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Three dimensional modeling of N-terminal region of galanin and its interaction with the galanin receptor

Marimuthu Parthiban[1] and Piramanayagam Shanmughavel[1, *]

[1]Department of Bioinformatics, Bharathiar University, Coimbatore - 641046, India; Piramanayagam Shanmughavel* - Phone: 91 422 2422655; Fax: 91 422 2422387; E-mail: shanvel_99@yahoo.com; * Corresponding author

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Abstract:
The neuropeptide galanin comes under the powerful and versatile modulators of classical neurotransmitters and is present in brain tissues, which are intimately involved in epileptogenesis. It acts as appealing targets for studying basic mechanisms of seizure initiation and arrest, and for the development of novel approaches for various neurodegenerative diseases. Galanin is widely distributed in the mammalian brain which controls various processes such as sensation of pain, learning, feeding, sexual behaviour, carcinogenesis, pathophysiology of neuroendocrine tumors and others. The function of galanin can be exploited through its interaction with three G-protein coupled receptors subtypes such as GalR1, GalR2 and GalR3. The N-terminal region of galanin comprises about highly conserved 15 amino acid residues, which act as the crucial region for agonist-receptor binding. We have constructed a theoretical structural model for the N-terminal region of galanin from *Homo sapiens* by homology modeling. The stereochemistry of the model was checked using PROCHECK. The functionally conserved regions were identified by surface mapping of phylogenetic information generated by online web algorithm ConSurf. The docking studies on the pharmacologically important galanin receptors with the theoretical model of N-terminal region of galanin predicted crucial residues for binding which would be useful in the development of novel leads for neurodegenerative disorders.

Keywords: binding site; agonist-receptor; docking; homology modeling

Background:
The neuropeptide galanin which is isolated by Tatemoto *et al* in 1983 is widely distributed in central and peripheral nervous systems. **[1]** Galanin, whose function is mediated through its interaction with G-protein coupled receptors (GPCR) is directly involved in the regulation of the release of neurotransmitters or hormones. Galanin comprises a total length of about 29 residues in most mammals with its C-terminal region amidated. In the case of *Homo sapiens*, galanin constitutes about 30 amino acid residues. The presence of galanin could modulate the action of classical neurotransmitters like acetylcholine, noradrenaline, serotonin and dopamine; inhibit the secretion of insulin, somatostatin and glucagon in pancreas, stimulate the release of growth hormone and prolactin in the pituitary. **[2]** Physiological and behavioral actions of galanin include the release of pituitary hormones, gastrointestinal and cardiovascular effects, up-regulation following neuronal injury, inhibitory effects on neuronal firing and neurotransmitter release, inhibitory effects on pain transmission, inhibitory effects on learning and memory, overexpression in the basal forebrain in Alzheimer's disease, and modulation of seizures, anxiety-related behaviors, depression-related behaviors, sexual behaviors, and feeding. **[3]** The activity of galanin is modulated by its interaction with three galanin receptor subtypes such as GalR1, GalR2 and GalR3, which belong to GPCR family. The N-terminal region of galanin (N-gal) which constitutes about 15 amino acids is highly conserved among mammals. **[4]** The absence of three dimensional structure for galanin acts as hindrance in the study of interaction of galanin with its receptors. Therefore, this work is an attempt to predict structural information on galanin at the N-terminal region (N-gal) and studying its interaction with galanin receptors, which would be useful for exploring the functional aspects of galanin.

Methodology:
The protein sequence of N-gal was extracted from the UniProtKB/Swiss-prot (http://www.expasy.org/uniprot) **[5]** database release 54.0. The main reason behind the choosing of N-terminal region was that, it acts as the crucial region of agonist receptor binding constituting about 15 amino acids, which are highly conserved. **[4, 6]** The sequence obtained from the Swiss-prot was submitted to NCBI-Blast (http://www.ncbi.nlm.nih.gov/blast/) **[7]** and searched against Protein Data Bank (PDB) database (http://www.rcsb.org/pdb/) **[8]** to extract suitable structural templates (Table 1 in supplementary material). The

