

RESEARCH ARTICLE

A molecular dynamics study of adenylyl cyclase: The impact of ATP and G-protein binding

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Abstract

Adenylyl cyclases (ACs) catalyze the biosynthesis of cyclic adenosine monophosphate (cAMP) from adenosine triphosphate (ATP) and play an important role in many signal transduction pathways. The enzymatic activity of ACs is carefully controlled by a variety of molecules, including G-protein subunits that can both stimulate and inhibit cAMP production. Using homology models developed from existing structural data, we have carried out all-atom, microsecond-scale molecular dynamics simulations on the AC5 isoform of adenylyl cyclase and on its complexes with ATP and with the stimulatory G-protein subunit G α . The results show that both ATP and G α binding have significant effects on the structure and flexibility of adenylyl cyclase. New data on ATP bound to AC5 in the absence of G α notably help to explain how G α binding enhances enzyme activity and could aid product release. Simulations also suggest a possible coupling between ATP binding and interactions with the inhibitory G-protein subunit G α i.

Introduction

Cyclic adenosine monophosphate (cAMP) is a universal second messenger in signal transduction based on G-protein coupled receptors (GPCR) in eukaryotes [1]. It is responsible for amplifying stimuli received by the cell [2–6]. It binds and regulates kinases and ion channels, whose activity subsequently determines how the cell will respond to the stimuli [7,8].

It is consequently not surprising that cAMP levels must be tightly controlled and the enzymes responsible for cAMP synthesis are indeed highly regulated [9]. This family of enzymes, the adenylyl cyclases (also commonly known as adenylate cyclases), has nine members (hereafter termed AC1–9). Each member of the family has specific regulatory properties and tissue distributions [10,11], however they all convert adenosine triphosphate (ATP) into cAMP via a cyclization reaction. Eukaryotic ACs share a similar topology with a variable N-terminus (NT) and two repeats of a membrane-spanning region followed by a cytoplasmic region [12,13]. The latter is divided into two pseudosymmetric domains, termed C1 and C2, each containing approximately 230 amino acid residues and sharing roughly 40% sequence

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identity. The N-terminal and C-terminal portions of C1 and C2 are the most variable regions among the different AC isoforms and among different organisms (see Fig 1).

The catalytic site of ACs is located at the C1/C2 interface and binds a molecule of ATP accompanied by two magnesium ions. The adenine moiety of ATP forms hydrophobic interactions in the cleft between the C1 and C2 domains and its N1 and N6 sites form H-bonds with Lys 938 and Arg 1018 deep in the active site cavity (the residue numbering used here is from the chimeric rat/dog X-ray structure of AC5, PDB entry: 1CJK [14]). The triphosphate moiety of ATP forms extensive H-bond interactions with residues of both the C1 (Thr 401, Phe 400, Gly 399, Arg 484) and C2 domains (Lys 1065, Arg 1029). The two magnesium ions within the active site are octahedrally coordinated by the O3' oxygen of the ribofuranose alcohol, the triphosphate group, and the residues Asp 396, Asp 440 and Ile 397 of the C2 domain. Despite this ensemble of interactions, the triphosphate-Mg complex remains exposed to the solvent and interacts with several water molecules (see Fig 1).

Upon GPCR activation [15–17], the stimulatory G-protein subunit alpha ($G\alpha$) is released from its cognate receptor and binds to and activates the AC enzyme via the subunit's interaction with the C2 domain [11,15,18,19]. A number of other AC modulators, either stimulators or inhibitors of cAMP synthesis, are known. These include the inhibitory G-protein subunits $G\alpha_i$ and $G\alpha_o$, calcium ions, calmodulin and a variety of kinases. This indicates that the AC enzymes are capable of integrating diverse signals in a subtle and specific manner [20–23].

Structural information on a single domain of the cytoplasmic catalytic core of AC [24] and on a complex containing both AC catalytic domains bound to an active conformation of the stimulating $G\alpha$, with or without a bound ATP analog, is available [25]. However, the structure and function of the transmembrane regions, each predicted to contain six membrane-spanning helices, is unknown and we also lack structural data on the N- and C-terminal portions of the C1 and C2 domains. In both structures, AC was indeed co-crystallized with forskolin, a plant-derived activator of cAMP production by AC [26,27]. However, no mammalian forskolin analogues have been identified that could regulate ACs via the forskolin binding pocket [28,29]. Moreover, there is no data on the enzyme bound to ATP (or an ATP analog) in the

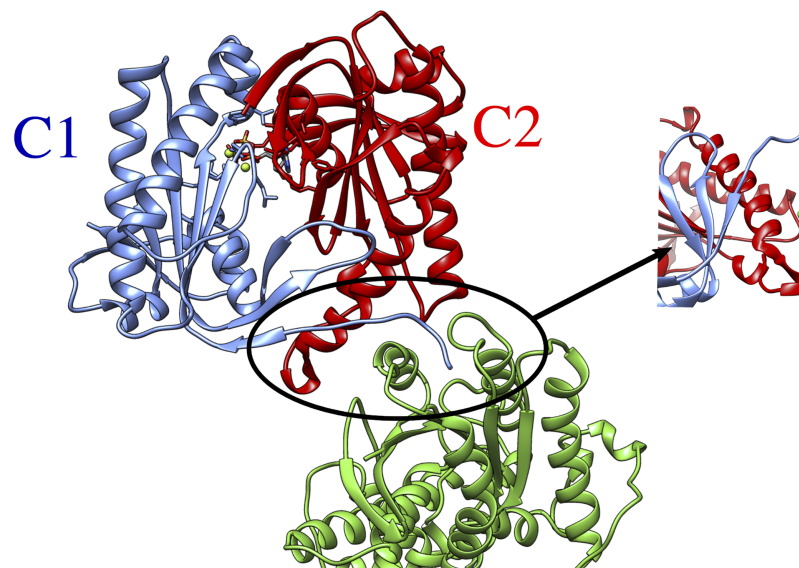


Fig 1. Structure of the cytoplasmic segment of the AC5 isoform of adenylyl cyclase in complex with ATP and the activating G-protein $G\alpha$ viewed from the side closest to the cell membrane. The C1 and C2 subunits of AC5 are colored blue and red respectively and $G\alpha$ is colored green. ATP is shown in a CPK representation with standard chemical coloring. A close-up view of the helix-helix interactions between AC5 and $G\alpha$ is provided (circle).

