Hinge region mediates signal transmission of luteinizing hormone and chorionic gonadotropin receptor

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Keywords: Glycoprotein hormone receptors, G protein-coupled receptors, Molecular dynamics simulation, Protein dynamics, Evolutionary analysis

1. Introduction

As a subfamily of class A G protein-coupled receptors (GPCRs), glycoprotein hormone receptors (GPHRs) were membrane proteins activated by endogenous agonists on their unique large extracellular domain (ECD) and induce intracellular signals through their transmembrane domain (TMD) [1,2]. GPHRs include gonadotropin receptors, namely follicle-stimulating hormone (FSH) receptor (FSHR) and luteinizing hormone (LH) /choriogonadotropin (CG) receptor (LHCGR). Gonadotropin receptors are situated at the surface of gonadal cells and play pivotal roles in the function of the reproductive system [3]. In particular, LHCGR is activated by both LH and CG, and regulates the maturity of germ cells and embryo development, respectively [4,5]. Human CG is used as the gold standard for pregnancy diagnosis. Moreover, it is also employed as a tumor marker, amyloid-β precursor, as well as a potential pre-drug for cancers [6–8]. The dysfunction of LHCGR causes hormone secretion disorders. For example, its inactivating mutation E354K induces inherited pseudohermaphroditism [9], while the activating mutation L457R causes testotoxicosis and oligozoospermia [10]. Thus, the elucidation of the LHCGR mechanism not only helps the understanding of GPCR function, but also provides hints for infertility and glycoprotein hormone-related drug design.

Compared with other class A GPCRs, a unique property of LHCGR is the large ECD that contains 340–420 amino acids and binds to a large agonist of glycoprotein hormone with around 200 amino acids [3,11]. The ECD consists of a leucine-rich repeat (LRR) and a hinge region. The LRR contains a structure composed of repeated β-sheets and loose loops, which is different from the combined α-helices and β-sheets in ECD of class B GPCRs [12,13]. The hinge region is composed of a hinge helix, a long hinge loop and the ECD-TMD interface. With the hormone, the hinge region showed a characteristic rotation and displayed an active-like conformational landscape of the ECD-TMD interface with an extended TMD. The active-like hinge region conformation transduces the hormone binding signal downwards from ECD to TMD. The relationship between the hinge region and the intracellular G protein-binding pocket was also inferred. The hinge region-mediated signal transmission mechanism offers a deeper understanding of LHCGR and provides insights into the elucidation of GPCR activation.

https://doi.org/10.1016/j.csbj.2022.11.039

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loop, and a highly conserved P10 loop in GPHRs [14]. The hinge helix and P10 loop directly interact with TMD [15]. The unique ECD and hinge region suggest a distinct signal transmission mechanism of LHCGR in class A GPCRs. Recently, the full-length active LHCGR-CG-G protein structures were solved, suggesting a push-and-pull model in the activation of LHCGR [15]. In the push-and-pull model, the CG may activate the receptor by pushing the ECD to be vertical to the membrane plane, and the hinge loop pulls the CG-bound ECD to stabilize the activated conformation. However, the complex lacks the hinge loop due to its flexibility but the integrity of the hinge loop is necessary for LHCGR activation [15,16]. The mechanism of how signal transfers through the hinge region remains unknown for this important drug target.

Here, molecular dynamics (MD) simulation and evolutionary analysis were combined to characterize the hinge region movements of LHCGR. Repeated 500 ns MD simulations were performed for LHCGR-only, CG-bound LHCGR, and CG-bound LHCGR-Gs complex. We also explored the dynamics of important LHCGR mutants (S277I and E354K) to elucidate the activation or inactivation mechanism. Besides, the relationship between hinge and TMD movements was evaluated by statistic coupled analysis and protein dynamics.

2. Materials and methods

2.1. System setup

The cryo-EM structure of the CG-bound LHCGR-Gs complex (PDB ID: 7FIG) was downloaded from the Protein Data Bank. Then, mutations on LHCGR were reverted to the wild type using maestro of the Schrödinger suite and missing loops were made up referring to the homology model provided by the Swissmodel web server [17]. The protonation state of each residue was calculated by propka3 and the post-translational modifications of the system were kept the same as the cryo-EM structure [18].

To build the LHCGR-CG system, we deleted the G protein in the CG-bound LHCGR-Gs complex, and further deleted CG for the construction of the LHCGR-only system. From the LHCGR-only system, we made S277I and E354K mutations for the construction of corresponding mutated systems.

