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Modelling the interaction of catecholamines with the α_{1A} Adrenoceptor towards a ligand-induced receptor structure

Gemma K. Kinsella[a], Isabel Rozas[a,b,*] & Graeme W. Watson[a,*]
[a]*Department of Chemistry, Trinity College, University of Dublin, Dublin 2, Ireland;* [b]*Centre for Synthesis and Chemical Biology, Trinity College, University of Dublin, Dublin 2, Ireland*

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Abstract

Adrenoceptors are members of the important G protein coupled receptor family for which the detailed mechanism of activation remains unclear. In this study, we have combined docking and molecular dynamics simulations to model the ligand induced effect on an homology derived human α_{1A} adrenoceptor. Analysis of agonist/α_{1A} adrenoceptor complex interactions focused on the role of the charged amine group, the aromatic ring, the *N*-methyl group of adrenaline, the *beta* hydroxyl group and the catechol *meta* and *para* hydroxyl groups of the catecholamines. The most critical interactions for the binding of the agonists are consistent with many earlier reports and our study suggests new residues possibly involved in the agonist-binding site, namely Thr-174 and Cys-176. We further observe a number of structural changes that occur upon agonist binding including a movement of TM-V away from TM-III and a change in the interactions of Asp-123 of the conserved DRY motif. This may cause Arg-124 to move out of the TM helical bundle and change the orientation of residues in IC-II and IC-III, allowing for increased affinity of coupling to the G-protein.

Introduction

The α_1 adrenoceptors (α_1-AR), which belong to Class A of the super family of G protein coupled receptors (GPCRs), are of particular therapeutic interest due to their important roles in the control of blood pressure and in the contraction and growth of smooth muscle. More specifically the α_{1A} adrenoceptor (α_{1A}-AR) located in large abundance in the prostate is thought to be influential in the condition Benign Prostatic Hyperplasia (BPH), a considerable health problem for aging men [1].

Class A GPCRs consist of a heptahelical bundle (TM I-VII), which transverses the cell membrane. They share key structural features including a disulfide bond between TM-III and the extracellular (EC) region, a tripeptide Glu/Asp-Arg-Tyr (E/DRY) motif located at the intracellular end of TM-III and a common Asn-Pro-X-X-Tyr (NPxxY) motif in TM-VII [2]. To advance our understanding of the molecular structure of the α_{1A}-AR we recently built a homology model based on the *1l9h* [3] 2.6 Å resolution crystal structure of bovine rhodopsin in the inactive state [4].

Mutagenesis studies suggest specific binding interactions of the endogenous catecholamines, adrenaline (AD) and noradrenaline (ND), see Figure 1, with the α_{1A}-AR [5–7]. These include an interaction between the amino group of the catecholamines and Asp-106 in TM-III; and of the catechol hydroxyl groups with Ser-188 and Ser-192 of TM-V [8, 9]. Once GPCRs receive an agonist stimulus at their extracellular side they transfer the signal across the cell membrane and initiate a

*To whom correspondence should be addressed. Fax: +353-1-671-2826, E-mail: rozasi@tcd.ie/watsong@tcd.ie

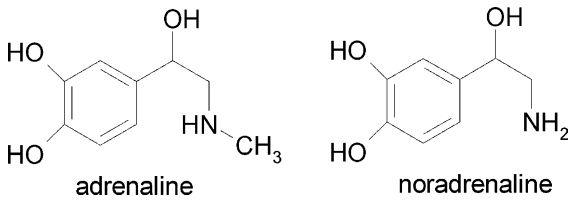

Figure 1. α_1-AR catecholamine agonists, adrenaline (on left) and noradrenaline (on right). The principal interacting groups include a basic nitrogen, a *meta* and *para* hydroxyl of the catechol ring, a *beta* hydroxyl and for adrenaline a *N*-methyl group.

variety of intracellular biochemical events. However, an activation mechanism has only been postulated for a few Class A receptors including the α_{2A}-AR and β_2-AR [10, 11]. Agonist binding may induce specific rearrangements in Pro kink regions or rigid body motion of TM-V and TM-VI [12]. Experimental studies also support a movement of TM-VI relative to TM-III as a consequence of agonist induced receptor activation.

Obtaining structural information about agonist/α_{1A}-AR complexes has been hampered by the lack of a GPCR crystal structure in the activated state [13]. The direct use of the Rhodopsin crystal structure as a template for modelling activation remains questionable. The crystal structure is a snapshot of Rhodopsin in its inactive state; while upon agonist binding an active form will be induced. To address this issue Bissantz et al. [14] attempted to produce an activated form of other GPCRs such as dopamine D_3, the β_2-AR and the δ opioid receptors, by manually rotating TM-VI anticlockwise by 30^o around its helical axis. Chambers et al. [15], developed an active form of the 5-HT_{2A} receptor through homology modelling using a theoretically active template of rhodopsin obtained by isomerisation of the 11-cis-retinal chromophore followed by constrained MD. However, Carmine et al. [16], suggested that the agonist-binding pocket of the β_2-AR is not rigid but is dynamically formed as the ligand builds an increasing number of contacts with the receptor. Therefore, as the receptor is ''en route'' to the active form, it could also change the configuration of the binding cavity as the key agonist binding interactions and the active receptor conformation develop in parallel.

