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Protein-protein Interaction Analysis of Glucagon-like Peptide-2 Receptor with Its Native Ligand Glucagon-like Peptide-2

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Abstract

Glucagon like pepide-2, one of the GLPs, is involved in various metabolic functions in the gastrointestinal tract. It plays a major role in the regulation of mucosal epithelium and the intestinal crypt cell proliferation. Because of their therapeutic importance towards the diseases in the gastrointestinal tract, it becomes necessary to study their interaction with its receptor, GLP-2R. In this study, we have developed protein-protein docking complexes of GLP-2 – GLP-2 receptor. Homology models of GLP-2 are developed, and a reliable model out of the predicted models was selected after model validation. The model was bound with the receptor, to study the important interactions of the complex. This study could be useful in developing novel and potent drugs for the diseases related with GLP-2.

Keywords: Glucagon-like Peptide-2 Receptor, GPCR, GLP-2, Protein-protein Docking

1. Introduction

Glucagon and glucagon like peptides (GLPs) which are secreted in the pancreas, gut, CNS and PNS, regulates various metabolic functions[1], including the control of nutrient assimilation and hepatic glucose production[2]. GLPs are derived from a common precursor, proglucagon, and later matured in the endocrine cells present in the gastronstestinal tract and glucagon in the pancreatic cells into GLPs-1 and -2 respectively[3]. They share a considerable sequence homology among themselves. The aminoacid identity of GLP-1 and GLP-2, with glucagon ranges from 21% to 48%. Glucagon receptors, a subfamily of G-protein coupled receptors, mediate the functions of GLPs. Glucagon-like peptide 2 receptor (GLP-2R) is a one of the glucagon receptor, mediates the role of the GLP-2[4].

GLP-2, a pleiotropic intestinotropic hormone, is expressed in the gastrointestinal tract. It controls the nutrient homeostasis proximal to nutrient assimilation by controlling the stasis of mucosal epithelium. GLP-2 controls the intestinal crypt cell proliferation which directly affects the cellular response to external injury, through signalling via GLP-2 receptor[5]. GLP-2 was identified to be consistently effecting villus growth of the jejunm and ileum and an increase in the bowel weight which leads small bowel epithelial proliferation[6]. GLP-2 is therapeutically attractive towards diseases related to the regulation of mucosal health in the gastrointestinal tract. It has to be noted that GPCRs have taken centre stage in the recent drug discovery, and nearly a third of all the marketed therapeutics are targeted towards GPCR[7]. GLP-2, the native ligand of the GPCR, GLP-2R, does not have a co crystallised structure, in spite of its therapeutic potential. Hence, in this study we have predicted the three dimensional structure of GLP-2 using homology moedeling.

Homology modelling is an alternate tool helps in predicting the three-dimensional conformation of a protein, when only the sequence data of the protein is available. Due to the enormous amount of time required to prepare protein for crystallization using experimental process such as protein expression, purification and crystallization, the number of protein structures resolved experimentally lags behind the sequence data available[8]. Homology modelling 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 GLP-2 based on homology modelling and validated them. The developed models were then docked with the models of

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GLP-2R. The crucial residues of the binding interaction identified using this study could be helpful in identifying novel and potent drugs for the treatment of the diseases related to GLP-2R.

2. Material and Methods

2.1. Homology Modelling

The amino acid sequence of the human Glucagon-like peptide-2 is HADGSFSDEMNTILDNLAARDFINWLIQTKITD. Using the modelling platforms, QUARK and PEP-FOLD3, the three dimensional structure of the peptide was predicted. QUARKS is an online server for ab initio protein structure prediction[9], was used to model the three dimensional structures of human neuromedin U protein. QUARK aims to model the correct protein three dimensional structure from amino acid sequence, using a computer algorithm which includes ab initio protein folding and protein structure prediction. They develop models from small fragments (1-20 residues long) by replica-exchange Monte Carlo simulation using an atomic-level knowledge-based force field. It was the No 1 ranked Free-modeling (FM) online server in CASP9 and CASP10 experiments. As the QUARK uses no global template information, it is suitable for proteins without any homologous templates. PEP-FOLD3 is a *de novo* structure prediction method, based on Hidden Markov Model[10].