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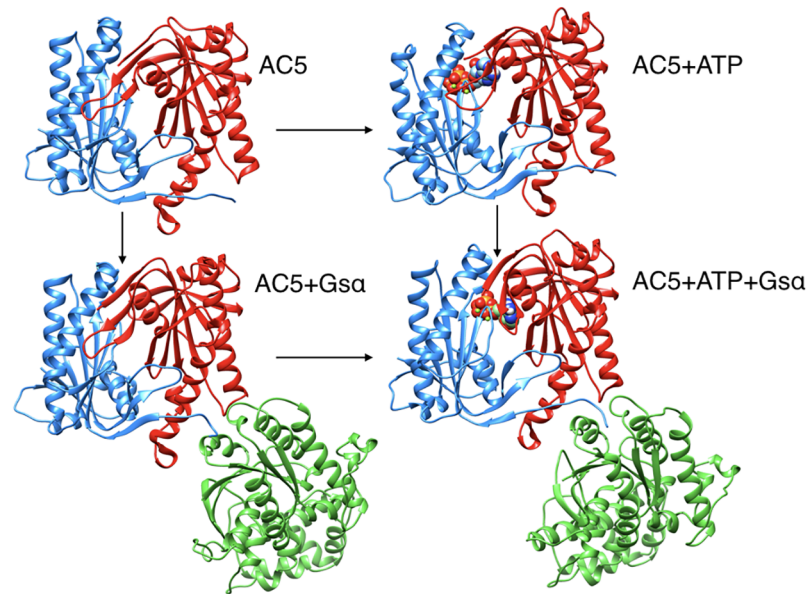


Fig 2. Structure of the cytoplasmic segment of the AC5 isoform of adenylyl cyclase and of its complexes with ATP and the activating G-protein $G\alpha$ viewed from the side closest to the cell membrane. Proteins are shown as backbone ribbons. The C1 and C2 subunits of AC5 are colored blue and red respectively and $G\alpha$ is colored green. ATP is shown in a CPK representation with standard chemical coloring. In each case, the structures are averages taken from the molecular dynamics simulations described in this work.

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absence of activating $G\alpha$, so it is difficult to understand how the latter protein actually activates adenylyl cyclase.

In order to address the latter question, we have used molecular dynamics simulations to study the impact of $G\alpha$ and also ATP binding on the structure and flexibility of AC. It is worth stressing that the cytoplasmic domains of AC5, structurally characterized by X-ray crystallography, are capable of reproducing many of the regulatory properties of the wild type enzyme and therefore can be used as working models to investigate the regulation mechanisms of AC [30,31]. Since different AC isoforms also respond differently to the same stimuli, we have used homology modeling to be able to work with a single isoform from a single organism. We have chosen to study the mouse AC5 isoform. This isoform notably plays a key role in a variety of neuronal GPCRs-based signal cascades [21,32,33].

The all-atom microsecond-scale simulations of the AC5 and its complexes with ATP and/or $G\alpha$ studied here (Fig 2) help to explain how binding changes the properties of AC5 and notably to understand the stimulatory effect of $G\alpha$. For comparison, we also carry out some studies of the impact of the plant stimulating agent forskolin (see below).

While we are interested in the stimulation of AC5 function, we remark that recent work from Van Keulen and Rothlisberger [34] provides information of the inhibition of AC5 function, by carrying out simulations on $G\alpha_i$ bound to a putative site within C1 (by analog with the $G\alpha$ site on C2). Note that, in contrast to $G\alpha$, the active form of $G\alpha_i$ is myristoylated on its N-terminal [35–37] (see also the conclusions to this article).

Materials and methods

Starting structures

Homology models for the mouse sequences of AC5 with or without ATP (adenosine triphosphate) and/or $G\alpha$ (the α subunit of the guanosine nucleotide binding protein) were built as

follows. We only take into account the cytoplasmic domains of AC5 (C1 and C2 in Fig 1) because no structural data exist on the linkers to the membrane. It has also been shown that the soluble domains of AC5 are enzymatically competent [30,31] and no impact of the membrane anchorage on the stimulation of AC5 has been found [38,39]. We also removed forskolin from the crystallographic structures, because no mammalian forskolin analogues have been yet identified that might regulate ACs [21]. Lastly, we did not include any modification to Gs α (like the majority of G protein α subunits, Gs α is not N-myristoylated) [39,40].

The model of the cytoplasmic segment of mouse AC5 without ATP or Gs α was generated with Modeller v9.12 [41] using the PDB structure 1AZS (chimera AC5 rat/ AC2 dog) [25]. This protein has a 98% sequence identity with the domain C1 and a 57% identity with C2 and is therefore a reliable template. The model of mouse AC5 with ATP and magnesium ions was generated using the same protocol using 1CJK as template [14]. The model of the mouse Gs α protein in its bound form with Mg²⁺ and GTP (guanosine triphosphate) as ligands was also generated with Modeller starting from bovine Gs α (80% sequence identity with the mouse protein) again taken from 1CJK. In each case, 100 homology models were generated and the structure with the lowest DOPE score [42] was selected for the simulations. The starting conformations for the AC5+Gs α and the AC5+ATP+Gs α complexes were obtained by molecular docking starting from the homology models of isolated AC5 and of Gs α using the Cluspro webserver [43]. The resulting positions of Gs α were close to those observed in the 1AZS and 1CJK complexes (0.8–1.5 Å for the binding α -helix of Gs α and 2.3–2.6 Å for the full backbone), although some change in its position is to be expected given residue substitutions at the ends of the α -helices of the C2 domain that bind to the G-protein (introduced during the homology modeling used to develop a non-chimeric AC5 structure).

