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Dimerization of the lutropin receptor: insights from computational modeling

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Abstract

A computational approach based upon rigid-body docking, ad hoc filtering, and cluster analysis has been carried out to predict likely interfaces in LHR homodimers. Quaternary structure predictions emphasize the role of helices 4, 5 and 6, with prominence to helix 4, in mediating inter-monomer interactions. Intermolecular interactions essentially involve the transmembrane domains rather than the hydrophilic loops and do not implicate disulfide bridges.

Collectively, Molecular Dynamics simulations on the isolated receptor and computational modeling of LHR homodimerization suggest that mutation-induced LHR activation favors H4-H4 contacts involving the highly conserved W491 from both the receptors monomers.

Keywords

GPCR dimerization; rigid-body docking; quaternary structure predictions; comparative modeling; molecular dynamics simulations

INTRODUCTION

Increasing piece of evidence indicate that G protein-Coupled Receptors (GPCRs) exist as homo- and heterodimer/oligomers in the cell membrane (reviewed in refs. (Agnati et al., 2003;Bouvier, 2001;Bulenger et al., 2005;Franco et al., 2003;George et al., 2002;Kroeger et al., 2003;Maggio et al., 2005;Milligan, 2001;Park et al., 2004;Rios et al., 2001;Terrillon and Bouvier, 2004)). A number of studies have shown that dimerization occurs early after biosynthesis, suggesting that it has a primary role in receptor maturation (reviewed in refs. (Bulenger et al., 2005;Terrillon and Bouvier, 2004)). For many proteins, oligomeric assembly has an important function in endoplasmic reticulum (ER) quality control because it masks specific retention signals or hydrophobic patches that would otherwise retain the proteins in ER. (Reddy and Corley, 1998) G protein coupling, downstream signaling and regulatory processes, such as internalization, have also been shown to be influenced by the dimeric nature of the receptors (reviewed in refs. (Maggio et al., 2005;Terrillon and Bouvier, 2004)). The question whether dimerization influences ligand-induced activation/regulation of GPCRs still remains to be answered. In fact, some studies suggest that ligand binding can regulate the dimer by either promoting or inhibiting its formation, whereas many others conclude that homodimerization and heterodimerization are constitutive processes that are not modulated by ligand binding (reviewed in ref. (Terrillon and Bouvier, 2004)). More resolved information on GPCR oligomerization has been inferred by Atomic Force Microscopy (AFM) measurements,

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which showed that rhodopsin and opsin form constitutive dimers in dark-adaptive retinal membrane (Fotiadis et al., 2003; Park et al., 2004). Thus, regulated protein-protein interactions are key features of many aspects of GPCR function and there is now increasing evidence for GPCRs acting as part of multi-component units comprising a variety of signaling and scaffolding molecules (Brady and Limbird, 2002; Pierce et al., 2002).

The glycoprotein hormone receptors are not exceptions to this rule as an increasing number of reports is coming out providing evidence that they form supramolecular assemblies (Horvat et al., 2001; Hunzicker-Dunn et al., 2003; Nakamura et al., 2004; Roess et al., 2000; Roess and Smith, 2003; Tao et al., 2004; Urizar et al., 2005). Whether dimerization of these receptors, in particular, of the lutropin (LH) receptor (LHR) depends on LH or hCG binding is unclear (Horvat et al., 2001; Hunzicker-Dunn et al., 2003; Nakamura et al., 2004; Roess et al., 2000; Roess and Smith, 2003; Tao et al., 2004; Urizar et al., 2005). More clear is the evidence that higher order aggregates of LHR form upon agonist-induced receptor desensitization (Hunzicker-Dunn et al., 2003; Roess and Smith, 2003).

Recently, a homo-dimerization model of the crystal structure of the follicle-stimulating hormone (FSH) receptor (FSHR) ectodomain in complex with FSH has been proposed, which is almost incompatible with inter-monomer contacts mediated by the transmembrane (TM) helices (Fan and Hendrickson, 2005). In apparent disagreement with that model, a couple of evidences from in vitro experiments support the hypothesis that the TMs participate in the intra-dimer interface both in the thyrotropin (TSH) and lutropin receptors (Nakamura et al., 2004; Urizar et al., 2005).

So far, predictions of likely interfaces of GPCR dimers essentially relied on sequence-based methods ((Dean et al., 2001); reviewed in (Filizola and Weinstein, 2005)).