10 models and 5 models were developed using the QUARK server and PEP-FOLD3 respectively. The predicted models were validated using Ramachandran plots, ERRAT plots and ProSA. RAMPAGE web server was used to plot the Ramachandran plots[11]. 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. ERRAT plots are plotted as a function of the position of a sliding 9-residue window[12]. The error function is based on the statistics of non-bonded atom-atom interactions present in the structure. QMEAN is a comprehensive scoring function for model quality assessment, which determines the compatibility of the predicted model by assessing the local structural quality of transmembrane protein models using statistical potentials[13].

2.2. Protein-protein Docking

ClusPro 2.0, a protein-protein docking server was used to perform protein-protein docking of Glucagon-like peptide-2 with the Glucagon-like peptide-2 receptor 2[14,15]. ClusPro is identified as the best web server to perform protein-protein docking and has performed well in the critical assessment of prediction of interac-

Table 1. Homology model validation results

Model No	Ramachandran Plot			QMEAN4 value	ERRAT Overall quality factor
	Number of residues in favored region (%)	Number of residues in allowed region (%)	Number of residues in outlier region (%)		
1	100.0	0.0	0.0	0.95	100.000
2	100.0	0.0	0.0	0.73	92.000
3	96.8	0.0	3.2	-0.77	100.000
4	100.0	0.0	0.0	-0.78	84.000
5	96.8	3.2	0.0	-0.60	56.000
6	96.8	3.2	0.0	-0.23	88.000
7	96.8	3.2	0.0	-0.70	80.000
8	100.0	0.0	0.0	-0.51	84.000
9	100.0	0.0	0.0	0.92	100.000
10	100.0	0.0	0.0	0.31	100.000
11	93.3	3.3	3.3	-1.84	100.000
12	90.0	3.3	6.7	-2.06	100.000
13	83.3	13.3	3.3	-1.95	100.000
14	73.3	20.0	6.7	-4.09	62.500
15	83.3	13.3	3.3	-2.99	100.000

tions (CAPRI)[16,17]. ClusPro works on a correlation method known as PIPER[18] which calculates the docked conformation energy in a grid using fast Fourier transform (FFT) coupled with pairwise interaction potentials. As a result of the more accurate pairwise interaction potential of PIPER, much fewer near-native structures were only retained. The structures were clustered based on the pairwise RMSD as the distance measure and were optimized.

3. Results and Discussion

3.1. Model Validation

The predicted models were validated using various

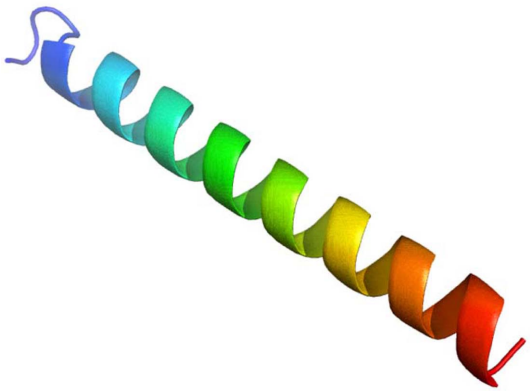

Fig. 1. Best model (Model 1) selected after validation.

