domain in the H-conformation was performed using the PDB ID 2QCS structure. Then, using spatial alignment of the 2QCS and 3PVB structures, 3′:5′-AMP was transferred into the binding site of the 3PVB A-domain.

In accordance with the existing data, G169(G287) as part of the β2β3-loop forms CH–π interaction with the guanidinium group of R209(R333) [13], which maintains the peculiar conformation of the sought loop. As shown by preliminary calculations, the CHARMM27 force field has no parameters correctly describing this interaction; therefore, the β2β3-loop, in spite of the presence of R209(R333), passes into another conformation during the first nanosecond of modeling. To avoid this unfavorable effect, the atoms of the main chain of the β2β3-loop were exposed to the forces compelling the loop to stay in the position corresponding to the B-conformation (force constants were 4-5 kcal/mol per Å^2).

Preparation of A-domains of PKA Iα with point mutations of 3′:5′-AMP-binding site and deletions of the N3A-motif and B-helix. Study of transitions of these domains from H- to B-conformation using molecular dynamics. In addition to the wild type A-domain with arginine residue at position 209, the domains carrying point mutations R209I, R209G, R209E, and R209K were modeled to better understand the roles of hydrophobic and electrostatic switches. The above-mentioned article of Steinberg et al. was the background of such calculation and the basis for analysis of the results [12]. All mutations were made using the standard options of NAMD 2.7 b3.

The A- but not B-domain was chosen to reduce the computing time, because only the crystal structure of 3PVB in the H-conformation already contains the A202–G206 hydrogen bond typical of the α-helix of PBC. Moreover, the N3A-motif and the B-helix were removed from each of the studied A-domain structures for the same purpose. The systems with deletions were stable under the modeling conditions though possessed some insignificant features untypical of the systems without deletions. It is important that the removal of the N3A-motif and the B-helix did not prevent the conformational transition of PBC but even hastened it. The A-domain with the R209K point mutation was an exception to the above rule. In the presence of the B-helix, the carbonyl group of D170 forms a hydrogen bond with the amide group of one of the amino acid residues of the B-helix. Elimination of the B-helix in combination with the presence of the positively charged side chain of K209 close by compels the carbonyl group of D170 to turn to K209, distorting the native conformation of the β2β3-loop. To avoid this effect, the first twist of the B-helix in this case was preserved, and the native hydrogen bond with the involvement of the carbonyl group of D170 was stabilized by imposition of external harmonic potential.

As a result, four calculations were made using molecular dynamics for each of the point mutants and for the protein carrying arginine at position 209. The modeling conditions were the same as for the systems without mutations.

The first calculation was the modeling of the A-domain transition from the H- to B-conformation in the presence of 3′:5′-AMP in the binding site. The second one modeled the possibility of the same transition in the absence of 3′:5′-AMP. Both calculations were made in three repeats according to the following plan: heating the system for 300 psec in 20 K increments from 0 to 300 K, modeling its behavior until completion of the conformational transition (1-5 nsec depending on the R209 point mutation), and equilibration of the system for an additional 5 nsec.

Since transitions from one conformation into the other in the systems under consideration were rather rapid, the trajectories obtained during the first and second calculations were suitable only to establish the very fact of transition but not to obtain the energy values averaged by the trajectory. As a result, additional calculations were made to determine some energy parameters of the conformational transition from the H- to B-conformation.

During the third calculation, the atoms of the PBC main chain were exposed for 10 nsec to forces keeping PBC at a position corresponding to the H-conformation (force constants were 2-3 kcal/mol per Å^2). During the fourth calculation, a stable B-conformation of the A-domain was obtained similarly.

In all of the analyzed cases of modeling the systems that carry a non-mutant arginine residue, the β2β3-loop position characteristic of the H- or B-conformation depending on the calculation was maintained by external forces as described in the previous section.