Through molecular modelling studies we aim to facilitate the integration of the available experimental observations and biophysical data into a scheme to examine receptor structure and function. Hence, in this paper, we present a computational strategy to investigate the ligand-induced conformation of the α_{1A}-AR.

Computational methods

Our computational approach to model the α_{1A}-AR conformation induced by the interaction with a ligand involves:

(1) Docking of agonists (AD and ND) to examine their interactions with our previously developed homology-derived model of the α_{1A}-AR [4].
(2) Molecular Dynamics (MD) simulations were performed in a $H_2O/CHCl_3/H_2O$ membrane mimic to refine the agonist/α_{1A}-AR complexes. We explore the structural changes during the dynamics and monitor the resultant conformational changes to the receptor.
(3) Redocking of the agonists into the 'agonist-induced' receptor form developed in step 2.
(4) Further MD simulations of the agonist/α_{1A}-AR complexes to determine agonist binding modes and the agonist induced receptor structures.

A repeated cycling of steps 3 and 4 can be used to further refine the binding site.

Molecular docking

Docking studies between the catecholamines and our α_{1A}-AR model [4] were performed using the programs Dock 4.0 [17] and FlexiDock [18]. Both agonists were protonated and charges were taken from the electrostatic potential obtained during Density Functional Theory (DFT) structural optimisations using the B3LYP hybrid functional and the 6-31G* basis set in Gaussian 98 [19]. The binding site was defined as all residues within 10 Å of Asp-106 in TM-III and Ser-188 and Ser-192 in TM-V, as suggested in mutagenesis studies [5–7]. The agonists were divided into rigid fragments and conformations were built into the receptors binding pocket (incremental construction). The docking modes were further optimised using a genetic algorithm to determine the optimum geometry. The parameters used in this work were the default parameters of Dock 4.0 and FlexiDock.

Molecular dynamics simulations

All MD simulations were performed with Amber 7.0 [20] using a time step of 1 fs, and the Amber force field [21]. The simulation cell was heated gradually to 300 K over 5 ps with equilibration performed using backbone restraints for 5 ps at each of 15, 10 and 5 kcal mol^{-1} $(Å^2)^{-1}$ followed by 65 ps without restraints. Periodic boundary conditions were applied in all three dimensions with the Particle Mesh Ewald (PME) method being used to treat the long-range electrostatic interactions. Non-bonded interactions were calculated for 1–4 interactions and higher using a cutoff radius of 9 Å. Further analysis was performed with the Carnal module of Amber 7.0 and the Gromacs (v3.1.4) tools [22]. Root mean square deviations (RMSDs) were calculated for the backbone heavy atoms after fitting the Cα carbons to the starting homology structure as a reference. Hydrogen bonds (HBs) were defined geometrically; the donor–acceptor-hydrogen angle having to be less than 60° and the donor to acceptor distance (*d*[donor...acceptor]) being less than 4 Å. Amber was also used to optimise the docked and final dynamical structures using steepest descent (250 steps) and conjugate gradient (750 steps) energy minimization methods.

Following a similar procedure to our previous study of the uncomplexed, apo-receptor [4], the dynamical behaviour of the agonist/receptor complexes were examined in a membrane mimic (H_2O/$CHCl_3$/H_2O). Such an environment allows the simulations to sample more conformational space than a phospholipid model, while being more appropriate than a gas phase environment [4, 23]. Counter-ions (Cl^-) were added to ensure a charge neutral cell, by replacing solvent molecules at sites of high electrostatic potential.

For AD and ND the atom types and parameters were obtained from the General Amber Force Field (GAFF) [24]. Two angular terms, *ca-c3-oh* (67.1 kcal mol^{-1}, 110.7°) and *ca-c3-h1* (47.3 kcal mol^{-1}, 109.1°), were added to GAFF by analogy to similar parameters already present. In addition, two improper dihedral terms, (*ca-ca-ca-ha, ca-ca-ca-oh*) were added to enforce the planarity of the aromatic rings (1.1 kcal mol^{-1}, 180.0°). These parameters were tested by comparison of the experimental geometry and vibrational frequencies to those of DFT calculations [25]. The parameters were further tested by performing MD simulations (150 ps) in water and the structures were found to be dynamically stable.