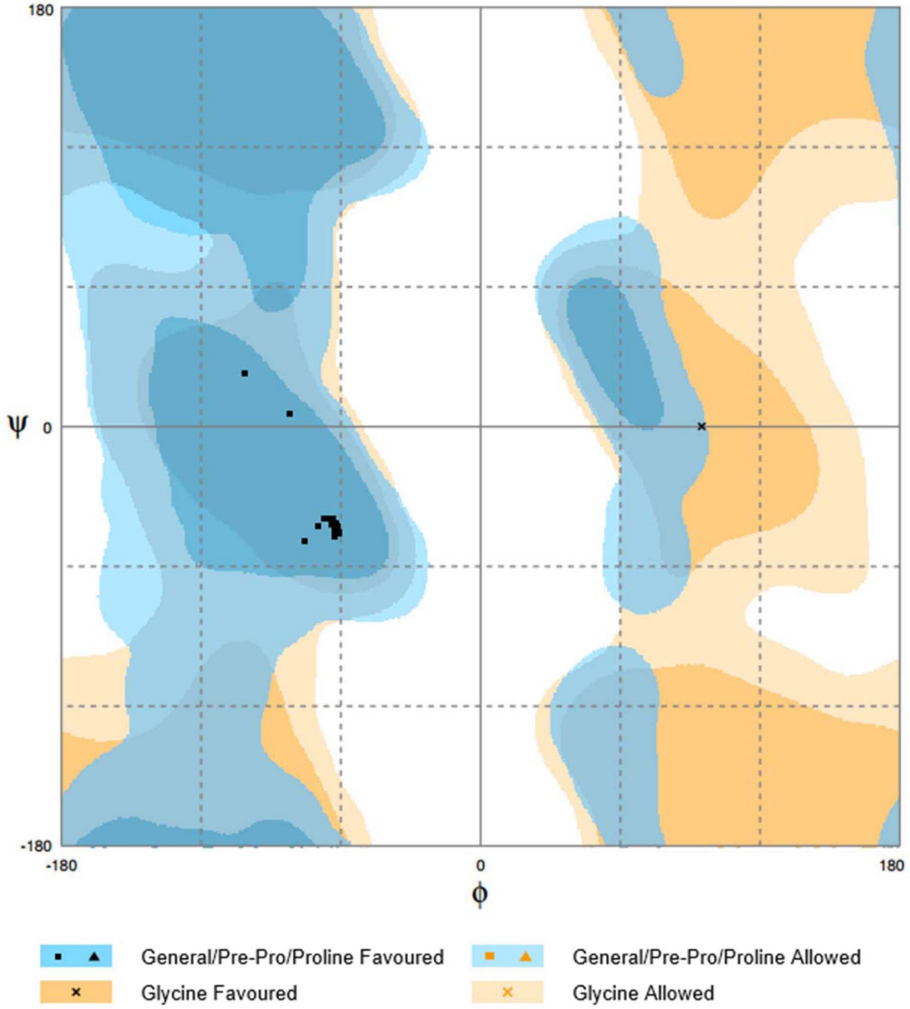

Fig. 2. RC plot for selected model – Model 1.

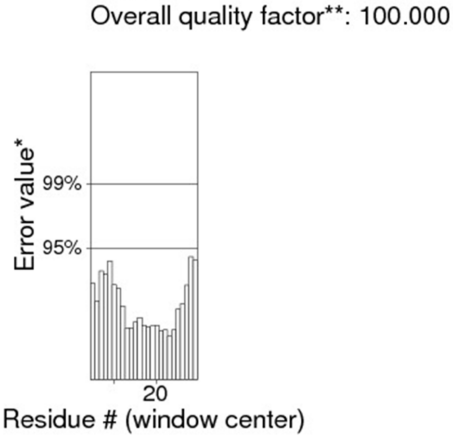

Fig. 3. ERRAT plot developed for the selected model – model 1. *on the error axis, two lines are drawn to indicate the confidence with which it is possible to reject regions that exceed that error value, ** Expressed as the percentage of the protein for which the calculated error value falls below the 95% rejection limit. Good high resolution structures generally produce values around 95% or higher. For lower resolutions (2.5 to 3Å) the average overall quality factor is around 91%

validation techniques. Ramachandran plot was generated for each model and the number of residues in favourable, allowed and disallowed region was identified. ERRAT plots were developed for the models. Using Swiss-Model web server QMEAN4 values were calculated. The statistics of model validation are represented in the Table 1. Based on the statistics, the model 1 was found to be the best models. Model 1 scored well in all the validation and found to be the most reliable among the developed models (Fig. 1). RC plot and ERRAT plots of the selected models were represented in Fig. 2 and Fig. 3 respectively.

