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Conformational flexibility and binding interactions of the G protein $\beta\gamma$ heterodimer

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Abstract

Previous NMR experiments on unbound G protein $\beta\gamma$ heterodimer suggested that particular residues in the binding interface are mobile on the nanosecond timescale. In this work we performed nanosecond-timescale molecular dynamics simulations to investigate conformational changes and dynamics of $G\beta\gamma$ in the presence of several binding partners: a high-affinity peptide (SIGK), phosducin, and the GDP-bound α subunit. In these simulations, the high mobility of $G\beta W99$ was reduced by SIGK, and it appeared that a tyrosine might stabilize $G\beta W99$ by hydrophobic or aromatic stacking interactions in addition to hydrogen bonds. Simulations of the phosducin- $G\beta\gamma$ complex showed that the mobility of $G\beta W99$ was restricted, consistent with inferences from NMR. However, large-scale conformational changes of $G\beta\gamma$ due to binding, which were hypothesized in the NMR study, were not observed in the simulations, most likely due to their short (nanosecond) duration.

A pocket consisting of hydrophobic amino acids on $G\alpha$ appears to restrict $G\beta W99$ mobility in the crystal structure of the $G\alpha\beta\gamma$ heterotrimer. The simulation trajectories are consistent with this idea. However, local conformational changes of residues $G\beta W63$, $G\beta W211$, $G\beta W297$, $G\beta W332$ and $G\beta W339$ were detected during the MD simulations. As expected, the magnitude of atomic fluctuations observed in simulations was greater for α than for the $\beta\gamma$ subunits, suggesting that α has greater flexibility. These observations support the notion that to maintain the high mobility of $G\beta W99$ observed by solution NMR requires that the $G\beta$ - α interface must open up on time scale longer than can be observed in nanosecond scale simulations.

Keywords

G-protein alpha beta gamma subunits; molecular dynamics; hot spot; subunit interactions

Introduction

G proteins play an important role in cellular signal transduction and are involved in many processes including sensory perception, modulation of cardiac rhythm, neurotransmission, and regulation of mitosis.¹⁻³ They are heterotrimers consisting of α , β , and γ subunits, and are maintained in an inactive state by association in a bound complex.^{4,5} In the standard model for signaling, the exchange of GDP for GTP on the $G\alpha$ subunit leads to a conformational change and a dissociation or conformational rearrangement of the $G\alpha$ and

G $\beta\gamma$ subunits.^{4,6} The subunits are then free to interact with diverse binding partners for downstream signal transduction.^{4,6}

Several studies have suggested that disruption of interactions of G $\beta\gamma$ with downstream binding partners might be a valuable strategy for pharmaceutical development.^{3,7-10} Recently, small molecule inhibitors of G $\beta\gamma$ subunit signaling have been discovered that bind G $\beta\gamma$ and inhibit G $\beta\gamma$ protein-protein interactions.¹¹ These function in cellular and animal models as a potential therapeutic target in pain¹², inflammation¹³, and cancer¹⁴. These small molecules were discovered using a competition binding assay with a high-affinity peptide ligand (SIGK). An X-ray crystal structure of a high affinity peptide ligand (SIGK) bound to G $\beta_{1\gamma 2}$ has been determined.¹⁵ It shows that SIGK binds to the same region of the G β subunit as the switch II region of G α . Also, SIGK inhibits activation of phospholipase C β and phosphoinositide 3-kinase γ by G $\beta\gamma$, suggesting that it occupies a surface region on G $\beta\gamma$ that is shared by these binding partners.^{15,16} This region, at the center of the G β beta propeller structure, has been postulated to be a “hot spot,” mediating interactions between G $\beta\gamma$ and G α and downstream signaling molecules. It is of great interest to understand the molecular basis for the interactions between G $\beta\gamma$ and its various binding partners, and in particular the ability of G $\beta\gamma$ to accommodate a wide range of structurally diverse binding partners using a common interface or hot spot.