RESULTS

Docking of 3′:5′-AMP into binding sites of A- and B-domains of PKA Iα. According to the results of docking in both domains in the B-conformation, 3′:5′-AMP formed five hydrogen bonds with amino acids residues of the PBC (with the amide groups of G199(G323), A202(A326) and A210(A334) and with the side chains of E200(E324) and R209(R333)), which is in full agreement with the data of X-ray structure analysis [6, 8]. However, the presence of only three hydrogen bonds in the B-domain (G323, A334, R333) and four hydrogen bonds in the A-domain (G199, A210, R209, E200) was shown in the course of 3′:5′-AMP docking into the binding sites of these domains in the H-conformation. The only hydrogen bond that could not be realized in principle in the H-conformation was the bond between 3′:5′-AMP and the amide group of A202(A326). The distance between the nitrogen atom of A202(A326) and the exocyclic oxygen atom of 3′:5′-AMP in both cases was about 5.5 Å, i.e. the amide group of A202(A326) was displaced for the bond formation. However, such displacement in the H-conformation was impossible: the amide group of alanine under consideration was a part of framework of the PBC 3_{10}-helix and formed a hydrogen bond with the G199(G323) carbonyl group in accordance with the helix type.

Modeling of transitions of 3′:5′-AMP-binding domains from the H- to B-conformation using molecular dynamics. According to the docking results, it is displacement of the A202(A326) amide group that must change the conformation of the PBC and, as a consequence, of the entire domain. However, in light of the literature data on significance of the R209(R333) residue [12] and for understanding the role of the β2β3-loop, we analyzed conformational transitions of the domains carrying both arginine and other amino acid residues at position 209(333).

The protein with native 3′:5′-AMP-binding site. Both in the presence and absence of 3′:5′-AMP, transition of the A-domain with deletions from the H- to B-conformation occurred during the first nanosecond of modeling. In the remaining time, the systems preserved the B-conformation; however, the PBC was located slightly higher above the β2β3-loop than it is observed in the crystal structure (Fig. 1b) [6, 8]. The latter effect is probably associated with the influence of the entropy factor and formation of two non-native hydrogen bonds between the protein and 3′:5′-AMP (with the amide groups of E200 and I201). As shown by further calculations, the PBC did not change its position after the conformational transition in the presence of the B-helix and N3A-motif.

Irrespective of the presence of 3′:5′-AMP in the binding site, conformational transition of the domain was accompanied by reorganization of the PBC helix: the turn of its axis (which is clearly seen in Fig. 2, a and b) and transition from 3_{10}- to α-form. The G199–A202 hydrogen bond in this case was broken; instead, however, a new bond was formed between the amide group of T207 and the carbonyl group of A202, which additionally stabilized the α-helix. Reorganization of the PBC helix obviously resulted in displacement of the amino acid residues forming the latter, with particular attention given to the A202 and L203 residues.

The side chain of A202, which in the H-conformation is located "to the right" of the methylene group of G169 (Fig. 2a), in the B-conformation is placed "to the left" of this group (Fig. 2b), which enables the formation of a hydrogen bond between the amide group of A202 and 3′:5′-AMP. It seems to be important that the transfer of the A202 side chain above the G169 methylene group is accompanied by overcoming the energy barrier; however, it is impossible to determine the height of this barrier in the framework of our calculations.

As a result of displacement of L203 residue, its side chain was found above the β2β3-loop (Fig. 2b) and, as a consequence, could be "packed" into a hydrophobic pocket formed by this loop and the PBC (Fig. 2b). The existence of the sought pocket in both conformations is a consequence of the β2β3-loop twist (Fig. 2, a and b), which, in turn, depends on the presence of CH–π interaction between G169 and R209. In the B-conformation, the PBC helix turn slightly shifted the β2β3-loop "downward" (Fig. 2, a and b) without disturbing the CH–π interaction, thereby making a pocket deeper and facilitating the "packing" of the L203 side chain.

In the new position of the β2β3-loop it was also possible to close the ionic bond between the D170 and R209 side chains (Fig. 2b).