Molecular dynamics simulations

Molecular dynamics simulations were performed with the GROMACS 5 package [44–48] using the Amber 99SB-ILDN force field for proteins that has been shown to yield an accurate description of many structural and dynamical properties of proteins [49–52]. Side chain protonation states of titratable amino acids were assigned using a value of pH = 7.4 with the help of the pdb2pqr software [53]. Capping acetyl and methyl-amino groups were added to the N and C termini of both AC5 domains and of Gs α . The four states we study (AC5, AC5+ATP, AC5+Gs α and AC5+ATP+Gs α) were each placed in a truncated octahedral box and solvated with TIP3P water molecules [54] to a depth of at least 11 Å. The solute was neutralized with potassium cations and then K⁺Cl⁻ ion pairs [55] were added to reach a physiological salt concentration of 0.15 M. Parameters for ATP and GTP were taken from [56]. The parameters for Mg²⁺ came from [57]. This new set of parameters was developed to improve the kinetic properties of Mg²⁺ ions with water and with the phosphate ion and it was implemented in Amber99. This new set of parameters also provided a better description of the structure of Mg²⁺-phosphate binding than previous sets (these interactions are naturally important in our simulations in the presence of ATP) [57]. Hence, the combination of Amber 99SB-ILDN and the new set of parameters of Mg²⁺ ions is currently the best choice to reproduce the dynamics of AC5 and Gs α , and to properly describe the interactions of Mg²⁺ with AC5 and ATP.

Long-range electrostatic interactions were treated using the particle mesh Ewald method [58,59] with a real-space cutoff of 10 Å. We used virtual interaction sites for the hydrogens and bond lengths were restrained using P-LINCS [47,60], allowing a time step of 4 fs [61]. Translational movement of the solute was removed every 1000 steps to avoid any kinetic energy build-up [62]. After energy minimization of the solvent and equilibration of the solvated system for 10 ns using a Berendsen thermostat ($\tau_T = 1$ ps) and Berendsen pressure coupling ($\tau_P = 4$ ps)

[63], the simulations were carried out in an NTP ensemble at a temperature of 310 K and a pressure of 1 bar using a Bussi velocity-rescaling thermostat [64] ($\tau_T = 1$ ps) and a Parrinello-Rahman barostat ($\tau_P = 1$ ps) [65]. Simulations were carried out using typically between 72 and 120 computer cores depending on the system size, which allowed a production rate of about 100 ns/ day. Analysis was carried out on a 1.1 μ s production segment for each simulation, following a 400 ns equilibration period.

Molecular dynamics simulations in the presence of forskolin

Although no mammalian forskolin (FOK) analogues have been yet identified that might regulate ACs, FOK is known to stimulate cAMP production by adenylyl cyclase. We can therefore ask if forskolin and Gs α have similar conformational and dynamic impacts on AC5. To do this, we began with the central structure of the largest clusters obtained in the last 500 ns of the trajectories of AC5 and AC5+ATP and we placed the forskolin in the pocket identified crystallographically. For FOK, we used RESP charges [66] and Amber 99SB with GAFF for the topology parameters. Using the protocol described above for the molecular dynamics simulations, we performed 1000 ns of simulation for each system (AC5+FOK and AC5+ATP+FOK). Analysis was carried out on the final 600 ns production segment for each simulation.

Analysis of the simulations

We analyzed our MD simulations using average structures, time series and average values of RMSD (root mean square deviation) and RMSF (root mean square fluctuations), specific geometrical measurements described below, protein-protein and protein-ligand interface characteristics and, in some cases, residue-by-residue conformational and dynamic properties.

The C1/C2 interface was characterized using three quantities: the gap volume, the change of accessible surface area upon binding (Δ ASA), and the gap index [67,68]. The gap volume was calculated using the procedure developed by Laskowski [67], which estimates the volume enclosed by any two molecules. The change of solvent accessible surface area on complexation is defined as:

$$\Delta\text{ASA} = \frac{(\text{ASA}_A + \text{ASA}_B - \text{ASA}_{AB})}{2} \quad (1)$$

where A and B represent the monomeric states, AB is the dimeric state. The solvent accessible surface area was calculated by using the same radii as the gap volume calculation [67]. The gap index evaluates the complementarity of the interacting surfaces in a protein-protein complex and is defined as Gap index = Gap volume / Δ ASA. Typical values of the gap index range from 1 \rightarrow 5. Lower values characterize interfaces with better structural complementarity. In general, protein homodimers have significantly smaller values than heterodimers [68].

In order to characterize potential G protein binding sites we computed the angle α_{C2} between the pairs of α -helices within domain C2 that bind Gs α (termed α_3 and α_4 in Fig 3) and also the angle α_{C1} between the quasi-symmetric pair of helices within domain C1 (termed α_1 and α_2 in Fig 3). The latter helices may constitute a binding site for the inhibitory G-protein subunit G α_i under the hypothesis of G α_i binding in a symmetrical configuration [36]. The angles were measured using helical axes derived from the residues that remain in stable α -helical conformations throughout the simulations (C1: 408–420 and 468–475, C2: 910–918 and 978–988, see Fig 3).

In order to address the collective motions involved in the stimulation pathway, we carried out a principal component analysis (PCA) on the all-atom molecular dynamics simulations. We considered only the backbone heavy atoms (C, C α and N) and computed a least squares fit

