J. Chosun Natural Sci.
Vol. 9, No. 4 (2016) pp. 234 – 240
https://doi.org/10.13160/ricns.2016.9.4.234

Theoretical Structure Prediction of Bradykinin Receptor B2 Using Comparative Modeling

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Abstract

Bradykinin receptor B2, a GPCR protein, binds with the inflammatory mediator hormone bradkynin. It plays an important role in cross-talk between the renin-angiotensin system (RAS) and the kinin-kallikrein system (KKS). Also, it is involved in many processes including vasodilation, edema, smooth muscle spasm and pain fiber stimulation. Hence, studuying the structural features of the receptor becomes important. But the unavailability of the three dimensional structure of the protein makes the analysis difficult. Hence we have performed the homology modelling of Bradykinin receptor B2 with 5 different templates. 25 different homology models were constructed. Two best models were selected based on the model validation. The developed models could be helpful in analysing the structural features of Bradykinin receptor B2 and in pathophysiology of various disorders related to them.

Keywords: Bradykinin Receptor B2, GPCR, GPR54, Bradkynin, Homology Modelling

1. Introduction

Bradykinins are short and structurally similar peptides which involve in the contraction of the venous smooth muscle, activation of sensory fibers, stimulating the release of cytokines, inducing the proliferation of connective tissue and mediating the endothelium-dependent vasodilation[1,2]. The antagonists of bradykinins are helpful in treating asthma, inflammation, mild pain and endotoxic shock.

Bradykinin receptors belong to the rhodopsin-like G-protein coupled receptor family. They are abundant in the peripheral tissues. The bradykinins receptors are subdivided into two subtypes: B1 and B2. Also, there could be a tracheal B3 receptor, but, they are yet to be confirmed[3]. Bradykinin receptor B2 is the predominant subtype which is ubiquitously and constitutively expressed in healthy tissues. The receptor is coupled to G_q and G_i, in which G_q stimulates phospholipase C to increase intracellular free calcium and G_i helps in inhibiting the adenylate cyclase. Also, the receptor stimulates the mitogen-activated protein kinase pathways[3,4].

The B2 receptor mediates slow contraction of various smooth muscles including veins, intestine, uterus, trachea, and lung, inducing endothelium-dependent relaxation of arteries and arterioles. It also stimulates the natriuresis/diuresis in the kidney[5]. They receptors are involved in the induction and maintenance of cytokine-induced hyperalgesia, along with B1 receptor. However, the B2 receptor to a lesser extent than B1[6]. The B2 receptor forms a complex with angiotensin converting enzyme (ACE) which is thought to play a role in cross-talk between the renin-angiotensin system (RAS) and the kinin-kallikrein system (KKS)[7,8].

Since B2 receptor greatly influences the various processes, from muscle contraction to activating pathways like MAPK pathway, it is plausible to consider them as a potential target for treatment of the conditions related to them. Exploring the structural features of B2 receptor thus becomes necessary. However, there are no available crystal structures of the B2 receptor. Homology modeling provides an alternate way of predicting the three-dimensional structure of the protein when only the sequence data of the protein is available. The number of protein structures resolved experimentally lags behind the sequence data available[9]. The main reason for this is the enormous amount of time required to pre-

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(Received : October 17, 2016, Revised : December 17, 2016, Accepted : December 25, 2016)

pare protein for crystallization as experimental process such as protein expression, purification followed by crystallization, requires years to perform. In this case, homology modeling based on comparative modeling can provide as a tool for the experimental procedures in finding the structure of the protein in a rather short time. In this study, we have developed three-dimensional models of B2 receptor based on comparative modeling and validated them. The developed models could provide as a tool for further studies on the structural features and binding features of B2 receptor/bradykinin interaction.

2. Material and Methods

2.1. Template Selection

The amino acid sequence of the human B2 receptor (Accession No: P30411) was retrieved from the UniProt database. Protein BLAST[10] search was performed against PDB[11] to find suitable templates for modeling the receptor. Five different templates were selected based on sequence identity, query coverage, and E-value. The selected templates were – 4ZUD, 4YAY, 2LNL, 4XT1, and 3OE0. If the level of sequence identity is above 30%, then up to 90% of the polypeptide conformation tends to be modeled well[12-14]. All the templates were having sequence identity ≥ 30%. Query coverage for the templates was greater than 70%. Also, all of the templates retained the seven transmembrane helix regions, which is the characteristic feature of the GPCR proteins.

