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Protein-Protein Interaction Analysis of Corticotropin – Releasing Hormone Receptor 1 with Corticotropin-Releasing Hormone and Sauvagine

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Abstract

Corticotropin - releasing hormone receptor 1 (CRHR1) forms an integral part of the pathophysiology of disorders like post-traumatic stress disorder, stress, anxiety, addiction, and depression. Hence it is essential to look for new, potent and structure-specific inhibitors of CRHR1. We have analysed the protein-protein interaction complexes of the CRHR1 receptor with its native ligand CRF and full agonist Sauvagine. The structure of Sauvagine was predicted using homology modelling. We have identified that the residues TYR253, ASP254, GLU256, GLY265, ARG1014 and LY1060 are important in the formation of protein-protein complex formation. Future studies on these residues could throw light on the crucial structural features required for the formation of CRHR1-inhibitor complex and in studies that try to solve the structural complexities of CRHR1.

Keywords: Corticotropin - Releasing Hormone Receptor 1, CRHR1, CRF, Sauvagine, Protein-Protein Docking

1. Introduction

Corticotropin - releasing hormone (CRH), a peptide hormone with 41 amino acid residues, plays a major role as a neurotransmitter in response to stress[1]. It is expressed in the placenta, peripheral tissues, T lymphocytes and paraventricular nucleus (PVN) of the hypothalamus as a stress response[1]. The function of CRH is mediated via a GPCR called as Corticotropin -releasing factor receptor (CRFR or CRHR)[2]. CRHR has two different types: CRHR1 and CRHR2 each encoded by separate genes[3]. CRHR1 receptor controls the expression of ACTH in the pituitary, thereby mediating the stress response. CRHRs controls the hypothalamic pituitary adrenal axis (HPA axis), a major part of the fight or flight response to stress[4]. In major depression and Alzheimer's disease, there is an increase in the level of CRH level has been observed[5]. Early life stress could lead to the chronic activation of CRHR1 by CRH and could result in anxiety, learning impairments and memory deficits in adulthood. CRF is involved in the pathophysiology of various disorders that includes addiction, stress, anxiety, depression, and post-traumatic stress disorder in the central nervous system. In the stress-induced phosphorylation of tau, CRF could be a possible link between Alzheimer's disease pathology and stress[6], and cancer and stress in the peripheral nervous system. It plays a key role in the activation of neurons that leads to the development and maintenance of bone cancer pain.

Hence, the need for look-out for the novel and potent inhibitors of CRHR for the treatment of stress-related disorders becomes essential. But the possible positives of CRHR blocking is still to be studied. The available antagonists for CRHR1 include CP-154,526, CP-316311, Antalarmin and Pexacerfont. Clinical trials of Pexacerfont[7] and animal studies of Antalaramin as a treatment for disorders linked with anxiety are currently under study. However, the studies have been less successful on comparison with standard antidepressant drugs[8]. In a double-blind study for depression, CP-316311 showed unsuccessful results[9] and CP-154,526 is currently being studied for its possible capability to curb alcoholism[10]. Therefore there is an essential reason to identify new, structure specific CRHR inhibitors and to understand their structural complexity for better comprehension of the pathophysiologies related to them. In previous

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studies, we have performed 3D QSAR and molecular docking of CRHR1 with its ligands, to study the important physiochemical features responsible for the ligand bioactivity and the crucial residues playing major role in the protein-ligand complex formation[11,12]. In this study, we have performed protein-protein docking of CRHR1 with its native ligand, CRF and agonist Sauvagine.

2. Material and Methods

2.1. Homology Modelling

As there is no three dimensional structure available for the Sauvagine in the Protein Data Bank, using homology modelling the structure of the peptide was predicted. The amino acid sequence of the Sauvagine peptide GPPISIDLSLELLRKMIEIEKQEKEKQQAANNRLLLDTI. PEP-FOLD3[13], an online server *ab initio* protein structure prediction server which works based on Hidden Markov Model, was used to predict the three dimensional structure of the peptide l[13]. Five top ranked models were selected from the PEP-FOLD3 and model validation based on ProSA, ERRAT and Ramachandran plots was followed. Ramachandran plots were developed using the RAMPAGE web server[14], which helped in identifying the sterically allowed regions for the backbone dihedral angles ψ against φ of the amino acid residues. ERRAT plots helped in analysing the non-bonded atom-atom interactions present in the structure by plotting them as a function of the position of a sliding 9-residue window[15]. ProSA, is an interactive web server which helps in identifying the errors in three dimensional structures of the protein, was also used to calculate quality scores for the models[16].

