

Study of V₂ vasopressin receptor hormone binding site using *in silico* methods

Yeganeh Sebti^{1,2}, Soroush Sardari^{2,*}, Hamid Mir Mohammad Sadeghi¹,
Mohammad Hossein Ghahremani³, and Giulio Innamorati⁴

¹Department of Pharmaceutical Biotechnology and Isfahan Pharmaceutical Sciences Research Center, School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, I.R. Iran.

²Drug Design and Bioinformatics Unit, Medical Biotechnology Department, Biotechnology Research Center, Pasteur Institute of Iran, Tehran, I.R. Iran.

³Department of Pharmacology and Toxicology, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, I.R. Iran.

⁴Section of Immunology, Department of Pathology, University of Verona, Verona, Italy.

Abstract

The antidiuretic effect of arginine vasopressin (AVP) is mediated by the vasopressin V₂ receptor. The docking study of AVP as a ligand to V₂ receptor helps in identifying important amino acid residues that might be involved in AVP binding for predicting the lowest free energy state of the protein complex. Whereas previous researchers were not able to detect the exact site of the ligand-receptor binding, we designed the current study to identify the vasopressin V₂ receptor hormone binding site using bioinformatic methods. The 3D structure of nonapeptide hormone vasopressin was extracted from *Protein Data Bank*. Since no suitable template resembling V₂ receptor was found, an *ab initio* approach was chosen to model the protein receptor. Using protein docking methods such as Hex protein-protein docking, the model of V₂ receptor was docked to the peptide ligand AVP to identify possible binding sites. The residues that involved in binding site are W293, W296, D297, A300, and P301. The lowest free energy state of the protein complex was predicted after mutation in the above residues. The amount of gained energies permits us to compare the mutant forms with native forms and help to assess critical changes such as positive and negative mutations followed by ranking the best mutations. Based on the mutation/docking predictions, we found some mutants such as W293D and A300E possess positively inducing effect in ligand binding and some of them such as A300R present negatively inducing effect in ligand binding.

Keywords: V₂ vasopressin receptor; Arginine vasopressin; Binding site; Docking; Structure similarity; Sequence alignment

INTRODUCTION

Arginine vasopressin (AVP), possess various physiological functions, such as water reabsorption, blood volume, blood pressure, cellular proliferation and adrenocorticotrophic hormone secretion. In the kidney, water reabsorption is mainly regulated by the binding of arginine vasopressin to vasopressin type 2 receptors (1). Arginine vasopressin receptor (V₂ receptor) is a *G protein-coupled receptor* that acts as a receptor for AVP. V₂ receptor is expressed in the kidney tubule, predominantly in the distal convoluted tubule

and collecting ducts, in fetal lung tissue and lung cancer, the last two being associated with alternative splicing (2). Some mutants V₂R, such as Y128S, R181C, ΔR202, R202C, and P286R are defective in ligand binding (3-6). Few mutant V₂ receptors have been found to affect multiple aspects of receptor function. For example, R113W was found to affect cell surface expression (20% of weight), ligand binding (20-fold decrease in affinity) and signaling (3,7). Although D85N is expressed normally on the cell surface, it binds to AVP with 6-fold lower affinity, which together with a 20-fold decrease in coupling efficiency,

*Corresponding author: S. Sardari
Tel: 0098 21 66480780, Fax: 0098 21 66480780
Email: ssardari@hotmail.com

resulted in a 50-fold increase in EC_{50} (3,8). Chimeric human V2 receptor in which the first and the second extracellular loops were replaced by the corresponding loops of the murine V2 receptor, showed high-affinity binding to the ligand (9).

Mutant V2 receptors such as G12E, A61V and Δ R247-G250 have normal ligand binding, cAMP stimulation and signaling which suggests that they might not be the cause of nephrogenic diabetes insipidus and do not play a main role in receptor-ligand binding site (3,4,10). However; the substitution of the aspartate at position 136 by alanine leads to agonist-independent activation of the mutant form (11).