We have developed a computational procedure for predicting the supramolecular organization of TM α -helical proteins by rigid-body docking (Casciari et al., 2005). Benchmarks of the approach were done on selected oligomers with known structure. In all the test cases, native-like quaternary structures, i.e. with Root Mean Square Deviation of the α -carbon atoms (C_α -RMSDs) lower than 2.5 Å from the native oligomer were achieved (Casciari et al., 2005). The effectiveness of the prediction protocol makes it suitable for *ab initio* quaternary structure predictions of other integral membrane proteins, including GPCRs. An attempt in this respect has been already reported, though based on an early and different version of the computational protocol, proving usefulness in aiding the interpretation of biophysical data and the design of novel in vitro experiments (Canals et al., 2003).

In this study, the computational protocol has been challenged in predictions of the LHR portions more likely involved in homodimerization.

METHODS

Computational modeling of the LHR

A novel model of the LHR was built by means of the comparative modeling software MODELER (Sali and Blundell, 1993), by using the latest rhodopsin structure as a template (i.e. PDB code: 1U19 (Okada et al., 2004)). The modeled sequence includes the TM helices (abbreviated as H), the three intracellular (i.e. I1, I2 and I3) and three extracellular (i.e. E1, E2 and E3) loops, as well as the 323–358 ectodomain sequence, which can be reasonably modeled based upon the N-terminus of rhodopsin (Fig. 1). A modified rhodopsin template was employed, in which the sequences 100–101 and 106–107 were deleted, which correspond to the H2/E1 junction and the first two amino acids of H3, respectively. The sequence 236–242, corresponding to the C-term of I3, was deleted as well. During comparative modeling, α -helical

restraints were imposed on the LHR sequences 420–423 and 432–439. Using the sequence alignment shown in Fig. 1, 200 models were obtained by randomizing the Cartesian coordinates of the model through a random number uniformly distributed in an interval from $-4 \text{ \AA}$ to $4 \text{ \AA}$ (Sali and Blundell, 1993). Among the 450 models finally obtained, the two were selected, which showed low restraint violations associated with high values of the 3D-Profile score, computed by means of the Protein Health module in the QUANTA 2000 package, and low numbers of main-chain and side-chains in non allowed conformation and close contacts. These models were first completed by the addition of the polar hydrogens and then subjected to automatic and manual rotation of the side chain torsion angles when in non allowed conformations, leading to 73 different input structures for further calculations. These input structures were subjected to energy minimization and Molecular Dynamics (MD) simulations by means of the CHARMM program (Brooks et al., 1983), using an implicit membrane-water model (i.e. IMM1) recently implemented in CHARMM (Lazaridis, 2003). This is an extension of the EEF1 implicit water solvation model to heterogeneous membrane-aqueous media (Lazaridis, 2003). In this respect, the adjustable parameter “a” and the non polar core thickness were set equal to 0.85 and $32 \text{ \AA}$, respectively. Minimizations were carried out by using 1500 steps of steepest descent followed by a conjugate gradient minimization, until the root mean square gradient was less than $0.001 \text{ kcal/mol \AA}$. A disulfide bridge patch was applied to C439 and C514, respectively located in H3 and E2.

The “united atom approximation” was employed. The lengths of the bonds involving the hydrogen atoms were restrained by the SHAKE algorithm, allowing an integration time step of 0.001 ps . The systems were heated to 300 K with 5 K rise, every 100 steps per 6000 steps, by randomly assigning velocities from the Gaussian distribution. After heating, the system was allowed to equilibrate for 64 ps .

The secondary structure of the helix bundle was preserved, by assigning distance restraints (i.e. minimum and maximum allowed distances of $2.7 \text{ \AA}$ and $3.0 \text{ \AA}$, respectively) between the backbone oxygen atom of residue i and the backbone nitrogen atom of residue $i+4$, except for prolines. The scaling factor of such restraints was 10 and the force constant at 300 K was $10 \text{ kcal/mol \AA}$. The application of these intra-helical distance restraints was instrumental in: (a) reducing the system degrees of freedom, (b) inferring the structural/dynamics role of prolines, and (c) letting the helices move as rigid bodies, consistent with the experimental evidences on rhodopsin activation (Farrens et al., 1996). The receptor amino acids, which were found in non canonical α -helical conformation in the input structure, condition inherited from the rhodopsin template, were not subjected to any intra-backbone distance restraint. Short (100 ps) equilibrated MD runs were carried out, probing different input structures and different combinations of intra-helical distance restraints. The latter tests consisted in applying distance restraints to different amino acid stretches in each helix. Finally, the computation conditions and the input receptor structure were chosen, which, following MD simulation, produced average arrangements characterized by good stereochemical quality, together with structural similarity to rhodopsin. Three different input structures were, therefore, selected for 1 ns MD production phases. For each MD run, the structures averaged over the first and last 500 ps , as well as over the entire 1 ns trajectory were considered for docking simulations.

