Two Model System of the α_{1A}-Adrenoceptor Docked with Selected Ligands

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In this study, we have developed a two model system to mimic the active and inactive states of a G-protein coupled receptor specifically the α1A adrenergic receptor. We have docked two agonists, epinephrine (phenylamine type) and oxymetazoline (imidazoline type), as well as two antagonists, prazosin and 5-methylurapidil, into two α1A receptor models, active and inactive. The best docking complexes for both agonists had hydrophilic interactions with D106, while neither antagonist did. Prazosin and oxymetazoline had hydrophobic interactions with F308 and F312. We predict from our study that the active state is stabilized by the interaction of F193 with I114, L197, V278, F281, and V282. The active state is further stabilized by the interaction of F312 with L75, V79, and L80. We also predict that the inactive state of the receptor is stabilized by the interaction of F312 with W102, F288, and M292.

INTRODUCTION

G protein coupled receptors (GPCRs) are a super family of receptors containing more than 1000 known receptors that include rhodopsin, serotonin receptors, dopamine receptors, and adrenergic receptors. To date, only rhodopsin has been crystallized.[1] The binding site for agonists is in the upper one-third to one-half of the receptor that includes the alpha helical and extracellular domains. Antagonists are believed to bind either in this same region or at the extracelluar surface.[2] Due to the lack of structural information for these receptors, they have been the subject of many computational studies dating back to the 1980s.[3−8]

The adrenergic receptors (ARs) respond to the biogenic amines including epinephrine and norepinephrine. The receptors have been classified into three classes, α_1, α_2, and β, with three members in the α_1 subfamily, α_{1A}, α_{1B}, and α_{1D}.[9] The ARs control many physiological events including vasoconstriction, bronchodilation, sedation, and analgesia.[10] Several classes of antagonists have been developed including arylpiperazines,[11] 4-piperidyl oxazoles,[12] and derivatives of known antagonists including BMY7378,[13] WB4101,[14] and niguldipine.[15] The mode of interaction is not clearly understood making these receptors a significant drug target.

Since crystallization of GPCRs is difficult at best, another method of studying the interactions between the receptor and the ligand is useful. Homology modeling affords the opportunity to develop in silico models of difficult to crystallize proteins. We have developed two homology models for the α_{1D} receptor[16] to represent the active and inactive conformations. Two models are needed since the receptor changes conformation when binding with agonists or antagonists. In this study we have developed models of the α_{1A} receptor and docked epinephrine, oxymetazoline, prazosin, and 5-methylurapidil. This study also investigates ligands with multiple nitrogens to determine which is most likely to be protonated. We predict that protonation will increase the affinity of antagonists for the inactive form of the receptor and will increase the affinity of agonists for the active form of the receptor. We can make no prediction of which nitrogen will most likely be protonated since the pK_as of some nitrogens are very similar.

METHODS

Sequences for the adrenergic receptors were downloaded from the National Center for Biotechnology Information (NCBI). Ascension numbers for those sequences are listed in Table 1. The sequences were aligned using the default settings in MOE.[17] Also aligned with the adrenergic receptors were the crystal structure of bovine rhodopsin[1] and the previously published sphingosine 1-phosphate receptor ($S1P_1$).[18,19] The alignment was manually adjusted to eliminate gaps in the transmembrane domains and to align the most conserved residues for each transmembrane domain as elucidated by Ballesteros and Weinstein (Ballesteros 1995). Two homology models were developed for the α_{1A} human sequence as in our previously published work.[16] The active model used the $S1P_1$ model as a template, and the inactive model used the crystal structure of bovine rhodopsin as a template. Each model was minimized to a root-mean-square gradient (RMSG) of 0.1 kcal/mol A to prevent collapse of the pocket within the helical bundle using the AMBER94 force field. Protein reports were calculated for both models to verify the integrity of the models versus biochemical parameters. Outliers to these norms were in loop regions that were not used in any conclusion in this research and were therefore ignored. Protein reports are provided in the Supporting Information.

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Table 1. Accession Numbers for Adrenergic Receptors Aligned[a]

sequence	accession number
alpha$_{1A}$	NP_150646
rat alpha$_{1A*}$	NP_058887
alpha$_{1B}$	NP_000670
hamster alpha$_{1B*}$	P18841
alpha$_{1D}$	P25100
alpha$_{2A}$	NP_000672
alpha$_{2B}$	NP_000673
alpha$_{2C}$	P18825
beta$_1$	P08588
beta$_2$	P07550
beta$_3$	P13945
bovine rhodopsin	1F88*

[a] Downloaded from the National Center for Biotechnology Information (NCBI).[30] All species are human unless noted with an *.