Results and discussion

Analysis of catecholamine/α_{1A}-AR complexes

Both agonists were docked into our homology model of the α_{1A}-AR. For the adrenaline complex, interactions were formed between the protonated nitrogen and a carboxylate oxygen of Asp-106 (*d*[N...O] = 3.23 Å); and between the *meta* hydroxyl and the Ser-188 hydroxyl (*d*[O...O] = 2.16 Å). Alternatively, for the noradrenaline complex, the protonated nitrogen to Asp-106 distance is long (*d*[N...O] = 6.04 Å), while the *para* hydroxyl interacts with the Ser-188 hydroxyl group (*d*[O...O] = 2.93 Å). These initial complexes provide starting structures for further structural refinement through MD simulations (1 ns).

While little structural change occurred during the optimisation step, a marked RMSD rise was observed for both simulations over the heating step of the equilibration runs, as the receptor structures move from a 'rhodopsin-like' conformation (see Figure 2). Over both simulations small structural changes were observed for the short TM-VIII, while the largest deviations were, for the AD complex, TM-VI at 3.30 Å and for the ND complex, TM-IV at 3.04 Å.

The time dependent RMSD data of the seven TM helices were next examined for further structural changes over the MD production runs (see trendlines in Figure 1). For the adrenaline complex, TM-I underwent a decrease in RMSD over 100–300 ps of the production run; TM-VII underwent a structural change between 300–400 ps while TM-IV changed between 600–800 ps (see Figure 2a). A HB interaction forms between Ser-188 and the *meta* hydroxyl after 200 ps, which corresponds to a slight movement of TM-V at this time. For the noradrenaline complex, most of the structural changes occur in the region of 200–600 ps including marked rises in RMSD for TM-IV and TM-V (Figure 2b).

Hence, the RMSD trendlines over the production runs for both agonist complexed simulations indicate that a structural rearrangement of the

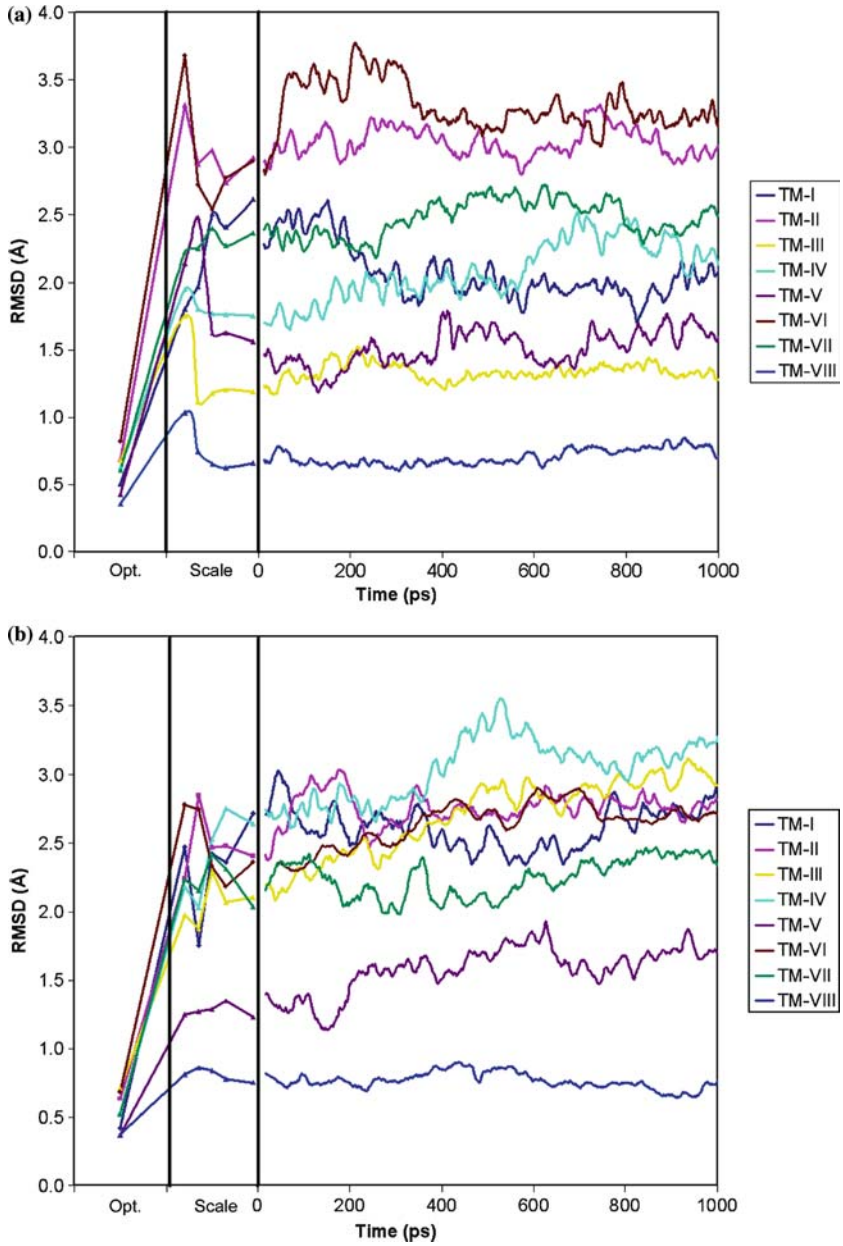

Figure 2. RMSD values of the (a) adrenaline/homology α1A-AR complex and the (b) noradrenaline/homology α1A-AR complex simulations; Optimisation and scaling steps (85 ps) followed by moving average trendline (period of 50 ps) over the production run (1 ns).