sequence of N-gal and transportan was aligned using EBI-ALIGN server (http://www.ebi.ac.uk/emboss/align/). **[9]** The program MODELLER (http://salilab.org/modeller/) **[10]** was employed in the construction of theoretical three-dimensional structure of N-gal. The theoretical model was subjected to energy minimization in SwissPdbViewer **[11]** software for correcting the stereochemistry of the model. The geometric inaccuracies of the theoretical model was corrected and submitted the model to RCSB ADIT Validation server, a online web interface (http://deposit.pdb.org/validate/) which provides validation reports from PROCHECK **[12, 13]** to ensure the sterochemical quality. The program PROCHECK concentrates on the parameters such as bond length, bond angle, main chain and side chain properties, residue-by-residue properties, RMS distance from planarity and distorted geometry plots. The theoretical model was submitted to ConSurf (http://consurf.tau.ac.il/) **[14]** an automated web based server for the identification of functional region in proteins of known three dimensional structures by estimating the degree of conservation of the amino-acid sites among their close sequence homologues. The conservation grades are projected onto the molecular surface of these proteins to reveal the patches of highly conserved residues that are often important for biological function.ModBase (http://modbase.compbio.ucsf.edu/modbase-cgi/search_form.cgi) **[15]** is a queryable database of annotated comparative protein structure models where the theoretical 3D structures of galanin receptors GalR1 (P47211), GalR2 (O43603) and GalR3 (O60755) were obtained (Table 2 in supplementary material). The molecular interaction studies of theoretical model of N-gal with its three receptor types GalR1, GalR2, GalR3 were performed by program GRAMM (Global RAnge Molecular Matching **[16]** a program for protein docking. GRAMM predicts the structure of a complex using the atomic coordinates of the two molecules without information about the binding sites. The GRAMM methodology involves an empirical approach to smooth the intermolecular energy function by changing the range of the atom-atom potentials and locates the area of the global minimum of intermolecular energy for structures of different accuracy. The residue wise contacts between the complexes were tabulated to know about the role of key residues involved in the interaction between the neuropeptide and receptor.

Discussion:

Sequence of N-gal

The protein sequence of neuropeptide N-gal sequence (Uniprot/SwissProt ID: P22466) extracted from the UniProtKB/Swiss-prot database is 123 amino acids in total length, in which mature region of galanin is 30 residues and the source of the neuropeptide was of *Homo sapiens* in origin.

Template identification

The NCBI-BLAST results yielded a structure of transporton in phospholipid bicellar solution (PDB ID: 1SMZ) **[17]** as a template with a length of 28 residues. The N-gal (1-15) showed 100% identity with transportan (Figure 1). The structure of the template, transportan that is constructed from the peptides galanin and mastoparan is a chimeric cell-penetrating peptide.

Homology modeling

The lack of 3D structure for N-gal in PDB and in MODBASE interested us to construct the 3D model for neuropeptide N-gal. The most successful techniques for prediction of three dimensional structures of protein rely on aligning the sequence of a protein of to a homolog of known structure. In order to understand the binding characteristics as well as the structural and molecular level properties of the N-gal neuropeptide, we carried out homology modeling based on the NMR structure of transporton in phospholipid bicellar solution (PDB ID: 1SMZ) as the template.

Modeling of N-gal

The structure of N-gal was modeled using the program MODELLER, (Figure 2) produced different conformations using three input files such as alignment file in PIR format, template coordinate file and file containing sequence of the galanin to be modeled. These files were subjected to the program MODELLER with default parameters.

Refinement and evaluation of the quality of the model

The stereochemistry of the theoretical model of N-gal was done by subjecting it into energy minimization in SwissPDB Viewer. The model was subjected to structure verification and evaluation using PROCHECK. The Ramachandran plot for the model showed all residues in favored regions (100%) and other parameters for PROCHECK was in the allowed range (Figure 3).

Analysis on the crucial residues of theoretical model of N-gal

The ConSurf server was used to extract information about important residues, which are of functional value. This server provides evolutionary related conservation scores for residues, which could be correlated with biological function. In our case, the server predicted residues thr3, asn5, ser6, ala7, his14 and ala15 with high conservation scores (Table 3 in supplementary material). Among these, thr3 and ser6 were found to be having high score values indicating evolutionary conservation and hence its importance for function (Figure 4).