DOCKING LIGANDS

Ligands selected for this study were epinephrine, oxymetazoline, prazosin, and 5-methylurapidil. Each structure was minimized in the Molecular Operating Environment (MOE) using the Merck Molecular force field[20] to a root mean squared gradient of 0.01 kcal/mol Å. A neutral and protonated form of epinephrine and 5-methylurapidil were developed and minimized. Oxymetazoline has 2 nitrogens and prazosin has 5 nitrogens. A series of protonation states was developed and docked to determine which compound was responsible for activity for both antagonists. See Figure 1 and Table 2, Supporting Information.

DOCKING PARAMETERS

All ligands were docked into both models using MMFF94 force field. Each docking run was analyzed as in our previously published work.[16] The database was sorted based on the scoring functions, and the top 10 were analyzed. Each complex was visually inspected to determine if the ligand was placed within the transmembrane domain and in the upper 1/3 of the receptor. Any positions that met with these criteria were further evaluated based on hydrophobic and hydrophilic interactions. The best complexes were minimized to a root mean squared gradient (RMSG) of 0.1 kcal/mol Å.

RESULTS

The active model was based on the experimentally validated $S1P_1$ model.[16,19] The resulting model was minimized using AMBER94 force field.[21] Biochemical parameters for bond angles, bond lengths, and dihedral angles were calculated, as in the Supporting Information. The length of each helix was determined. The helices ranged from 20 to 30 residues. Helices 5 and 7 are the two shortest helices and were significantly shorter than the corresponding helices in the inactive model. Refer to the Supporting Information. However, the active model's helix begins within 2 residues of the inactive model's helix and terminates significantly earlier. This difference may be due to a deficiency in the active model or a difference in the two states of the receptor. The region that is not alpha helical is not used in any findings for this study since both agonists and antagonists bind either in the upper 1/3 or in the extracellular loop regions.

The template for the inactive model was the crystal structure of bovine rhodopsin.[1] The resulting model was minimized using the AMBER94 force field to an RMSG of 0.1 kcal/mol Å. Biochemical parameters for bond angles, dihedrals, and bond distances were calculated, as in the Supporting Information. The helix lengths varied from 27 to 33 residues and are reported in the Supporting Information. In all cases, the inactive model has longer helices than the active model with the exception of helix 1.

The models presented in this study are very similar to the previously published α_{1D} models. Unlike the α_{1D} models, helix seven in both α_{1A} models have intact alpha helical structures for helix seven due to having only one proline.[16] The inactive α_{1A} model does have a region of α helix that is perpendicular to the other helices and is often referred to as helix 8. The active model does not have this feature. The absence or presence of this helix is identical in both the α_{1A} and α_{1D} models. The active and inactive models of the α_{1A} AR can be seen in the Supporting Information.

DOCKING RESULTS

The 16 compounds of prazosin were docked into each receptor. The top 5 scoring results are reported for the protonated state. Two complexes had a +2 charge and three complexes had a +1 charge. The other antagonist, 5-methylurapidil, had only one nitrogen protonated which resulted in only two ligand types. Oxymetazoline has two nitrogens which resulted in four ligands. Epinephrine has 1 nitrogen which resulted in 2 ligand forms for docking. This study will discuss 6 prazosin compounds, 4 oxymetazoline compounds, and 2 each for epinephrine and 5-methylurapidil (see Table 2 and Figure 1).

Protonated epinephrine had two side-chain hydrophilic interactions with D106. The complex also had four hydrophobic interactions with V107, W165, V185, and W285. This complex had the ligand interacting with helices 3, 5, 6, and 7 of the receptor. The ligand was within the helical bundle and centralized between the helices. The neutral epinephrine also had two hydrophilic interactions with S83 and K309. The interaction with K309 involved side-chain atoms. There was only one hydrophobic interaction with I27. The ligand was positioned at the top of helix 1 toward the extracellular space. The protonated epinephrine ligand was deemed superior to the neutral form, Figure 2 and Table 2. See the Supporting Information for additional epinephrine data.