These results were fully confirmed by the calculations on domains without the deletions. However, only the changes in the 3′:5′-AMP-binding site of the A-domain had time to be completed during the modeling. This is probably due to existence of the A202–G206 bond in the A-domain structure used.

It should be particularly noted that the description of conformational changes in the 3′:5′-AMP-binding domain is given in this section as though the transition of the PBC helix into the α-form is the very first event initiating other changes, including the displacement of the A202 residue. We believe that it is really true for the free domain. However, in the presence of the C-subunit, the 3′:5′-AMP-induced transition is obviously driven by formation of a hydrogen bond between A202 and 3′:5′-AMP entailing reorganization of the PBC helix.

The energy change accompanying the conformational transition was estimated in the first approximation on the basis of data on the mobility of different regions of A-domain carrying the deletions. Hence, the trajectory-averaged energies of the interaction between amino acid residues 198-208 (the main part of PBC), 209, and 167-171 (the main part of the β2β3-loop) were calculated and their changes during the transition from the H- to B-conformation were estimated.

The energy of interaction between the 167-171 and the 209 regions was -40 ± 6 kcal/mol in the H-conformation and -95 ± 7 kcal/mol in the B-conformation, and the energy decrease was associated only with the formation of D170–R209 ionic interaction. Conformational transition did not result in energy change for region 198-207 relative to residue 209: in both cases it was about -10 ± 4 kcal/mol. On the other hand, the interaction between sequences 198-208 and 167-171 became less advantageous (-30 ± 6 and -16 ± 3 kcal/mol for the H- and B-conformations, respectively). Energy change during the transition of the PBC helix into the α-form was considered separately. In the H-conformation, the energy of the helix was 160 ± 10 kcal/mol; in the B-conformation it decreased to 145 ± 10 kcal/mol as expected. These estimates show that both conformations of the A-domain carrying the deletions are similarly advantageous in the first approximation and only formation of the D170–R209 ionic bond stabilizes the B-conformation. Subsequent estimations of energy changes obviously should take into account not only the N3A-motif and hinge region but also the entropy factor.

A-domain carrying R209I mutation. As shown [12], point mutation R209I involves minimal consequences for the A-domain functioning. Our calculations have clearly