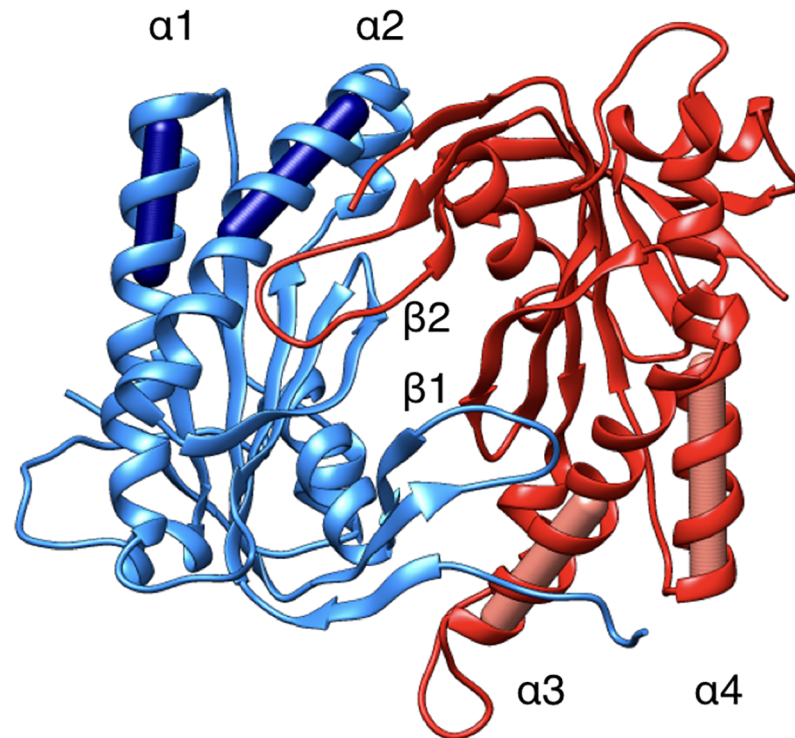


Fig 3. Illustration of the α -helices involved in binding the stimulatory protein $G_{s\alpha}$ ($\alpha3$ and $\alpha4$) and potentially binding the inhibitory protein $G_{i\alpha}$ ($\alpha1$ and $\alpha2$). The cylinders represent the helical axes used in determining the angles between the pairs of helices. The C1 and C2 domains are colored blue and red respectively. Two relevant β -loops ($\beta1$ in domain C1:494–503 and $\beta2$ in domain C2:1010–1016) are also indicated.

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on the molecular system and the mass-weight covariance matrix using the GROMACS package [48]. In order to analyze the motions corresponding to individual eigenvectors, we filtered the original trajectory and projected out the part along the selected eigenvector. We then analyzed the movements associated with the first five eigenvectors that contribute most to the global movements of the system.

Results

In the absence of any structural information on a single non-chimeric isoform of adenylyl cyclase, or on the catalytic domains of the enzyme without the stimulating G-protein subunit $G_{s\alpha}$, we use a combination of homology modeling and molecular dynamics to try to understand how protein or ligand binding can modify the conformation or the dynamics of AC5 and subsequently impact on its enzymatic function.

We have studied the behavior of four molecular species (see Fig 2): isolated AC5, AC5 with ATP and two Mg^{2+} ions in its active site (AC5+ATP), AC5 bound to the activating G-protein subunit $G_{s\alpha}$ (AC5+ $G_{s\alpha}$ +GTP) and AC5 bound to both ATP and $G_{s\alpha}$ (AC5+ATP+ $G_{s\alpha}$ +GTP). For each of these species, we generated 1.5 μ s MD trajectories in an aqueous environment with a physiological salt concentration (0.15 M KCl). The first 400 ns of each trajectory were treated as equilibration of the system and analysis was carried out only on the remaining 1.1 μ s. Data shown in S1–S3 Tables confirm the stability of the simulations during the segment used for analysis.

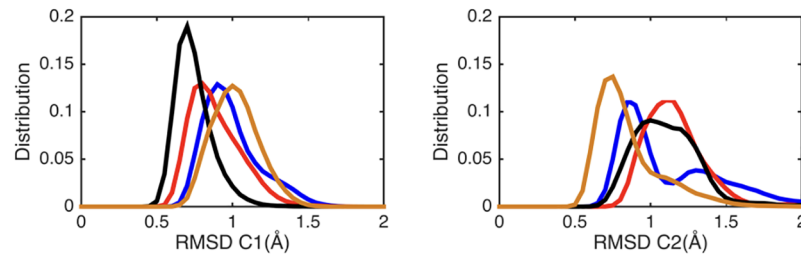


Fig 4. Probability distributions of RMSD for C1 (left) and C2 domains (right) calculated along the MD trajectories with respect to the corresponding average structure: AC5 (blue), AC5+ATP (red), AC5+G α (ochre) and AC5+ATP+G α (black).

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We begin by considering the global impact of ATP or G α binding on AC5. RMSD calculations with respect to the average MD structure of each AC5 domain show that the isolated protein (blue lines in Fig 4) is moderately flexible. Most of the flexibility in domain C1 concerns the unstructured N-terminal. However, as shown in Fig 4, domain C2 visits three conformational substates that involve large movements of loop β 2 (see Fig 3), changing its distance from the unoccupied ATP binding site.

Adding ATP to the AC5 binding site has a significant effect on both the structure and the dynamics of the protein. Although the ATP binding site involves both domains of AC5, ATP has stronger interactions with domain C1 via its associated Mg $^{2+}$ ions, notably with residues Asp 396 and Asp 440. It is thus not surprising that ATP binding slightly rigidifies domain C1 (red lines in Fig 4). However, it also leads to an increased overall flexibility in domain C2, coupled with the selection of a single substate for the β 2 loop (the longest-lived substate in isolated AC5, see Fig 5). ATP remains mobile and internally flexible within its binding site with the adenine base regularly changing its orientation with respect to the triphosphate tail. Although interactions between the terminal phosphate group of ATP and Lys 1065 (belonging to loop β 2) are maintained, interactions between the penultimate phosphate and Arg 1029, a key

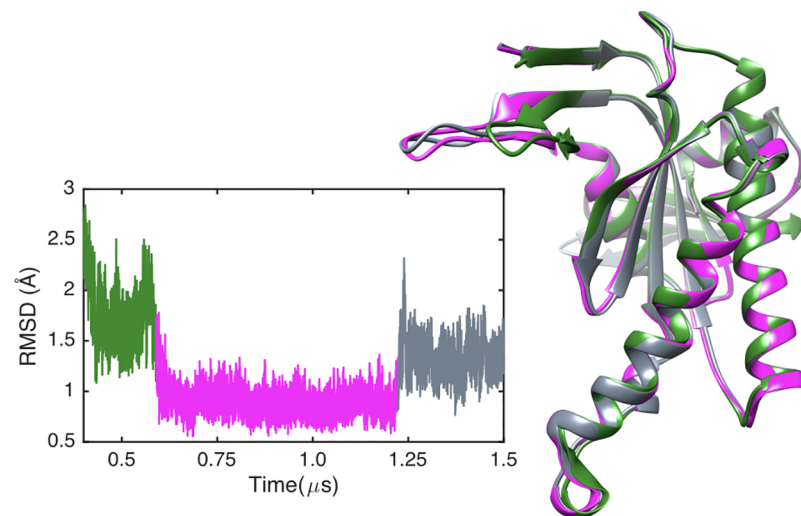


Fig 5. Left: RMSD time series of domain C2 of AC5. Right: Three average substate structures of C2 (green, magenta and grey) show that changes mainly involve the β 2 loop.