The docking result selected as best overall for oxymetazoline was protonated at nitrogen 1 (N1) only. The other results are presented in the Supporting Information. Oxymetazoline +1 N1 had three hydrophilic interactions with D106 (2X side-chain atoms) and F308 (backbone atoms). This complex also had hydrophobic interactions with V107, L186, F281, W285, F308, V311, and F312. The ligand was positioned in the center of the helical bundle and interacted with helices 3, 5, 6, and 7. See Table 2 and Figure 3.

The best complexes were analyzed for prazosin with the inactive model. Since 16 complexes would be too much to present in this paper, we have limited our results to the top scoring complexes for six complexes including the neutral form. This analysis included two complexes with a +2 charge and three complexes with a +1 charge.

Prazosin +2 N1, nitrogen 3 (N3) had six hydrophilic interactions and was selected as best overall. The remaining

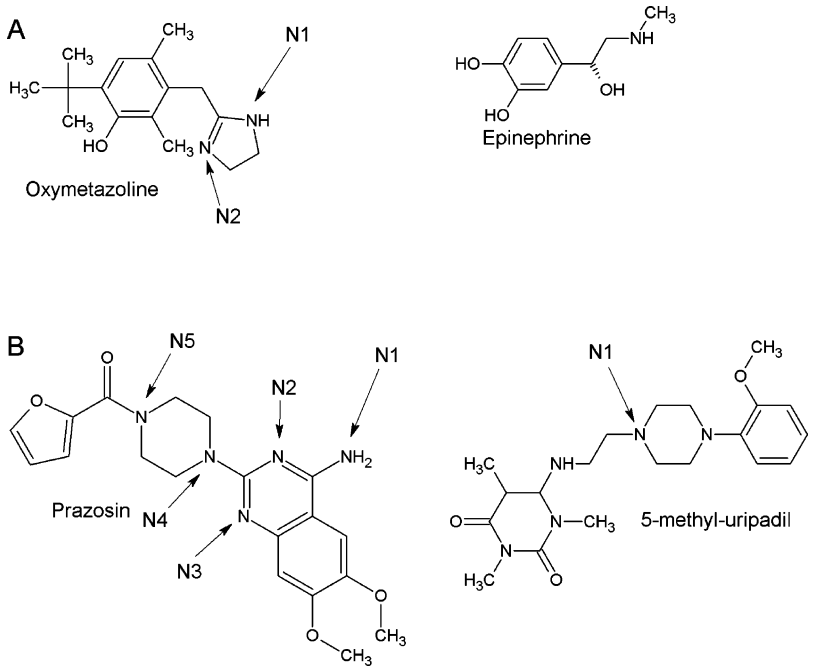

Figure 1. Ligands used for docking. Panel A, agonists oxymetazoline (left) and epinephrine (right). Two forms of epinephrine were docked; one neutral as shown and one with the nitrogen protonated and an overall +1 charge. Oxymetazoline was protonated at N1, N2 or both and neutral for four ligands for docking. Panel B, antagonists prazosin (left) and 5-methylurapidil. Two forms of 5-methyluripadil were docked, neutral, as shown, and protonated at the indicated nitrogen atom. Prazosin was docked in 16 different combinations of N1−N4 being protonated and the neutral form (total charge from 0 to +4).

Table 2. Overall Best Complexes for Active and Inactive Models[a]

ligand	pK_i	hydrophilic interactions	hydrophobic interactions
		Active Model	
agonist[31]			
epinephrine protonated	6.05 ± 0.14	D106 (2X) sc	V107, W165, V185, W285
oxymetazoline +1 (N1)	7.80	D106 (2X) sc, F308 bb	V107, L186, F281, W285, F308, V311, F312
		Inactive Model	
antagonist[7]			
prazosin +2 (N1, N3)	9.15	R166 sc, P170 bb, E173 bb, E180 (2X) sc, E181 sc	V107, F187, F288, M292, F308, F312
5-methylurapidil protonated	8.69 ± 0.02	E180 (2X) sc, D172 sc	V2, I175

[a] Sc indicates side-chain interaction. bb indicates backbone interaction. (2X) indicates two interactions with the same residue. The complete results are listed in the Supporting Information.

complexes are presented in the Supporting Information. The residues involved in these interactions were R166, P187, E173, E180 had 2 interactions (2X), and E181. Two residues involved used side chains, P170 and E173, and the remaining used backbone interactions. Hydrophobic interactions involved V107, F187, F288, M292, F308, and F312. The placement of this ligand was interesting. Half of the ligand was in the helical bundle, and half was extending into the extracellular space. Helices 3, 5, 6, and 7 were involved. See Table 2 and Figure 4.