Docking simulations of LHR dimerization

Predictions of oligomeric structures were done by means of the rigid-body docking program ZDOCK 2.1, a version of the Fast Fourier Transform-based algorithm, which utilizes the Pairwise Shape Complementarity (PSC) scoring function, neglecting desolvation (Chen and Weng, 2003).

For each protein system, the first docking run consisted of docking two identical copies of the monomer, i.e. one monomer was used as fixed protein (i.e. target) and the other as mobile

protein (i.e. probe). Different average minimized structures were employed in docking simulations.

A rotational sampling interval of 6° was employed, i.e. dense sampling, and the best 4000 solutions were retained and ranked according to the ZDOCK score. These solutions were subjected to a filter, i.e. the “membrane topology” filter, which discards all the solutions that violate the membrane topology requirements. In detail, the filter discards all the solutions characterized by a deviation angle from the original z-axis, i.e. tilt angle, and a displacement of the geometrical center along the z-axis, i.e. z-offset, above defined threshold values. For the tilt angle and the z-offset, thresholds of 0.4 radians and 6.0 Å were, respectively, employed. The filtered solutions from each run were merged with the target protein, leading to an equivalent number of oligomers that were subjected to cluster analysis. The C_α-RMSD threshold for each pair of superimposed dimers was set equal to 2.5 Å. All the amino acid residues in the dimer were included in C_α-RMSD calculations.

To identify the cluster of solutions with the best membrane topology, i.e. with the lowest values of both tilt angle and z-offset, the MemTop index was defined according to the following formula: $MemTop = \sqrt{\langle tilt \rangle^2 + \langle Z_{off} \rangle^2}$, where $\langle tilt \rangle$ and $\langle Z_{off} \rangle$ are, respectively, the tilt angle and the z-offset averaged over all the members of a given cluster. In general, MemTop indexes close to 0.0 are indicative of good membrane topology.

RESULTS AND DISCUSSION

In this study, a computational approach based upon rigid-body docking, ad hoc filtering, automatic cluster analysis and visual inspection of the cluster centers (i.e. the structure with the highest number of neighbors in a cluster) has been employed to predict likely interfaces in LHR homodimers. Docking simulations have been done on a novel model of the LHR, which, differently from the previous model (Fanelli et al., 2004), holds the 323–358 ectodomain sequence that forms a β-sheet with E2, feature inherited from rhodopsin structure.

The filtering of the 4000 best ZDOCK solutions from each run reduced by more than 98% the number of reliable solutions, i.e. the solutions that fulfill the membrane-topology requirements, thus improving the effectiveness of the following automatic cluster analysis and visual inspection of the cluster centers. The number of reliable solutions was less than 2% independently of the receptor structure employed and hence too low for giving significance to the cluster population. Therefore, the membrane topology parameter MemTop, rather than the cluster population, was used as major criterion for solution selection, i.e. such index has to be close to 0.0 for an optimal membrane topology.

The receptor configuration, which gave the highest number of reliable solutions (i.e. 67 out of 4000), was an energy minimized average over the 2000 structures collected during one of the different 1 ns MD production phases. Automatic cluster analysis divided the 67 reliable solutions into 15 clusters. Among these clusters, the ones showing a MemTop index lower than 1.0 were essentially characterized by H4-H4 (Fig. 2a, MemTop score equal to 0.69), or H4-H6 (Fig. 2b, MemTop score equal to 0.34), or H5-H6 (MemTop score equal to 0.80) contacts. As for the H4-H4 dimer, also H1 and H3, from one monomer, make contacts with H4 from the other monomer (Fig. 2a). This dimer is characterized by the approaching of the highly conserved W491 from both monomers, providing a possible explanation for the fact that W491, despite its very high conservation, is almost exposed to the lipids in the inactive receptor forms. Interestingly, previous computations highlighted the conserved tryptophan as a marker of the orientation changes of H4 associated with mutation-induced activation of the LHR. In fact, in the structure representative of the ground state, the tryptophan is involved in H-bonding interaction with N400 (in H2), whereas in the structures of the constitutively active mutants it

becomes more exposed to the lipids losing its interaction with N400 (Fanelli et al., 2004). Collectively, MD simulations on the isolated receptor and computational modeling of LHR homodimerization suggest that mutation-induced LHR activation favors H4-H4 contacts involving the highly conserved tryptophan from both the receptors monomers. These inferences agree with in vitro cysteine cross-linking experiments on the D2 dopamine receptor, indicating a dynamic involvement of H4 in forming a symmetrical dimer interface (Guo et al., 2005). As for the H4-H6 dimer, also H5, from one monomer, participates in the interaction with H4, from the other monomer (Fig. 2b). Finally, for the H5-H6 dimer, also H7, from one monomer, participates in the interaction with H5, from the other monomer. Another possible docking solution but extracted from a cluster with a MemTop score higher than 1.0 (i.e. 1.62) is characterized by contacts between H4, from one monomer, and both H5 and H3, from the other monomer.