Referring to class A GPCRs, we oriented the structures locally and then submitted them to CHARMM-GUI to insert them into a membrane, composed of 137×137 Å² POPC (palmitoyl-2-oleoyl-sn-glycerol-3-phosphocholine) lipids [19–21]. TIP3P (transferable intermolecular potential 3P) water molecules with a length of 22.5 Å were added to the top and bottom of the system. Then, 0.15 mol/L KCl plus the same counterions were placed in each system. Sodium and potassium counterions were dissolved in water at this site. The calculation refers to

\[ D^i_j = f^i_j \times \ln \left( \frac{f^i_j}{q^i} \right) + (1 - f^i_j) \times \ln \left( \frac{1 - f^i_j}{1 - q^i} \right) \]

In each position, \( D^i_j \) was calculated by the most frequent residue. Then, the correlations between positions i and j with the mostly emerged residue a and b, namely \( C^a_b \), was estimated by Equation [2].

\[ C^a_b = \frac{\partial D^a_j}{\partial f^i_j} \frac{\partial D^b_j}{\partial f^i_j} \left| f^a_j - f^b_j \right| \]

in which \( f^a_j \) means the frequency that position i has residue a and in the meantime, position j has residue b. \( C^a_b \) values made up of the SCA matrix in Fig. 5.
The eigenvectors of the SCA matrix were then calculated to identify the evolutionary-related residues (sectors). Since the first mode shows the global fluctuations between species and mutants, it was deprecated in the search for sectors [34]. In the application of LHCCR and FSHR, we set the threshold value $\varepsilon$ as 0.035–0.05 dependent on the distribution of sectors. We identified the positions whose weight of the 2nd eigenvector is larger than the weight of the 4th eigenvector and larger than $\varepsilon$ as one sector, the positions whose weight of the 2nd eigenvector is smaller than the weight of the 4th eigenvector and $-\varepsilon$ as the other sector, and the positions whose weight of 4th eigenvector is larger than the weight of the 2nd eigenvector and larger than $\varepsilon$ as the last sector. Then, the sectors were renumbered to match the relative position in LHCCR and FSHR. The FSHR structure model was obtained from AlphaFold2 database [35]. The sector choosing and SCA calculation were confirmed in previous publications [32,34,36].

3. Results

3.1. Rotation of hinge loop with agonist binding

To investigate the effects of agonist binding on LHCCR, we performed MD simulations of the LHCCR with and without the endogenous agonist CG, termed LHCCR-CG and LHCCR-only, respectively. We also conducted simulations of LHCCR bound to CG and G protein as a control group, termed LHCCR-CG-G. All protein models were generated using the cryo-EM structure of the LHCCR-CG-Gs complex as the template (PDB ID: 7FIG) [15].

LHCCR uses a LRR to receive the agonist signal, a hinge region to transfer the signal, and a TMD to bind G protein (Fig. 1A, B). Particularly, the hinge region includes a hinge helix (residues 276–282), a hinge loop (residues 284–340), and a P10 loop (residues 350–359) (Fig. 1A). To characterize the system stability, we calculated the root mean square deviation (RMSD) from the starting conformation of the whole receptor and its subdomains, respectively (Figure S1 and Table S1). RMSD of all systems became stable after 50 ns, indicating the equilibrium of systems. Compared with

![Fig. 2. Hinge helix-P10 loop in LHCCR-only and LHCCR-CG systems. (A, B) The free energy landscape for the minimal distance between E354$P_{10}$ and Y426$ECL1$ ($dP_{10}$-$ECL1$) and the minimal distance between A281$hinge$ helix and N428$ECL1$ ($dhinge$ helix-$ECL1$) in LHCCR-only (A) and LHCCR-CG (B) systems. Rectangles show the dominant conformations of the landscapes. (C, D) Representative models showing interactions between hinge helix, P10 loop, and ECL1 in LHCCR-only (C) and LHCCR-CG (D) systems. Protein and key residues are shown in cartoon and stick depicts, respectively.](image)
CG-bound systems, LHCGR-only had a higher ECD RMSD value of 9.5 ± 1.1 Å, but a similar TMD RMSD value of 3.2 ± 0.4 Å (Table S1). As a component of ECD, the hinge loop showed remarkably high RMSDs (9.6 ± 1.2 Å for LHCGR-CG-G, 8.8 ± 2.0 Å for LHCGR-CG, and 11.5 ± 2.5 Å for LHCGR-only). The ECD RMSDs decreased by removing the hinge loop in all systems (Table S1). These findings suggested large movements or conformational changes of the hinge loops in simulations. As for the P10 loop and hinge helix, RMSD increased from LHCGR-CG-G protein, LHCGR-CG, to LHCGR-only systems (Table S1), suggesting both CG and G protein stabilized these regions.