Only two forms of 5-methylurapidil were docked, neutral and protonated at N1. The protonated form had three hydrophilic interactions with residues Glu180 2X and D172. These interactions all involved side-chain atoms. Hydrophobic interactions involved V2 and I175. As with the prazosin complexes, this position was central to helices 3, 5, 6, and 7 with one end extending into the extracellular space. See Table 2 and Figure 5. Refer to the Supporting Information for additional 5-methylurapidil data.

Antagonists did not dock well with the active model. The interactions were decreased over the agonist complexes. The results can be reviewed in the Supporting Information.

DISCUSSION

In this study we have expanded our previous work to the α_{1A} receptor using the same methods. We have developed a two model system to describe the α_{1A} AR. The active model was based on our previously experimentally validated $S1P_1$ receptor.[16,19] The inactive form is based on the crystal structure of bovine rhodopsin [PDB ID 1F88].[1] This study is unique in that we have also explored protonation of compounds with multiple nitrogens and the interactions that stabilize the active and inactive conformations.

The α_{1A} model is very similar to the α_{1D} model when comparing our data or the experimental literature. Both epinephrine and oxymetazoline (both protonated) have hydrophilic interactions with D106. These data are in agreement with Strader (1987).[22] In addition, F308 and F312 interact

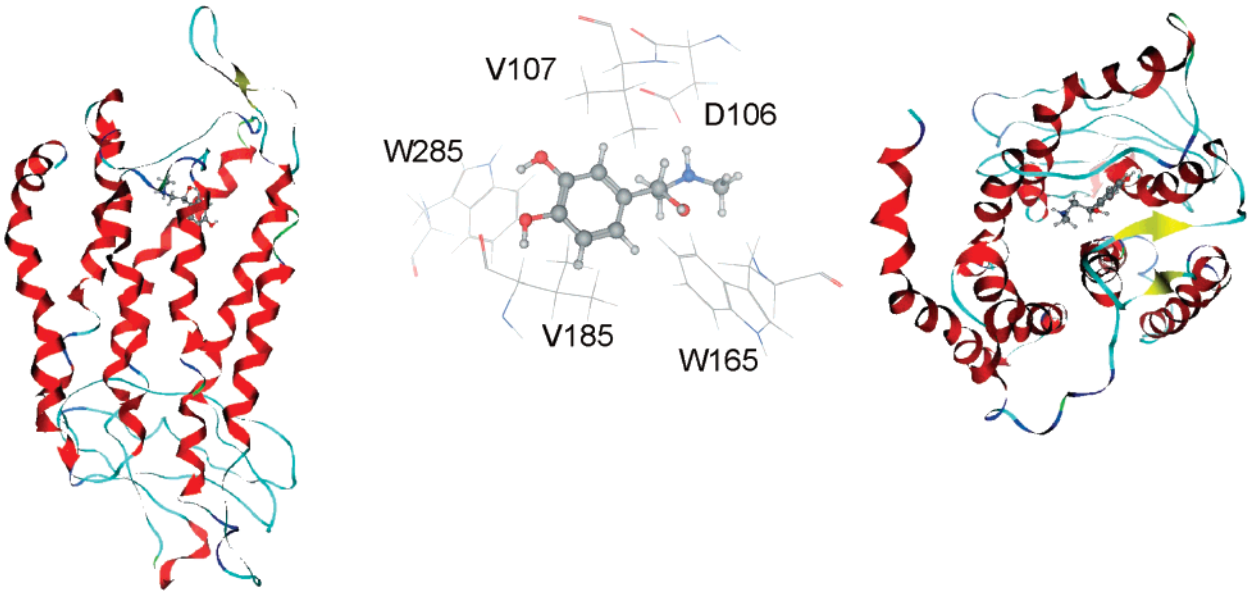

Figure 2. Active model with protonated epinephrine. Left shows protonated epinephrine in the center of the helical bundle. Center shows the specific interactions with the ligand and receptor. Right shows the ligand placement with the helical bundle from extracellular space.