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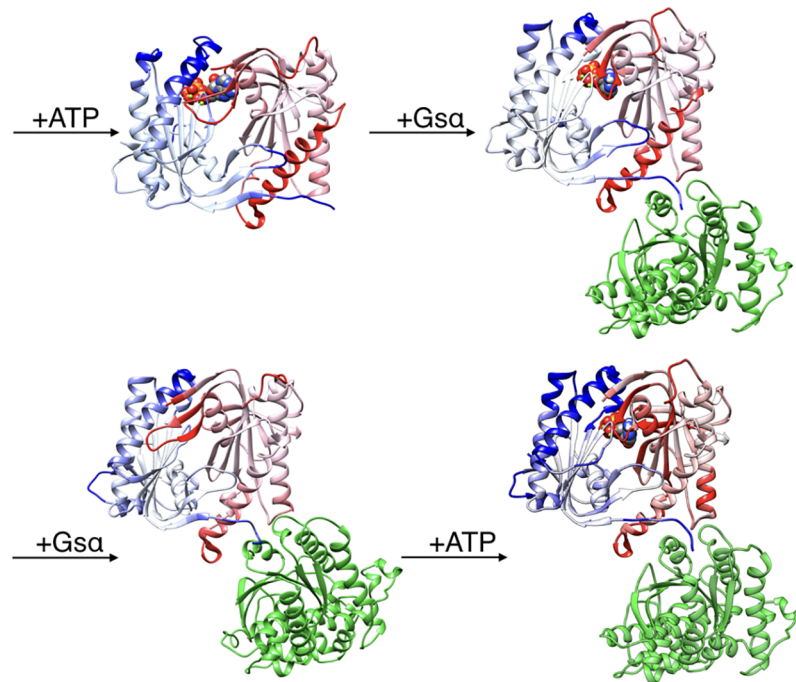


Fig 6. Changes in the conformation induced by ATP and G α binding. More intense colors (blue for domain C1 and red for domain C2) correspond to larger movements compared to the preceding structure (i.e. AC5 and AC5+ATP for the top line and AC5 and AC5+G α for the bottom line) on a scale of 0 \rightarrow 4 Å.

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functional residue, are absent, the arginine side chain being separated from its target oxygen atom by roughly 11 Å.

ATP binding also turns out to have more global effects on AC5. First, the angles between the pairs of α -helices in both AC5 domains are modified: significantly increased in domain C1 (by 18°, see supplementary S1 Table) and reduced in C2 (by 7°), involving a global movement of helix α_3 (see Fig 5). We also see an overall tightening of the C1/C2 interface, with a decrease in the average gap index from 3.1 Å for isolated AC5 to 2.8 Å once ATP is bound. In terms of flexibility, ATP binding mainly rigidifies the binding site region of AC5, although it also increases the flexibility of the C-terminals of helices α_1 and α_3 and the N-terminal of α_4 .

If we now add G α to the AC5+ATP complex we see a further movement of helix α_3 (see Fig 6), although this has little effect on the angle α_{C2} where the G-protein binds (see S1 Table). In the more distant C1 domain, the angle α_{C1} is also only slightly affected despite some movement in the helices α_1 and α_2 . The most striking effects of G α binding involve the ATP active site, where a β -sheet movement brings the key residue Arg 1029 back into its active location binding the primary phosphate group of ATP and globally rigidifies the binding site (with a concomitant effect on domain C1, see Figs 4 and 7). Simultaneously, we see an opening of loop β_2 away from the active site, re-adopting the first substate seen in isolated AC5 and increasing its flexibility (see Fig 7). This change is coupled to a looser C1/C2 interface whose gap index increases from 2.8 to 3.8, putting it in the realm of non-obligate protein-protein interfaces [67,68].

As well as modifying the ATP binding site, G α also modifies the mobility of ATP whose average RMSD decreases by 50% from 0.6 Å to 0.3 Å (see S3 Table and S1 Fig). In this state, ATP has strong interactions with both domains. As well as stable binding of the triphosphate tail to the key residues Arg 1029 and Lys 1065, there are also stable interactions of Asp 460 and