receptor has occurred due to the presence of an agonist, which has stabilised by 800 ps of simulation. The overall profiles differ from the previously reported time dependent RMSD for the uncomplexed apo-receptor structure [4] indicating that an agonist induced receptor form has been produced. Structural changes are observed for TM-V and TM-VI in both agonist complexed simulations in agreement with experimental postulates of such helical movements over activation [11].

Throughout the 1 ns simulations, interactions between the positively charged amine of the agonists and the negatively charged side chain carboxyl of Asp-106 in TM-III, anchors the catecholamines in the binding pocket. The second extracellular loop (EC-II), moves further into the

TM framework allowing for the participation of EC-II residues (Thr-174, Cys-176, Gln-177 and Ile-178) in the binding site as observed by Pedretti et al. [26].

The final structures of the complexes were averaged over the last 200 ps of the simulations and were optimised. Both the complexed receptor structures underwent a loss of inter-helical interactions and hydrogen bonds relative to the uncomplexed structure [4]. However, the AD complex experienced a larger movement of TM-VI and this receptor structure was chosen for redocking of adrenaline and crossdocking of noradrenaline.

Further docking of catecholamines utilising the agonist induced α_{1A}-AR

For the redocked adrenaline and crossdocked noradrenaline complexes, the HB distance from the protonated nitrogen to the carboxylic group of Asp-106, (d[H...O]) was 2.43 and 1.42 Å, respectively. For the adrenaline complex, the protonated nitrogen also interacts with Thr-174, (d[H...O] = 3.07 Å). For the noradrenaline complex, the *beta* hydroxyl is orientated towards Asp-106 (d[H...O] = 3.46 Å), while no other close hydroxyl interactions were determined in either docked conformation. Finally, the *N*-methyl group of adrenaline is near Cys-176 (d[H...O] = 3.57 Å) and Tyr-316 (d[H...O] = 2.67 Å). Unlike the previously docked complexes, no interactions were formed with the Ser residues of TM-V and new interactions have formed with EC-II residues. To optimise the agonist binding modes, further MD simulations (2 ns) were performed on both complexes.

Further agonist induced changes to the α_{1A}-AR

Once again the optimisation step causes almost no change in the position of the atoms as illustrated by the small RMSDs (Figure 3), once again indicating that optimisation of docked complexes can not be used to obtain information on protein conformational changes. The largest rise in RMSD is over the heating period, while over the production run a maximum Cα RMSD of 2.59 Å was observed for the adrenaline complex and a higher value of 3.59 Å for the noradrenaline complex. The helical RMSD deviations are smaller than for the original MD simulations (see Figure 3), as the structural rearrangement from a 'rhodopsin-like' conformation occurred over the original MD runs.

Specifically, for the AD/α_{1A}-AR complex simulation, there is a gentle structural drift for TM-I, which has stabilised by 1.5 ns. For TM-VI, there is a structural change at 1.6 ns, which again appears to stabilise quickly. For the ND/α_{1A}-AR complex simulation, there were no structural changes of significance with largely stable helical RMSDs throughout the simulation. The overall analysis indicates that there are no structural changes of significance, which signifies that most changes induced by the ligand have already occurred.

The HB interactions have stabilized by ~1 ns and the following HB analysis was performed over the second ns of the simulations. For the AD complex, the protonated nitrogen interacts frequently with the carboxylate group of Asp-106 (66.7%) and Cys-176 (34.3%) of EC-II. Initially the *meta* hydroxyl interacts with the Ser-188 hydroxyl group but it also forms an interaction with the Ser-192 hydroxyl group by this time period. The *N*-methyl group forms occasional interactions with Trp-102 (22.9%) and the Thr-174 hydroxyl group (10.9%). For the ND complex, similar interactions occur for the protonated nitrogen with Asp-106 (50.5%) and Cys-176 (35.9%). The nearby *beta* hydroxyl also forms a strong interaction with the hydroxyl group of Thr-174 (93.2%).

Final binding mode analysis of the catecholamine/ α_{1A}-AR complexes

The two final complexed structures were averaged over the last 200 ps of the simulations, optimised and examined. Both receptor structures proved to be stereochemically satisfactory, with 316 residues (99.3%) in sterically allowed regions of the Ramachandran plot. Despite the energy minimisation, an EC-II and an IC-III loop residue were in sterically disallowed regions for the AD complex; while additionally for the ND complex, Val-282 of TM-VI was in a sterically disallowed region.