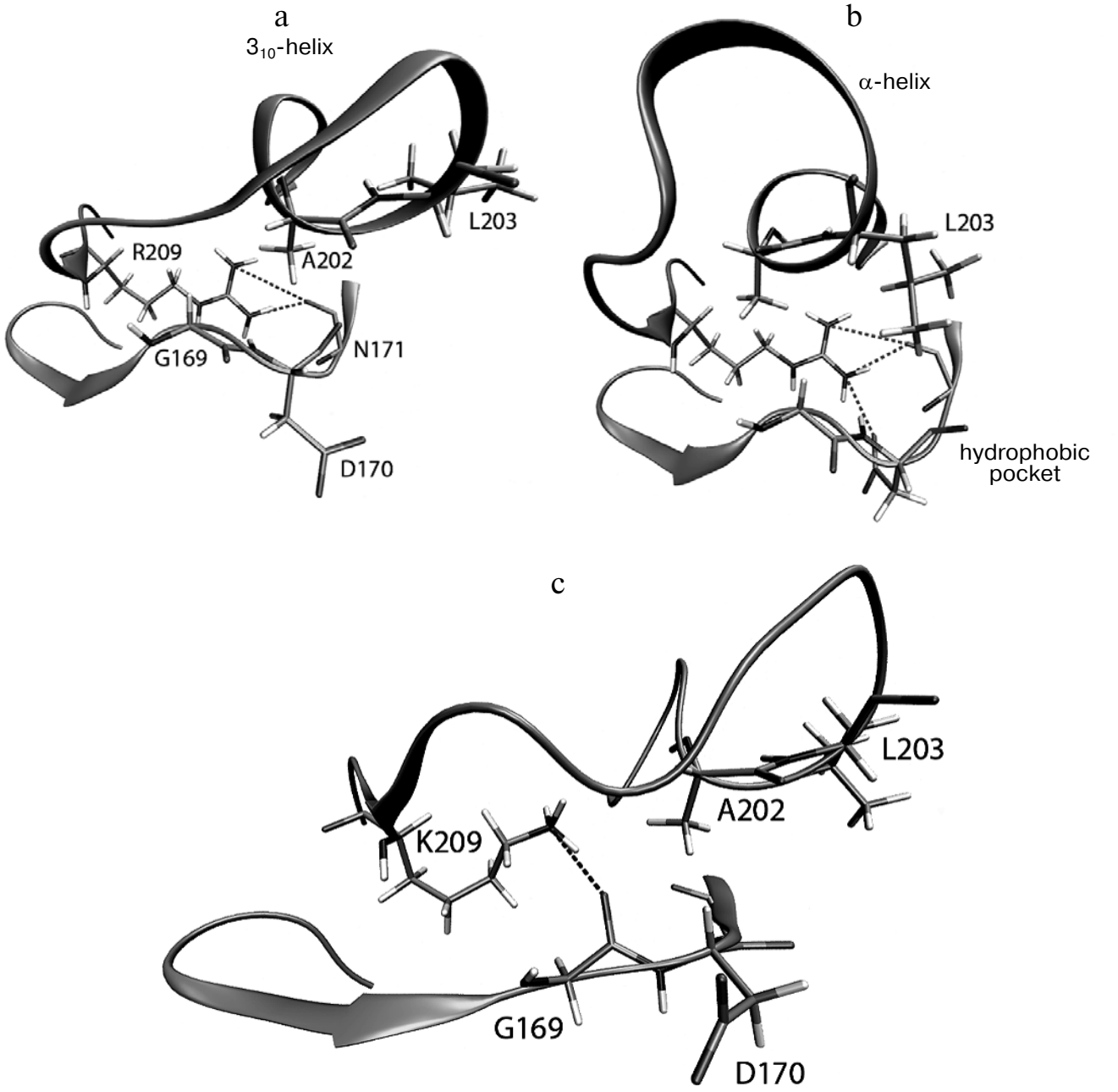

Fig. 2. Changes in PBC and the β2β3-loop during the transition of the A-domain of PKA Iα from H- to B-conformation. a) H-conformation; b) B-conformation; c) H-conformation of the A-domain carrying the point mutation R209K. Hydrogen bonds are shown by dotted lines.

confirmed this experimental fact. Notwithstanding the presence of 3′:5′-AMP in the binding site, the transition proceeded in exactly the same way as for the domains with the R209 residue. The absence of CH–π interaction between residues 169 and 209 in this case was partially compensated by electrostatic and van der Waals interactions contributing to the maintenance of correct orientation of the β2β3-loop. As a result, residue L203 was "packed" into the hydrophobic pocket.

It should be noted that, as in the case of the A-domain with residue R209, the pure B-conformation of the A-domain with R209I substitution did not exist for a long time. This fact is supposedly explained by the influence of the entropy factor.

Because of short times of modeling, we could not demonstrate the losses of 3′:5′-AMP from the binding site. However, comparison of the energies of interaction between the residue at position 209 and 3′:5′-AMP (−13 ± 2 and −80 ± 3 kcal/mol for I209 and R209, respectively) shows much lower affinity of the ligands to the site with the R209I mutation. Obviously, the domains with this site must be characterized by the lower capacity for

3′:5′-AMP-induced activation, which has been shown experimentally [12].

A-domain carrying R209G mutation. The A-domain carrying point mutation R209G was analyzed only to show the importance of the residue at position 209 for conformational transition of the 3′:5′-AMP-binding domain. As a result, it was demonstrated that in the absence of 3′:5′-AMP[1] the system made a transition into but did not remain in the B-conformation and stayed most of the time between the conformations, periodically passing from one to the other.

