Modeling of Human Thromboxane A_2 Receptor and Analysis of the Receptor–Ligand Interaction

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In order to elucidate the mode of the thromboxane A_2 (TXA_2) receptor–ligand interaction at the molecular level, a model for the human TXA_2 receptor, a member of the G protein-coupled receptor family with seven transmembrane segments, was constructed on the basis of its amino acid sequence, which was determined recently (Hirata, M.; et al. *Nature* **1991**, *349*, 617–620). First, we made a model for the human β_2-adrenergic receptor using its amino acid sequence and the known helix arrangement of bacteriorhodopsin. Then, a TXA_2 receptor model was constructed based on the β_2 receptor model and was used to analyze the receptor–ligand interaction. The ligand-binding pocket of the TXA_2 receptor includes a serine residue from segment V, an arginine residue from segment VII, and a large hydrophobic pocket between these two residues. These results are consistent with the known properties of TXA_2 and TXA_2 antagonists having a hydrogen-bonding group such as hydroxyl, a carboxyl group, and a hydrophobic moiety. This model should be helpful for rational design of potent TXA_2 antagonists.

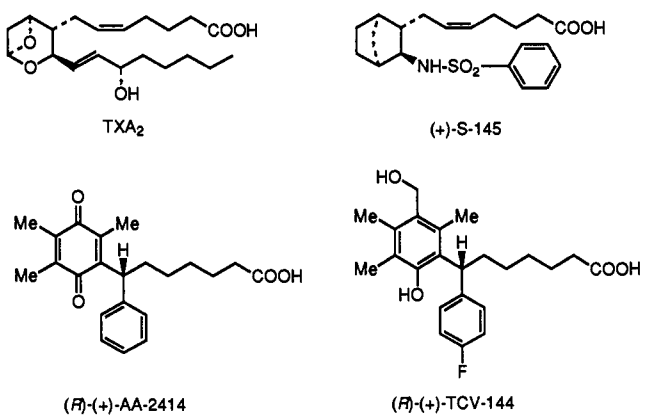
Introduction

Thromboxane A_2 (TXA_2, Chart I) is a potent stimulator of platelet aggregation and a constrictor of vascular and respiratory smooth muscles.[1] Its function is counterbalanced with that of prostacyclin, which inhibits platelet aggregation and elicits vasorelaxation. Disruption of that balance in favor of TXA_2 has been suggested to be responsible for diseases such as thrombosis, asthma, and myocardial infarction.[2] Antagonists of the TXA_2 receptor are therefore expected to have therapeutic importance, and some compounds which block the TXA_2 receptor are actually in clinical trials.[3]

Recently, the amino acid sequence of the human TXA_2 receptor has been determined from its cDNA sequence.[4] It belongs to the G protein-coupled receptor (GPCR) family and consists of seven transmembrane segments, presumably adopting an α-helical conformation. As this feature is common to all GPCRs and some residues are conserved in all GPCRs, it is expected that these receptors have similar three-dimensional (3D) architecture. However, no 3D structure has been determined for any of the GPCRs. On the other hand, the 3D structure of bacteriorhodopsin (bR), which also consists of seven transmembrane segments, has been revealed by electron cryo-microscopy,[5] and the arrangement of its helices as well as the position of side chains has been determined. Although there is little sequence homology between bR and visual rhodopsin, a member of GPCR family, both proteins bind a retinal molecule at a lysine residue in segment VII, and both proteins are activated by light with cis–trans isomerization of retinal.[6] Therefore, it seems reasonable to assume that visual rhodopsin and other GPCRs including the TXA_2 receptor have a helix arrangement similar to that of bR. In fact, modeling of GPCRs based on this assumption has been reported from a few groups.[6–9]

These considerations prompted us to study the features of the TXA_2 receptor–ligand interaction at the molecular level on the basis of a 3D model of the receptor. As the first step, we constructed a model for the β_2-adrenergic receptor using the structure of bR as a template, because abundant mutational data, which are helpful for reliable modeling, are available for this receptor.[10] Then, a model for the TXA_2 receptor was constructed from the model for the β_2 receptor, and the mode of the receptor–ligand interaction was analyzed. This model would be useful in developing more potent TXA_2 antagonists.

Chart I



Results and Discussion

1. Modeling of Human β_2-Adrenergic Receptor. A model for the human β_2-adrenergic receptor complexed with isoproterenol, a typical β agonist, was constructed from its amino acid sequence[11] (Figure 1) according to the following assumptions: (1) The transmembrane segments adopt α-helical conformations. (2) The arrangement of the helices is essentially the same as that of bR, which has been determined by electron cryo-microscopy.[5] (3) The residues conserved in almost all GPCRs or in the adrenergic receptor family are directed inward. (4) Charged or polar residues are also directed inward. (5) The model should be consistent with mutational data (see below).

In the present modeling, loop regions connecting individual helices are omitted, because it is difficult to predict

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hB2AR:  --W V V G M G I V M S L I V L A I V F G N V L V I T A I A      59
hTXR:   --R L I A S P W F A A S F C V V G L A S N L L A L S V L A G A  52
bR:     --P E W I W L A L G T A L M G L G T L Y F L V K G M G V S D P  37
                                                    II
                                  ----------------------------
hB2AR:        K F E R L Q T V T N Y F I T S L A C A D L V M G L A V V  87
hTXR:   R Q G G S H T R S S F L T F L C G L V L T D F L G L L V T G  82
bR:                             D A K K F Y A I T T L V P A I A F  54