We observed movements of the hinge loop in the LHCGR-only simulations. To quantify the movements, we projected the Cα arrows in Fig. 1E) to the membrane (XY-plane) to quantify the positions of the hinge loop. As shown in Fig. 1C, the hinge loop of LHCGR-only had a large population around (0 Å, −27 Å). However, LHCGR-CG had a relative X value larger than 10 Å and a relative Y value larger than −20 Å in most populations (Fig. 1D). Similar dominant conformations of the hinge loop were also observed in the LHCGR-CG-G system (Figure S2). Compared with that of LHCGR-only, the hinge loop of LHCGR-CG moved from the membrane side of TM6 to the position above TM5 and had a 90° clockwise rotation (Fig. 1E).

We observed that the hinge loop formed coiled conformations in the LHCGR-only system, characterized by decreased solvent accessible surface areas (SASA) of the hinge loop. In LHCGR-only simulations, the SASA of the hinge loop decreased from 7276 Å² to approximately 5500 Å² within 300 ns (Fig. S2A). Meanwhile, with an extended conformation, the hinge loop of CG-bound LHCR maintained a stable large SASA around 6700 Å² (Fig. S3B). In the LHCR-only system, the reduced SASA indicated a diminished surface exposed to solvent, due to the coiled hinge loop of LHCR-only. Particularly, R283, L326, W329, C336, P338, and K339 showed smaller SASAs in the LHCR-only system by forming additional interactions (Fig. S3C, D).

3.2. Alteration of ECD-TMD interface

As two components of the hinge region, the hinge helix and P10 loop are linked by the hinge loop and form the interface of ECD-TMD (Fig. 1A, B). In simulations, we observed the movements of this interface (termed hinge helix-P10 loop) with respect to extra- cellular loop 1 (ECL1) in Fig. 2. We observed different interactions between A281\(^{\text{Hinge helix}}\) and N428\(^{\text{ECL1}}\) as well as between E354\(^{\text{Hinge helix}}\) and Y426\(^{\text{ECL1}}\) in LHCGR-only and LHCR-CG systems, suggesting conformational changes of ECD-TMD interfaces. E354 plays an important role in receptor activation [9]. Thus, the minimal distance between A281\(^{\text{Hinge helix}}\) and N428\(^{\text{ECL1}}\) and between E354\(^{\text{Hinge helix}}\) and Y426\(^{\text{ECL1}}\) were measured to show the position of the hinge helix-P10 loop with respect to ECL1 (Fig. 2). 79.31% conformations of the LHCR-only have \(d_{\text{hinge helix-ECL1}}\) larger than 4 Å (Fig. 2A, C), suggesting reduced interactions between hinge helix and ECL1. In contrast, in LHCR-CG, most conformations had \(d_{\text{hinge helix-ECL1}}\) less than 4 Å, suggesting stable direct interactions between hinge helix and ECL1 (Fig. 2B, D). Meanwhile, \(d_{\text{h10-ECL1}}\) was mostly smaller than 4 Å in the LHCR-only (Fig. 2A, C), but larger than 4 Å in LHCR-CG (Fig. 2B, D). These findings indicate that LHCR-only had hinge helix move away from ECL1 but had P10 loop directly interact with it, which was an inactive-like conformation compared with the active-like CG-bound LHCR. It is worth mentioning that most LHCR with a rotated hinge loop had large \(d_{\text{h10-ECL1}}\) and small \(d_{\text{hinge helix-ECL1}}\) (Figure S4, S5), suggesting that such movements of the hinge helix-P10 loop were accompanied by the hinge loop rotation.

Previous work had revealed a constitutively active mutation, i.e. S277I, on the hinge helix [15,37]. Moreover, a mutation on the P10 loop, i.e. E354K, was identified to diminish the activity of LHGR [9]. To characterize these functionally important mutations in the hinge helix-P10 loop domain, we performed MD simulations for S277I and E354K mutants (termed LHCGR-S277I and LHCGR-E354K), respectively. The positions of these two residues are shown in Fig. 3A. In LHCGR-S277I, we observed a population of receptor conformations with \(d_{\text{hinge helix-ECL1}}\) between 3 Å and 4 Å and \(d_{\text{h10-ECL1}}\) between 4 Å and 6 Å (Fig. 3B), similar to those of active-like LHCR-CG (Fig. 2B). In contrast, LHCGR-E354K had its P10 move towards ECL1 (Fig. 3C), which was distinct from active-like conformations of LHCR-CG (Fig. 2B). We then investigated how S277I and E354K mutations influence hinge helix-P10 conformation (Fig. 3D-I and Figure S6). For the S277I mutation, the WT representative model extended the sidechain of 277\(^{\text{Hinge helix}}\) opposite with 143\(^{\text{ECL1}}\) (Fig. 3D). The minimal distance between 277\(^{\text{Hinge helix}}\) and 143\(^{\text{ECL1}}\) is 7.6 Å. In contrast, I277\(^{\text{Hinge helix}}\) made hydrophobic contact with I431\(^{\text{ECL1}}\) in the mutated system (Fig. 3E). Globally, LHCGR-S277I had more \(d_{\text{277-143}}\) sampled in 3–5 Å (96.0 %) than LHCR-only (75.5 %), suggesting increasing hydrophobic contacts between 277\(^{\text{Hinge helix}}\) and I431\(^{\text{ECL1}}\) (Fig. 3F).