2.2. Protein-Protein Docking

ClusPro 2.0[17,18] is an online server was used to perform the protein-protein docking of CRHR1 with the peptides CRF and Sauvagine. ClusPro is considered to the best protein-protein docking web server, as it has ranked well in the critical assessment of prediction of interactions (CAPRI)[19,20]. PIPER, a correlation method based on which the ClusPro server works[21-23], uses fast Fourier transform (FFT) coupled with pairwise interaction potentials to calculate the binding conformation energy. Hence, much fewer near-native structures were only retained and the structures were clustered and optimized using pairwise RMSD as the distance measure.

3. Results and Discussion

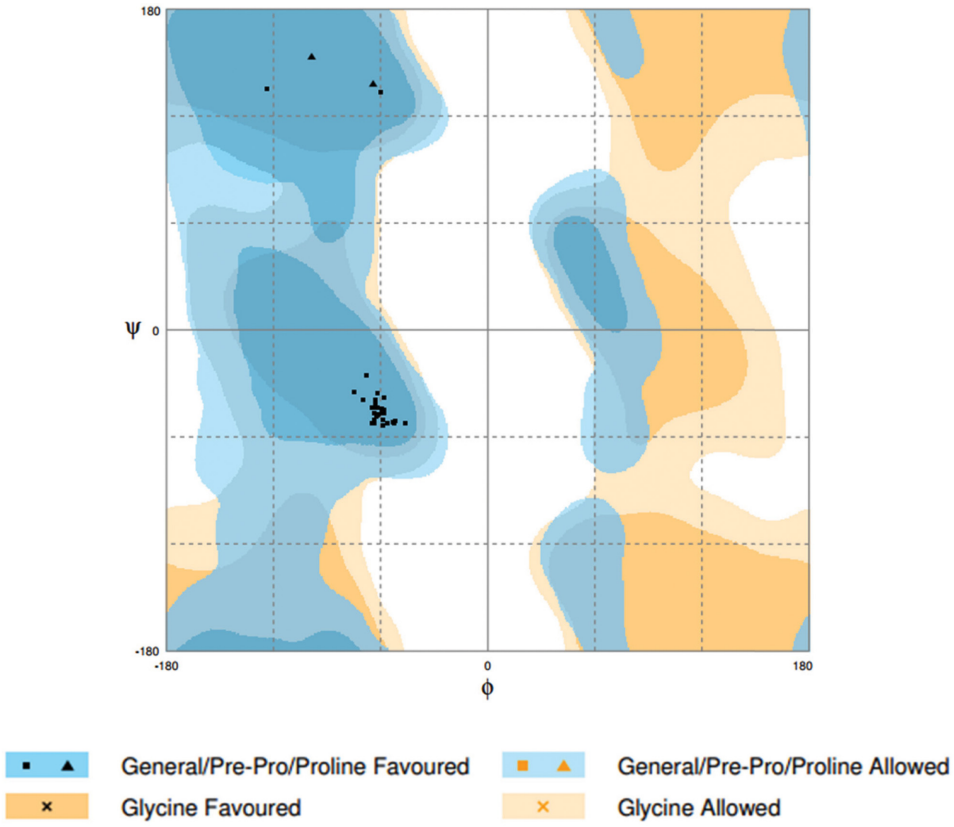
3.1. Model Validation

The model validation of all the selected models was performed using Ramachandran(RC) plot, ProSA and ERRAT plot. Based on RC plot, we have identified that top three models had 100.00% of their amino acid residues in the favourable region. All the models scored a quality score of 100 based on the validation by ERRAT plot. The ProSA quality scores for the models were -2.00, -1.82, -1.87, -2.07 and -2.12 respectively. Table 1 represents the statistics of model validation. Based on the statistics, we have identified the model **1** as the most reliable model (Fig. 1). RC plot and ERRAT plot of the selected model are represented in Fig. 2.