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hB2AR:  P F G A A H I L   M K M W       T F G N F W C E F W T S I D 113
hTXR:   T I V V S Q H A A L F E W H A V D P G C R L C R F M G V V M 112
bR:     T M Y L S M L L G YGLTMVPFGGE Q N P I Y W A R Y A D W L F T  89
                III
        ----------------------------
hB2AR:  V L C V T A S I E T L C V I A V D R Y F A I T S P F K Y Q S 143
hTXR:   I F F G L S P L L L G A A M A S E R Y L G I T R P F S R P A 142
bR:     T P L L L L D L A L L V D A D Q                          105
                                    IV
                        ----------------------------------------
hB2AR:  L L T K N K A R V I I L M V W I V S G L T S F L P I Q M H W 173
hTXR:   V A S Q R R A W A T V G L V W A A A L A L G L L P L L G V G 172
bR:                     G T I L A L V G A D G I M I G T G L V G A  126

hB2AR:  Y R A T H Q E A I N C Y A N E T C C D            F F T N Q 197
hTXR:   R Y T V Q Y P G                 S W C F L T L G A E S G D V A 195
bR:     L T K V Y S Y                                   R F V W W 138
                                  V
        ----------------------------------------------------
hB2AR:  A Y A I A S S I V S F Y V P L V I M V F V Y S R V F Q E A K 227
hTXR:   F G L L F S M L G G L S V G L S F L L N T V S V A T L C H V 225
bR:     A I S T A A M L Y I L Y V L F F G F T S K A E S M R      164

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hB2AR:  R Q L Q   -- 33aa --  C L K E H K A L K T L G I I M G T F T 283
hTXR:   Y H G Q E A A Q Q R P R D S E V E M M A Q L L G I M V V A S 255
bR:                             P E V A S T F K V L R N V T V 179
                        VI
        ----------------------------
hB2AR:  L C W L P F F I V N I V H V I Q D N L I R K              305
hTXR:   V C W L P L L V F I A Q T V L R N P P A M S P A G Q L S R T 285
bR:     V L W S A Y P V V W L I G S E G A G I V P L N I          203
                                VII
        ----------------------------------------
hB2AR:        E V Y I L L N W I G Y V N S G F N P L I Y C R S P D F-- 332
hTXR:   T E K E L L I Y L R V A T W N Q I L D P W V Y I L F R R A-- 314
bR:     E T L L F M V L D V S A K V G F G L I L L R S R A I F G E-- 232
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Figure 1. Alignment of amino acid sequences of human β_2-adrenergic receptor (hB2AR),[12] TXA_2 receptor (hTXR),[4] and bacteriorhodopsin (bR).[6] Residues conserved in almost all GPCRs including β_2 and TXA_2 receptors are boxed, and those suggested to be important for ligand binding are circled. Roman numerals indicate the transmembrane segments.