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Interaction of N-gal with three galanin receptors

Previous studies done on the interaction of N-gal with galanin receptor-2 (GalR2) by Lundstrom *et al* **[18]** revealed Trp2, Asn5, Gly8 and Tyr9 as the important ones. The hypothetical docking studies on the theoretical model of N-gal with three types galanin receptors of was performed to explore the importance of galanin residues involved in the interaction with the receptor (Table 4 and Table 5 in supplementary material). The theoretical 3D structures of galanin receptors GalR1, GalR2 & GalR3 obtained from MODBASE were subjected to docking studies in GRAMM with modified parameters. The insilico docking studies indicated that residues Trp2, Thr3, Asn5, Ser6, His14, and Ala15 of N-gal were found to be interacting with GalR1 (Figure 5a and Figure 6a). Trp2, Thr3, Asn5, Ser6, Ala7, and Tyr9 of N-gal were the important residues of galanin interacting in case of GalR2 (Figure 5b and Figure 6b). For GalR3 (Figure 5c and Figure 6c), the Ser6, Ala7, Tyr9, Ala15 of galanin were involved in binding. The ConSurf server studies predicted residues Thr3, Asn5, Ser6, and Ala7 of galanin as the evolutionarily conserved residues, which were reflected in our docking studies of galanin with the three galanin receptors. Moreover, Ser6 having highest conservation score was interacting with all three receptors. Thus, the residues that were predicted as important by ConSurf server were in concurrence with our hypothetical docking studies showing the importance of N-gal**.**

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N-Gal: GWTLNSAGYLLGPHA
1SMZ: GWTLNSAGYLLGK- -
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Figure 1: The sequence alignment between N-gal and transportan (PDB code: 1SMZ) performed using EBI-ALIGN server

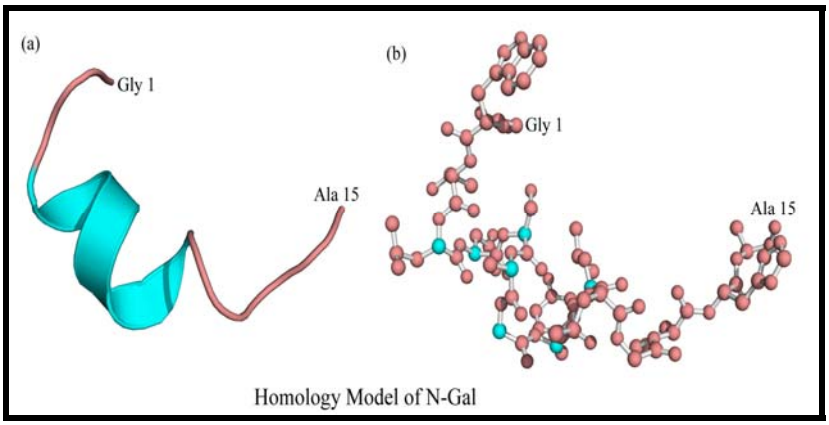

Figure 2: Theoretical model of N-gal. The modelling was done in MODELLER using default parameters. (a) Secondary structure (b) Ball and Stick. The images were generated using PYMOL. **[19]**