As for E354K, K354\(^{\text{Hinge helix}}\) made P10 loop closer to ECL1 (Fig. 3G, 3H). The WT system had its \(d_{\text{E354-K605}}\) concentrated on 2–4 Å (58.9 %, Fig. 3I). Conversely, the E354K mutation led to 90.2 % snapshot’s with a \(d_{\text{E354-K605}}\) value larger than 4 Å (Fig. 3J). The cryo-EM structure showed a similar tendency with WT trajectories, including far 277\(^{\text{Hinge helix}}\)-143\(^{\text{ECL1}}\) and close E354\(^{\text{Hinge helix}}\)-I431\(^{\text{ECL1}}\) (Figure S7). Thus, activating mutation S277I led to a closer hinge helix and ECL1 via hydrophobic contact with I431\(^{\text{ECL1}}\). The inactivating mutation E354K caused P10 conformation closer to ECL1. The effect can be attributed to the electrostatic repulsion effect with K605\(^{\text{ECL1}}\). Taken together, our definitions of interface conformations matched the mutation effects and thus explain the mutations in this domain.

3.3. TMD expansion during activation

Since both the hinge helix and P10 loop directly interact with ECLs, we investigated the effects of the hinge helix-P10 domain on TMD conformations. In simulations, LHCR-CG showed a larger distance between TM6 and TM7, compared with the LHCGR-only (Fig. 4A, B, Table 1). At the extracellular side of TM6 and TM7, the average distance between the Ca atoms of A592\(^{\text{TM6}}\) and K605\(^{\text{TM7}}\) was 7.7 ± 3.5 Å in LHCR-only simulations, which was approximately 5 Å shorter than that in LHCR-CG simulations (12.9 ± 1.3 Å for CG, 12.1 ± 1.5 Å for CG-G) (Fig. 4A). The movements of TM6 and TM7 also transferred to the middle of TMD (Fig. 4B). At the middle regions of these helices, the Cα atom distance between C581\(^{\text{TM6}}\) and N615\(^{\text{TM7}}\) was 7.2 ± 0.9 Å of LHCR-only, which was also shorter than that of LHCR-CG (8.5 ± 0.2 Å) and LHCR-CG-G (8.7 ± 1.2 Å) (Fig. 4B). As shown in Figure S8, LHCGR-only had a shorter extracellular TM6-TM7 distance than LHCR-CG in the dominant hinge helix-P10 conformation, suggesting the coupling between the TMD expansion and hinge helix-P10 conformation.

The TM6 and TM7 participate in forming a transmembrane pocket, corresponding to the major small molecule-binding pocket of classical family A GPCRs [38,39]. The size of such pocket directly correlates with GPCR activation [40]. We found that the size of the small molecule-binding pocket increased with CG binding (Fig. 4C, Table 1). LHCR-CG had a long TM6-TM7 distance and an extended pocket towards extracellular TM2-TM3. The active-like P10 conformation also permitted an extra volume above the TMD of LHCR-
These properties led to an average volume of $253.7 \pm 121.1 \text{ Å}^3$. However, the upward movements of the hinge helix in LHCGR-only caused ECL1 and TM2-TM3 to depart from the bundle center, while the P10 loop failed to form a pocket with TMD due to its upward movement. The closer TM6-TM7 distance contributed to a more contracted pocket with an average volume of $188.4 \pm 111.3 \text{ Å}^3$. The large volume of pocket was also observed in the active-state simulation of LHCGR-CG-G ($313.03 \pm 162.09 \text{ Å}^3$).

