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Initiation of the 3′:5′-AMP-Induced Protein Kinase A Iα Regulatory Subunit Conformational Transition. Part II. Inhibition by Rp-3′:5′-AMPS

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Abstract—Protein–ligand docking and *ab initio* calculations have shown that the 3′:5′-AMP phosphorothioate analog (Rp-3′:5′-AMPS) blocks the A326 amide group displacement typical of transition from the H- to B-conformation within the B-domain of protein kinase A Iα R-subunit. This behavior of Rp-3′:5′-AMPS leads to the inhibition of initial stages of hydrophobic relay operation. In accordance with the proposed hypothesis, Rp-3′:5′-AMPS similarly to 3′:5′-AMP forms a hydrogen bond with the amide group of A326; however, the properties of this bond together with the position of the sulfur atom prevent the movement of A326. Finally, the Rp-3′:5′-AMPS-bound domain appears to be locked in the H-conformation, which is in agreement with the X-ray data.

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Key words: B-domain of PKA Iα R-subunit, 3′:5′-AMP, Rp-3′:5′-AMPS, A326, protein–ligand docking, quantum chemical analysis

The 3′:5′-AMP-binding domains are components of the R-subunits of protein kinase A (PKA), EPAC proteins, ion channels (CNG, HCN), and some transcription factors [1]. All these proteins can to some extent be activated by 3′:5′-AMP and other cyclic nucleotides, their derivatives, and analogs. However, there is a group of cyclic nucleotide derivatives being inverse agonists of all PKA isoforms [2, 3] and EPAC proteins [4]. All compounds of this group are characterized by substitution of a sulfur atom for the equatorial oxygen atom of the phosphate ring [2, 5]. One of representatives of such sulfur-substituted analogs of cyclic nucleotides is Rp-3′:5′-AMPS, a 3′:5′-AMP analog.

The practical significance of Rp-3′:5′-AMPS and its derivatives as PKA activation blockers consists first of all in their application as AIDS drugs [5]. Hence, it is necessary to understand the principles of Rp-3′:5′-AMPS action for creating more efficient preparations on its basis. In fundamental research, understanding of the mechanism of PKA and EPAC inhibition by Rp-3′:5′-AMPS is a key for description of conformational changes in the 3′:5′-AMP-binding domains. However, in spite of the great number of works [2-4, 6-8] devoted to the interaction between 3′:5′-AMP-binding domains and Rp-3′:5′-AMPS and exceptional importance of this problem, the mechanism of Rp-3′:5′-AMPS action is still unclear in many respects.

In accordance with the recent conceptions, the inhibitory properties of Rp-3′:5′-AMPS are accounted for by different capabilities of sulfur and oxygen atoms to form hydrogen bonds [3, 7, 8]. It is known that the oxygen atom of 3′:5′-AMP at the same position as the sulfur atom of Rp-3′:5′-AMPS forms two conservative hydrogen bonds in all 3′:5′-AMP-binding domains. One bond links the side chain of invariant arginine (R333 for the B-domain of PKA Iα) and the other bond links the amide group of invariant alanine (A326 for the B-domain of PKA Iα) [9]. The sulfur atom of Rp-3′:5′-AMPS cannot form these bonds, or the formed bonds are very weak [7]. As a result, Rp-3′:5′-AMPS prevents switching on of both electrostatic and hydrophobic relays, and the domain is blocked in the H-conformation [7]. The latter assump-

Abbreviations: 3′:5′-AMP, cyclic adenosine-3′,5′-monophosphate; C-subunit, catalytic subunit; PKA Iα, protein kinase A Iα; Rp-3′:5′-AMPS, cyclic adenosine-3′,5′-monophosphorothioate Rp isomer; R-subunit, regulatory subunit.

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tion is confirmed by the data of X-ray structure analysis. It was shown that, if the A-domain was initially in the H-conformation, the complex of the A-domain and Rp-3′:5′-AMPS also had the H-conformation [7]. However, there also exist complexes of Rp-3′:5′-AMPS and the 3′:5′-AMP-binding domain in the B-conformation [6].

It should be noted that it is impossible to speak about final understanding of the principle of Rp-3′:5′-AMPS inhibitory effect on PKA activation in the absence of precise knowledge of the roles of hydrophobic and electrostatic relays in conformational transition of the 3′:5′-AMP-binding domain. On the other hand, explanation of the effect of Rp-3′:5′-AMPS by the proposed mechanism of rearrangement of the phosphate binding cassette and the β2β3-loop (Part I of this series, see preceding paper) would be indirect verification of the actual implementation of this mechanism. Therefore, the goal of this work was to explain the inhibitory effect of Rp-3′:5′-AMPS on PKA Iα activation in light of the demonstrated (in Part I) conformational changes in the 3′:5′-AMP-binding sites.

MATERIALS AND METHODS

Preparation of B-domain of PKA Iα. The B-domain of PKA Iα was prepared for subsequent ligand–receptor docking as described in the "Materials and Methods" of Part I.

