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STRUCTURAL CHARACTERIZATION OF NPAS4-ARNT DIMERIZATION THROUGH COMPUTATIONAL SIMULATION

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Abstract: Neuronal PAS Domain Protein 4 (Npas4) is an activity dependent transcription factor harboring basic helix-loop-helix (bHLH)-PAS domain, mediating the expression of target genes involved in neuro-transmission. NPAS4 crucially regulates response to various excitatory stimuli and has a role in GABAergic neuronal synapse development. Functionally, NPAS4 as a transcription factor dimerizes with the ARNT protein to serve as complete transcription factor and start the transcription of downstream genes. However, NPAS4 dimerization characteristics with ARNT has not been studied so far. Hence the current study aimed to identify the interaction pattern of NPAS4-ARNT complex through computational docking via HADDOCK. The interaction pattern were determined through pdbSum. The electrostatic surface calculations were performed through APBS plugins in PyMOL. The results indicated that PASB domain of NPAS4 is involved in interactions with the PAS B domain of ARNT. A toll of 136 structures generated by HADDOCK were further grouped into 14 clusters. The cluster with the minimum energy value of -82.6 KJ/mol was then further selected for interaction analysis. The results showed that there is one salt bridge, 12 H-bonding interactions and 156 non-bonded contacts between two proteins. The important interactions among two proteins are Asp224:NPAS4 and Gln421:ARNT, Asp229:NPAS4 and Ser442:ARNT, Glu232:NPAS4 and Thr361:ARNT, Phe240, Glu241:NPAS4, and Arg440:ARNT. The electrostatic potential of these two proteins revealed the binding interface of NPAS4 and ARNT to be neutral hence favoring hydrophobic interactions. The findings can help elucidate Npas4 role in interacting with other neuronal proteins involved in neuronal signaling. Moreover, the interaction findings provide useful comparative insight with other bHLH proteins.

Keywords: NPAS4, bHLH proteins, dimerization, neurotransmission, molecular simulation

Introduction

NPAS4 is an immediate-early gene (IEG) which encodes a transcription factor specific to neurons. This transcription factor is involved in regulation of expression of a large number of genes that mediate diverse effects on synapses. It plays an important role as an early transcription factor which induces late response gene expression in both excitatory and inhibitory neurons. Structurally, NPAS4 belongs to basic Helix-Loop-Helix (HLH) PAS protein family. This protein is specific to, and is highly expressed in brain in response to excitatory activity in the synapse [1]. NPAS4 protein expression is partially induced by calcium influx in neurons. This protein is particularly important in development and repair of inhibitory synapses where it regulates the expression of genes depending on the activity of neuron. These activity-dependent genes are involved in controlling the number of GABA-releasing synapses formed on excitatory neurons. Inhibitory pathways require a balance between neuronal excitation and inhibition [2]. NPAS4 transcription factor regulate the expression of genes which participate in maintaining this balance. NPAS4 regulates and balances the modification of synapses formed on excitatory and inhibitory neurons according to their function within the neuronal circuit. In excitatory neurons NPAS4 promotes BDNF gene expression which controls GABA releasing synapses and increases number of inhibitory synapse formed on excitatory neurons. In inhibitory neurons NPAS4 promotes expression of genes which increase feedback inhibition thereby increasing excitatory input in inhibitory neurons [3]. This

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balance in both types of neurons is implicated in a number of processes including formation of long and short-term memory of experience, fear, stress and social behavior.

NPAS4 is a transcription factor that regulates the expression of downstream genes upon dimerization with its partner aryl nuclear hydrocarbon receptor (ARNT). But how NPAS4 interacts with ARNT is not known yet. Hence current study was designed to understand the interaction pattern between NPAS4 and ARNT.

Material and Methods

Structure Preparation

Human NPAS4 is a non-structured protein of 802 amino acids long with structured motif present in its N-terminal (1-350 a.a). NPAS4 harbors bHLH (10-52 a.a), PASA (72-155 a.a), and PASB (216-273 a.a) domains. For docking studies, the structure of NPAS4 was determined previously through ab-initio modeling technique [4]. NPAS4 is dimeric transcription factor and the other partner is ARNT which also harbors bHLH, PASA, and PASB domains. It has been reported for other members of bHLH family that the PASB domain involved in dimerization hence in the present study PASB domain of NPAS4 and ARNT were used for interaction analysis. The structure of ARNT-PASB domain has been downloaded from protein data bank (pdb ID: 4EQ1) with resolution of 1.6 Å. Both the structures were then energy minimized using Amber99 force field implemented in Molecular Operating Environment (MOE).