The extended TM6-TM7 also altered the sidechain contact of highly conserved M582$_{6.48}$ in GPHRs (Fig. 4D). In LHCGR-CG, M582$_{6.48}$ flipped upward to interact with L532$_{5.44}$ (Fig. 4D). However, in LHCGR-only, it flipped downward and contacted with F539$_{5.51}$ and L457$_{3.43}$ with minimal distances of 4.2 Å and 4.3 Å (Fig. 4D). In all simulations, LHCGR-only had more flipped-down M582$_{6.48}$ (67.7 %) than LHCGR-CG (50.9 %). In LHCGR-CG-G simulations, the population of flipped-down M582$_{6.48}$ further decreased to 43.6 % (Table 1). The interaction between the flipped-down M582$_{6.48}$ and L457$_{3.43}$ on the opposite of TMD might induce the closure of TM6. Consistently, previous clinical research reported that L457R mutation led to LHCGR activation, which may be attributed to the disruption of hydrophobic interaction with M582$_{6.48}$ [10].

3.4. Evolutionary and dynamics analysis show correlations between the hinge region and the other subdomains

We applied evolutionary and dynamics relationship analysis to build connections between the aforementioned movements and the intracellular pocket for G protein binding. The G protein-binding pocket locates in the intracellular side of TMD and its opening depends on the movement of TMs 5–7 [41,42]. Since LHCGR has a close homolog FSHR with similar structure composition and mechanism [15], we also did an evolutionary analysis on FSHR for reference. Statistic coupled analysis (SCA) models of LHCGR and FSHR were constructed. SCA is based on the deviation degree of residue frequency on the protein from the background frequency and calculates the correlation constant ($C_{ij}$) for position $i$ with residue $a$ and position $j$ with residue $b$ to show the coevolution level between them. Then, the correlation matrix can be denoised to show evolutional related sectors, which tend to be structurally and functionally related (see material and methods) [34,36,43]. With LHCGR and FSHR from all species, the correlation constant between each residue pair was shown in Fig. 5A and 5C, while the corresponding sectors were shown in Fig. 5B and 5D.

The conservation degree of each position in LHCGR and FSHR was shown in Figure S9 and Figure S10, respectively.

Fig. 3. The effects of mutations on the hinge helix-P10 loop conformations. (A) S277$_{6.27}$ Hinge helix and E354$_{7.10}$ of LHCGR. Protein and key residues are shown in cartoon and stick depicts, respectively. (B, C) The free energy landscape for the minimal distance between E354$_{7.10}$ and Y426$_{ECL1}$ ($d_{E354-ECL1}$) and the minimal distance between A281$_{6.21}$ Hinge helix and N428$_{ECL1}$ in LHCGR-CG-G (B) and LHCGR-E354K (C) systems. (D, E) Representative models showing interactions between hinge helix and ECL1 in LHCGR-only (D) and LHCGR-S277I (E) systems. (F) Distribution of the minimal distance between S277$_{6.27}$ Hinge helix and I431$_{ECL1}$ in LHCGR-only and LHCGR-S277I systems. (G, H) Representative models showing interactions between P10 loop, TMD, and ECL1 in LHCGR-only (G) and LHCGR-E354K (H) systems. (I) Distribution of the minimal distance between E354$_{7.10}$ and K605$_{7.35}$ in LHCGR-only and LHCGR-E354K systems.
Because ECD (including LRR and hinge region) and C-terminus are not conserved among species for GPHRs (Figure S9 and Figure S10), these two domains in both FSHR and LHCGR showed higher coevolution scores, which was not informative. Notably, several TMD residues showed coevolution with ECD residues. The mostly coevolved TMD parts were ECL1 and intracellular TM5-TM6 in both FSHR and LHCGR (Fig. 5A, 5C, shown by rectangles). Remarkably, previous GPCRs studies indicate the intracellular TM5-TM6 are determinant for the receptor activation [44,45].

In Fig. 5B, D, the residues were separated into different sectors according to their coevolution relationship. Blue sectors were distributed around CG- or FSH- binding pocket in LRR and hinge loop, functioning as a signal receptor domain in GPHRs. Red sectors located on hinge loop, ECL1, and TM5-TM6. They may play their role as a signal deliverer and the inclusion of the hinge loop confirmed that loop movements influenced signal transmission. Green sectors included the small molecule-binding pocket as well as the G protein-binding pocket for class A GPCRs. This coevolution analysis unraveled the relationship between ECD and ECL1, TM5-TM6, showed the importance of hinge loop in signal transfer, and reflected the retention of classical TMD signal pathway in GPHR.

Considering the conservation of the hinge helix and P10 loop, the coevolution analysis based on mutations was not able to capture their impact on TMD. We hence calculated the dynamical cross-correlation map (DCCM) to show the relationship of movements between residue pairs. The correlation between residue movements was shown as a DCCM in Fig. 6A and 6B [46]. Red and blue represented correlated and anti-correlated movements, respectively. To identify the correlations between the hinge helix and P10 to other parts, we labeled them in rectangles and visualized their movement relationships in Fig. 6C.