To examine the role of protein flexibility in molecular recognition by G $\beta\gamma$ TROSY-HSQC NMR studies with site-specific ¹⁵N labeling of G β tryptophan residue backbone and indole amines were performed.¹⁷ A very intense signal for the indole nitrogen on a particular tryptophan, G β W99, and a signal of lower intensity for G β W332 were observed in the vicinity of the hot spot. The intense signal was interpreted as being due to a high level of mobility on the nanosecond time scale compared with the other seven G β tryptophans. In the presence of SIGK and phosducin (which binds G $\beta\gamma$ and inhibits its biological activity¹⁸) the intense G β W99 indole peak was completely suppressed, suggesting that these binding partners restrict G β W99 mobility (Upon binding of SIGK a new resonance corresponding to G β W332 appears indicating that the motion is restricted. It is not clear what happens for G β W332 with phosducin because the resonances in complex have not all been identified¹⁷). Interestingly, the indole G β W99 peak was not suppressed by G α_{i1} -GDP. This was surprising given the X-ray crystal structure of the G $\alpha\beta\gamma$ heterotrimer complex, in which G β W99 protrudes into a hydrophobic pocket in the surface of G α subunit, which would appear to restrict its mobility.¹⁹ It was therefore suggested that the crystal structure of the G $\alpha\beta\gamma$ heterotrimer complex might differ from that found in solution; in particular, the inactive heterotrimer might adopt a conformation with an open subunit interface for a significant fraction of time.

To clarify the dynamics of the unbound G $\beta\gamma$ heterodimer, we performed molecular dynamics (MD) simulations.¹⁷ The backbone ϕ and ψ dihedral angles and the side chain χ_1 and χ_2 dihedral angles of the eight G β tryptophans were monitored during ten independent 8-ns molecular dynamics trajectories. Only the dihedral angles of G β W99 were observed to change substantially on the timescale of the simulations, in agreement with the interpretation of the NMR results.

Here, we present additional MD simulations aimed at further understanding the G $\beta\gamma$ binding interface, again focusing on the dynamics of 8 tryptophan residues on the G β subunit. We report simulations of G $\beta\gamma$ bound to SIGK and phosducin, as well as the G $\alpha\beta\gamma$ heterotrimer, and interpret these in conjunction with the NMR experiments.

Methods

Initial structures for the simulations were based on the crystallographic structures of the $G\alpha\beta\gamma$ heterotrimer (PDB code 1GP2), and $G\beta\gamma$ in a bound complex with the SIGK peptide ligand (PDB code 1XHM) or phosducin (PDB code 1A0R). As in previously reported work, the simulations did not include the geranyl-geranyl lipid attached to $G\beta\gamma$. In the structure with phosducin bound, only residues 13-37 and 68-230 of phosducin are well-resolved and coordinates included in the PDB entry. In our model structure, residues 37 and 68 (which are in close proximity) were connected to obtain a contiguous polypeptide chain. This model structure was in close agreement with the resolved portions of the experimental phosducin crystal structure.²⁰

To create an initial ensemble of conformations, Langevin dynamics simulations were performed at 300 K using the sander module of the AMBER9.0 software package. The parm99 force field parameters were used with the pairwise generalized Born (GBSA) implicit solvent model as parameterized by Tsui and Case.²¹ The crystal structure of the $G\alpha\beta\gamma$ heterotrimer has a GDP molecule bound to the $G\alpha$ subunit. GDP was described using parameters from the generalized AMBER force field (GAFF), with charges taken from the OPLS²² force field as assigned by the Maestro software package (Schrödinger, Inc). Prior to dynamics runs, the complexes were relaxed by steepest descent energy minimization, followed by conjugate gradient energy minimization. The system was then heated over 25 ps from 0 K to 300 K, followed by Langevin dynamics simulation at 300 K for 1 ns. A nonbonded cutoff distance of 12 Å and a timestep of 2 fs were used. All bonds involving hydrogen atoms were constrained using the SHAKE algorithm. Coordinates were saved every thousand steps, for a total of 500 snapshots. The snapshots were classified into nine groups on the basis of root-mean-square displacement to the starting structure by the using the GA fuzzy c-means clustering program (biorainbow.com). From each cluster, the structure closest to the center was chosen as the starting configuration for a subsequent molecular dynamics simulation. The conformation from the crystal structure was also chosen as a starting configuration, giving a total of ten initial conformations.