The conserved cysteine residues of the V2 receptor are not only important for the structure of the ligand binding domain but also for efficient intracellular receptor transport, for example C112S, C112A and C192S, C192A mutant V2 receptors were non-functional and located mostly in the cell's interior. Also functional expression in stably transfected Ltk-cells showed that single mutants C341S and C342S and the double mutant C341S, C342S have normal affinity for arginine vasopressin (12,13).

Extensive functional studies showed that mutant receptor G185C and R202C were efficiently transported to the plasma membrane but were defective in ligand binding, although ligand binding of R202C mutant receptor is not blocked completely (12).

Site directed mutagenesis of the cloned bovine and porcine V2 receptors identified a residue (D103) which is responsible for high affinity binding of dDAVP (14).

In contrast to the wild-type receptor, the naturally occurring mutant R337X failed to confer specific [3 H] AVP binding to transfected cells (15).

Recent advances in bioinformatics facilitate the use of structure prediction techniques such as homology modeling, combinational approaches, and partial *ab initio* methods. Structure prediction can be helpful in site directed mutagenesis experiments and predict a potential binding site. These studies play an important role in drug design and experimental research.

The amount of gained energy permits comparing the mutant forms and assessment of the critical positive and critical negative mutations, followed by ranking. 3D models can be built based on multiple-threading alignments by local meta-threading-server (LOMETS) and iterative template fragment assembly simulations.

The main purpose of this study was to develop a structural model for V2 receptor and to identify the residues responsible for ligand binding by *in silico* studies.

MATERIALS AND METHODS

3D structures of arginine vasopressin and arginine vasopressin receptor

The initial work was a database search using National Center for Biotechnology Information (NCBI) and the sequences of the AVP and V2 receptor were obtained from this source. *Basic Local Alignment Search Tool* (BLAST) was used for the alignment of the V2 receptor sequence with its templates (16).

Briefly there is sequence information for V2 receptor but there is no 3D information of this receptor. This led us to identify the usable 3D structure that can be applied in the docking experiments.

Therefore, an *ab initio* approach was chosen to model the receptor protein (16). For this purpose, by inserting the FASTA format of V2 receptor in I-TASSER online, top 5 models were predicted by I-TASSER and compared based on the template modeling score (TM-score) and confidence-score (C-score). Finally the best predicted model was selected on the basis of C-score, TM-score and *root-mean-square deviation* (RMSD) value for this research.

The associated root mean square (rms) error is frequently used to measure the extent of structure similarity. TM-score is a measure of structural similarity between the predicted model and the native structure. C-score is an estimate of the confidence and quality of the predicted models, C-score is typically in the range of -5 to 2, wherein a higher score reflects a model of better quality.

In general, models with C-score >-1.5 have a correct fold. I-TASSER server is an on-line

platform for protein structure and function predictions. 3D models are built based on multiple-threading alignments by local meta-threading-server (LOMETTS) and iterative template fragment assembly simulations; function insights are derived by matching the 3D models with BioLip protein function database (17).

The 3D structure of nonapeptide hormone vasopressin was extracted from *protein data bank (PDB)* so that chain B isolated from the crystal structure of trypsin-vasopressin complex as PDB format (PDB ID: 1YF4-chain B).

More analysis was done to approve the quality of V2 receptor model by using PDB sum analysis, Ramachandran plot and ProSA (protein structure analysis).

Minimization and visualization

Minimization was carried out by considering shape and shape/electrostatic by using Swiss PDB Viewer (SPDBV) using Gromos algorithm. Minimization leads to reduction of the protein energy and protein relaxation (16). Rasmol v2.7.3 (2005), Accelrys Discovery Studio Visualizer v2.5.1.9167 (2005), ArgusLab v4.0.1 (2004) and SPDBV v4.0.1 (2008) are some tools that were used for viewing and analyzing models.