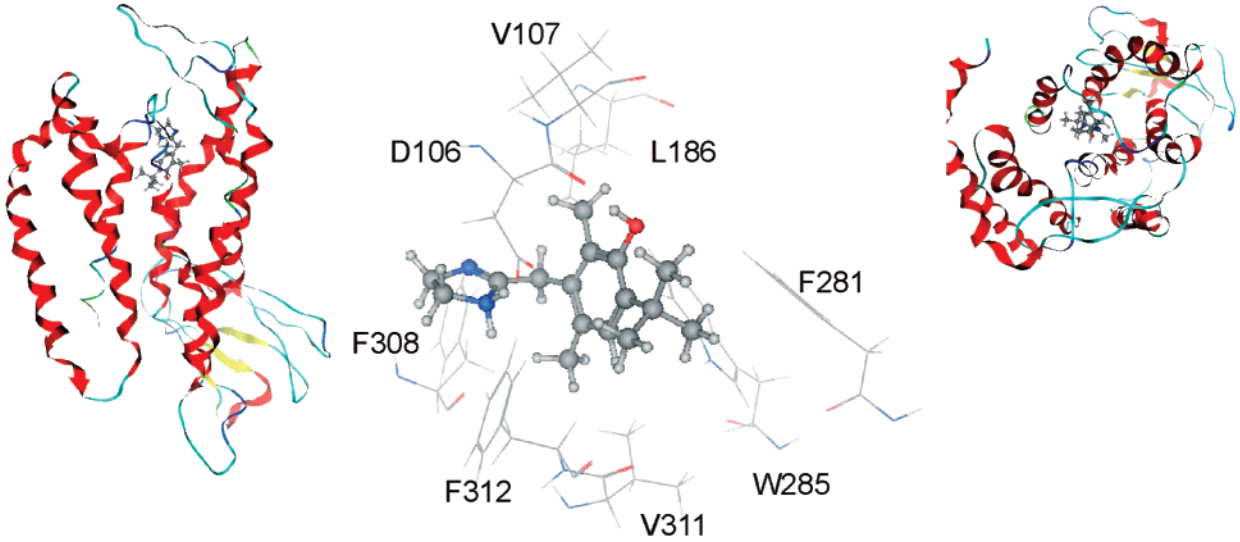

Figure 3. Active model with oxymetazoline protonated. Left shows the ligand in the center of the upper 1/3 of the alpha helices. Center shows the specific interactions between the ligand and receptor. Right shows the ligand placement in the center of the helical bundle from extracellular space.

with the aromatic moiety of both prazosin (+2), an antagonist, and with oxymetazoline, an agonist.[23] Waugh's work supports this finding for oxymetazoline. As in our previous work, neither S188 or S192 were found in agonist or antagonist binding.[22,24]

Pedretti recently published a homology model of the α_{1A} receptor.[25] This work included docking analysis with epinephrine. The model developed was based on the crystal structure of bovine rhodopsin as was our inactive model. However, based on the study of Bissantz, it is not expected that an agonist would interact well with a model based on that template.[5] It is interesting to note that several interactions found by these investigators were found in our active model. Both their study and our own found interactions of the agonist epinephrine with D106. Their work found interactions with epinephrine and S158, S188, S192, Q177, and W285. Our model had interactions with only W285 of the former residues. When an antagonist was used, WB4101, Pedretti et al. found interactions with D106, C110, Q177, F193, F312, and W316. Finding these residues may have been over selected for based on the manual docking method used in that study. The agonist and antagonist binding interactions from Pedretti's study identified one overlapping binding site since both the agonist and antagonist interacted with D106 and Q177. Our results indicate that the agonist and antagonist are binding in unique regions of the receptor since none of the amino acids identified in the agonist binding were also found in the antagonist binding.