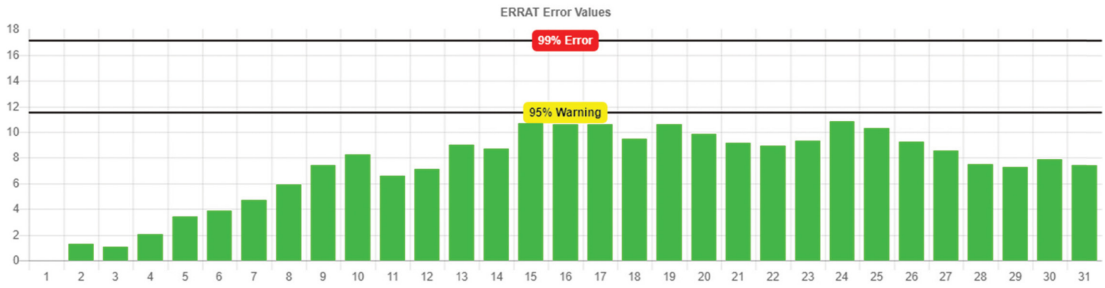
Fig. 1. Best model (Model **1**) of Sauvagine selected after validation

Table 1. Homology modelling validation results

Model No	Ramachandran Plot			ProSA Z-Score	ERRAT Overall quality factor
	Number of residues in favored region (%)	Number of residues in allowed region (%)	Number of residues in outlier region (%)		
1	100.00	0.00	0.00	-2.00	100.00
2	100.00	0.00	0.00	-1.82	100.00
3	100.00	0.00	0.00	-1.87	100.00
4	97.20	2.80	0.00	-2.07	100.00
5	97.20	2.80	0.00	-2.12	100.00



(a)



(b)

Fig. 2. Ramachandran plot (a) and ERRAT plot (b) of the selected model of Sauvagine.

3.2. Protein-Protein Docking

The crystal structure of human corticotropin-releasing factor receptor 1 (PDB ID: 4K5Y) and Corticotropin Releasing Hormone (PDB ID: 1GOE) were downloaded from the PDB. Using ClusPro, the two proteins were docked and 29 different clusters of docked complexes were generated. There were 126 members in the top cluster, and the lowest energy weighted score was -1519.5 (Table 2). In the case of Sauvagine, the selected model after model validation was docked with the crystal structure of CRHR1. 29 different clusters of docked complexes were generated and there were 97 members in the top cluster with a lowest energy weighted score of -1165.0 (Table 3). It is important to note that both

Table 2. Cluster Scores developed using ClusPro server for CRHR1 receptor – CRF complex

Cluster	Members	Representative	Weighted Score
0	126	Center	-1237.0
		Lowest Energy	-1519.5
1	85	Center	-1227.3
		Lowest Energy	-1423.2
2	59	Center	-1153.8
		Lowest Energy	-1375.1
3	44	Center	-1193.0
		Lowest Energy	-1442.7
4	43	Center	-1321.0
		Lowest Energy	-1349.5
5	36	Center	-1255.9
		Lowest Energy	-1357.7
6	35	Center	-1179.5
		Lowest Energy	-1313.8
7	32	Center	-1204.6
		Lowest Energy	-1344.5
8	31	Center	-1186.2
		Lowest Energy	-1332.0
9	31	Center	-1273.3
		Lowest Energy	-1302.1
10	31	Center	-1193.7
		Lowest Energy	-1303.7
11	31	Center	-1227.3
		Lowest Energy	-1352.2
12	29	Center	-1181.6
		Lowest Energy	-1380.7
13	28	Center	-1167.7
		Lowest Energy	-1337.9
14	27	Center	-1162.2
		Lowest Energy	-1262.9
15	23	Center	-1186.4
		Lowest Energy	-1343.0
16	22	Center	-1207.8
		Lowest Energy	-1285.5
17	21	Center	-1368.3
		Lowest Energy	-1368.3
18	20	Center	-1204.3
		Lowest Energy	-1337.7
19	20	Center	-1187.5
		Lowest Energy	-1259.2
20	17	Center	-1224.2
		Lowest Energy	-1294.0
21	15	Center	-1196.0
		Lowest Energy	-1355.9
22	15	Center	-1169.7
		Lowest Energy	-1243.4
23	14	Center	-1229.1
		Lowest Energy	-1229.1
24	14	Center	-1156.7
		Lowest Energy	-1218.6
25	13	Center	-1179.5
		Lowest Energy	-1361.7
26	13	Center	-1248.5
		Lowest Energy	-1248.5
27	12	Center	-1195.3
		Lowest Energy	-1229.6
28	11	Center	-1211.7
		Lowest Energy	-1306.2
29	11	Center	-1157.5
		Lowest Energy	-1199.7