Protein docking tools and prediction of the exact binding site of V2R-AVP complex

All of the dockings for this work was done using Hex protein-protein docking software 6.0 (2010). PDB file of the docking complex of V2R-AVP was opened in Argus-lab application v 4.0.1 (2004) to specify the residues that involved in binding site of the receptor.

Mutations and energy minimizations

By using Swiss PDB viewer software 4.0.1 (2008), each of the residues that involved in binding site of the receptor (W293, W296, D297, A300 and P301 pro) mutated to another 19 amino acid separately followed by energy minimization. Finally, the obtained structures were used for docking experiments by Hex protein-protein docking software 6.0 (2010).

RESULTS

Modeling of V2 receptor

The main goal in this study was to determine the binding domains between V2 receptor and vasopressin hormone using *in silico* methods. In this study, NCBI reference sequence of vasopressin V2 receptor isoform 1 (Homo sapiens) NP-000045.1 was used.

BLAST similarity search and multiple alignments exhibited no suitable template resembling V2 receptor. The multiple sequence alignment carried out by using T-Coffee (Tree-Based Consistency Objective Function for Alignment Evaluation) software in FASTA format (18). Regarding the value of identity in BLAST (sequence similarity less than 40%), structure similarity was used instead of sequence similarity for this surveying. Since the amounts of RMSD, for 3D similarity study, should be less than 2, the structure similarity method was not applicable. No suitable template resembling V2 receptor was found (16).

3D structure of arginine vasopressin

The 3D structure of the AVP is shown in Fig. 1. The 3D structure of nonapeptide hormone vasopressin was extracted from chain B of the crystal structure of trypsin-vasopressin complex as PDB format from Protein Data Bank. (PDB ID: 1YF4).

Construction of a structural model for V2 receptor using I-Tasser on line

V2 receptor sequence was used as an input for protein modeling server (I-tasser on line). The best predicted model is shown in Fig. 2.

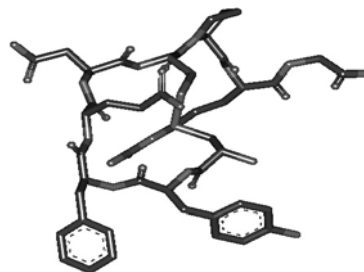


Fig. 1. The 3D structure of nonapeptide hormone vasopressin was extracted from chain B of the crystal structure of trypsin-vasopressin complex as *protein data bank* format Protein Data Bank (PDB ID: 1YF4_B).

Visualization, docking and identification of the receptor binding sites

Using Hex protein-protein docking application, the model of V2 receptor was docked to the peptide ligand, AVP to identify possible binding sites. The docking complex is shown in Fig. 3.

The ligand-receptor complex was analyzed by Argus-lab software V4.0.1 (2004) to determine the binding site residues. The residues involved in binding site are as below; W293, W296, D297, A300 and P301 pro.

Mutations and docking experiments

The residues involved in binding site were

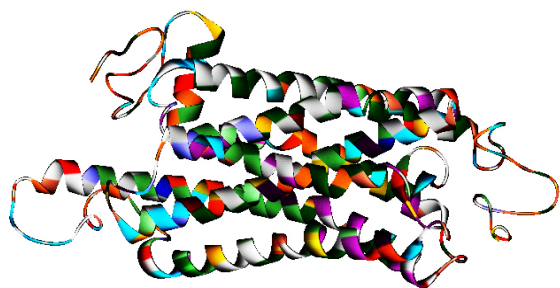


Fig. 2. The best selected model of V2 receptor on the basis of C-score, template modeling score, and root-mean-square deviation value.

investigated by mutations and assessment of the energy state of the ligand-receptor complex. The amounts of gained energy (E_{total}) are comparable between the native and mutated receptors.

The amount of E_{total} for each amino acid substitution that involved in binding site is shown in Table 1. The ligand residues neighboring (interact) to receptor binding site are shown in Table 2.