Docking simulations on an alternative average LHR structure predict another possible dimer characterized by contacts between H4, from one monomer, and both H6 and H7 from the other monomer.

Consistent with evidence from in vitro experiments (Tao et al., 2004), no disulfide linkages are predicted to participate in inter-monomer interactions. The latter essentially consist in weak van der Waals interactions between hydrophobic amino acids (Fig. 2). In summary, the results of this study emphasize the role of H4, H5 and H6, with prominence to H4, in mediating inter-monomer interactions in LHR homodimers. These results are consistent with the general inferences from sequence-based predictions on different GPCRs ((Dean et al., 2001); reviewed in (Filizola and Weinstein, 2005)). More LHR configurations need to be probed in docking simulations to improve the effectiveness of quaternary structure predictions. Docking simulations on the most likely dimers are expected to provide hints into the architecture of higher-order oligomers. However, unraveling the mechanism of the observed LHR aggregation in multimolecular complexes following hormone-induced desensitization (Hunzicker-Dunn et al., 2003; Roess and Smith, 2003) awaits computational modeling of the hormone-induced active states of the receptor.

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N-TER			
LHR	ESEL SGWDY EYGFCLPKTPRCAPEDAFNPCEDI	356	
rhodopsin	MNGTEGPNFYVPFSNKTGVVRS PFEAPQYYLAEP	34	
H1			
LHR	MGYDFLRVLIWLI NILAIMGNMTVLFVLLT	386	
rhodopsin	WQFSMLAAYMFLLIMLGFPINFLTLYVTVQ	64	
I1			
LHR	SRYKLT	392	
rhodopsin	HKKLRT	70	
H2			
LHR	VPRFLMCNLSFADF CMGLYLLLIASVDSQTK	423	
rhodopsin	PLNYILLNLAVADLFMVFGGFTTTLYTSL--	99	
E1			
LHR	GQYYNHA I	431	
rhodopsin	-YFVF---	105	
H3			
LHR	DWQTGSGCSTAGFFT VFAS ELSVYTLTVITLERWHTI	468	
rhodopsin	---GPTGCNLEGGFFATLGGEIALWSLVVLAIERVYVV	139	
I2			
LHR	TYA IHL DQKLRLR	481	
rhodopsin	CKPMSNFRFGEN-	151	
H4			
LHR	HAILIMLG GWLFSSLIAMLP LV	503	
rhodopsin	HAIMGVAFTWVMALACAAPLV	173	
E2			
LHR	GVSNYMKV---SICFPMDV---ETTLSQ	525	
rhodopsin	GWSRYIPEGMQCSCGIDYYTPHEETNNE	201	
H5			
LHR	VYILTILILN-VVAFFIICACYIKIYFYF	551	
rhodopsin	SFVIYMFVVHFI IPLIVIFFCYGQLVFVF	228	
I3			
LHR	AVRNPELMA	560	
rhodopsin	TVKEAAA--	235	
H6			
LHR	TNK-DTKIAKKMAILIFTDFTCMAPISFFAISA AFK	595	
rhodopsin	TQKAEKEVTRMVIIMVIAFLICWLPYAGVAFYIFTH	278	
E3			
LHR	VPLITVT	602	
rhodopsin	QGSDFGP	285	
H7		H8	
LHR	NSKVLLVLFYPINSCANPFLYAIFT	KTFQRDFFLLL	638
rhodopsin	IFMTIPAFFAKTSAVYNPVIYIMMN	KQFRNCMVTTL	321

Figure 1.

Alignment between bovine rhodopsin (i.e. template) and the human LHR (target) that has been employed for comparative modeling. The amino acid stretches 100–101, 106–107 and 236–242 have been deleted from the 1U19 template. The boxed sequences correspond to H8.

Figure 2.

Top: Cartoon representation of two different dimeric models of the LHR, seen from the intracellular side in a direction perpendicular to the membrane surface. The extracellular loops are not shown. H1, H2, H3, H4, H5, H6, H7 and H8 are, respectively, colored in blue, orange, green, pink, yellow, cyan, violet and magenta. I1, I2 and I3 are respectively colored in lime, slate and salmon. Only the helices participating in the inter-monomer interfaces are indicated by numbers. Bottom: amino acids that contribute to the inter-monomer interface. Squares are colored according to the amino acid location (see above in the legend). White squares indicate E2. Drawings were done by means of the software PYMOL 0.97 (<http://pymol.sourceforge.net/>).