In the presence of the R209G mutation, no definite state of the β2β3-loop was maintained by the residue at position 209 and, as a result, the β2β3-loop had higher conformational mobility. However, at certain moments of time there was a spontaneous appearance of the β2β3-loop twist, so that the B-conformation of the domain could be realized. Additional calculations showed that fixation of the necessary twist of the β2β3-loop by external forces retained the A-domain with the R209G mutation for a long time in the B-conformation and considerably reduced the time spent by the system between the conformations.

A-domain carrying R209E mutation. In 1996, Steinber et al. [12] showed that the introduction of a glutamic acid residue into position 209 of the A-domain almost completely deprived this domain of the capability for 3′:5′-AMP-induced activation. Our calculations are in agreement with this experimental fact, because all our attempts to obtain a 3′:5′-AMP-induced conformational transition failed due to the losses of 3′:5′-AMP from the binding site in 2-3 nsec of modeling.

At the same time, in the absence of 3′:5′-AMP the A-domain passed into the B-conformation during the first nanosecond. From the standpoint of energy estimation, such transition of the A-domain with the R209E mutation was advantageous because, in addition to reorganization of the PBC helix into the α-form, the energy of interaction between sequence 198-207 and residue 209 decreased (-1 ± 8 and -29 ± 3 kcal/mol for the H- and B-conformations, respectively). Besides, residue E209, due to the electrostatic and van der Waals interactions with residue G169, was able to maintain the correct twist of the β2β3-loop. The total energy of the E209–G169 interaction was -5 ± 3 kcal/mol.

A-domain carrying R209K mutation. According to the literature, the R209K and R333K substitutions considerably complicate conformational transitions of the 3′:5′-AMP-binding domains [10-12]. At present there is no explanation for this fact because it is obvious that the substitution of lysine for arginine cannot be an obstacle for 3′:5′-AMP binding. Indeed, the calculations have shown that 3′:5′-AMP does not change its position in the binding site under modeling conditions; however, even 15 nsec were insufficient to complete the transition from the H- to B-conformation. As it turned out, the inhibitory effect of K209 on conformational transition of the A-domain is caused by the fact that K209 does not maintain the β2β3-loop twist necessary for hydrophobic pocket formation (Fig. 2c). According to our results, which are in complete agreement with the data of X-ray structure analysis [4], the G169 residue acquired a position (Fig. 2c) such that the energy of electrostatic interaction between its carbonyl group and the side chain of K209 approached the minimum (-10 ± 5 kcal/mol), which in turn absolutely eliminated the hydrophobic pocket. It should be noted that reorganization of the PBC helix and formation of a bond between A202 and 3′:5′-AMP took place also in the presence of lysine residue at position 209.

The conformational transition under study was to a certain extent complicated by the fact that the energy of interaction between sequence 198-207 and residue 209 increased (-8 ± 5 and -1 ± 4 kcal/mol for H- and B-conformations, respectively) upon reaching the B-conformation. However, due to reorganization of the PBC helix, the H-conformation was not preferable in this case either.

It is noteworthy that the side chain of K209 in the absence of 3′:5′-AMP shifted toward the β2β3-loop and formed an ionic interaction with one of its acidic amino acid residues. In this case, the β2β3-loop could form the necessary twist with a higher probability, and conformational transition of the A-domain was almost the same as in the presence of the G209 mutation.

DISCUSSION

We have established using the methods of molecular modeling that the key event initiated by 3′:5′-AMP in the course of binding to the A- and B-domains of PKA Iα is formation of a hydrogen bond between the A202(A326) amide group and 3′:5′-AMP, which triggers the work of the hydrophobic switch [5, 15, 16]. The movement of the A202(A326) amide group, in the absence of which this bond cannot be formed, results in transformation of the short 3_{10}-helix of PBC into the longer α-helix and finally to displacement of the L203(L327) residue. The latter event, as is known [15, 16], becomes a prerequisite for the B-helix turn. The second no less important condition of displacement of the L203(L327) residue and its "packing" into the hydrophobic pocket formed by the PBC and the β2β3-loop lies in a special orientation of the β2β3-loop. Such orientation is maintained by residues R, I, E, but not K, at position 209(333). As a result, the presence of point mutations R209K and R333K impedes conformational transitions of the A- and B-domains of PKA Iα, which is confirmed by numerous experimental facts [10-12]. In light of these results, the role of the electrostatic switch seems to be supplementary and the invariance of arginine at positions 209(333) may be due to the fact that

[1] This system was not modeled in the presence of 3′:5′-AMP.

this residue best of all combines two functions: 3′:5′-AMP binding and maintenance of the β2β3-loop twist.