Each starting structure was then immersed in a truncated octahedral box of water. The TIP3P explicit water model was used. A buffer of 10 Å between the protein and the edge of the box was used for the SIGK- $G\beta\gamma$ complex, while a buffer of 7 Å was used for the $G\alpha\beta\gamma$ heterotrimer and $G\beta\gamma$ -phosducin complex. Sodium ions were added to neutralize each system. The water and ions in box were initially minimized by 500 steps of steepest descent followed by another 500 steps of conjugated gradient, keeping the proteins constrained to their initial positions. Each system was then minimized without restraints by 1500 steps of steepest descent minimization followed by 1000 steps of conjugated gradient minimization. In the first stage of equilibration, the systems were gradually heated up from 100 K to 300 K at constant volume. Next, each system was equilibrated at 300 K and 1 atm constant pressure for 200 ps, followed by a 1 ns constant volume and constant energy equilibration. Production simulations were then performed at constant energy and volume for a total of ten independent 8-ns, 7-ns, and 55-ns simulations for the $G\beta\gamma$ -SIGK, $G\beta\gamma$ -phosducin, and $G\alpha\beta\gamma$ complexes, respectively. Simulations used a 2 fs time step with bonds to hydrogen constrained with SHAKE. Long-range electrostatic forces were calculated using the particle-mesh Ewald (PME) algorithm with default parameters. A cutoff of 10 Å was used for Lennard-Jones interactions as well as the real-space part of the Ewald sum. All minimizations and equilibrations were performed using the sander module of the AMBER9 software package. Production simulations of the $G\beta\gamma$ -SIGK and $G\beta\gamma$ -phosducin complex were performed using the sander module of the AMBER10 package on Intel Xeon 3.0 GHz processors, while the longer simulations of the $G\alpha\beta\gamma$ complex used the pmemd module of the AMBER10 package on an IBM Blue Gene/P supercomputer.

Trajectory snapshots were collected every 1 ps (10 ps for G $\alpha\beta\gamma$), and all trajectory analyses were performed using the ptraj module in AMBER9.0. Hydrogen bonds between the indole nitrogen of each G β tryptophan and other residues were examined. A hydrogen bond was defined as a contact between the nitrogen and an acceptor atom closer than 3.0 Å and forming an angle within 120 degrees. To examine the mobility of tryptophans, the four dihedral angles ϕ , ψ , χ_1 , and χ_2 were monitored as a function of time. Ramachandran diagrams of ϕ vs. ψ and χ_1 vs. χ_2 were used for examining the degree of conformational freedom for each tryptophan during the simulations. For G $\alpha\beta\gamma$ complexes, the atomic fluctuations of each C-alpha atom were computed as B-factors from the MD trajectories of the ten independent simulations.

Results and Discussion

G $\beta\gamma$ -SIGK peptide simulations

There are 8 tryptophan residues in the G β subunit at the positions of 63, 82, 99, 169, 211, 297, 332, and 339. Previous simulations of the unbound G $\beta\gamma$ heterodimer showed that G β W99 is significantly more mobile than the other tryptophans on the nanosecond timescale (plots of the ϕ , ψ , χ_1 and χ_2 dihedral angles for G β W99 and G β W332 as a function of time for 10 independent 8 ns simulations are shown on Figure S5 and S6 in reference 17).

In all ten of the current simulations of G $\beta\gamma$ bound to SIGK, the high mobility of G β W99 was reduced, showing less fluctuation in the dihedral angles (Figure S1). However, G β W99 still appears to be the most flexible of the tryptophan residues, as shown in Ramachandran diagrams (Figure S2 and S3). The propensity to form hydrogen bonds between SIGK and G β W99 was also examined. However, the percent occupancy of hydrogen bonds in trajectories was relatively low (Figure 1). SIGK appeared not to form tight hydrogen bonds with G β W99. This is surprising because the oxygen of a tyrosine side chain (Y11) of SIGK is less than 3 Å away from the indole nitrogen of G β W99 in the X-ray crystal structure of SIGK-G $\beta\gamma$ complex.¹⁵ In the simulations, this distance increases, and the two residues appear to interact primarily by hydrophobic attraction or aromatic stacking rather than by the hydrogen bonds (Figure 2).

G $\beta\gamma$ -phosducin simulations

In the previously reported two-dimensional TROSY-HSQC NMR studies, phosducin binding significantly altered NMR peak intensities and/or chemical shifts for all tryptophan residues on G $\beta\gamma$ subunits, suggesting overall structural changes upon binding.¹⁷ In the 10 independent 7-ns simulations for phosducin-G $\beta\gamma$ complex, the four dihedral angles of all the G β W residues were relatively constant (Figure S5 and S6). In particular, the mobility of G β W99 was significantly suppressed by phosducin binding. Monitoring G β W residues that participate in hydrogen bonds during the simulations suggests that G β W99 could form strong hydrogen bonds with Glu85 of phosducin (Figure 1). In the simulation trajectories, the N-terminus of phosducin directly contacts G β W99 (Figure 5) and may therefore be responsible for the strong suppression of the TROSY-HSQC intensity. Large conformational changes were not observed in G $\beta\gamma$, nor in phosducin; most likely, these cannot be observed on the nanosecond timescale of the simulations. However, when the snapshots from the unbound G $\beta\gamma$ heterodimer and the phosducin-G $\beta\gamma$ complex were compared, some small variations of G β W82 were apparent (Figure 4).