Quantum-chemical analysis of parameters of hydrogen bonds formed by equatorial oxygen (sulfur) atom of ligand and amide group of the protein. The energy of the bonds under study was minimized using Gaussian 03 quantum chemistry software [10] by the Hartree–Fock–Roothan method [11] in the 6-31+G(d) basis [12-15] without considering the effect of the medium, $T = 0$ K. The peculiar features of the hydrogen bond between the amide group of the protein and 3′:5′-AMP were studied using the model compounds: formyl methylamine and trimethyl phosphate. The bond involving Rp-3′:5′-AMPS was analyzed with trimethyl phosphorothioate used in place of trimethyl phosphate.

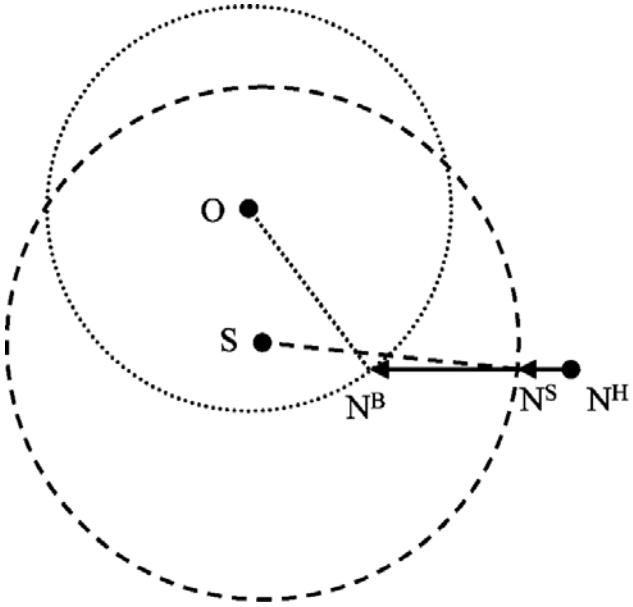
Docking of ligands into 3′:5′-AMP-binding site of PKA Iα B-domain. The docking of Rp-3′:5′-AMPS into the 3′:5′-AMP-binding site of the B-domain was performed in Quantum 3.3.0 (2008-2009) [16]. This software performs the docking of a flexible ligand into a rigid protein and then minimizes the energy of the resulting complex.

Alignment of spatial structures of H- and B-conformations of B-domain. The spatial structures of the B-domain were aligned using VMD software [17] by the main-chain atoms (N, CA, C) of the following amino acid residues: 272-281, 291-301, 314-319, 338-348. All of these amino acid residues are components of the most stable part of the domain called β-barrel [18].

RESULTS

Docking of Rp-3′:5′-AMPS into 3′:5′-AMP-binding site of PKA Iα B-domain. The results of Rp-3′:5′-AMPS docking into the binding site of the B-domain in the B-conformation were fully confirmed by the data of X-ray structure analysis [6]. Rp-3′:5′-AMPS forms five hydrogen bonds with the same amino acid residues of the protein as 3′:5′-AMP: the G323, A326, and A334 amide groups and the E324 and R333 side chains.

In the course of Rp-3′:5′-AMPS docking into the binding site of the B-domain in the H-conformation, it was established that Rp-3′:5′-AMPS interacts with a site similar to 3′:5′-AMP, forming three hydrogen bonds: with the G323 and A334 amide groups and with the R333 side chain. However, the alignment of Rp-3′:5′-AMPS- and 3′:5′-AMP-bound H-conformations showed different positions of the equatorial oxygen and sulfur atoms (figure). This difference was due to a slightly different position of the Rp-3′:5′-AMPS phosphate ring and the longer covalent P–S bond (1.95 Å) compared to the P–O bond



Scheme explaining the proposed model of Rp-3′:5′-AMPS-dependent inhibition of conformational transition of the PKA Iα B-domain. Points O and S show the positions of equatorial oxygen and sulfur atoms of the ligands determined by the docking of 3′:5′-AMP and Rp-3′:5′-AMPS, respectively, in the binding site of the B-domain in the H-conformation. The abilities of the sought oxygen and sulfur atoms to form hydrogen bonds of different length with the amide group of the protein are shown by the circles of different radii. Points N^H and N^B correspond to the positions of nitrogen atom of the A326 amide group in the H- and B-conformations, and vector $N^H N^B$ corresponds to the direction of movement of this atom during the transition from one conformation to the other. Point N^S indicates the supposed position of the nitrogen atom during the binding of the B-domain in the H-conformation with Rp-3′:5′-AMPS

(1.50 Å) [8]. We suppose that the difference in positions of the oxygen and sulfur atoms is one of the reasons why Rp-3′:5′-AMPS and its derivatives behave as inverse agonists [3] of PKA Iα.

Quantum-chemical analysis of parameters of hydrogen bonds formed by equatorial oxygen (sulfur) atom of ligand and amide group of the protein. According to the results of quantum-chemical calculations, the hydrogen bond between the equatorial oxygen atom of 3′:5′-AMP and the amide group of the protein is characterized by the energy of −7.7 kcal/mol and length defined as a distance between heavy atoms: 3.0 Å. Rp-3′:5′-AMPS also forms a hydrogen bond with the amide group of the protein; however, this bond is less advantageous energetically (−4.7 kcal/mol) and longer (3.9 Å). The difference between the lengths of hydrogen bonds under consideration, in accordance with our conceptions, is the second reason why Rp-3′:5′-AMPS and its derivatives block the activation of PKA Iα.