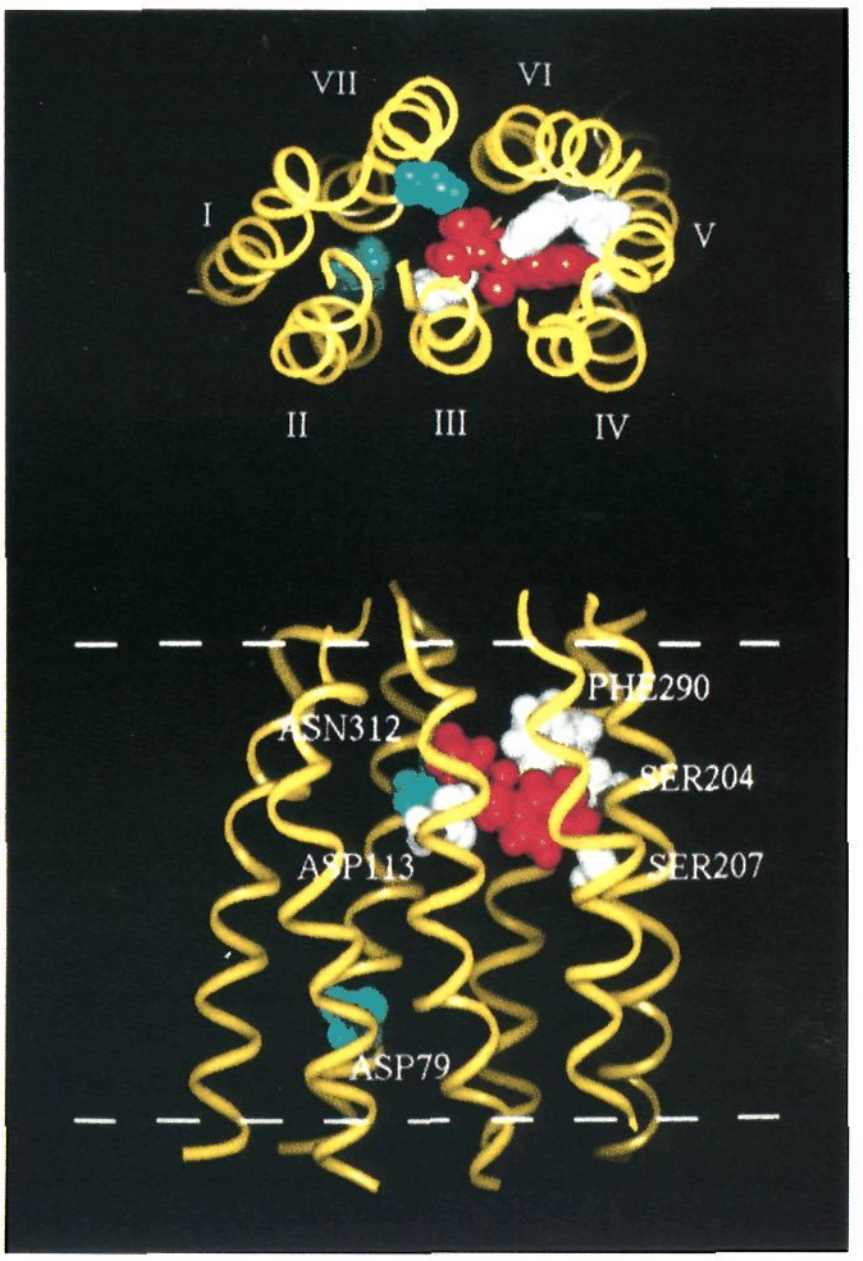

Figure 2. A ribbon model for the human β_2-adrenergic receptor complexed with isoproterenol (red) viewed from the surface of the cell (top) and from the side of the membrane (bottom). Residues suggested to be important for ligand binding by mutational analyses and used in the modeling are shown with white CPK models. In addition, Asp-79 and Asn-312 are shown with blue CPK models. Approximate positions of the membrane boundaries are represented with dashed lines. Cytoplasm is at the bottom of the figure.

the conformation of these regions. Omission of the loop regions, however, is not a big problem in the analysis of the receptor–ligand interaction as the ligand-binding pocket is supposed to be located in the region surrounded by transmembrane segments.[10] The termini of the helices were tentatively determined from a hydropathy plot,[12] but their precise locations were hard to define.

The inward/outward direction of each helix was determined by assumptions 3 and 4. These two assumptions were found to be consistent with each other except for a few residues, because many of the charged or polar residues in the transmembrane segments are conserved in GPCRs or in the adrenergic receptor family. In addition, many of these residues were on the same side of the helix. The relative positions of segments III, V, and VI along the normal of the membrane plane were determined to be consistent with the mutational data[10] summarized as follows: (1) Asp-113 in segment III interacts with the amino group of isoproterenol. (2) Ser-204 and Ser-207 in segment V form hydrogen bonds with *m*- and *p*-hydroxyls of the catechol group, respectively. (3) Phe-290 in segment VI interacts with the benzene ring of isoproterenol.

The conformation of isoproterenol bound to the receptor was assumed to be identical to that of a rigid analog.[13] Although other residues were suggested to be important for the receptor function, they were not included in the modeling, because their roles are not unambiguously determined. Asp-79 was also implicated in agonist binding, but not in antagonist binding.[14] This residue, however, is conserved in all GPCRs irrespective of the electrostatic property of the ligand. Therefore, rather than directly interacting with the ligand, its role is probably an indirect one. It might for example be necessary for agonist-induced conformational change of the receptor. The relative position of segment VII was determined from the location of retinal-bound Lys in visual rhodopsin, which is conserved in bR and visual rhodopsin.[6] In segments I, II, III, and VII, some polar residues such as Asn-51 and Asp-79 are well conserved near the cytoplasmic region. We assumed that these residues form a hydrogen bonding network; this made it possible to locate these segments. The relative position of segment IV, which was more ambiguous compared with the others, was determined in such a way that the packing interaction with adjacent helices was favorable.

Based on the above considerations, an initial structure for the β_2-adrenergic receptor complexed with isoproterenol was constructed and subjected to energy minimization. The resultant model is shown in Figure 2. The helices are not completely regular, but are distorted at proline and glycine residues. The mode of receptor–isoproterenol interaction is compatible with the mutational data mentioned above. It should also be pointed out that the ligand is located in the transmembrane region. This is consistent with physicochemical data concerning its location.[15] Recently, Suryanarayana et al.[16] have indicated that Asn-312 in segment VII, which is conserved as Phe in

α-adrenergic receptors, is the key residue for the distinction of α- and β-specific ligands. One notable difference between α- and β-specific ligands resides in the size of the substituent on the amino group (e.g. isopropyl for β-specific isoproterenol and hydrogen for α-specific norepinephrine). In our model, Asn-312 is located near the isopropyl group of isoproterenol (Figure 2). If it is replaced with Phe as in α receptors, steric hindrance is caused between the isopropyl group of isoproterenol and the side chain of this Phe (data not shown). Our model is therefore in agreement with this mutational data,[16] although it was not included in the modeling. This suggests that the model has well reproduced the essential features of the ligand-binding pocket of the receptor.

2. Modeling of Human TXA_2 Receptor. As the model for the β_2-adrenergic receptor was found to be reliable at least qualitatively, we then tried to construct a model for the human TXA_2 receptor using the model for the β_2 receptor as the template. The modeling procedure is as follows. (1) Amino acid sequences of the two receptors were aligned (Figure 1). No gap was introduced into the transmembrane segments. (2) Side chains of the β_2 receptor model were replaced with corresponding residues of the TXA_2 receptor without changing the main chain conformation, and the resultant model was energy-minimized.

A conspicuous feature of the ligand-binding pocket of the model (Figure 3) is the existence of Ser-201 in segment V, Arg-295 in segment VII, and a large hydrophobic pocket (shown in yellow) between these two residues. The location of Ser-201 is nearly identical to that of Ser-204 of the β_2-adrenergic receptor, a residue which is involved in ligand binding. Comparing this feature with the structures of TXA_2 and known antagonists, it is supposed that the ligand carboxyl group interacts with Arg 295 as suggested by Hirata et al.,[4] the ligand polar group such as hydroxyl with Ser-201, and the ligand hydrophobic moiety with the receptor hydrophobic pocket. In order to examine the mode of the receptor–ligand interaction, we first docked (*R*)-(+)-7-(4-fluorophenyl)-7-[2-hydroxy-5-(hydroxymethyl)-3,4,6-trimethylphenyl]heptanoic acid ((*R*)-(+)-TCV-144, Chart I),[17] a potent nonprostanoid antagonist with a relatively rigid conformation, into the receptor model. The compound was found to fit the pocket if its nonphenolic benzene ring was directed downward and the methylene chain was made to adopt an extended conformation (Figure 4). As expected, the hydroxyl group of CH_2OH formed a hydrogen bond with Ser-201, the carboxyl group interacted with Arg-295, and the nonphenolic benzene ring was surrounded by hydrophobic residues (Figure 5). The phenolic benzene ring was also in the hydrophobic pocket. The phenolic hydroxyl formed a hydrogen bond with the backbone oxygen of Trp-258. The receptor-bound conformation of the ligand was nearly identical to one of its stable conformations (data not shown). The hydroquinone form of (*R*)-(+)-7-(3,5,6-trimethyl-1,4-benzoquinon-2-yl)-7-phenylheptanoic acid[18] ((*R*)-(+)-AA-2414, Chart I), which is also a potent TXA_2 antagonist, fitted the pocket of the TXA_2 receptor as well, and nearly the same interactions were found (data not shown).