Table 3. Cluster Scores developed using ClusPro server for CRHR1 – Sauvagine complex

Cluster	Members	Representative	Weighted Score
0	97	Center	-1001.4
		Lowest Energy	-1165.0
1	80	Center	-1031.7
		Lowest Energy	-1267.2
2	59	Center	-1029.5
		Lowest Energy	-1251.5
3	56	Center	-1077.1
		Lowest Energy	-1239.0
4	46	Center	-1001.1
		Lowest Energy	-1088.6
5	37	Center	-965.7
		Lowest Energy	-1180.8
6	36	Center	-975.6
		Lowest Energy	-1114.2
7	31	Center	-990.6
		Lowest Energy	-1063.2
8	29	Center	-960.6
		Lowest Energy	-1140.3
9	29	Center	-984.8
		Lowest Energy	-1121.2
10	28	Center	-1032.8
		Lowest Energy	-1165.6
11	28	Center	-980.2
		Lowest Energy	-1142.5
12	28	Center	-1089.6
		Lowest Energy	-1089.6
13	27	Center	-1023.1
		Lowest Energy	-1187.2
14	26	Center	-962.4
		Lowest Energy	-1063.2
15	24	Center	-1128.1
		Lowest Energy	-1128.1
16	20	Center	-1016.6
		Lowest Energy	-1101.7
17	17	Center	-956.0
		Lowest Energy	-1071.3
18	17	Center	-1059.3
		Lowest Energy	-1059.3
19	16	Center	-965.0
		Lowest Energy	-1084.3
20	16	Center	-963.1
		Lowest Energy	-1073.8
21	16	Center	-1039.5
		Lowest Energy	-1071.7
22	14	Center	-993.1
		Lowest Energy	-1045.2
23	14	Center	-990.3
		Lowest Energy	-1033.3
24	13	Center	-1013.6
		Lowest Energy	-1026.6
25	13	Center	-975.8
		Lowest Energy	-1039.7
26	12	Center	-995.6
		Lowest Energy	-1107.3
27	12	Center	-987.7
		Lowest Energy	-1072.2
28	10	Center	-973.3
		Lowest Energy	-1024.7
29	10	Center	-956.7
		Lowest Energy	-1064.6

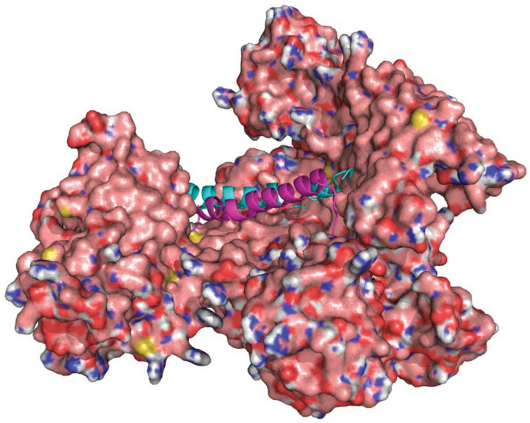


Fig. 3. Both the peptides, CRF and Sauvagine sharing the same binding spot is represented. Here, the CRF peptide is represented in cyan color and Sauvagine is represented in purple color.

the peptides shared the same binding space, which is represented in Figure 3. On analysing the complexes, we have identified that the residues TYR253, ASP254, GLU256, GLY265, ARG1014 and LY1060 formed hydrogen bonds with the peptides.

4. Conclusion

In this study, we have generated three dimensional conformation of Sauvagine peptide using homology modelling. After model validation, model **1** was selected as the most reliable model. Protein-protein interaction analysis of CRHR1 with the peptides CRF and Sauvagine was performed. The residues TYR253, ASP254, GLU256, GLY265, ARG1014 and LY1060 were identified to be the important residues that form the binding site of CRHR1. The results of this study could throw light on the complexities of the CRHR structure and in the pharmaceutical studies regarding the disorders related with CRHR.

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