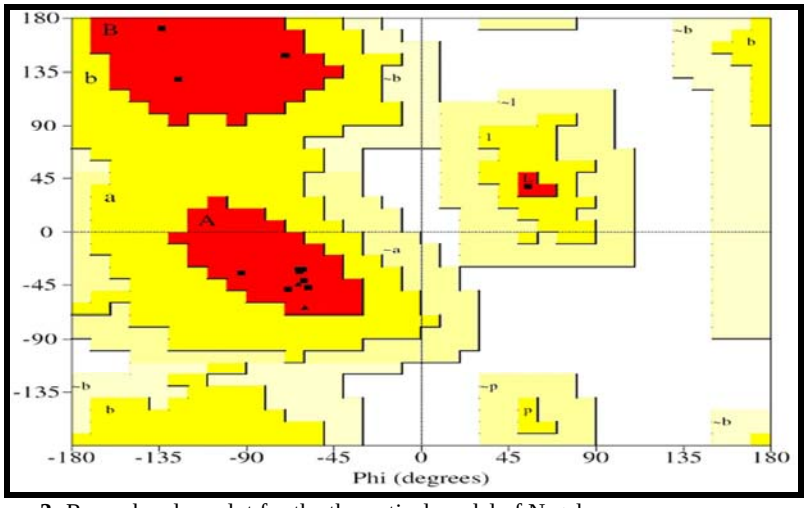

Figure 3: Ramachandran plot for the theoretical model of N-gal

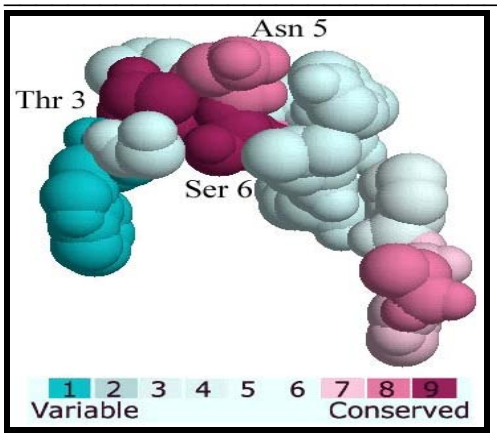

Figure 4: A model of N-gal according to ConSurf analysis: The aminoacids of N-gal are coloured in the range from turquoise to maroon based on conservation grades according to ConSurf description

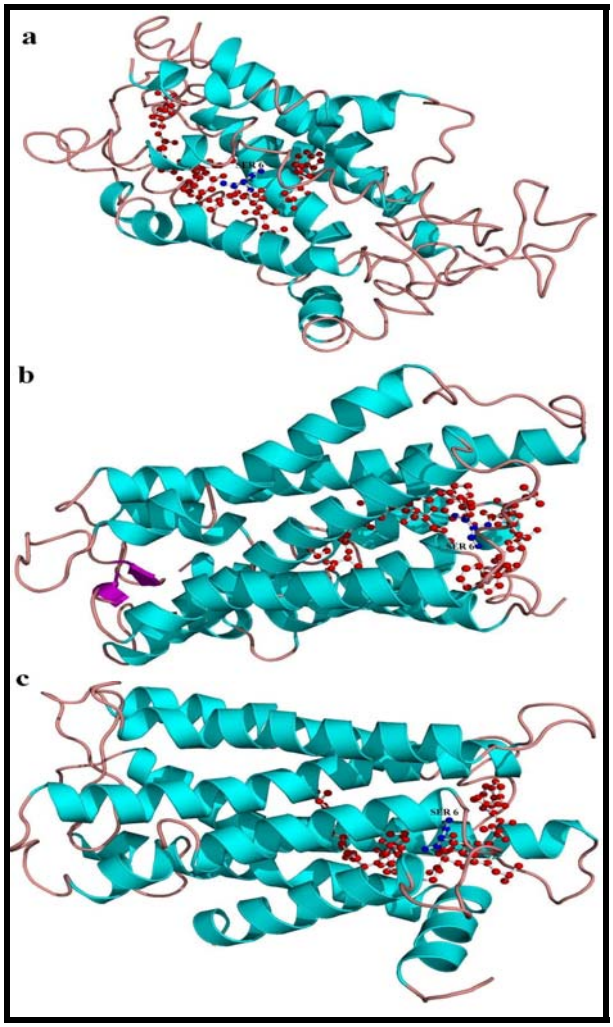

Figure 5: Representation of interaction between N-gal and three galanin receptors after docking. All the three receptors are represented in cartoon format and coloured according to secondary structure. The structure of N-gal is shown in ball and stick format in red colour and Ser6 in N-gal is highlighted using dark blue color. The docking was performed in GRAMM software. (a) N-gal with GalR1 (b) N-gal with GalR2 (c) N-gal with GalR3