G $\alpha\beta\gamma$ heterotrimer simulations

The switch II domain of G α subunit has high sequence and structural homology with SIGK and it binds to the same region on the G $\beta\gamma$ hot spot.¹⁵ In the X-ray crystal structure of the G $\alpha\beta\gamma$ heterotrimer, G β W99 protrudes into a hydrophobic pocket consisting of Ile184,

His188, Phe199, and Phe215 on the $G\alpha$ subunit.¹⁹ However, in NMR studies, the $G\alpha$ subunit suppressed only 30-50% of the $G\beta W99$ resonance (whereas the binding of SIGK resulted in complete suppression of the signal). This suggests that the solution structure of the $G\alpha\beta\gamma$ heterotrimer might be somewhat different from the crystal structure; in particular, that it can adopt a conformation with a relatively open interface between the subunits, allowing $G\beta W99$ to retain some flexibility.

During ten independent 55-ns simulations, only minor changes of the four dihedral angles for $G\beta W99$ were observed (Figure S7 and S9). On the timescale of the simulations, the indole moiety of $G\beta W99$ remains inside the hydrophobic pocket of $G\alpha$, even though the backbone dihedrals are somewhat flexible. Ramachandran diagrams from all ten simulations showed conformational changes of $G\beta$ tryptophan residues 63, 211, 297, 332, and 339 upon binding of the $G\alpha$ subunit (Figure S8 and S9). It should be noted that of these residues only residue 332 is in the immediate vicinity of the binding site of $G\alpha$, so these results are consistent with the proposition that different binding partners can cause global conformational changes in the $G\beta\gamma$ heterodimer. The propensity for $G\beta$ tryptophan residues to form hydrogen bonds was also examined; it was found to be reduced for residues 63, 211, and 332 in all trajectories (Figure 1). Taken in conjunction with the NMR results, the simulations suggest that in order for tryptophans near the binding interface to remain relatively mobile, the $G\alpha\beta\gamma$ heterotrimer must adopt a more open conformation that is somewhat different from the crystal structure.

In order to further examine the flexibility of different regions of the $G\alpha\beta\gamma$ heterotrimer, mean-square atom fluctuations (i.e. B-factors) of each C-alpha atom in all trajectories from the ten independent simulations were calculated and averaged (Figure 6 (a)). The specific regions of the $G\beta\gamma$ complex that are indicated as having high flexibility are shown in Figure 6 (b). Overall, the $G\alpha$ subunit has larger atomic fluctuations compared with the $G\beta$ subunit, and regions of higher flexibility are found in the periphery away from the binding interface. The $G\beta$ subunit appears to be relatively stable except for the N-terminal tail region that is parallel to the γ subunit.

Conclusions

This study presented molecular dynamics simulations of the SIGK- $G\beta\gamma$ complex, phosducin- $G\beta\gamma$ complex, and the $G\alpha\beta\gamma$ heterotrimer, to provide further interpretation of dynamics inferred from NMR experiments. For each complex, ten independent simulations were initiated using different starting conformations; in general, the results from all ten were very similar. In previous molecular dynamics simulations of the unbound $G\beta\gamma$ heterodimer, a particular tryptophan residue, $G\beta W99$, was observed to be highly mobile on the nanosecond timescale in comparison to other tryptophans, as suggested by NMR TROSY-HSQC experiments.¹⁷ In the simulations of the SIGK- $G\beta\gamma$ complex, $G\beta W99$ was far less mobile, again consistent with the observed diminished NMR peak intensity. The simulations suggested that a tyrosine residue on SIGK might stabilize $G\beta W99$ by hydrophobic or aromatic stacking interactions instead of or in addition to hydrogen bonds. It is also notable that SIGK-Phe6 was observed to be in close proximity to $G\beta W99$ in five of ten simulation trajectories (so that in essence, $G\beta W99$ was sandwiched between SIGK-Tyr11 and SIGK-Phe6), providing further conformational restriction. In addition, SIGK is highly flexible in the simulations (Figure 3) even though the $G\beta\gamma$ heterodimer does not undergo a significant global conformational change from the crystal structure.