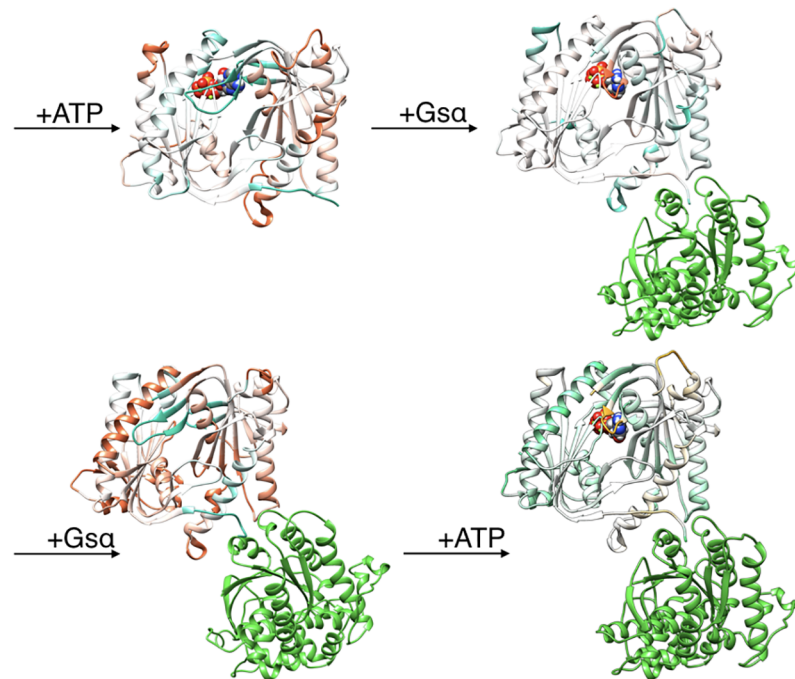


Fig 7. Changes in RMSF induced by ATP and Gsq binding. More intense colors (orange for increased flexibility and cyan for decreased flexibility) correspond to differences with respect to the preceding structure (i.e. AC5 and AC5+ATP for the top line and AC5 and AC5+Gsq for the bottom line) on a scale of $-1.2 \text{ \AA} \rightarrow +1.2 \text{ \AA}$.

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Asp 396 with the two Mg^{2+} ions. This leads to a 25% reduction on the exposed surface of ATP, mostly due to stronger interactions with domain C2 (see [S3 Table](#)).

At this stage, it is interesting to compare the impact of Gsq with that of the stimulating agent forskolin (FOK). We find that, like Gsq, FOK binding also induces a change in the helix α_3 (see [S3](#) and [S4](#) Figs), that has an effect on the angle α_{C2} where the G-protein binds (see [S2 Table](#)). The change of this angle depends on the presence or absence of the ATP. FOK binding also induces changes at the ATP active site in a similar fashion to Gsq: the movement of the α -helices at the interface brings the key residue Arg 1029 back into its active location and globally rigidifies the binding site. However, in the case of AC5+ATP+FOK, there is a slight rotation of the two domains and a movement of the helices at the interface that are coupled to a stronger C1/C2 interface and a small reduction of the gap index. Most interestingly, FOK binding reduces ATP mobility and establishes stable interactions with the key residues for cAMP production. These changes lead to a 10% reduction of the exposed surface of ATP, mostly due to stronger interactions with domain C2 (see [S3 Table](#) and [S3](#) and [S4](#) Figs).

Although it seems probable that ATP is already bound to AC5 before its enzymatic action is activated by Gsq binding, we have also considered the alternative scenario where Gsq binds prior to ATP. This involved simulating the AC5+Gsq complex shown in [Fig 2](#). As seen in [Figs 6](#) and [7](#), Gsq binding to isolated AC5 has a similar effect to its binding to the AC5+ATP complex, with two significant differences. First, the loop β_2 is rigidified and does not move away from the (empty) ATP binding site. However, the overall flexibility of domain C1 is increased by the lability of helix α_2 . Second, the C1/C2 interface remains as tight as in isolated AC5.

A further interesting observation is that the ATP/Gsq interface is considerably less stable when the G-protein is bound to AC5 alone than when ATP occupies its AC5 binding site. As

shown in [S3 Table](#), ATP decreases the AC5/Gs α gap index from 3.2 to 2.7, a value close to that of the AC5 C1/C2 interface in the presence of ATP.

Discussion

The first observation that can be made from these microsecond-scale simulations is that strong allosteric coupling exists within AC5. Thus, ATP binding modifies not only the position of the proximal α -helices of domain C1, but also those at the distal end of domain C2. It also significantly improves the steric complementarity of the C1/C2 interface. Subsequent Gs α binding to domain C2 modifies the pair of α -helices belonging to distal end of domain C1, weakens the C1/C2 interface, locks ATP into its active conformation by completing bonds with all the key AC5 residues and also displaces and renders flexible the β 2 loop (see [S2 Fig](#)). Equally, the AC5-Gs α interface is significantly stabilized by the presence of ATP in its AC5 binding site. All of these changes involve coupling through AC5 over distances of tens of angstroms. The mechanism behind this coupling can be better understood with the help of a principal component analysis of the corresponding trajectories. The results are illustrated in four videos included in the supplementary data to this article. By comparing the low frequency modes of AC5 alone ([S1 Video](#)) with AC5:ATP ([S2 Video](#)), one can see that ATP binding improves the C1/C2 domain interface, opens the α 1/ α 2 angle, the presumed G α i binding site, and closes the α 3/ α 4 angle at the Gs α binding site. As observed in the RMSF profile, the β -sheet β 2 is no longer mobile in the presence of ATP. In contrast, in the AC5:ATP:Gs α complex ([S4 Video](#)), as in apoAC5, β 2 is mobile and its movements are strongly linked to movements of the α 1- α 4 helices. Communication between the Gs α binding site in domain C2 and the postulated G α i site in domain C1, through the domain interface, particularly striking in the AC5:Gs α complex ([S3 Video](#)), but is in fact observed in all four trajectories studied here, although the relative movements of the two domains change in each case.

Turning now to the enzymatic function of AC5, it is known that a hydrogen bond between the highly conserved Arg 1029 residue and the primary phosphate group of ATP play an important role on the production of cAMP from ATP and substitution of arginine by alanine at this position leads to a 100-fold reduction in enzymatic activity [69]. Hybrid QM/MM free energy calculations have also stressed the role of this interaction and show a significant increase in the free energy barrier for the reaction when the same substitution is made [70].

The present simulations show that this interaction is not formed unless Gs α is bound to AC5, helping to explain the stimulatory mechanism of Gs α . The presence of the Arg 1029-ATP interaction in the AC5+ATP+Gs α complexes reproduces the interaction seen in the chimeric crystal structure of adenylyl cyclase with an ATP analog, 1CJK [14]. [Fig 8](#) shows why this interaction is absent in the simulated AC5+ATP complex. As mentioned above, there are significant differences in the overall structure of AC5 depending on the presence or absence of Gs α . These include a displacement of the β -sheet that carries the aspartic acid residues bound to ATP through the two chelated magnesium ions. In the absence of Gs α , the β -sheet movement thus also displaces ATP, placing the triphosphate tail too far away from the Arg 1029 side chain for an interaction to occur.