2.2. Homology Modelling

Using EasyModeller 4.0[15], the three-dimensional structures of the B2 receptor were developed. EasyModeller 4.0 uses MODELLER 9.12[16] and Python 2.7.1 in the backend. At first, the predicted models were assessed and validated using the RMSD values. Then, Using RAMPAGE web server, Ramachandran plots for the models were plotted[17]. Ramachandran plot provides a way to visualize backbone dihedral angles ψ against φ of amino acid residues in protein structure, which identifies the sterically allowed regions for these angles. Later, validation by Verify3D and ERRAT plots were carried out. Verify3D determines the compatibility of the predicted model with its amino acid sequence by

Fig. 1. Alignment between the query (BKRB2) and templates (4ZUD, 4YAY, 2LNL, 4XT1 and 3OE0).

assigning a structural class based on its location and environment (alpha, beta, loop, polar, and nonpolar) and comparing the results to good structures[18]. ERRAT plots are plotted as a function of the position of a sliding 9-residue window. The error function is based on the statistics of non-bonded atom-atom interactions present in the structure[19].

3. Results and Discussion

3.1. Model Generation

Using EasyModeller, five models are modeled for each of the five templates – 4ZUD, 4YAY, 2LNL, 4XT1, and 3OE0. Therefore totally 25 models were developed using EasyModeller. Using the CLUS-

Table 1. The query coverage and identity values of the templates

PDB ID	Max Score	Total Score	Query Coverage %	E-Value	Identity %
4ZUD	166	166	75%	6e-47	34%
4YAY	166	166	75%	7e-47	34%
2LNL	122	122	70%	8e-32	32%
4XT1	124	124	73%	9e-32	30%
3OE0	98.6	148	71%	4e-22	33%

Table 2. RMS Deviation values

Model No	Templates Used	Homology Modelling Validation			
		RMSD	Ramachandran Plot		
			Number of residues in favored region (%)	Number of residues in allowed region (%)	Number of residues in outlier region (%)
1	4ZUD	0.438	91.5	5.4	3.1
2		0.341	91.0	6.2	2.8
3		0.321	90.2	7.7	2.1
4		0.386	92.0	4.4	3.6
5		0.453	91.5	5.4	3.1
6	4YAY	0.887	91.8	3.9	4.4
7		0.706	92.0	5.7	2.3
8		0.474	91.0	5.4	3.6
9		0.980	94.1	3.9	2.1
10		0.638	92.8	5.1	2.1
11	2LNL	0.219	90.0	7.5	2.6
12		0.288	90.5	6.2	3.3
13		0.196	90.5	7.5	2.1
14		0.232	88.9	8.0	3.1
15		0.231	90.7	6.2	3.1
16	4XT1	0.558	88.2	8.7	3.1
17		0.574	89.7	7.5	2.8
18		0.559	91.0	6.4	2.6
19		0.539	89.2	6.9	3.9
20		0.407	90.5	6.7	2.8
21	3OE0	0.540	90.5	6.4	3.1
22		0.439	90.7	6.2	3.1
23		0.653	90.2	6.9	2.8
24		0.645	90.5	4.9	4.6
25		0.496	88.7	8.0	3.3

Table 3. ERRAT and Verify results

Model No	Templates Used	ERRAT Overall quality factor	Verify3D (% of the residues had an averaged 3D-1D score >= 0.2)
1		70.341	64.02
2		65.796	60.66
3	4ZUD	73.243	64.25
4		67.733	59.64
5		62.032	48.87
6		58.967	41.20
7		56.720	40.66
8	4YAY	56.836	40.66
9		55.645	34.30
10		58.981	39.67
11		54.354	34.30
12		53.743	35.32
13	2LNL	56.417	25.35
14		56.464	27.65
15		55.585	37.37
16		69.146	32.51
17		61.905	29.18
18	4XT1	66.485	28.67
19		65.746	37.11
20		65.651	30.97
21		54.974	39.92
22		55.643	40.69
23	3OE0	51.047	39.16
24		61.067	39.41
25		53.927	40.95

TALW[20] program, multiple sequence alignment was done to find conserved residues. Various models were developed. The alignment of the templates with the receptor B2 receptor was represented in Fig. 1.