The ability of oxygen and sulfur atoms to form hydrogen bonds of different length with the amide group of the protein is shown in the figure as the circles of different radii.

Rp-3′:5′-AMPS blocks movement of A326 amide group. As demonstrated by molecular dynamics (Part I), the nitrogen atom of the A326 amide group is displaced during the conformational transition from point N^H to point N^B (figure). In the H-conformation (point N^H), the distance between the oxygen and nitrogen atoms is 5.4 Å. Then it begins to decrease and reaches 3.0 Å in the B-conformation (point N^B). At this very distance, according to the results of quantum chemical calculations, the hydrogen bond between the A326 group and the equatorial oxygen atom of 3′:5′-AMP is characterized by the maximum energy.

It is obvious that the condition of maximum energy of the hydrogen bond for Rp-3′:5′-AMPS will be fulfilled already in point N^S (figure). Moreover, if we assume the displacement of the nitrogen atom from point N^H to point N^B, the distance between the sulfur and nitrogen atoms will decrease from 4.6 to 2.0 Å and will be less than the sum of van der Waals radii of these atoms. The response to the concomitant increase of system energy will be the return of the nitrogen atom into point N^S. Thus, Rp-3′:5′-AMPS blocks the movement of the A326 amide group characteristic of domain transition from the H- to B-conformation.

DISCUSSION

The present research resulted in construction of a model, according to which Rp-3′:5′-AMPS prevents displacement of the A326 amide group observed during transition of the B-domain from the H- to B-conformation and thereby blocks the initial stages of the hydrophobic relay operation. In accordance with the suggested hypothesis, the binding of Rp-3′:5′-AMPS, similar to that of 3′:5′-AMP, is accompanied by formation of a hydrogen bond with the A326 amide group; however, the parameters of this bond, in combination with the position of sulfur atom, make the movement of A326 impossible. As a result, the Rp-3′:5′-AMPS-bound domain is "locked" in the H-conformation, as is confirmed by the data of X-ray structure analysis [7].

Due to the impossibility of transition of the 3′:5′-AMP-binding domain from the H- to B-conformation in the presence of Rp-3′:5′-AMPS, there is only one way to form a complex, being the B-conformation of the PKA Iα R-subunit with Rp-3′:5′-AMPS in the binding site [6]. As been demonstrated (Part I and [7, 19]), the free 3′:5′-AMP-binding domain spends most of the time in the B-conformation, which, in accordance with our data, binds Rp-3′:5′-AMPS in the same position as 3′:5′-AMP. This means that in the absence of C-subunit or weak bonds with it, both 3′:5′-AMP and Rp-3′:5′-AMPS will stabilize the B-conformation of the 3′:5′-AMP-binding domain.

Stabilization of the B-conformation of free 3′:5′-AMP-binding domains in the presence of Rp-3′:5′-AMPS can obviously account for some experimental facts of behavior of this ligand as a partial weak agonist of PKA Iα. So, it was shown that Rp-3′:5′-AMPS is a weak agonist of PKA Iα with the G199E mutation [20] and with substitutions of some other residues for arginine at position 209 [21]. Moreover, at high substrate concentration, Rp-3′:5′-AMPS can be a partial agonist of PKA Iα not carrying the mutations [21]. The essence of potential mechanism is as follows. The PKA Iα B-domain is actually lacking the bonds with the C-subunit [22] and, therefore, spends most of the time in the B-conformation [23]. The free B-domain binds Rp-3′:5′-AMPS like 3′:5′-AMP, which enhances the probability of spontaneous ligand-independent conformational transition of the A-domain even in the presence of the C-subunit. There may be two reasons why Rp-3′:5′-AMPS only insignificantly inhibits the change in the A-domain conformation. In the presence of excessive substrate there may be competitive displacement of the A-domain from the surface of the C-subunit and, consequently, the binding of Rp-3′:5′-AMPS with the free A-domain being preferably in the B-conformation. On the other hand, mutations in the A-domain prevent the binding of ligands to the 3′:5′-AMP-binding sites, and the ligand-independent transition becomes the only possible variant of conformational changes for such domain.

The analogous mechanism can be assumed also for the Rp-3′:5′-AMPS-induced activation of PKA Iα with the R209K mutation [24]. However, there seems to be a more reasonable explanation. Due to its great length (5.6 ± 0.1 Å), the R209 side chain forms two hydrogen bonds with the carbonyl group of the N171 main chain; as a result, R209 is rigidly fixed in the binding site.

Obviously, the fixed arginine maintains the constant position of the ligand. The length of the side chain of lysine residue is less (4.7 ± 0.1 Å); therefore, it is much more mobile and cannot accurately fix the ligand. It is quite probable that in the case of Rp-3′:5′-AMPS the above circumstance partially removes obstacles for the movement of the A202 amide group and permits conformational transition.

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