Greasely et al. mutated several residues in the hamster α_{1B} receptor creating a constitutively active receptor.[26] They predicted that these residues were involved in stabilizing the interaction of helix 6 with helix 3 in the inactive state. These residues were A292 (270), L296 (274), V299 (277), V300 (278), and F303 (281). In our study, L274, V277, V278, and F281 interacted with residues in helix 3 (I114, L117, and I120). These residues also interacted with helices 4 (I199)

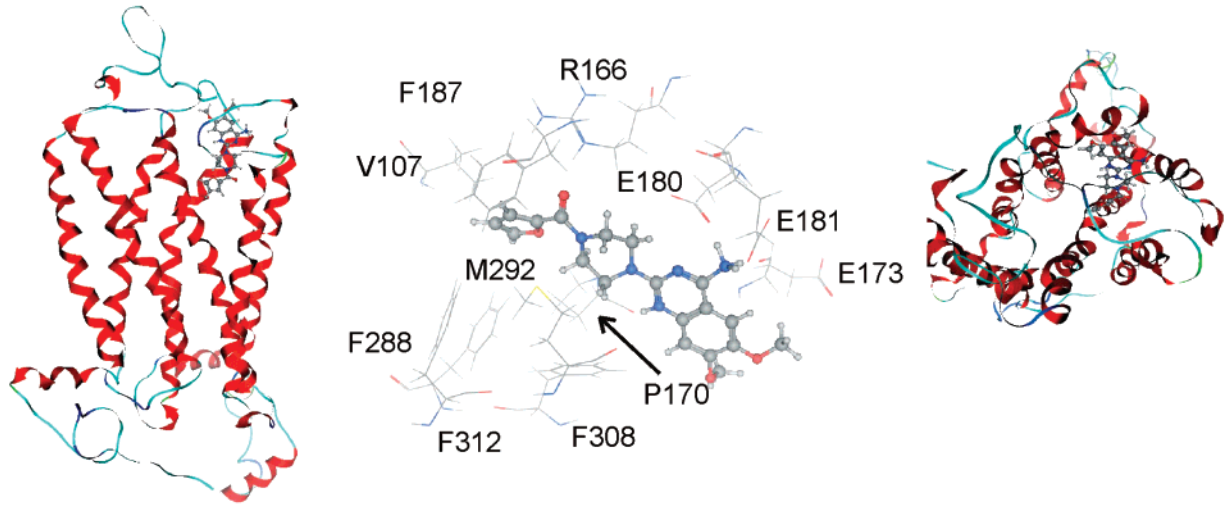

Figure 4. Inactive model with prazosin +2 protonated. Left shows the ligand in the upper portion of the alpha helices extending slightly into the extracellular loops. Center shows the specific interactions between the ligand and receptor. Right shows the ligand placement in the center of the helical bundle from extracellular space.

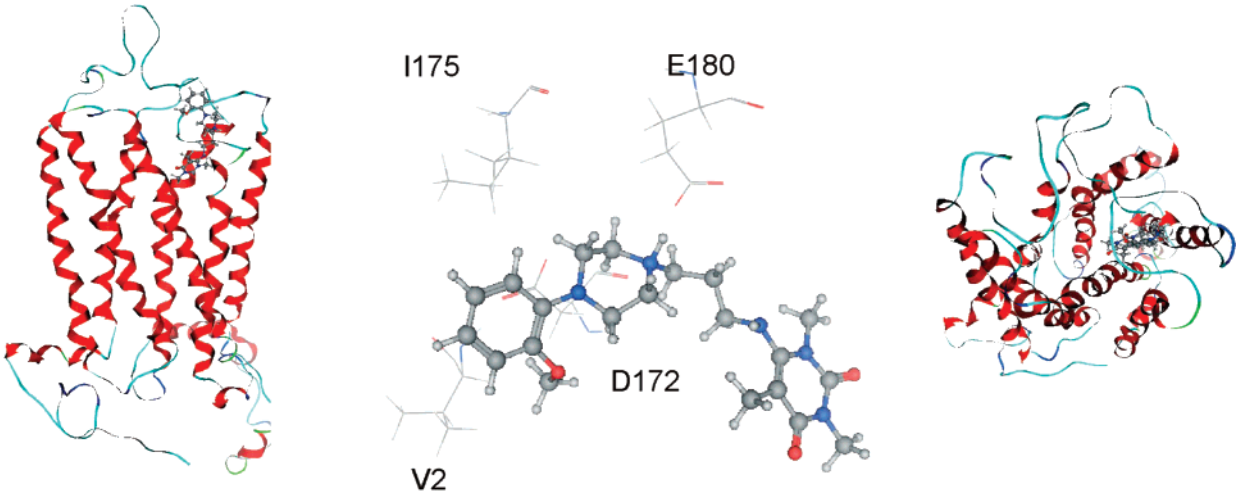

Figure 5. The inactive model with 5-methylurapidil protonated. Left shows the ligand near helices 3, 5, and 6 in the upper portion of the alpha helices extending slightly into the extracellular loops. Center shows the specific interactions between the ligand and receptor. Right shows the ligand placement in the helical bundle from extracellular space.