As already noted, FOK is a plant-derived agent known to stimulate cAMP production by adenylyl cyclase [26,27]. It is interesting to remark that our simulations show the Arg 1029-ATP interaction generated by Gs α binding is also recovered with FOK binding. Moreover, when FOK is bound to AC5, the primary phosphate group of ATP is more exposed to the solvent, thus suggesting the possibility to escape the products again similarly to our findings with Gs α . The only major difference with FOK involves the C1/C2 interface that become

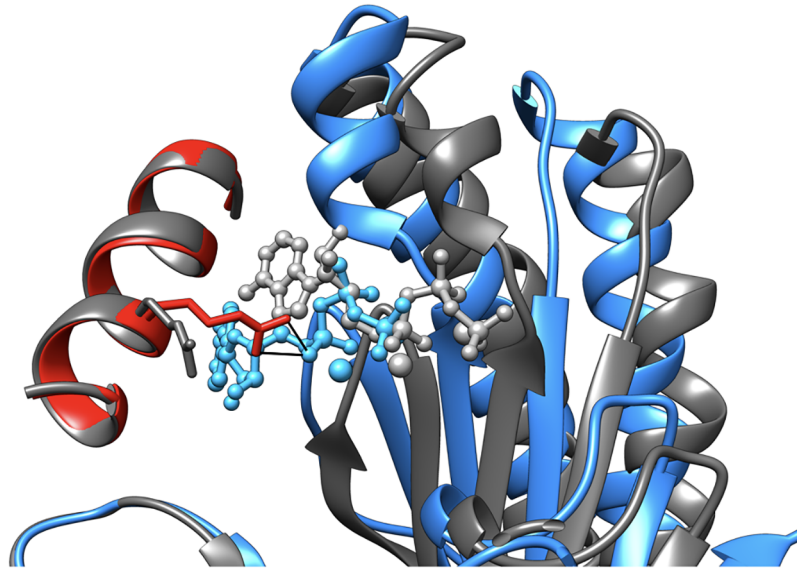


Fig 8. Movement of the β -sheet of AC5 domain C1 and ATP as a function of $G\alpha$ binding. The average conformation AC5 and ATP bound to $G\alpha$ is shown in blue (domain C1), red (α -helix of domain C2 carrying residue Arg 1029) and sky blue (ATP). The same elements of the average conformation without $G\alpha$ are shown in grey. The two complexes are superposed on the C2 domain of AC5. In the absence of $G\alpha$, the β -sheet of domain C1 is shifted toward the viewer, breaking the interaction between ATP with Arg 1029 (black lines).

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slightly stronger, in line with experimental observations [28,29], whereas it is weakened by $G\alpha$.

Lastly, following earlier work that suggested that decreasing the mobility of ATP within the enzyme active site would enhance catalytic efficiency [71], we recall that $G\alpha$ not only modifies the position of ATP, but also reduces its positional and internal mobility within the AC5 binding site and this is another factor helping to explain its stimulatory effect. Once again, FOK binding induces similar effect on ATP and its binding pocket.

A second possible role for $G\alpha$ is linked to changes in the AC5 C1/C2 domain interface. While firmly blocking ATP in its binding site, $G\alpha$ concurrently weakens the AC5 interface, changing its average gap index from 2.8 Å to 3.8 Å, a value in the range of weak non-obligate protein-protein interfaces [68]. This change, coupled with the displacement and the increase in flexibility of loop β 2 (S2 Fig) that is close to the ATP binding site could conceivably facilitate the escape of the products from the ATP binding site once the enzyme reaction has occurred.

In common with $G\alpha$ binding, ATP entering the AC5 binding site also impacts the conformation of the protein. The most interesting change is the opening up of the angle between the pair of α -helices of domain C1 (α_{C1}). ATP causes this angle to increase on average from 26° to 44°, a value close to that of the equivalent helix pair in domain C2 where $G\alpha$ binds. The α_{C1} angle remains open when $G\alpha$ binds, but is not opened by $G\alpha$ binding alone (see S1 Table). It is not yet clear where the inhibitory G-protein $G\alpha_i$ binds to AC5, or how it acts. Given its structural similarity to $G\alpha$, it could bind to domain C2 in the place of $G\alpha$, but it could also bind to the pseudo-symmetric site of domain C1—if the angle between the corresponding α -helices α_{C1} was sufficiently large to accommodate the protein. We can at least conclude that if domain C1 is involved in $G\alpha_i$ inhibitor binding, then this would be less likely to occur until an ATP molecule enters the AC5 active site (and whether $G\alpha$ is already bound to domain C2 or not).

Conclusions

We use molecular dynamics simulations in an attempt to better understand the behaviour of adenylyl cyclase, a key enzymatic player in cellular signalling cascades. Microsecond-scale simulations of the impact of binding ATP and/or the G-protein subunit $G_{s\alpha}$ to adenylyl cyclase help to explain features of this important signal transmission protein that are not easily derived from known experimental structures or from biochemical investigations of enzyme function. They notably provide information on a single, non-chimeric adenylyl cyclase isoform, AC5, and on the enzyme alone or bound to ATP, but in the absence of a stimulatory G-protein.

The simulations show that both ligand and protein binding create significant changes in structure, and in flexibility, throughout AC5 and due to a strong allosteric coupling existing within AC5, that is confirmed by a PCA analysis. They provide data that help to explain the stimulatory action of $G_{s\alpha}$, whose binding quenches the conformational and positional fluctuations of ATP in the active site of AC5 and leads to the formation of a key Arg 1029-ATP interaction.

Our results also show that ATP binding at the AC5 domain interface results in a shift in flexibility from C1 to C2 (although the most flexible substates of C2 are eliminated), strengthens the C1/C2 interface and significantly opens the angle between the C1 α -helices than can potentially bind $G_{\alpha i}$. $G_{s\alpha}$ binding to C2 has a lesser impact and the C1/C2 interface is unaffected. Lastly, simultaneous binding of ATP and $G_{s\alpha}$ resembles the state where ATP is bound alone, except that the C1/C2 interface is notably weakened and the C1 α -helices are somewhat less opened.