Based on mutation/docking predictions in this research, the results are classified into two categories including Highly influential (opposing effect) and Conserved (preserving the activity).



Fig. 3. The docking complex of arginine vasopressin as a ligand to its receptor (V2 receptor); the ligand arginine vasopressin and V2 receptor have been represented in black and gray respectively. Also W293, W296, D297, A300 and P301 pro residues are shown as black color in the ribbon structure of the receptor.

Table 1. Mutations and docking experiment results using Hex server. The amounts of E_{total} for each amino acid substitution involved in binding site are indicated.

Mutation	E total for amino acid position				
	Native W293	Native W296	Native D297	Native A300	Native P301
A	-560.7	-533.3	-327.8	-599.9	-594.1
R	-341.6	-340.8	0	-332.9	-408.6
N	-551.8	-545.6	-325.4	-581.7	-586.6
D	-1078.1	-977	-599.9	-1115.9	-876.1
C	-561.8	-556.1	-325.5	-564.4	-591.9
Q	-646.9	-554.1	-331.5	-580	-597.6
E	-1067.9	-880.7	-621.1	-1160.3	-840.3
G	-598.7	-533	-331.1	-591	-562.3
H	-567.8	-553.5	-325.2	-586.6	-587.6
I	-555.3	-553.9	-325.4	-581	-590.5
L	-561	-507.4	-325.1	-551.1	-583
K	-403.9	-383.6	0	-330.4	-400.5
M	-566.3	-546.1	-324.2	-557.6	-589.5
F	-549.1	-586	-324.2	-586.1	-594
P	-581.8	-554.4	-328.8	-602.9	-599.9
S	-561.9	-551.7	-324	-588.5	-604.6
T	-557.7	-573.5	-331	-566.7	-603.9
Y	-555.9	-577.1	-324.8	-559.1	-587.1
V	-556.3	-557.8	-326	-575.8	-590.9
W	-599.9	-599.9	-413.7	-535.2	-592.9

Table 2. Mutations and docking experiment results using Hex server. The ligand residues that neighbor (interact) with receptor binding site are shown.

Mutation	Native W293	Native W296	Native D297	Native A300	Native P301
A	N5-C6	R8	non		P7
R	Y2	non	non	non	P7
N	R8-G9	R8-C1	non	N5-P7	P7
D	R8-G9	R8-C6-G9-P7		N5-C6-P7	R8-C6
C	R8-G9	R8-C1	non	C6-P7	P7
Q	F3	R8-F3	non	N5-C6-P7	P7
E	N5-C6-P7	R8-C6-G9-P7	R8-C6-P7	N5-C6-P7	R8
G	R8	R8	non	non	P7
H	F3	R8	non	N5-P7	P7
I	F3	R8-C1	non	N5-C6-P7	P7
L	non	non	non	N5-C6-P7	P7
K	F3-Y2	non	non	non	P7
M	F3	R8	non	N5-C6-P7	P7
F	F3	R8	non	N5-P7	P7
P	R8	R8	non	P7	
S	R8-G9	R8-C1	non	C6-P7	P7
T	F3	R8-C1	non	C6-P7	P7
Y	F3	R8	non	C6-P7	P7
V	F3	R8	non	P7	P7
W			F3	non	P7

DISCUSSION

The main goal of this study was to determine the exact amino acids are involved in V2 vasopressin receptor binding to its ligand arginine and to compare the energy state of the ligand-receptor complex between the native and mutated receptors.

Our bioinformatic studies revealed that W293, W296, D297, A300, and P301 pro are important residues for binding. The amino acid substitution in the predicted binding domain affects the E total for ligand-receptor complex. The results of the present study indicated that replacement of amino acids on receptor binding residues such as W293D, W293E, W296D, W296E, A300D, A300E, P301D, P301E caused positive effect on ligand-receptor binding, while this resulted in negative effect on ligand-receptor binding of some other receptor binding residues such as W293R, W293K, W296R, W296K, D297X, (X is any amino acid other than aspartic acid), A300R, A300K, P301R, P301K. Finally in several other receptor binding residues like D297E, mutation has no significant effect on ligand-receptor binding.