Next, this model was compared with known structure–activity relationships for this series of compounds[17,18] as summarized below. (1) For the $-(CH_2)_nCOOH$ moiety, the activity is highest when $n = 5$. (2) When nonphenolic benzene is replaced with hydrogen or methyl, the activity is significantly lowered. (3) Introduction of methyl or isopropyl into the para position of this benzene ring lowers the activity. Introduction of methyl into the ortho position causes a larger decrease in activity. (4) The hydroquinone form of AA-2414 is more active than the quinone form (Chart I). (5) Replacement of CH_2OH with CHO on phenolic benzene of TCV-144 does not lower the activity. (6) Concerning the absolute configuration, the *R*-isomer is favorable for the activity.

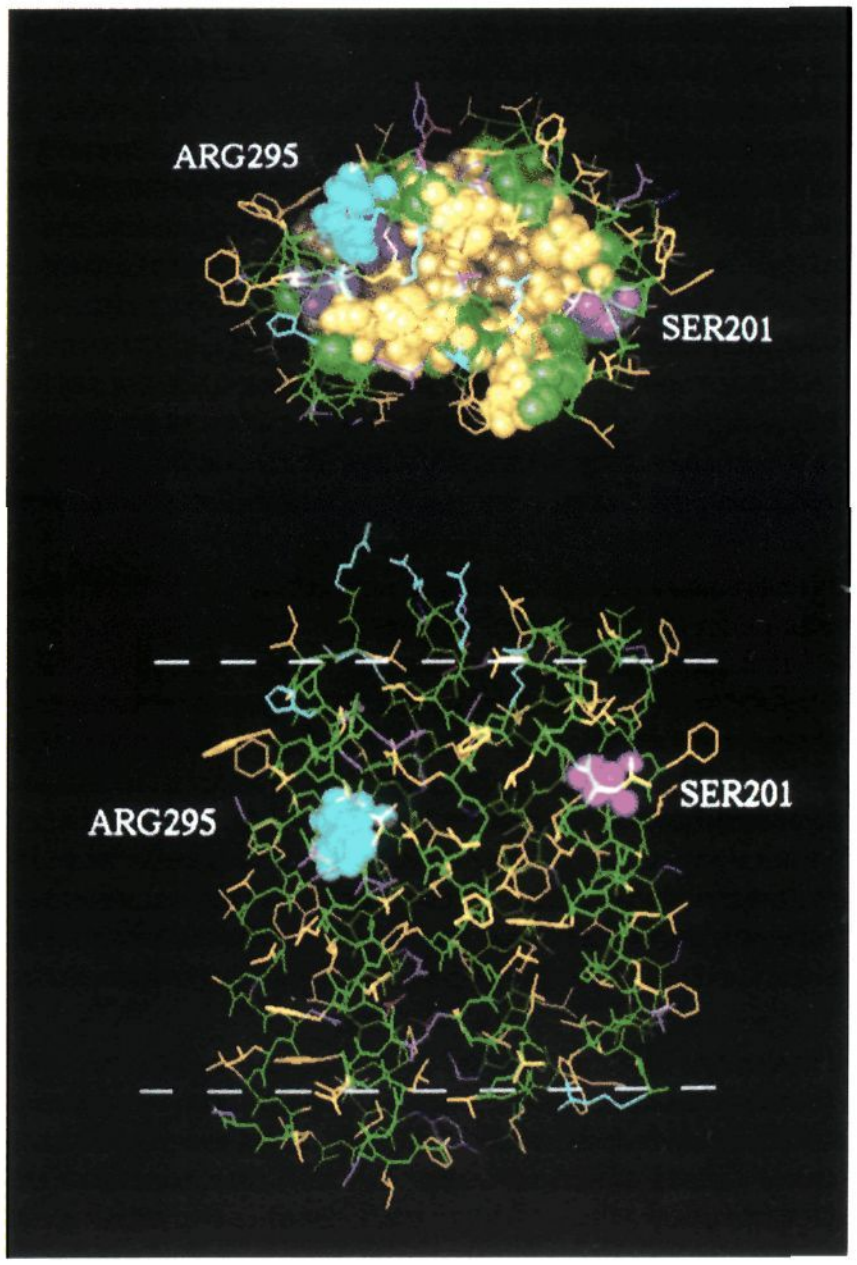

Figure 3. A model for the human TXA_2 receptor viewed from the surface of the cell (top) and from the side of the membrane (bottom). Color codes: green, main chains; red, acidic side chains; cyan, basic side chains; magenta, other hydrophilic side chains; yellow, hydrophobic side chains. In the top figure, residues constituting the ligand-binding pocket are represented with CPK models.