3.2. Model Validation

The predicted models were validated using various validation techniques. Root mean square deviation (RMSD) of all the predicted models with their respective template was calculated. Ramachandran plot was generated for each model, and the number of residues in the favorable, allowed, and disallowed region was identified. The statistics of both RMS deviation and Ramachandran plots are represented in Table 2. Only models scoring acceptable results are displayed and are numbered. Verify3D was also performed for all the models. Finally, ERRAT plots were developed for the

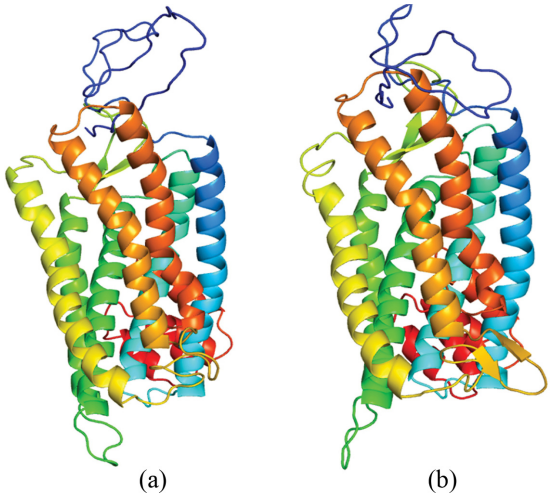

Fig. 2. Best models (Model13 and Model 28) selected after validation.

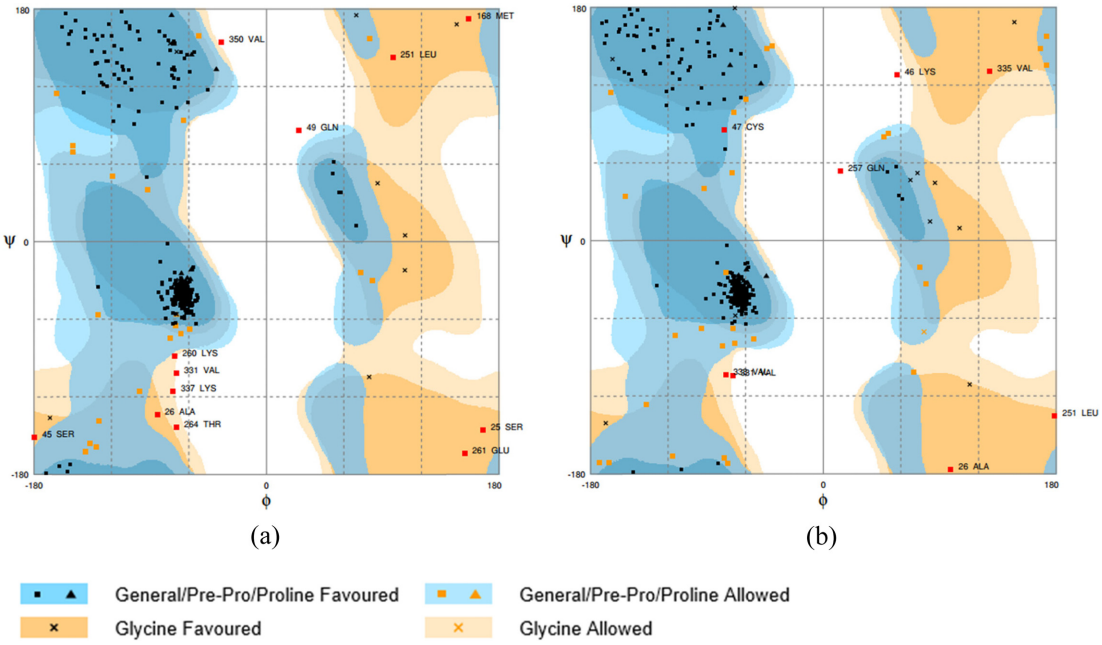

Fig. 3. RC plot for selected models 1(a) and 3(b).