3.2. Molecular Docking of GLP-2 Receptor – GLP-2

We have performed protein-protein docking to identify the crucial residues involved in the interaction of the natural agonist, GLP-2, with the receptor GLP-2R. CLUSPRO 2.0 server was used to do protein-protein docking, and 29 different clusters of docked complexes were generated. The top cluster consists of 87 members, and lowest energy weighted score was -836.8. The cluster scores are represented in the Table 2. The top cluster was chosen was studying the interaction between the

Table 2. Cluster Scores developed using ClusPro server

Cluster	Members	Representative	Weighted Score
0	87	Center	-824.2
		Lowest Energy	-836.8
1	76	Center	-774.1
		Lowest Energy	-901.3
2	66	Center	-763.8
		Lowest Energy	-812.1
3	65	Center	-759.1
		Lowest Energy	-823.5
4	56	Center	-778.3
		Lowest Energy	-801.1
5	49	Center	-792.7
		Lowest Energy	-799.7
6	48	Center	-784.2
		Lowest Energy	-815.1
7	46	Center	-825.2
		Lowest Energy	-825.2
8	45	Center	-765.3
		Lowest Energy	-808.6
9	45	Center	-755.2
		Lowest Energy	-870.6
10	36	Center	-837.7
		Lowest Energy	-837.7
11	34	Center	-775.6
		Lowest Energy	-835.8
12	32	Center	-772.5
		Lowest Energy	-804.1
13	30	Center	-777.3
		Lowest Energy	-820.5
14	30	Center	-760.1
		Lowest Energy	-800.9
15	28	Center	-803.3
		Lowest Energy	-818.4
16	24	Center	-794.4
		Lowest Energy	-811.2
17	20	Center	-779.0
		Lowest Energy	-799.1
18	18	Center	-757.9
		Lowest Energy	-805.9
19	18	Center	-816.8
		Lowest Energy	-836.5
20	17	Center	-795.7
		Lowest Energy	-795.7
21	14	Center	-777.0
		Lowest Energy	-791.1

Table 2. Continued

Cluster	Members	Representative	Weighted Score
22	12	Center	-767.5
		Lowest Energy	-773.2
23	11	Center	-760.3
		Lowest Energy	-811.6
24	11	Center	-790.7
		Lowest Energy	-790.7
25	8	Center	-758.1
		Lowest Energy	-785.8
26	8	Center	-793.1
		Lowest Energy	-804.3
27	7	Center	-775.0
		Lowest Energy	-792.0
28	6	Center	-754.7
		Lowest Energy	-776.7
29	4	Center	-756.8
		Lowest Energy	-765.4

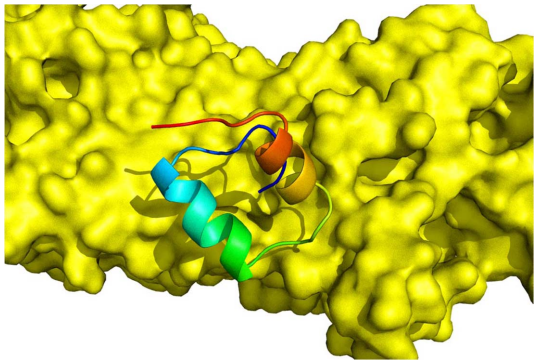

Fig. 4. Binding mode of the native ligand (GLP-2) with the receptor (GLP-2 receptor).

receptor and the ligand. We have identified the important residues involved in the interaction. Fig. 4 displays the binding mode of the peptide with the receptor.

4. Conclusion

Three dimensional models for GLP-2 were generated using the QUARK and PEPFOLD-3 web servers. Model number 1 was selected as the best model, based on their Ramachandran plot, ERRAT plot and QMEAN4 values. Based on the results after model validation, it is found that all the generated models are similar and the structures are reliable. The selected was then docked with a homology model of GLP-2 receptor. The resultant docked complex could help in identifying the crucial residues involved in the receptor-ligand complex formation.

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