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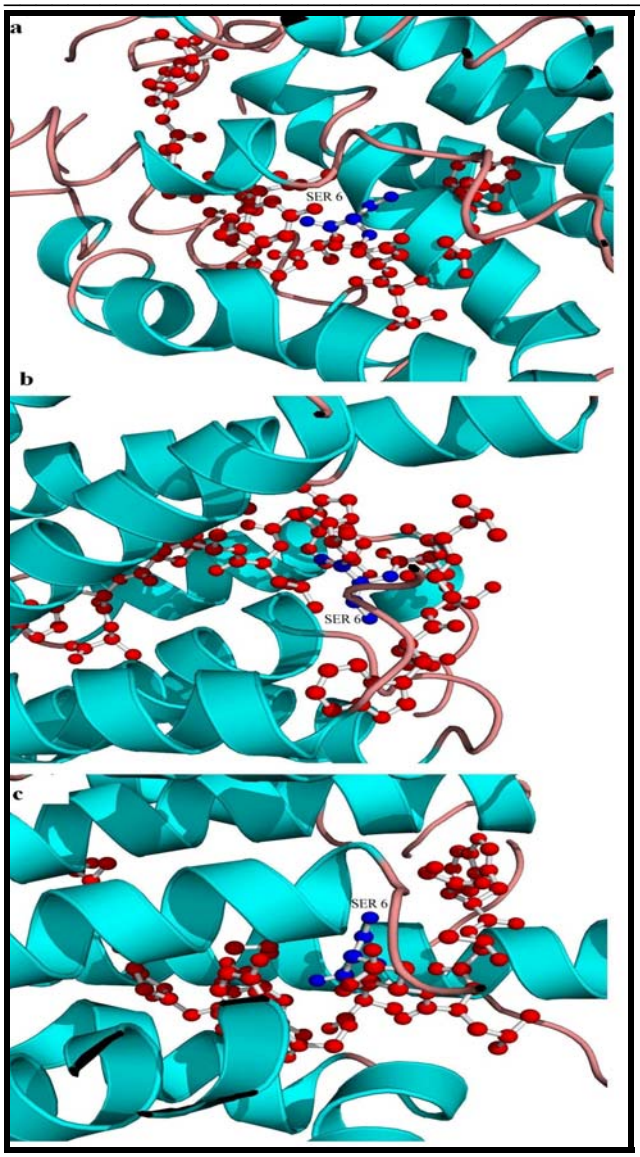

Figure 6: Representation of interaction between N-gal and three galanin receptors by close view. All the three receptors are represented in cartoon format and coloured according to secondary structure. The structure of N-gal is shown in ball and stick format in red colour. The Ser6 of N-gal is highlighted in dark blue color. The docking was performed in GRAMM software. (a) N-gal with GalR1 (b) N-gal with GalR2 (c) N-gal with GalR3

Conclusion:

The docking studies as well as the ConSurf analysis reveal the important residues involved in the interaction of N-gal region with various receptors and these residues might provide some valuable insight into the role of galanin in controlling or regulating various biological processes. Our work involved the homology modeling on N-gal, which would be useful in concentrating the binding feature of this neuropeptide. The hypothetical docking studies of N-gal with galanin receptor, ConSurf analysis and previous experimental studies are in concurrence with each other reflecting the importance of crucial residues. Thus the computational work on N-gal provides valuable information for future studies on galanin which could provide insight into the role of galanin in various biological processes.

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Supplementary material

Protein	SwissProt ID	Sequence length	Source	PDB code
Galanin	P22466	29	*Homo sapiens*	
Transportan		28	Scientific construct	1SMZ

Table 1: N-gal and its template data

Protein Name	Galanin receptor type 1 (GalR1)	Galanin receptor type 2(GalR2)	Galanin receptor type 3(GalR3)
SwissProt ID	P47211	O43603	O60755
Source	*Homo sapiens*	*Homo sapiens*	*Homo sapiens*
Sequence length	349	387	368

Table 2: Data on three galanin receptors

Residue No.	Name	Conservation score
1	GLY	3
2	TRP	1
3	THR	9
4	LEU	3
5	ASN	8
6	SER	9
7	ALA	8
8	GLY	3
9	TYR	3
10	LEU	3
11	LEU	3
12	GLY	3
13	PRO	4
14	HIS	7
15	ALA	8

Table 3: The amino acid conservation scores for N-gal predicted by ConSurf server

Complex	Residues of N-Gal involved in interaction
N-gal & GalR1	Trp2, Thr3, Asn5, Ser6, Leu11, Pro13, His14, Ala15
N-gal & GalR2	Gly1, Trp2, Thr3, Leu4, Asn5, Ser6, Ala7, Tyr9
N-gal & GalR3	Gly1, Ser 6, Ala7, Tyr9, Ala15

Table 4: The amino acid residues involved in interaction between N-gal and three galanin receptor types

Complex	Residues of GalR involved in interaction
N-gal & GalR1	TYR96, CYS108, MET119, SER121, SER122, SER122, LEU126, SER163, ASN183, VAL255, HIS267
N-gal & GalR2	THR91, ALA94, VAL95, SER96, ASP98, ARG99, ARG99, LEU125, GLY180, MET213, CYS222
N-gal & GalR3	VAL99, HIS101, LEU177, GLY191, GLY191, PRO192

Table 5: The amino acid residues involved in interaction between N-gal and three galanin receptor types