Although $G_{s\alpha}$ alone has a relatively small impact on AC5 dynamics it has a major effect when ATP binds, by limiting the conformational freedom of the bound ligand, correctly configuring it within its binding site and establishing key interactions between ATP and AC5. As discussed above, this change also significantly weakens the C1/C2 interface, opening the two domains at the ATP binding site and potentially facilitating the escape of the enzymatic reaction products from the ATP binding site. Similar results were also found when forskolin, a plant-derived agent known to stimulate cAMP production by adenylyl cyclase, is bound to AC5 (with the exception of the C1/C2 interface which remains strong, in line with experimental data). The similar changes induced within the ATP site by two very different molecular species argue in favor of their importance in the functional mechanism of adenylyl cyclase.

Our simulations also show that ATP binding could influence the binding of the inhibitory G-protein subunit $G_{\alpha i}$, if the potential binding site within domain C1 (pseudo-symmetric with the domain C2 site used by $G_{s\alpha}$) were to be involved. This work may be juxtaposed with the recent simulation study of AC5 inhibition by $G_{\alpha i}$ made by Van Keulen and Rothisberger [34]. By binding $G_{\alpha i}$ to a postulated C1 binding site, they find structural modifications that would disfavor both ATP and $G_{s\alpha}$. Given the results presented here, it would be interesting to see what impact $G_{\alpha i}$ would have if ATP were already bound in its AC5 pocket and also whether $G_{s\alpha}$ and $G_{\alpha i}$ could nevertheless bind simultaneously to AC5. We are continuing our studies in this direction. Overall the results presented here stress the importance of obtaining structural data on adenylyl cyclase in the absence of stimulatory factors (forskolin or $G_{s\alpha}$), and also of obtaining information on the impact of these same factors on dynamics of bound ATP (or rather of a close, non-reactive analogue).

Supporting information

S1 Table. Average and standard deviation of RMSD for the C1 and C2 domains of AC5, α -helix angles for both domains and characterization of the C1/C2 interface: Gap volume, change in accessible surface area (Δ ASA) and gap index, for the systems studied with

molecular dynamics simulation. To compute the RMSD we used the average structure as the reference state.

(PDF)

S2 Table. Average and standard deviation of RMSD for the C1 and C2 domains of AC5, α -helix angles for both domains and characterization of the C1/C2 interface: Gap volume, change in accessible surface area (Δ ASA) and gap index, for the systems studied with molecular dynamics simulation. To compute the RMSD we used the average structure as the reference state.

(PDF)

S3 Table. Average and standard deviation of RMSD, ASA and Δ ASA with domains C1 and C2 for ATP bound to AC5 in the absence or in the presence of Gs α and in the presence of FOK.

(PDF)

S4 Table. Average and standard deviation of the gap volume, Δ ASA and the gap index of the AC5/Gs α interface in presence and in the absence of ATP.

(PDF)

S1 Fig. Change in mobility of ATP due to Gs α binding. Top: AC5+ATP, Bottom: AC5+ATP+Gs α . In both cases snapshots from the MD trajectories are superposed on the average structure of domain C1 of AC5 (blue). ATP is shown with standard chemical coloring.

(TIFF)

S2 Fig. Change in position and flexibility of the β 2 loop as a result of Gs α binding. The average structure of the AC5+ATP complex is shown in dark grey (ATP not shown). The same structure after Gs α binding is shown with coloring representing the change in RMSF (the cyan-white-orange variation covering variations of -1.2 Å to +1.2 Å).

(TIFF)

S3 Fig. Changes in the conformation induced by FOK on AC5 and AC5+ATP. More intense colors (blue for domain C1 and red for domain C2) correspond to larger movements compared to the preceding structure on a scale of 0 \rightarrow 4 Å.

(PNG)

S4 Fig. Changes in RMSF induced by FOK on AC5 and AC5+ATP. More intense colors (orange for increased flexibility and cyan for decreased flexibility) correspond to differences with respect to the preceding structure on a scale of -1.2 Å \rightarrow +1.2 Å.

(PNG)

S1 Video. PCA on the MD simulation of AC5. Global movement obtained by PCA on the MD simulation of AC5 alone (mode 1). The beta-sheet β 2 is highly flexible and its movement is coupled with the relative movement of the domain C1 (blue) and C2 (red). The opening of the angle between the helices α 1 and α 2 (top left) is coupled with the closing of the angle between the helices α 3 and α 4 (bottom right). The important regions are highlighted with orange arrows.

(MOV)

S2 Video. PCA on the MD simulation of AC5+ATP. Global movement obtained by PCA on the MD simulation of AC5+ATP (mode 2, note that the first two modes have very similar eigenvalues in this case). The beta-sheet β 2 is no longer mobile. The opening of the angle between the helices α 1 and α 2 is coupled with the closing of the angle between the helices α 3

and $\alpha 4$ and with the closing of interface between the two domains. Loops in domain C2, at either end of helix $\alpha 3$, also undergo coupled movements.

(MOV)

S3 Video. PCA on the MD simulation of AC5+Gs α . Global movement obtained by PCA on the MD simulation of AC5+Gs α (mode 1). The translation of helix $\alpha 2$ along its axis is coupled with the change of position of the beta-sheet $\beta 2$. The movement of helix $\alpha 2$ also opens the angle with helix $\alpha 1$ and this is coupled with closing of the angle between the helices $\alpha 3$ and $\alpha 4$ and the relative rotation of the two domains, opening the domain interface towards the viewer and closing it on the far side.

(MOV)

S4 Video. PCA on the MD simulation of AC5+ATP+Gs α . Global movement obtained by PCA on the MD simulation of AC5: ATP+Gs α (mode 1). The beta-sheet $\beta 2$ is highly mobile. Its movement is mainly coupled with intra-domain movements, although some changes occur in helix $\alpha 3$ and with the loops at the closed end of helices $\alpha 3$ and $\alpha 4$.

(MOV)

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