The mobility of $G\beta W99$ was restricted in the simulation of the phosducin- $G\beta\gamma$ complex, also consistent with the interpretation of the NMR data. However, global conformational changes in the beta-sheet propeller blades of $G\beta\gamma$, which were hypothesized in the NMR study, were

not observed on the simulation timescale. As mentioned above, amino acids 38-67 for phosducin are missing in the X-ray crystal structure of phosducin-G $\beta\gamma$ complex and the amino acids 38 and 67 were connected to create a contiguous polypeptide chain in the construction of the model structure used for the simulations, which closely resembled the actual crystal structure. However, it is possible that the missing amino acid chains may be involved in contacts with the distant G $\beta\gamma$ residues.

A hydrophobic pocket consisting of hydrophobic amino acids on G α appears to restrict the G $\beta\gamma$ W99 mobility in the X-ray crystal structure of the G $\alpha\beta\gamma$ heterotrimer. On the relatively short timescale of the simulations (~ 50 ns), we did not observe significant disagreement with the crystal structure, so the simulations do not directly suggest that the G $\alpha\beta\gamma$ heterotrimer might adopt a substantially different conformation in solution than the crystal structure, as suggested by the NMR experiments. However, the global conformational changes upon binding of G α subunit on G $\beta\gamma$ may be explained in part by the local environmental changes of residue G β W63, G β W211, G β W297, G β W332, and G β W339 observed during the MD simulations. Taken in conjunction with the NMR results, the simulations suggest that in order for tryptophans near the binding interface to remain relatively mobile, the G $\alpha\beta\gamma$ heterotrimer must adopt a relatively open conformation that is somewhat different from the crystal structure.

Simulations for longer timescales might help clarify the hypothesis that G $\beta\gamma$ subunits may accommodate various binding partners through distinct conformational changes, sharing the same/or similar binding region. Furthermore, simulations with additional binding partners with co-crystallized structures, such as G-protein-coupled receptor kinase²³ and parathyroid hormone receptor peptide²⁴, might be useful to further understand interactions between G $\beta\gamma$ and its various binding partners.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Average percentage hydrogen bonding occupancies for various G β -tryptophans observed in trajectories taken from ten independent 8-ns simulations for the free G $\beta\gamma$ heterodimer (blue), SIGK-G $\beta\gamma$ complex (red), phosducin-G $\beta\gamma$ complex (yellow), and ten independent 55-ns simulations of the G $\alpha\beta\gamma$ heterotrimer (green).

Figure 2. Superposition of the X-ray crystal structure (cyan) and the last snapshot (tan) of an 8-ns simulation of the SIGK-Gβγ complex, focusing on the side chains of Y11 of SIGK and GβW99. The SIGK backbone is depicted by cyan and tan ribbons, Trp side chains by sticks, and other portions of Gβ by gray ribbons.

Figure 3.

A superposition of the binding conformations of the SIGK peptide on the $G\beta\gamma$ heterodimer obtained from ten independent simulations, illustrating the high flexibility of SIGK. One representative of the relatively rigid $G\beta\gamma$ heterodimer is depicted by blue ribbons.

Figure 4.

Superimposed structures from two snapshots at 8-ns of the simulations. One was initiated from the crystal structure of the phosducin-G $\beta\gamma$ complex (white and tan ribbons depict phosducin and G $\beta\gamma$, respectively). The other was initiated from the structure of the unbound G $\beta\gamma$ heterodimer (pink ribbons). The presence of phosducin does not significantly change the global conformation of G $\beta\gamma$ heterodimer. Trp residues on G β are shown as sticks (blue for unbound G $\beta\gamma$ red for the phosducin-G $\beta\gamma$ complex). Only G β W82 has a substantially different conformation in these two structures.

Figure 5.

A representative snapshot at 8-ns from the phosducin-G $\beta\gamma$ complex simulation. White and tan ribbons depict phosducin and G $\beta\gamma$, respectively. A hydrogen bond exists between G β W99 and E85 of phosducin. This hydrogen bond is present about half the time during the simulations.

(a)

(b)

Figure 6.

(a) Mean-square atomic fluctuations (B-factors) for each C-alpha atom, averaged over ten independent 55-ns simulations for the G $\beta\gamma$ heterodimer. Red, green, and blue indicate the α , β , and γ subunits, respectively. (b) The crystal structure of the G $\alpha\beta\gamma$ heterotrimer with alpha carbons colored based on the corresponding B-factors from MD simulations. Color range is from blue (low B-factor) to red (high B-factor), and a ball of larger radius indicates a larger B-factor. The G α subunit is at the top of the figure; the binding interface is at the center, and the G β N-terminal tail and G γ subunit are at the bottom.