Different HB interactions were observed for the two catecholamine agonists as they induced different structural variations to the binding site. The final adrenaline binding mode (Figure 4a) had both, the *beta* hydroxyl (d[H...O] = 1.61 Å) and the protonated nitrogen (d[H...O] = 1.78 Å) orientated towards the carboxylate group of

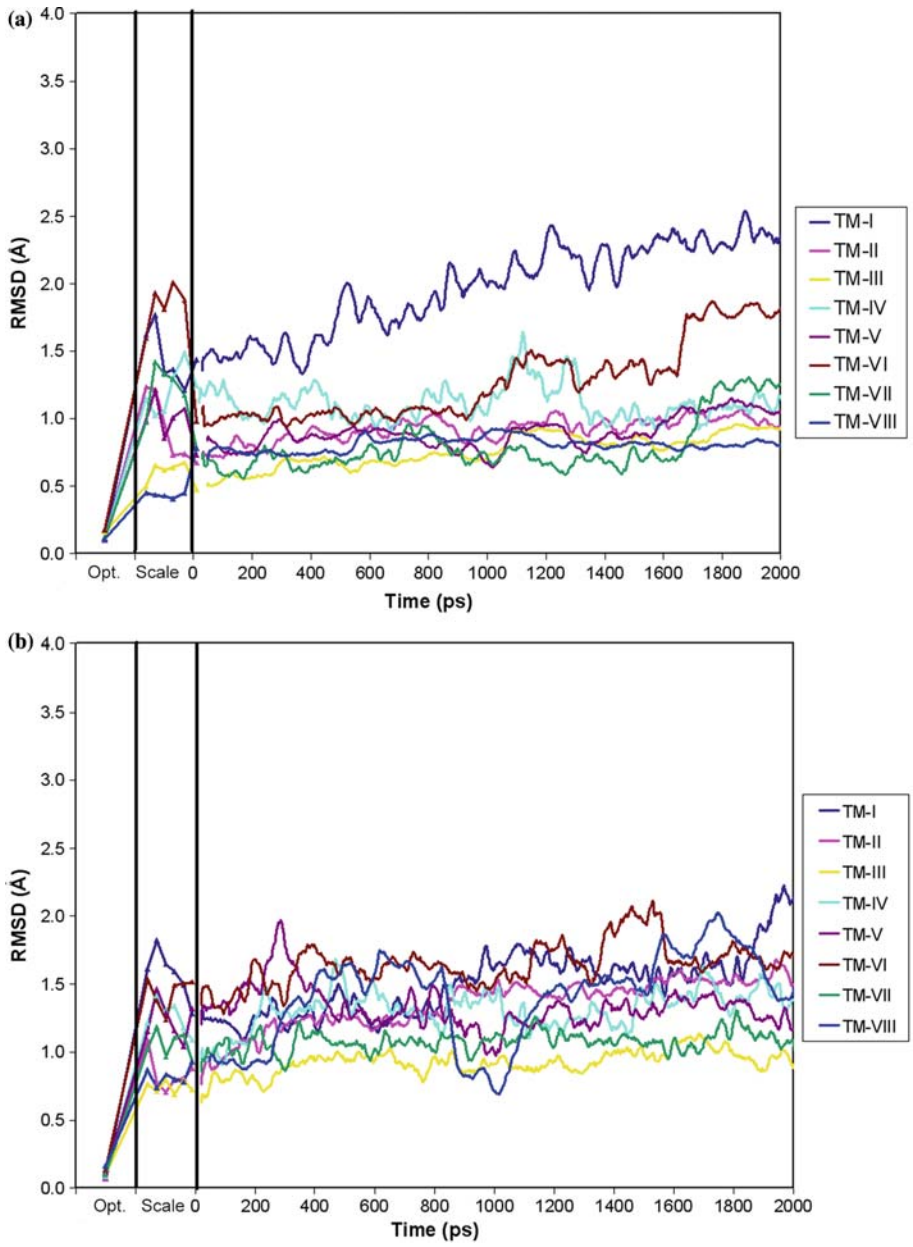

Figure 3. RMSD values of the (a) adrenaline/ligand-induced α1A-AR complex and the (b) noradrenaline/ligand-induced α1A-AR complex simulations; Optimisation and scaling steps (85 ps) followed by moving average trendline (period of 50 ps) over the production run (2 ns).