Based on mutation and docking experiment, we deduced that acidic amino acid (aspartic acid and Glutamic acid) substitution in receptor binding residues may lead to stronger

ligand-receptor binding (lower E total), while basic amino acid (arginine and lysine) substitution in receptor binding residues may lead to a weaker ligand-receptor binding (higher E total). In other words, replacement of negatively-charged amino acid caused positive inducing effect on receptor-ligand binding, while positively-charged amino acid replacement had negative inducing effect.

With regards to the above information, it is possible to accurately determine the ligand-receptor binding site that leads to identifying significant steps required for synthesis of the effective therapeutic compounds (19,20).

According to the previous studies, the receptor amino acid residues, potentially important in ligand binding, are mainly in the TM3-TM7 helices (21). Though more than 190 V2 receptor mutations have so far been studied (3), none of them identified the exact receptor binding site residues that interact with ligand AVP.

Bioinformatic studies on vasopressin receptor using homology modeling showed that glutamine residue of V2 receptor (Q48) is the only amino acid that involved in AVP (Y2) binding. The structural model presented in aforementioned study is based on a *G protein-coupled receptor* template (bovine rhodopsin) (20), whereas our method for modeling is different, and the model was constructed using protein modeling server (I-tasser on line).

In another study that investigated V2 receptor antagonist binding site by site-directed mutagenesis, V2 receptor structure was predicted through homology modeling. The 41% overall sequence identity between V2 receptor and V1 receptor is related to the extracellular loops el1, el2, and el3, but not to the whole protein sequence. K100D, A110W, M120V, L175Y, R202S, and F307I mutations found not to alter the affinity for arginine vasopressin. The investigated mutations affect only the subpocket for nonpeptide antagonists, whereas the determined amino acids did not alter the affinity for arginine vasopressin and therefore would not be important in binding of AVP (22).

CONCLUSION

In the present work, the effect of amino acid substitution in the predicted binding domain was investigated. Using bioinformatic methods, it seems that W293, W296, D297, A300 and P301 residues of the V2 vasopressin receptor are involved in receptor binding domain. This information may be helpful in developing effective therapeutic compounds.

ACKNOWLEDGMENTS

Financial support of Drug Design and Bioinformatics Unit of Medical Biotechnology Department of Pasteur Institute of Iran is gratefully acknowledged.