and 7 (I325). In fact, oxymetazoline interacts with F281 in the complex with the active model. When comparing the active and inactive model contacts for these five residues, we find that A270 is not involved in any contacts. We propose that this residue is too small to be detected with the current algorithm. The active model had increased interactions over the inactive model with contacts with F193, L197, I200, N318, and I312 involving helices 5 and 7. It is interesting to note that no residues from helix 5 were found in the inactive model. We propose that the activation of the α_{1A} requires the breaking of the interaction of V278 with I199 and V277 with I328. We further propose that the formation of interactions between V277 with N318 and I321 and V278 with F193, L197, and I200 are also required for activation of the α_{1A} receptor. Interactions with I114, L117, I120, and W285 were maintained in both models and may be needed for correct helical packing.

In 2004, Kinsella, Rozas, and Watson published a homology model of the human α_{1A} AR.[27] In that study, they suggested that nine residues, F193, Y194, W285, F288, F289, F308, F312, W313, and Y316, formed a hydrophobic network that stabilized the helical packing of the receptor. The ARs differ from most other GPCRs because there is a large intracellular loop between helices 5 and 6. Careful attention must be used to align the most conserved residue in helix 6 to account for this variation.[28] The alignment in that work has the most conserved residue misaligned by 4 residues, but even that small difference will move the entire helix by one complete turn. By examining the indicated residues, it was apparent that the interactions vary significantly for F193 and F312 in our two model system. In the active model, F193 had interactions with I114, L197, V278, F281, and V282. In the inactive model F193 had interactions with only L197. F312 had interactions in the active model with L75, V79, and L80. In the inactive model, F312 had interactions with W102, F288, and M292 as shown in Figure 6. Clearly these two residues, F193 and F312, had very different interactions in the active versus inactive models. The making and breaking of the various interactions may be responsible for activation of the receptor.