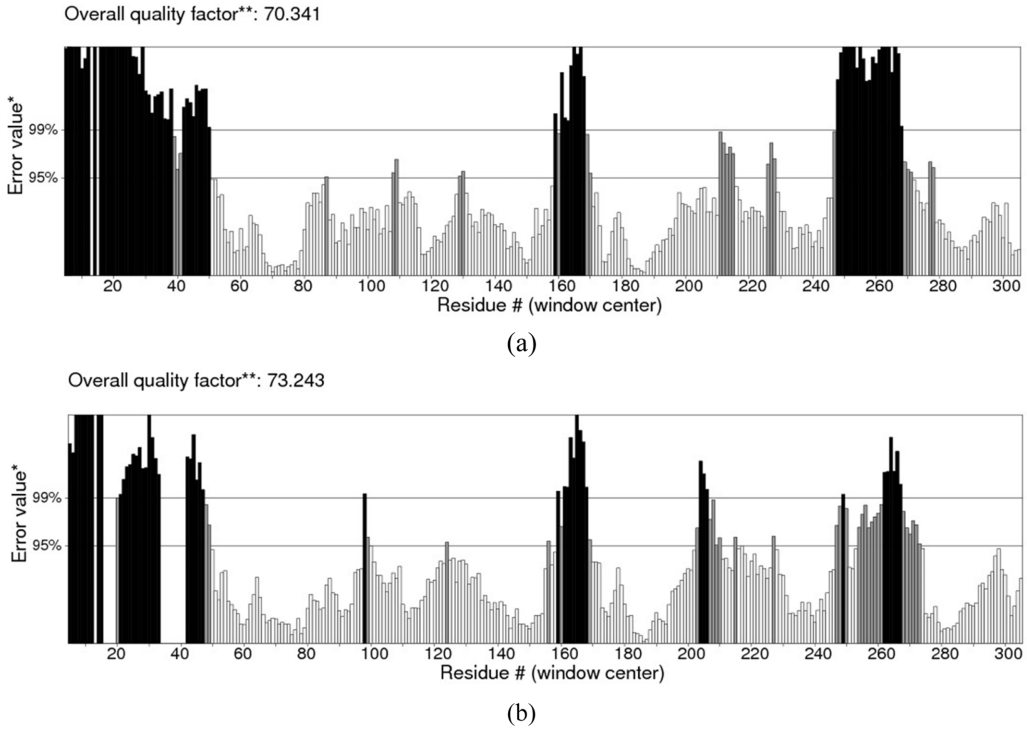

Fig. 4. ERRAT plot developed for the selected models 1(a) and 3(b).
*on the error axis, two lines are drawn to indicate the confidence with which it is possible to reject regions that exceed that error value

models. The results from Verify3D and ERRAT plots are represented in Table 3. Based on the statistics, from the models developed using Easymodeller, models 1 and 3 were found to be the best models. Especially, model 3 scored well in all the validation and is found to be the most reliable among the developed models. Also, all the developed models have a similar structure. The best models – Model 1 and Model 3 are represented in Fig. 2. Ramachandran plot and ERRAT plots of the selected models were represented in Fig. 3 and Fig. 4 respectively.

4. Conclusion

Three-dimensional models for B2 receptor were generated using multiple template based approaches. Model numbers 1 and 3 were selected as best, based on their RMS deviation, Ramachandran plot, ERRAT plot and Verify3D values. The selected models showed similar structures. Depending on the results of model validation, it is found that all the generated models are similar and the structures are reliable. These predicted models would be useful in the studying the interaction of the B2 receptor with bradykinin in future. Also, these models may serve as a reliable tool for analyzing the essential structural features and function of B2 receptor.

References

[1] J. M. Hall, "Bradykinin receptors" General Pharmacology: The Vascular System, Vol. 28, pp. 1-6, 1997.

[2] S. Yamaguchi-Sase, I. Hayashi, H. Okamoto, Y. Nara, S. Matsuzaki, S. Hoka, and M. Majima, "Amelioration of hyperalgesia by kinin receptor antagonists or kininogen deficiency in chronic constriction nerve injury in rats", Inflamm. Res., Vol. 52, pp. 164-169, 2003

[3] D. Regoli, S. N. Allogho, A. Rizzi, and F. J. Gobeil, "Bradykinin receptors and their antagonists", Eur. J. Pharmacol., Vol. 348, pp. 1-10, 1998.