These data can be explained as follows according to the present model. (1) When $-(CH_2)_nCOOH$ with $n = 5$ adopts an extended conformation, COOH, phenolic OH, and CH_2-OH of the ligand come to ideal positions for interacting with the receptor. If n is smaller, some of these interactions will be disfavored, and if n is larger, the methylene chain has to be bent. In any case, binding to the receptor will be retarded. (2) The nonphenolic benzene ring is located in the hydrophobic pocket. If it is replaced with hydrogen or methyl, a gap will be formed between the receptor and ligand, causing decrease in affinity to the receptor. (3) If a substituent is introduced into the benzene ring at the para position, steric hindrance will be caused between the substituent and receptor. In the case of the ortho position, conformational change of the ligand might affect the binding, in addition to the steric hindrance. (4) Phenolic OH of TCV-144 and the corresponding OH of the hydroquinone form of AA-2414 act as a proton donor in the interaction with the receptor. If the latter OH is

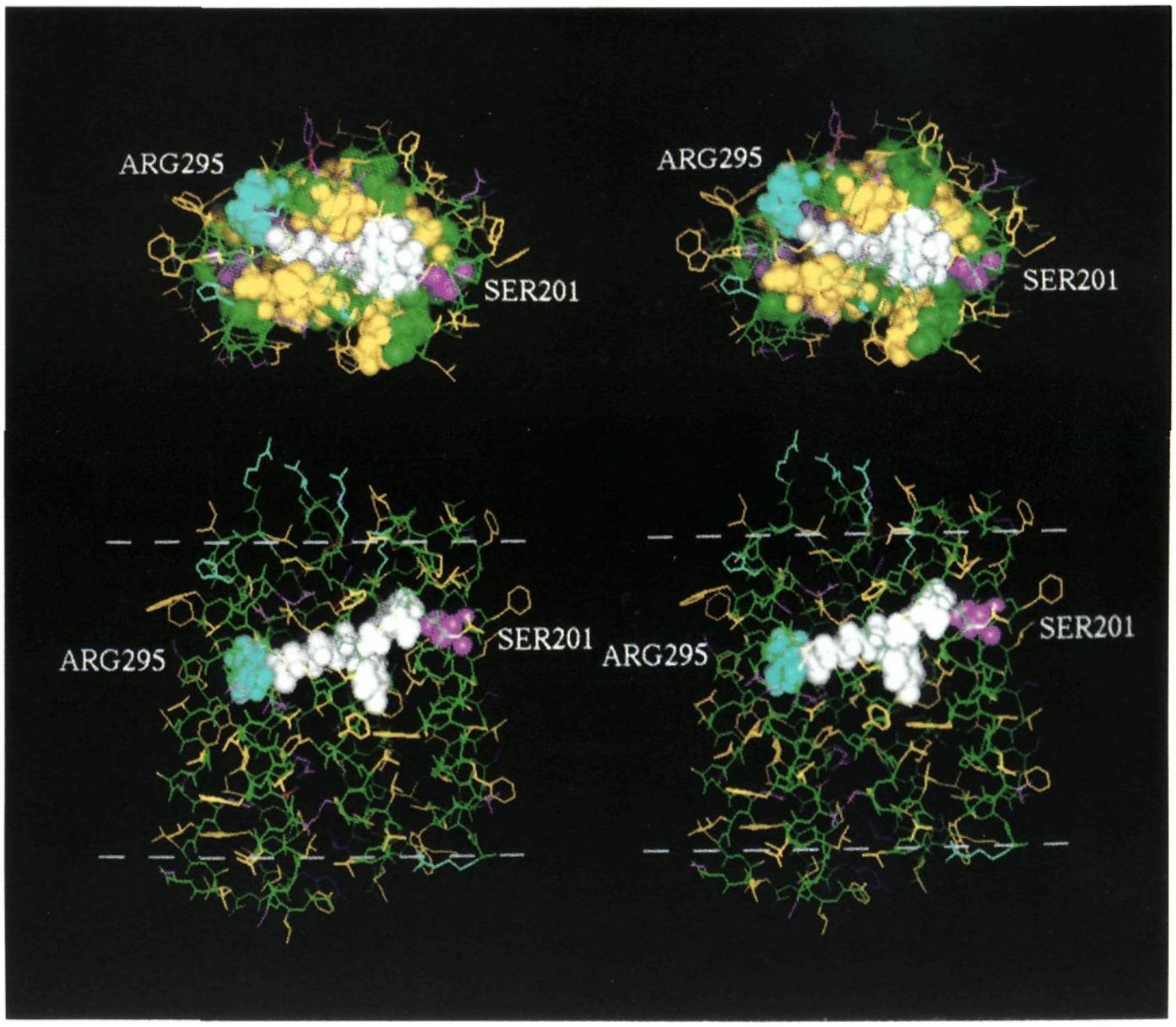

Figure 4. A model for the human TXA_2 receptor complexed with (*R*)-(+)-TCV-144 (stereoviews). The orientation of the receptor and color codes are the same as those in Figure 3. TCV-144 is represented with white CPK models.