In spite of nonobligatory presence of the electrostatic switch in most of the 3′:5′-AMP-binding domains, one cannot underestimate the significance of formation of the D170–R209 bond for the A-domain of PKA Iα, which obviously must result in stabilization of the B-conformation. The above statement can be confirmed by a number of experimental works [14, 26, 27]. It is known that regulatory subunits carrying the D170A mutation are unable to retain 3′:5′-AMP in the site in the presence of C-subunits and rapidly pass into the H-conformation, forming an inactive complex with the latter.

Our data demonstrating the capability of 3′:5′-AMP-binding domains for spontaneous, not 3′:5′-AMP-induced transition into the B-conformation in the absence of C-subunit, are in complete agreement with the results of X-ray structure analysis [5] and NMR [28]. In this context, it should be noted that if the A-domain of PKA Iα forms many bonds with the C-subunit, then the B-domain is actually free [4] and, in accordance with our conceptions, must spend most of the time in the B-conformation, losing a few bonds with the C-subunit. This hypothesis is confirmed by data obtained by small-angle X-ray scattering [29] and demonstrating, in the case of PKA Iα (but not PKA IIβ), the absence of a bond between the B-domain and the C-subunit. Unsuccessful attempts to crystallize the inactive PKA Iα complex may also be indirect evidence of this [4].

Crystal structures could be obtained only for PKA IIα [30] and PKA Iα carrying the R333K mutation of the R-subunit [4]. The latter fact, at first glance, contradicts our results, because we have shown that the R333K mutation in the absence of 3′:5′-AMP has little influence on the course of conformational transition. In other words, the B-domain with the K333 mutation as part of the R:C complex must also be in the B-conformation and lose contacts with the C-subunit. However, more thorough consideration of the sought crystal structure showed the presence of inorganic phosphate in the 3′:5′-AMP-binding site, which maintained the K333 side chain in the same position as 3′:5′-AMP. Because of the positive charge of K333, it should be expected that the 3′:5′-AMP-binding site of the B-domain will actually invariably be occupied by organic or inorganic anions and, consequently, its transition into the B-conformation will be complicated.

In the case with PKA IIα, the following argument can be suggested as a hypothesis explaining the absence of B-domain transition into the B-conformation. PKA IIα does not have a glycine, but rather has an alanine residue in the position homologous to position 169 of PKA Iα. It has already been mentioned that the transfer of the A202 side chain even via the methylene group of G169 is accompanied by an energy barrier. The larger methyl group of alanine undoubtedly increases this barrier. Thus, at least in the presence of the C-subunit, there can be no transition of the B-domain of PKA IIα into the B-conformation.

The explanation of inhibitory effect of the sulfur-substituted 3′:5′-AMP analog (Rp-3′:5′-AMPS) on PKA activation may be important indirect evidence of the mechanism of the hydrophobic switch proposed in this paper and its key role in the conformational transitions of 3′:5′-AMP-binding domains [8]. Such explanation based on the demonstrated conformational changes in 3′:5′-AMP-binding domains is presented in the following paper.

The authors are grateful to Prof. M. L. McKee, Department of Chemistry and Biochemistry, Auburn University, USA, and to T. Lee, research worker of the same university, for their assistance with calculations.

This work was supported by a grant for students and post-graduate students of institutions of higher education and academic institutes situated on the territory of St. Petersburg (2010), a grant of Federal Target Program NK-541 (3), and grants of St. Petersburg State University (projects No. 11.37.141.2011 and 1.0.130.2010).

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