[4] W. Gu, Z. Li, Z. Wang, Y. Liu, J. Liu, and S. Wen, "Association of the bradykinin receptors genes variants with hypertension: a case-control study and meta-analysis", Clin. Exp. Hypertens., Vol. 38, pp. 100-106, 2016.

[5] J.-X. Ma, D.-Z. Wang, D. C. Ward, L. Chen, T. Desai, J. Chao, and L. Chao, "Structure and chromosomal localization of the gene (BDKRB2) encoding human bradykinin B2 receptor", Genomics, Vol. 23, pp. 362-369, 1994.

[6] M. N. Perkins, D. Kelly, and A. J. Davis, "Bradykinin B1 and B2 receptor mechanisms and cytokine-induced hyperalgesia in the rat", Can. J. Physiol. Pharm., Vol. 73, pp. 832-836, 1995.

[7] Z. Chen, P. A. Deddish, R. D. Minshall, R. P. Becker, E. G. Erdös, and F. Tan, "Human ACE and bradykinin B2 receptors form a complex at the plasma membrane" FASEB J., Vol. 20, pp. 2261-2270, 2006.

[8] C. Tschöpe, H. P. Schultheiss, and T. Walther, "Multiple interactions between the renin-angiotensin and the kallikrein-kinin systems: role of ACE inhibition and AT1 receptor blockade", J. Cardiovasc. Pharm., Vol. 39, pp. 478-487, 2002.

[9] G. C. Baker, J. J. Smith, and D. A. Cowan, "Review and re-analysis of domain-specific 16S primers", J. Microbiol. Meth., Vol. 55, pp. 541-555, 2003.

[10] S. F. Altschul, W. Gish, W. Miller, E. W. Myers, and D. J. Lipman, "Basic local alignment search tool", J. Mol. Biol., Vol. 215, pp. 403-410, 1990.

[11] H. M. Berman, J. Westbrook, Z. Feng, G. Gilliland, T. N. Bhat, H. Weissig, I. N. Shindyalov, and P. E. Bourne, "The Protein data bank", Nucleic Acids Res., Vol. 28, pp. 235-242, 2000.

[12] S. K. Nagarajan and T. Madhavan, "Protein phosphatase 1D (PPM1D) structure prediction using Homology Modeling", J. Chosun Natural Sci., Vol. 9, pp. 35-40, 2016.

[13] B. Sathya and T. Madhavan, "Homology modeling of cysteinyl leukotriene1 receptor", J. Chosun Natural Sci., Vol. 8, pp. 13-18, 2015.

[14] S. K. Nagarajan and T. Madhavan, "3D Structure prediction of thromboxane A2 receptor by homology modeling", J. Chosun Natural Sci., Vol. 8, pp. 75-79, 2015.

[15] B. K. Kuntal, P. Aparoy, and P. Reddanna, "EasyModeller: A graphical interface to MODELLER", BMC Research Notes, Vol. 3, pp. 226, 2010.

[16] N. Eswar, M. A. Marti-Renom, B. Webb, M. S. Madhusudhan, D. Eramian, M. Shen, U. Pieper, and A. Sali, "A Comparative protein structure modelling with Modeller", Current Protocols in Bioinformatics, Vol. 5, pp. 1-5, 2006.

[17] S. C. Lovell, I. W. Davis, W. B. Arendall III, P. I. W. de Bakker, J. M. Word, M. G. Prisant, J. S. Richardson and D. C. Richardson, "Structure validation by Cα geometry: Φ, Ψ and Cβ deviation," Proteins, Vol. 50, pp. 437-450, 2002.

[18] J. U. Bowie, R. Lüthy, and D. Eisenberg, "A method to identify protein sequences that fold into a known three-dimensional structure", Science, Vol. 253, pp. 164-170, 1991.
[19] C. Colovos and T. O. Yeates, "Verification of protein structures: patterns of nonbonded atomic interactions", Protein Sci., Vol. 2, pp. 1511-1519, 1993.
[20] J. D. Thompson, D. G. Higgins, and T. J. Gibson, "CLUSTAL W: improving the sensitivity of progressive sequence weighting, position-specific gap penalties, and weight matrix choice," Nucleic Acids Res., Vol. 22, pp. 4673-4680, 1994.