Globally, the movement relationship was slightly stronger in LHCGR-CG than which in LHCGR-only, indicating that CG promoted correlated movement in LHCGR and contributed to signal transmission. In Fig. 6A and 6B, as the rectangles showed, both hinge helix (276–282) and P10 loop (350–359) are anti-correlated with TM5-TM6 movements. Fig. 6C also had deep blue

![Figure 4](image_url)

**Fig. 4.** Different conformations of TMD in simulations. (A, B) Representative models showing the extracellular TM6-TM7 movements. Protein and key residues are shown in cartoon and stick depicts, respectively. (C) The major small-molecule binding pockets in LHCGR-only and LHCGR-CG systems. (D) M582 in LHCGR-only and LHCGR-CG systems.

| Table 1 | The properties showing different TMD conformations in simulation systems. |
|---------|--------------------------------------------------------------------------|
| Properties | LHCGR-CG-G | LHCGR-CG | LHCGR-only |
| δ_{A592-K605} | 12.1 ± 1.5 Å | 12.9 ± 1.3 Å | 7.7 ± 3.5 Å |
| δ_{C581-N615} | 8.7 ± 1.2 Å | 8.5 ± 0.8 Å | 7.2 ± 0.9 Å |
| volume | 313.03 ± 162.09 Å³ | 253.7 ± 121.1 Å³ | 188.4 ± 111.3 Å³ |
| Flipped-up M582 | 56.4 % | 45.1 % | 32.3 % |
| Flipped-down M582 | 43.6 % | 50.9 % | 67.7 % |
color in the transducer-binding pocket for both the hinge helix and P10, suggesting that the movements of these domains were related to the movements of the G protein-binding pocket. Taken together, evolutionary and dynamics relationship analysis suggested the correlation between the hinge domain and the other subdomains, including LRR, ECL1, and G protein-binding pocket, consistent with our trajectory observations.

4. Discussion

Here, our all-atom MD simulations showed a unique hinge loop rotation in LHCGR upon the binding of its endogenous agonist CG. The rotation occurs with increased surface area and reduced interactions of the hinge loop compared with LHCGR-only. With loop rotation, the hinge helix-P10 region has specific downward movements, including the P10 loop with respect to TMD and the hinge helix with respect to ECL1, indicating an active-like conformation. In contrast, inactive-like conformation in LHCGR-only shows upward movements of the P10 loop and hinge helix. These movements correspond with known activation and inactivation mutations in the hinge helix-P10 domain. Also, the active-like conformation is related to the TMD expansion. Evolutionary and dynamics analysis suggest that conformational changes of hinge helix loop and ECD-TMD interface are correlated to TM5-TM6 movements at the intracellular side, inferring their roles in signal transmission. This work supplements the push-and-pull model of LHCGR activation by elucidating the roles of the hinge region in signal transmission using computational models.

Our simulation results underscore the importance of hinge helix-P10 conformation and provide a potential molecular basis for previous mutagenesis studies on the ECD-TMD interface [9,15,37]. Although P10 is shown as a flexible loop, its residue composition is critical to the activation of LHCGR and a single mutation is enough to decrease LHCGR activity [1,14,47]. Mutations other
than S277I on the hinge helix also significantly change the activation property for LHCGR and its homolog thyrostimulating hormone receptor (TSHHR) [48]. Notably, hydrophobic mutations on S277 commonly lead to activation, while polar mutations cause inactivation [37]. It supports our assumption that 277–431 hydrophobic interactions make the hinge helix closer to ECL1 to promote active-like conformation in LHCGR.

A recent cryo-EM structure of TSHHR shows a similar signal transmission induced by the endogenous glycoprotein hormone [49]. The interactions between hormone and hinge loop constrain the conformation of the loop to activate TSHHR [49,50]. Upon the agonist binding, the hinge helix of TSHHR also moves towards ECL1, while its P10 loop stays away from ECL1, highly consistent with the ECD-TMD alteration observed in our MD simulations. In addition, an antibody interacting with the bottom of LRR activates TSHHR without the dependence on the hinge loop [49]. It indicates the possibility to activate GPHRs via directly regulating the ECD-TMD interface.

From our signal transmission computational model, the hinge region plays functional roles in GPHR via interacting with ECL1. As N-terminal residues, the hinge region has been identified as a tethered agonist and their residue composition is critical to GPHR activation [48]. The N-termius has been shown to interact with ECL3 and initiate activation of class A GPCRs, including melanocortin and chemokine receptors [51–53]. For class B and class C GPCRs, the N-terminus can also regulate ligand binding and activation [54–56]. Due to its flexibility, however, the N-terminus is not always solved in GPCR structures [31]. The observed activation effect of the N-terminus reminds attention to such a “vanished” part.