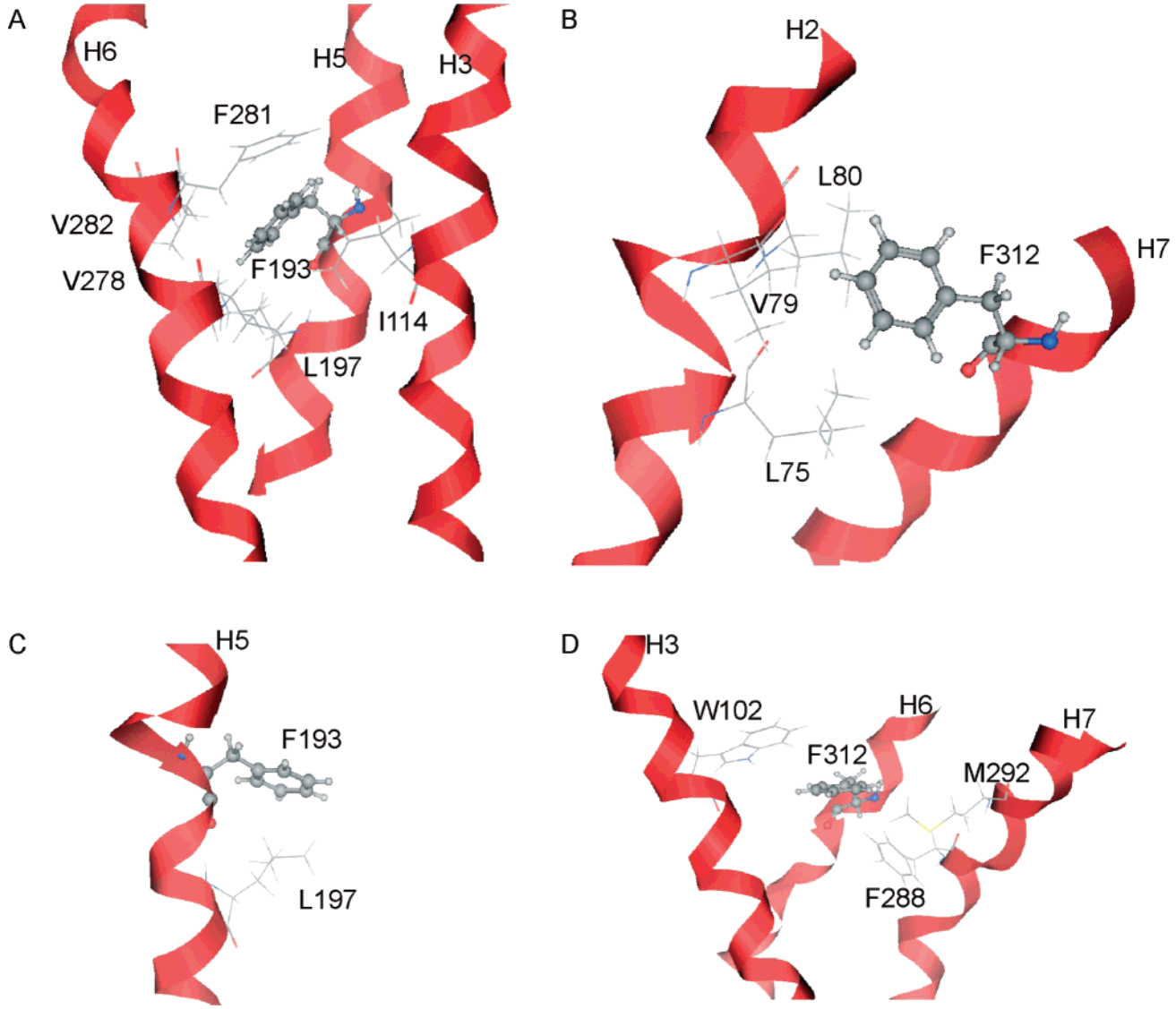

Figure 6. Key interactions stabilizing active and inactive models. Panel A: active model with only helices 3, 5, and 6 (H3, H5, and H6, respectively) shown. F193 interacts with F281, V282, and V278 of helix 6. L197 (helix 6) and I114 (helix 3) also interact with F193. Panel B: active model with interactions for F312 shown. Helices 2 and 7 are shown with interactions of F312 with L80, V79, and L75 (all in helix 2). Panel C: inactive model showing the interaction between F193 and L197 both of helix 5. Panel D: inactive model showing interactions of F312 and W102 (helix 3) and F288 and M292 (both in helix 7).

Hwa and workers mutated several residues in α_{1B} to the corresponding residues in α_{1A}.[29] They were able to increase affinity of the α_{1B} receptor from a pK_i of 6.05−6.90 by mutation of A204V. The pK_i for α_{1A} is 7.34. Clearly this did not reverse binding affinity, but it did impact greatly the affinity for oxymetazoline. The same mutation had less effect on the binding of epinephrine as binding affinity was only modestly increased from 5.29 to 5.50. Our models indicated that V185 interacted with epinephrine and L186 interacted with oxymetazoline. Clearly both models indicated that the agonist was located near V185 or L186 and mutation at that location which changed the size of the side chain could impact affinity for agonists. The antagonist location was not near these two residues for either prazosin or for 5-methylurapidil. The mutation by Hwa and workers of L314M impacted the affinity of 5-methylurapidil only. In our models, 5-methylurapidil did not interact with M292 but prazosin did. It is possible that mutation of L314 impacted the helical packing since M292 was indicated in interactions with F288 and F308 in the active model and with F288, F308, and F312 in the inactive model.

CONCLUSION

We conclude that the models presented here are consistent with mutagenesis data from the literature. The active model had hydrophilic interactions with both agonists and D106 which is supported by the experimental study of Strader.[22] The inactive models have interactions with both antagonists involving residues F312 and F308 as in the study by Waugh.[23] Our active model had interactions with F308 and F312 with oxymetazoline but not with epinephrine. This is consistent with Waugh.[23] We predict from our study that the active state is stabilized by interaction of F193 with I114, L197, V278, F281, and V282. The active state is further stabilized by the interaction of F312 with L75, V79, and L80. We also predict that the inactive state of the receptor is stabilized by the interaction of F312 with W102, F288, and M292.

ACKNOWLEDGMENT

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Supporting Information Available: Alignment, protein reports for both models, table of all ligands docked into the two models, figure of helix 7, helical domains, models of all complexes not shown in the paper, and table of all model and ligand interactions. This material is available free of charge via the Internet at http://pubs.acs.org.

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