Asp-106, while the *N*-methyl group is close to the Thr-174 (d[H...O] = 2.62 Å) hydroxyl group. Two Ser interactions were also formed in the final bound mode where the *meta* hydroxyl interacts with the Ser-188 (d[H...O] = 1.80 Å) hydroxyl group and the *para* hydroxyl is d[H...O] = 1.94 Å from the Ser-192 hydroxyl group. For the noradrenaline complex (see Figure 4b) the main interaction formed was between the protonated nitrogen and a carboxylate oxygen of Asp-106 (d[H...O] = 1.80 Å). It appears that Noradrenaline has twisted in the active site allowing the *para* hydroxyl to be orientated away from the Ser residues in the final binding mode.

Furthermore, in the simulations there are three aromatic residues (Trp-285, Phe-288 and Phe-289)

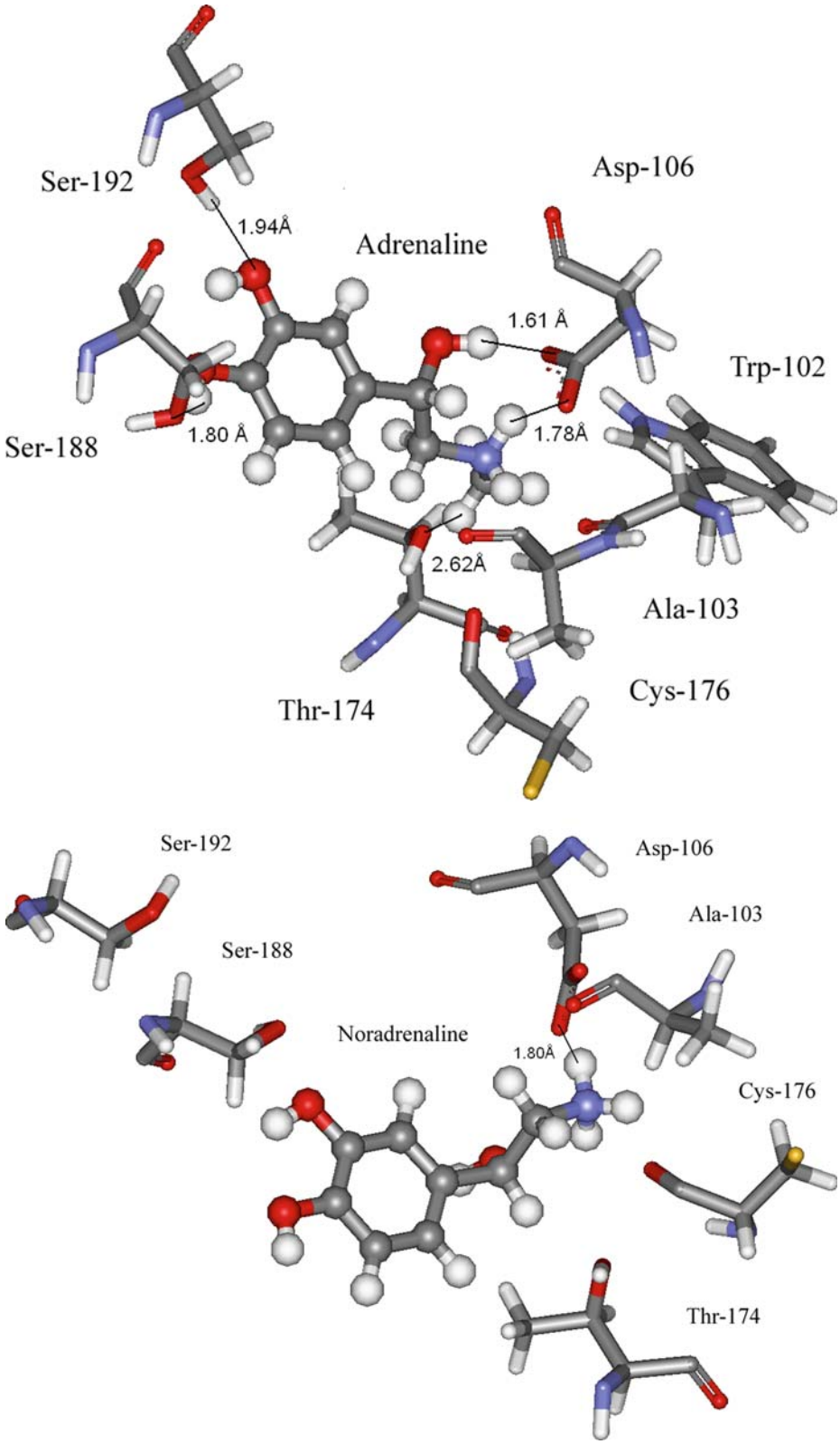

Figure 4. Final binding mode of Adrenaline with Trp-102, Asp-106, Thr-175, Ser-188 and Ser-192 (upper) and Noradrenaline with Asp-106, Thr-175, Gln-177, Ser-188 and Ser-192 (lower).

in TM-VI clustered around the binding site. Interactions of agonists with residues in this aromatic cluster are hypothesised to further induce or stabilize an altered configuration of the side chains within this cluster that may promote receptor activation, consistent with the findings of Visiers et al. [27].