Our simulations have explored the conformational ensemble of the hinge loop of a typical GPHR. Since the clockwise rotation of the hinge loop is a unique property of LHCGR-CG and downstream signaling, maintaining such rotation might provide a new strategy for LHCGR activation. Besides, our sampling has shown the distinct dynamics of the ECD-TMD interface and investigated their influence on extracellular TMD. Considering the flexibility of the hinge domain and extracellular TMD, ensemble docking can be a proposed way to design targeting compounds [57,58]. In addition, a covalent molecule connecting the hinge loop and ECD may perform a similar function as LH or CG and activate the downstream signaling [31]. The observed activation effect of the N-terminus reminds attention to such a “vanished” part.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was partially supported by the Shanghai Municipal Science and Technology Major Project (X.C. and H.E.X.); the Ling-gang Laboratory grant (LG202102-01-01 to X.C.); the Ministry of Science and Technology (China) grants (2018YFA0507002 to H.E. X.); the CAS Strategic Priority Research Program (XDB37030103 to H.E.X.); the National Natural Science Foundation of China (32130022 to H.E.X., 32171187 to Y.J., 82121105 to H.E.X. and Y.J.).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.csbj.2022.11.039.

References

[1] Narayan P, Ulloa-Aguirre A, Dias JA. Chapter 2 - Gonadotropin Hormones and Their Receptors. In Strauss JF, Rathbun RLBT-Y and JRE (Eighth E, editors., Philadelphia: Elsevier; 2019, p. 25–57.
[2] Choi J, Smitz J. Luteinizing hormone and human chorionic gonadotropin: origins of difference. Mol Cell Endocrinol 2014;383:203–13.
[3] Jiang X, Liu H, Chen X, Chen P-H, Fischer D, Sriraman V, et al. Structure of follicle-stimulating-hormone in complex with the entire ectodomain of its receptor. Proc Natl Acad Sci U S A 2012;109:12491–6.
[4] Ulloa-Aguirre A, Zariñán T, Jardón-Valadez E, Gutiérrez-Sagal R, Dias JA, Wu EL, Cheng X, Jo S, Rui H, Song KC, Dávila-Contreras EM, et al. CHARMM-GUI input generator for NAMD, GROMACS, AMBER, OpenMM, and CHARMM/SPC.
[5] Cole LA. HCG variants, the growth factors which drive human malignancies. Søndergaard CR, Olsson MHM, Rostkowski M, Jensen JH. Improved Treatment of Classical T-Cell Lymphomas: Role of Allosteric Modulators. Curr Opin Pharmacol 2013;13:727–34.
[6] Hansel W, Leuschner C, Enright F. Conjugates of lytic peptides and LHRH or LHRH analogs. Cunha-Silva M, Brito VN, Macedo DB, Bessa DS, Ramos CO, Lima LG, et al. Leucine-rich repeat (LRR) proteins: integrators of pattern recognition and signaling in immunity. Autophagy 2011;7:1082–4.
[7] Jia Duan, Xi Song, Kun Jiang, X. He, J. Duan, Y. Ji et al. Computational and Structural Biotechnology Journal 20 (2022) 6503–6511.
[8] Scalise AM, Torio C, Basile OG, Franchini G, Brancaccio RN, Cattaneo AM. Crystal structure of human chorionic gonadotropin. Nature 1994;369:455–61.
[9] Ng A, Xavier RJ. Leucine-rich repeat (LRR) proteins: integrators of pattern recognition and signaling in immunity. Autophagy 2011;7:1082–4.
[10] Waterhouse A, Bertoft M, Bienert S, Studer G, Tauriello G, Gumminery R, et al. SWISS-MODEL: homology modelling of protein structures and complexes. Nucleic Acids Res 2018;46:W296–303.
[11] Sandbergard CR, Olsson MHH, Rostkowski M, Jensen JH. Improved Treatment of Ligands and Coupling Effects in Empirical Calculation and Rationalization of pKa Values. J Chem Theory Comput 2011;7:2284–95.
[12] Wu EL, Cheng X, Jo S, Rui H, Song KC, Dávila-Contreras EM, et al. CHARMM-GUI membrane builder toward realistic biological membrane simulations. J Comput Chem 2014;35:1997–2004.
[13] Wu EL, Cheng X, Jo S, Rui H, Song KC, Dávila-Contreras EM, et al. CHARMM-GUI membrane builder toward realistic biological membrane simulations. J Comput Chem 2014;35:1997–2004.
OpenMM simulations using the CHARMM36 additive force field. J Chem Theory Comput 2016;12:405–13.