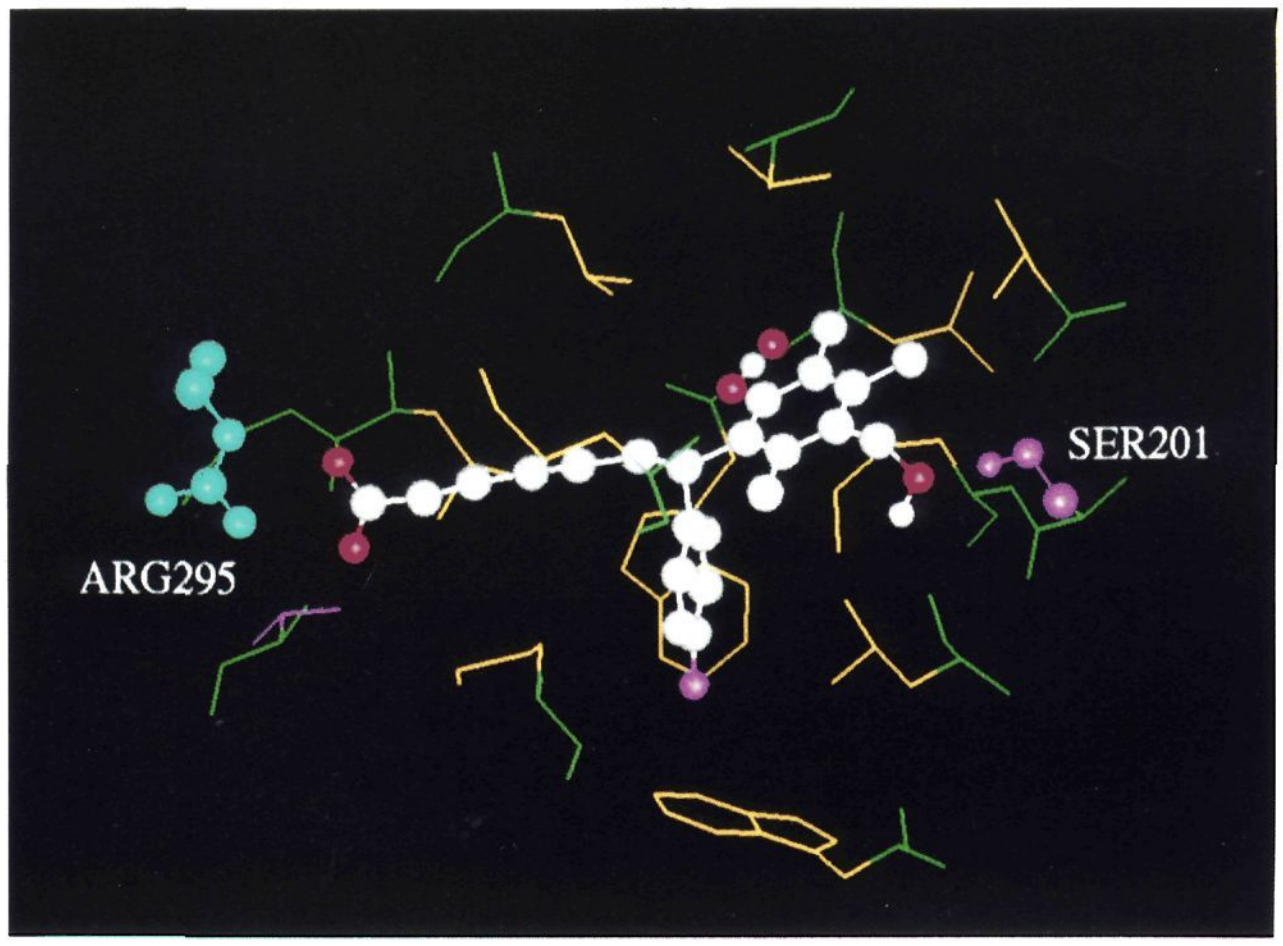

Figure 5. Detailed picture of the ligand-binding site of the TXA_2 receptor complexed with (*R*)-(+)-TCV-144. Color codes are the same as those in Figure 3 except that oxygen and fluorine atoms of TCV-144 are shown in red and magenta, respectively.

oxidized, it can no longer form a hydrogen bond with the receptor, and the affinity to the receptor will be lowered. (5) CH_2OH on phenolic benzene of TCV-144 serves as a proton acceptor, and therefore CHO is also feasible. (6) If the absolute configuration is inversed, it is impossible to satisfy all the three interactions mentioned above, and affinity to the receptor will be lowered.

Accordingly, this model is reasonable enough to explain the structure–activity relationships for TCV-144-related compounds.

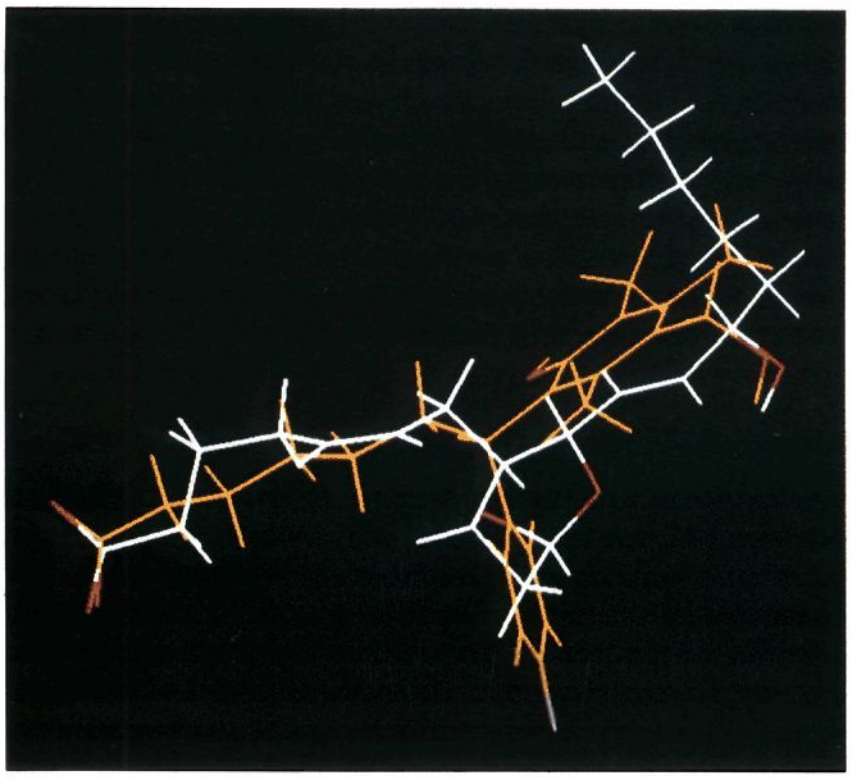

Figure 6. Comparison of receptor-bound conformations of TXA_2 (white) and (*R*)-(+)-TCV-144 (orange). The receptor moieties of the two complex structures were superimposed, but only ligand molecules are displayed here. Oxygen and fluorine atoms are shown in red and magenta, respectively.