Due to the interesting differences in the poses of the adrenaline and noradrenaline ligands in the binding site, two further MD simulations were performed. These involved mutating the initial docked adrenaline to noradrenaline so that both simulations would commence with the ligands in the same starting mode. Simulations of 2 ns were performed on the mutated complexes and the ligands again exhibited different final orientations in the binding pocket similar to that above. This highlights that the differences noted for these two structurally similar ligands is not due to inadequencies in the initial docking but due to their interaction with binding site residues.

Comparison between inactive and ligand induced α_{1A}-AR models

So far, comparisions have been made with respect to our homology model with which we obtained the initial binding modes through docking. This has allowed us to examine the degree of rearrangement during the dynamics and hence determine when structural change has occurred. However, determination of the differences between the inactive and our ligand induced activated forms of the receptor requires comparisions with an inactive form which has been equilibrated under the same conditions [4]. In this study, two components of receptor activation deduced from prior experimental and computational studies of other GPCRs were investigated for the α_{1A}-AR. These were (1) conformational changes in the 'agonist-bound' form of the receptor that are different from the inactive form and (2) changes in the interactions of the conserved DRY and NPxxY motifs.

Conformational changes involving TM-III, TM-V and TM-VI

To quantify the overall structural effect caused by the agonists the final RMSDs were examined. The Cα RMSDs are similar at 5.68 Å for the AD complex and at 5.25 Å for the ND complex (Table 1). In terms of the helical regions, TM-I has the highest RMSD difference for the adrenaline complex to the uncomplexed at 4.53 Å, while for the noradrenaline complex it is TM-VII at 4.20 Å. This indicates that an overall structural change has occurred due to the presence of the agonists.

For the uncomplexed receptor, the binding site has interactions formed between Ser-192 and both Asp-106 and Ser-188, which do not occur for the activated receptors. Hence, the binding site is closed in the inactive receptor form and can not accommodate an agonist, highlighting the need for our protocol to develop an 'agonist-induced' receptor structure. We observe upon agonist binding a repositioning of the hydroxyl side chains of the Ser residues of TM-V with Ser-188 moving slightly towards the binding pocket, while Ser-192 has a slight movement away from the pocket (see Figure 5).

Furthermore, Figure 5 indicates a slight movement of TM-V away from TM-III on the intracellular side, which was monitored by measuring the distances between the alpha carbons of internal facing residues on each turn of these helices. The intracellular opening between TM-III and TM-V is most marked for the helical turn on the intracellular side of Pro-196 in TM-V. For the uncomplexed receptor the reference separation distance between the Cα of Ile-199 in TM-V and of Leu-117 in TM-III is 6.96 Å, while in the complexes it is notably larger at 11.42 Å for the AD complex and 10.55 Å for the ND complex.

In rhodopsin and in the inactive α_{1A}-AR homology model, TM-VI exists in a kinked configuration, with its cytoplasmic end near to the cytoplasmic end of TM-III [28]. In our complexed receptor, flexibility about a Pro residue (Pro-287) in

Table 1. Comparison of uncomplexed receptor structure and final agonist complexed receptor structures via RMSD (in Å).

	AD complex	ND complex
TM-I	4.42	3.28
TM-II	2.79	2.93
TM-III	2.64	2.58
TM-IV	2.55	2.76
TM-V	2.19	2.55
TM-VI	4.09	3.70
TM-VII	3.97	3.99
TM-VIII	0.86	0.89
Cα (all)	5.68	5.25

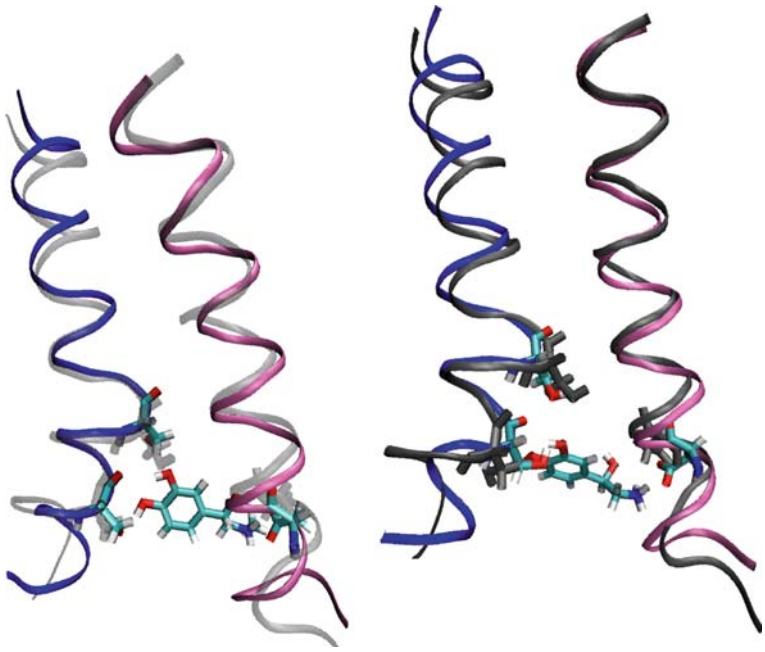

Figure 5. The original uncomplexed α1A-AR (in grey), with TM-III (in mauve) and TM-V (in blue) for the final adrenaline complex (on left) and final noradrenaline complex (on right).