Huang J, Krasavits S, Nrawicki C, Ran T, Feig M, de Groot BL, et al. CHARMM36: an improved force field for folded and intrinsically disordered proteins. Nat Methods 2017;14:71–3.

Nose S. A unified formulation of the constant temperature molecular dynamics methods. J Chem Phys 1984;81:511–9.

Aoki KM. Yonezawa F. Constant-pressure molecular-dynamics simulations of the crystal-smectic transition in systems of soft parallel spherocylinders. Phys Rev A, At Mol Opt Phys 1992;46:6541–5.

Hess B. P-UNCS: A Parallel Linear Constraint Solver for Molecular Simulation. J Chem Theory Comput 2008;4:116–22.

Darden T, York D, Pedersen L. Particle mesh Ewald: An N log(N) method for Ewald sums in large systems. J Chem Phys 1993;98:10089–92.

Abraham MJ, Murtola T, Schütz R, Páll S, Smith JC, Hess B, et al. GROMACS: High-performance molecular simulations through multi-level parallelism from laptops to supercomputers. SoftwX 2015;1–2:19–25.

Roe DR, Cheatham III TE, PTRAJ and CPTTRAJ: software for processing and analysis of molecular dynamics trajectory data. J Chem Theory Comput 2013;9:3084–95.

Weisser J, Shenkin PS, Still WC. Approximate atomic surfaces from linear combinations of pairwise overlaps (LCPO). J Comput Chem 1999;20:217–30.

Durrant JD, Votapka L, Sörensen J, Amaro RE. POMME 2.0: An Enhanced Tool for Determining Pocket Shape and Volume Characteristics. J Chem Theory Comput 2014;10:5047–56.

Munk C, Mutt E, Isberg V, Nikolajsen LF, Bibbe JM, Flock T, et al. An online resource for GPCR structure determination and analysis. Nat Methods 2019;16:151–62.

Seo MJ, Heo J, Kim K, Chung KY, Yu W. Coevolution underlies GPCR-G protein selectivity and functionality. Sci Rep 2021;11:7858.

Crooks GE, Hon G, Chandonia J-M, Brenner SE. WebLogo: a sequence logo generator. Genome Res 2004;14:1188–90.

Halabi N, Rivoire O, Leibler S, Ranganathan R. Protein sectors: evolutionary units of three-dimensional structure. Cell 2009;138:774–86.

Tungusavunakool K, Adler J, Wu Z, Green T, Zielinski M, Zidek A, et al. Highly accurate protein structure prediction for the human proteome. Nature 2021;596:390–6.

Cong X, Zhang X, Liang X, He X, Tang Y, Zheng X, et al. Delineating the conformational landscape and intrinsic properties of the angiotensin II type 2 receptor using a computational study. Comput Struct. Biotechnol J 2022.

Nakabayashi K, Kudo M, Kobilka B, Hsueh AJW. Activation of the luteinizing hormone receptor following substitution of Ser-277 with selective hydrophobic residues in the ectodomain hinge region. J Biol Chem 2000;275:30264–71.

Zhong W, Pei J, Lai L. Statistical Analysis and Prediction of Covalent Ligand Receptors and Follicle Stimulating Hormone Receptors. Dis (Basel, Switzerland) 2020;8:E35.

Schulze A, Kleinau G, Neumann S, Scheerer P, Schönberg T, Brüser A. The structural diversity of G protein-coupled receptors. J Chem Phys 1984;81:511–9.

Shulman AI, Larson C, Mangelsdorf DJ, Ranganathan R. Structural determinants of allosteric ligand activation in RXX heterodimers. Cell 2004;116:417–29.

Zhou Q, Yang D, Wu M, Guo Y, Guo W, Zhong L, et al. Common activation mechanism of class A GPCRs. Elife 2019;8:e50279.

Lu S, He X, Yang Z, Chai Z, Zhou S, Wang J, et al. Activation pathway of a G protein-coupled receptor uncoveres conformational intermediates as targets for allosteric drug design. Nat Commun 2021;12:4721.

Swaminathan S, Harte Jr WE, Beveridge DL. Investigation of domain structure in proteins via molecular dynamics simulation: application to HIV-1 protease dimer. J Am Chem Soc 1991;113:2717–21.

Althumairy D, Zhang X, Baez N, Barisas G, Roess DA, Bousfield GR, et al. Glycoprotein G protein-Coupled Receptors in Disease: Luteinizing Hormone Receptors and Follicle Stimulating Hormone Receptors. Dis (Basel, Switzerland) 2020;8:E35.