Finally, the mode of the receptor–TXA_2 interaction was analyzed on the basis of the above model. The carboxyl group of TXA_2 was assumed to interact with Arg-295, and the hydroxyl group at position 15 with Ser-201. Docking of TXA_2 into the receptor model, manual adjustment of the conformation of TXA_2, and subsequent energy minimization resulted in a conformation which satisfied the above interactions and was energetically reasonable. When this conformation was compared with the receptor-bound conformation of TCV-144, the correspondence shown in Figure 6 was obtained. The carboxyl and hydroxyl groups of TXA_2 corresponded to the same groups of TCV-144, respectively, and the bicyclic moiety of TXA_2 was roughly superimposed on the nonphenolic benzene ring of TCV-144. Although there was no counterpart of TCV-144 to the TXA_2 ω-chain, this moiety was surrounded by hydrophobic side chains of the receptor. Neither of the two oxygen atoms in the bicyclic moiety were involved in hydrogen bonding interactions with the receptor. This is not unreasonable, however, because the TXA_2 analog in which these oxygens were replaced with CH_2 was also reported to have affinity to the receptor.[19] Moreover, this binding mode is consistent with the fact that TXB_2, which has a more hydrophilic ring structure, is biologically inactive. On the other hand, Fukumoto et al.[17] made the phenolic hydroxyl of TCV-144 correspond to one of the two oxygen atoms in the bicyclic moiety of TXA_2. Judging from the present model, the correspondence shown in Figure 6 seems to be most likely, but other possibilities cannot be ruled out at present.

Another feature of the TXA_2 receptor model is the existence of several basic residues (shown in cyan in Figure 3) near the entrance of the ligand-binding pocket. Although the inward/outward directions of these residues are not definite, some of them may be directed inward, providing a positive electrostatic field at the entrance of the ligand-binding pocket. This would help attract the negative charge of the ligand molecule.

Although we do not go into details, the binding mode of other ligands can also be analyzed the same way. In the case of (+)-(5*Z*)-7-[3-*endo*-[(phenylsulfonyl)amino]bicyclo-[2.2.1]hept-2-*exo*-yl]heptenoic acid ((+)-*S*-145, Chart I),[20] for example, a sulfonyl oxygen atom formed a hydrogen bond with Ser-201, the benzene ring interacted with hydrophobic residues like the ω-chain of TXA_2, and other interactions were essentially the same as those for TXA_2 (data not shown). It is also interesting whether or not the agonist-bound conformation of the receptor is the same as the antagonist-bound form. However, this problem is difficult to answer from the present model considering its moderate accuracy.

Conclusions

The present model is preliminary, and further modification is necessary for quantitative analysis of the receptor–ligand interaction. It is, however, at least qualitatively useful for drug design. Inclusion of additional mutational data would improve the reliability of the model considerably. Moreover, the modeling method described in the present paper can be applied to other GPCRs whose amino acid sequences have been determined. In fact, modeling of a prostaglandin E_2 receptor (subtype EP_3), whose amino acid sequence is homologous with that of the TXA_2 receptor,[21] is now in progress in our laboratory.

Experimental Section

Molecular modeling including energy minimization was carried out on Iris 4D/220GTX (Silicon Graphics) with software Discover/Insight II (Biosym Technologies). The coordinates (entry 1BRD) of bacteriorhodopsin[5] were from the Protein Data Bank[22] at Brookhaven National Laboratory. The receptor models were optimized using molecular mechanics calculations with CVFF force field in Discover. An initial model for the β_2 receptor–ligand complex was constructed by using bR as a template (see Figure 1 for the alignment) and docking the ligand into the receptor. Some manual adjustments were made to remove bad steric interactions and to make the model consistent with the mutational data. Then, the whole complex was energy minimized for 500 steps with the steepest descent minimizer and subsequently for 3000 steps with the conjugate gradient minimizer. A cutoff of 10 Å was used, and no solvent molecules were included in the calculation. The other models were also optimized with the same procedure.

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Supplementary Material Available: Tables compiling the results of the calculations (bond lengths, bond angles, and torsion angles) for TXA_2, TCV-144, and S-145 (7 pages). Ordering information is given on any current masthead page.

References

(1) Samuelsson, B.; Goldyne, E.; Granström, E.; Hamberg, M.; Hammarström, S.; Malmsten, C. Prostaglandins and Thromboxanes. *Annu. Rev. Biochem.* **1978**, *47*, 997–1029.
(2) Ogletree, M. L. Overview of Physiological and Pathophysiological Effects of Thromboxane A_2. *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **1987**, *46*, 133–138.
(3) Hall, S. E. Thromboxane A_2 Receptor Antagonists. *Med. Res. Rev.* **1991**, *11*, 503–579.
(4) Hirata, M.; Hayashi, Y.; Ushikubi, F.; Yokota, Y.; Kageyama, R.; Nakanishi, S.; Narumiya, S. Cloning and Expression of cDNA for a Human Thromboxane A_2 Receptor. *Nature* **1991**, *349*, 617–620.
(5) Henderson, R.; Baldwin, J. M.; Ceska, T. A.; Zemlin, F.; Beckmann, E.; Downing, K. H. Model for the Structure of Bacteriorhodopsin Based on High-resolution Electron Cryo-microscopy. *J. Mol. Biol.* **1990**, *213*, 899–929.
(6) Findlay, J. B. C.; Pappin, D. J. C. The Opsin Family of Proteins. *Biochem. J.* **1986**, *238*, 625–642.
(7) Findlay, J. B. C.; Eliopoulos, E. Three-Dimensional Modeling of G Protein-Linked Receptors. *Trends Pharmacol. Sci.* **1990**, *11*, 492–499.