TM-VI again results in a movement of the cytoplasmic end of TM-VI away from TM-III although different motions are observed for the two agonist complexes. We observe a marked lengthening of the distance separating Leu-117 in TM-III from Val-278 in TM-VI from 9.48 Å in the uncomplexed receptor to 12.84 Å for the ND complex, while the AD complex remains at 10.02 Å. Conversely, for the distance separating Ile-120 in TM-III from Gly-275 in TM-VI, the uncomplexed distance is 13.24 Å while the AD complex distance is longer at 15.19 Å and the ND complex distance is similar at 13.45 Å.

The observed changes in the intracellular end of TM-V and TM-VI are consistent with conformational changes in activation that have been detected by computational [29–31] and experimental studies [32–36]. It is generally understood that IC-III plays the greatest role in receptor activation [37, 38] and a change in TM-V and TM-VI would naturally cause a change in the IC loop connecting them.

Interactions of the conserved motifs DRY and NPxxY in the agonist induced conformation

In the uncomplexed α_{1A}-AR structure, Asp-123 of the DRY motif donates to both neighbouring Arg-124 and Tyr-125. Arg-124, a residue entirely conserved in all Rhodopsin-like GPCRs, also interacts with residues in TM-I, TM-III and IC-III. However, for the agonist complexed receptors, novel interactions are formed with Asp-123 donating to Ile-119 and in the noradrenaline induced structure, interactions between Arg-124 and TM-I and IC-III are lost, with interactions only occurring with other TM-III residues. We, therefore, hypothesise that agonists promote conformational changes, which release interactions of Arg-124 of the DRY motif, which is then free to reposition and possibly facilitate G protein binding.

In the uncomplexed form, Asn-322 of the NPxxY (Asn-322, Pro-323, Ile-324, Ile-325, Tyr-326) motif, thought to be important in receptor activation [39, 40], forms various interactions with Asp-72 of TM-II, Ser-113 of TM-III and Ser-319 and Cys-320 of TM-VII. We again observe changes in these interactions upon agonist complexation. For the AD complex, Asn-322 only interacts with its neighbouring Ile-321, while the ND complex loses interactions with TM-III and gains interactions with TM-VI. For the ND complex, Asn-322 interacts with Asp-72 of TM-II, Val-277 of TM-VI and Asn-318 of TM-VII. Hence, in our analysis, a Ser-113/Asn-322

interaction is formed in the uncomplexed receptor and broken in the complexed forms, possibly freeing Asn-322 for other interactions such as with other TM-VII residues. This is consistent with the postulate of Bruysters et al. [41], which suggests that a Ser residue of TM-III acts as a molecular switch in the activation of the Histamine H_1 receptor. Such a movement may change the orientation of residues in IC-II and IC-III that allows for increased affinity of coupling to the G-protein.

Conclusions

Despite a large number of experimental studies on GPCRs, knowledge of how agonist binding to receptors results in G protein activation remains unclear. We have developed a computational procedure, involving docking to generate a number of plausible agonist/α_{1A}-AR complexes and structural refinement through MD simulations using the Amber force field. In this way, ligand induced receptor structures were produced, which were subsequently used for redocking and further MD simulations. Analysis of agonist interactions with the ligand induced receptor structures are in agreement with mutagenesis experiments and suggest novel residues involved in the agonist-binding site, namely Cys-176 and Thr-174. The role of the charged amine group, the aromatic ring, the *N*-methyl group (for adrenaline), the *beta* hydroxyl group and the catechol *meta* and *para* hydroxyl groups have been examined and the most critical interactions for the binding of the agonists is consistent with many earlier reports.

From this work, we have determined a number of structural changes that occur as a consequence of agonist binding. These include a separation of TM-V away from TM-III, and increased flexibility about a Pro kink, which facilitates a movement of TM-VI. Upon examining the conserved residues in α_{1A}-AR, activation may be a consequence of a change in the interactions of Asp-123 of the DRY motif that causes Arg-124 to move out of the TM helical bundle and change the orientation of residues in IC-II and IC-III, allowing for increased affinity of coupling to the G-protein. GPCR models such as ours can be used to generate hypotheses regarding the binding and signaling functions of these receptors for further experimental testing. However, we await the production of an experimental crystal structure of an adrenoceptor in the inactive or active state for a thorough comparison with experiment.

Acknowledgements

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