REFERENCES

1. Wu N, Macion-Dazard R R, Nithianantham S, Xu Z, Hanson SM, Vishnivetskiy SA, *et al.* Soluble mimics of the cytoplasmic face of the human V1-vascular vasopressin receptor bind arrestin2 and calmodulin. *Mol. Pharmacol.* 2006;70:249-258.
2. Postina R, Ufer E, Pfeiffer R, Knoers NVAM, Fahrenholz F. Misfolded vasopressin V2 receptors caused by extracellular point mutations entail congenital nephrogenic diabetes insipidus. *Mol Cell Endocrinol.* 2000;164:31-39.
3. Tao YX. Inactivating mutations of G protein-coupled receptors and diseases: Structure-function insights and therapeutic implications. *Pharmacol Ther.* 2006;111:949-973.
4. Pan Y, Wilson P, Gitschier J. The effect of eight V2 vasopressin receptor mutations on stimulation of adenylyl cyclase and binding to vasopressin. *J Biol Chem.* 1994;269:31933-31937.
5. Ala Y, Morin D, Mouilia B, Sabatier N, Vargas R, Cotte N, *et al.* Functional studies of twelve mutant V₂ vasopressin receptors related to nephrogenic diabetes insipidus: molecular basis of a mild clinical phenotype. *J Am Soc Nephrol.* 1998;9:1861-1872.
6. Tsukaguchi I, Matsubara H, Taketani S, Mori Y, Seido T, Inada. Binding, intracellular transport and biosynthesis defective mutants of vasopressin type 2 receptor in patients with x-linked nephrogenic diabetes insipidus. *J. Clin. Invest.* 1995;96:2043-2050.
7. Robben JH, Knoers NV, Deen PM. Characterization of vasopressin V2 receptor mutants in nephrogenic diabetes insipidus in a polarized cell model. *Am J Physiol Renal Physiol.* 2005;289:F265-F272.
8. Sadeghi H, Robertson GL, Bichet DG, Innamorati G, Birnbaumer M. Biochemical basis of partial nephrogenic diabetes insipidus phenotypes. *Mol Endocrinol.* 1997;11:1806-1813.
9. Oksche A, Leder G, Valet S, Platzer M, Hasse K, Geist S, *et al.* Rosenthal. variant amino acids in the extracellular loops of murine and human vasopressin v2 receptors account for differences in cell surface expression and ligand affinity. *Mol Endocrinol.* 2002;16:799-813.
10. Wenkert D, Schoneberg T, Merendino JJ, Rodriguez Pena MS, Vinitsky RR, Goldsmith PK, *et al.* Functional characterization of five V2 vasopressin receptor gene mutations. *Mol Cell Endocrinol.* 1996;124:43-50.
11. Morin D, Cotte N, Balestre MN, Mouillac B, Manning M, Breton Ch, *et al.* The D136A mutation of the V2 vasopressin receptor induces a constitutive activity which permits discrimination between antagonists with partial agonist and inverse agonist activities. *FEBS Lett.* 1998;441:470-475.
12. Schulein R, Zuhlke K, Oksche A, Hermosilla R, Furkert J, Rosenthal W. The role of conserved extracellular cysteine residues in vasopressin V2 receptor function and properties of two naturally occurring mutant receptors with additional extracellular cysteine residues. *FEBS Lett.* 2000;466:101-106.
13. Schulein R, Liebenhoff U, Muller H, Birnbaumer M, Rosenthal W. Properties of the human arginine vasopressin V2 receptor after site-directed mutagenesis of its putative palmitoylation site. *Biochem J.* 1996;313:611-616.
14. Ufer E, Postina R, Gorbulev V, Fahrenholz F. An extracellular residue determines the agonist specificity of V₂ vasopressin receptors. *FEBS Lett.* 1995;362:19-23.
15. Oksche A, Dehe M, Schulein R, Wiesner B, Rosenthal W. Folding and cell surface expression of the vasopressin V2 receptor: requirement of the intracellular C-terminus. *FEBS Lett.* 1998;424:57-62.
16. Marcus FB. *Bioinformatics and systems biology.* Brussels:Springer; 2008. p. 75-78, 140-150.
17. Zhang Y. I-TASSER server for protein 3D structure prediction. *BMC Bioinformatics.* 2008; 9:40.

18. Bonneau R, Baker D. Ab initio protein structure prediction: progress and prospects. *Annu. Rev Biophys Biomol Struct.* 2001;30:173-189.
19. Sardari S, Sebti Y, Mir Mohammad Sadeghi H. Study of V2 vasopressin receptor hormone binding site using *in silico* methods. *Res Pharm Sci.* 2012;7:S493.
20. Sakethapuram S. Identification of the ligand binding site of human vasopressin receptor (V2R) using homology modeling and docking techniques. New York: Long Island University, the Brooklyn Center; 2007. p. 34-35.
21. Czaplewski C, Kazmierkiewicz R, Ciarkowski J. Molecular modeling of the vasopressin V₂ receptor/antagonist interactions. *Acta Biochimi Pol.* 1998;45:19-26.
22. Macion-Dazard R, Callahan N, Xu Zh, Wu N, Thibonnier M, Shoham M. Mapping the binding site of six nonpeptide antagonists to the human V2-renal vasopressin receptor. *J Pharmacol Exp Ther.* 2006;316:564-571.