(8) Grötzinger, J.; Engels, M.; Jacoby, E.; Wollmer, A.; Straussburger, W. A Model for the C5a Receptor and for Its Interaction with the Ligand. *Protein Eng.* **1991**, *4*, 767–771.
(9) Hibert, M. F.; Trumpp-Kallmeyer, S.; Bruinvels, A.; Hoflack, J. Three-Dimensional Models of Neurotransmitter G-Binding Protein-Coupled Receptors. *Mol. Pharmacol.* **1991**, *40*, 8–15.
(10) Strader, C. D.; Sigal, I. S.; Dixon, R. A. F. Structural Basis of β-Adrenergic Receptor Function. *FASEB J.* **1989**, *3*, 1825–1832.
(11) Kobilka, B. K.; Dixon, R. A. F.; Frielle, T.; Dohlman, H. G.; Bolanowsky, M. A.; Sigal, I. S.; Yang-Feng, T. L.; Francke, U.; Caron, M. G.; Lefkowitz, R. J. cDNA for the Human β_2-Adrenergic Receptor: A Protein with Multiple Membrane-Spanning Domains and Encoded by a Gene Whose Chromosomal Location is Shared with That of the Receptor for Platelet-derived Growth Factor. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 46–50.
(12) Kyte, J.; Doolittle, R. F. A Simple Method for Displaying the Hydropathic Character of a Protein. *J. Mol. Biol.* **1982**, *157*, 105–132.
(13) Nishikawa, M.; Kanno, M.; Kuriki, H.; Sugihara, H.; Motohashi, M.; Itoh, K.; Miyashita, O.; Oka, Y.; Sanno, Y. Selective β-Adrenoceptor Activities of Tetrahydronaphthalene Derivatives. *Life Sci.* **1975**, *16*, 305–314.
(14) Strader, C. D.; Sigal, I. S.; Candelore, M. R.; Rands, E.; Hill, W. S.; Dixon, R. A. F. Conserved Aspartic Acid Residues 79 and 113 of the β-Adrenergic Receptor Have Different Roles in Receptor Function. *J. Biol. Chem.* **1988**, *263*, 10267–10271.
(15) Tota, M. R.; Strader, C. D. Characterization of the Binding Domain of the β-Adrenergic Receptor with the Fluorescent Antagonist Carazolol. *J. Biol. Chem.* **1990**, *265*, 16891–16897.
(16) Suryanarayana, S.; Daunt, D. A.; Zastrow, M. V.; Kobilka, B. K. A Point Mutation in the Seventh Hydrophobic Domain of the α_2 Adrenergic Receptor Increases Its Affinity for a Family of β Receptor Antagonists. *J. Biol. Chem.* **1991**, *266*, 15488–15492.
(17) Fukumoto, S.; Shiraishi, M.; Terashita, Z.; Ashida, Y.; Inada, Y. Synthesis and Thromboxane A_2/Prostaglandin H_2 Receptor Antagonistic Activity of Phenol Derivatives. *J. Med. Chem.* **1992**, *35*, 2202–2209. TCV-144 was originally synthesized as a racemic form, but *R*-(+)-isomer (shown in Chart I) was later found to be active (Shiraishi, M.; Fukumoto, S. Personal communication).
(18) Shiraishi, M.; Kato, K.; Terao, S.; Ashida, Y.; Terashita, Z.; Kito, G. Quinones. 4. Novel Eicosanoid Antagonists: Synthesis and Pharmacological Evaluation. *J. Med. Chem.* **1989**, *32*, 2214–2221.
(19) Lefer, A. M.; Smith, E. F. III; Araki, H.; Smith, J. B.; Aharony, D.; Claremon, D. A.; Magolda, R. L.; Nicolaou, K. C. Dissociation of Vasoconstrictor and Platelet Aggregatory Activities of Thromboxane by Carbocyclic Thromboxane A_2, a Stable Analog of Thromboxane A_2. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 1706–1710.
(20) Narisada, M.; Ohtani, M.; Watanabe, F.; Uchida, K.; Arita, H.; Doteuchi, M.; Hanasaki, K.; Kakushi, H.; Otani, K.; Hara, S. Synthesis and in Vitro Activity of Various Derivatives of a Novel Thromboxane Receptor Antagonist, (±)-(5*Z*)-7-[3-*endo*-[(Phenylsulfonyl)amino]bicyclo[2.2.1]hept-2-*exo*-yl]heptenoic Acid. *J. Med. Chem.* **1988**, *31*, 1847–1854.
(21) Sugimoto, Y.; Namba, T.; Honda, A.; Hayashi, Y.; Negishi, M.; Ichikawa, A.; Narumiya, S. Cloning and Expression of a cDNA for Mouse Prostaglandin E Receptor EP_3 Subtype. *J. Biol. Chem.* **1992**, *267*, 6463–6466.
(22) Bernstein, F. C.; Koetzle, T. F.; Williams, G. J. B.; Meyer, E. F., Jr.; Brice, M. D.; Rodgers, J. R.; Kennard, O.; Shimanouchi, T.; Tasumi, M. The Protein Data Bank: A Computer-based Archival File for Macromolecular Structures. *J. Mol. Biol.* **1977**, *112*, 535–542.