Docking Studies:

The docking of NPAS4 with ARNT was performed through HADDOCK web server [5]. The PASB domain (a.a 220-250) of NPAS4 was selected as active site while the residues surrounding the active site were selected as passive site residues. In case of ARNT the active site was selected in the PASB domain (a.a 420-450) while the residues surrounding the active site were selected as passive site residues. Figure 1 is showing the active site of NPAS4 as well as ARNT.

Protein-protein Interaction Analysis:

The top ranking cluster generated by HADDOCK was further analyzed for protein-protein interaction analysis through pdbSUM [6] and PyMOL (Molecular Graphics System, Version 2.0 Schrodinger, LLC).

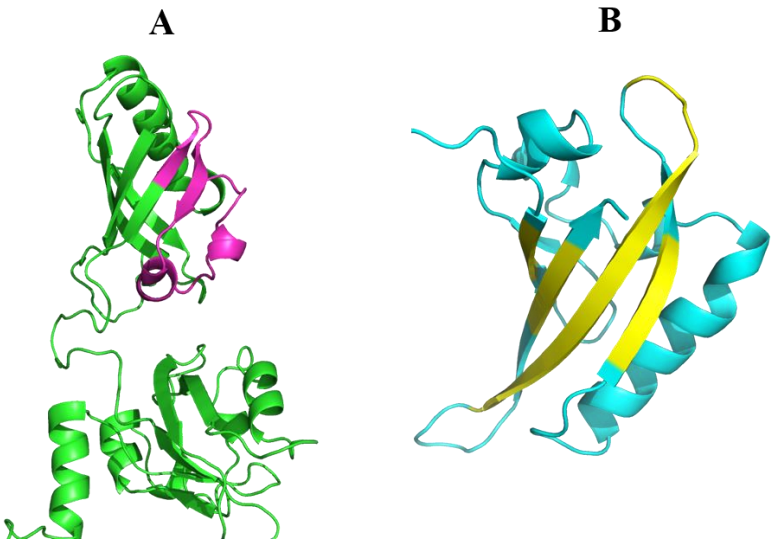

Figure 1: Active site of (A) NPAS4 highlighted as purple; (B) ARNT highlighted as yellow.

Research results

Protein-protein interactions of NPAS4 with ARNT: For protein-protein interaction analysis, the docking studies were performed. The PASB domain of NPAS4 and ARNT was employed for docking. A total of 137 structures are generated that grouped into 14 clusters. The top ranking cluster is having the minimum HADDOCK score (-82.6) and Z-score (-2.0). There were total 13 structures present in that cluster and the top four structures were then employed for further analysis. The HADDOCK score, RMSD, Z-score and different energy values (Vander walls, electrostatic, desolvation and restraint violation energy) of all the clusters is shown in Table 1.

Table 1: Parameters of top ranking clusters generated by HADDOCK.

Cluster No.	HADDOCK score	Cluster size	RMSD	Van der Waals energy (KJ/mol)	Electrostatic energy (KJ/mol)	Desolvation energy (KJ/mol)	Restraints violation energy (KJ/mol)	Buried Surface Area	Z-score
1.	-66.5	27	14.1	-51.3	-234.7	-6.1	379.5	1823.4	-1.1
2.	-53.3	17	11.5	-52	-234.5	-1.8	473.9	1787.3	-0.3
3.	-82.6	13	0.6	-71.9	-178	-36.8	617	2221.8	-2
4.	-29.8	12	12.2	-39.8	-180	-3.7	497.2	1517.5	1.1
6.	-53.3	9	12.9	-61.3	-146	-8.8	459.5	1849.2	-0.3
8.	-40.1	8	12.4	-52.4	-185.6	-8.2	576.5	1851.9	0.5
9.	-55.8	8	15.2	-60.7	-149.0	-15.9	505.8	1805.1	-0.5
10.	-24.4	6	8.9	-45.8	-118.5	-4.3	494.7	1489.8	1.4
11.	-32.9	6	18.8	-54.2	-100.9	-2.2	437	1563.2	0.9
13.	-40	4	7.7	-51.3	-138.1	-2.7	416.1	1461.5	0.5

By analyzing the interaction between these two proteins we found 20 residues of NPAS4 and 18 residues of ARNT at the dimer interface out of these residues we found 1 salt bridge and 12 H-bonding interactions while 155 non-bonded contacts among these residues. These interactions include: Asp224:NPAS4 and Gln421:ARNT, Asp229:NPAS4 and Ser442, Thr460:ARNT, Glu232, Ser233:NPAS4 and Glu362:ARNT, Phe240, Glu241, Arg242 :NPAS4, and Arg440:ARNT, Arg242:NPAS4 and Ser442,Thr460:ARNT, Glu304:NPAS4 and Tyr450:ARNT (Figure 2; Table 2).

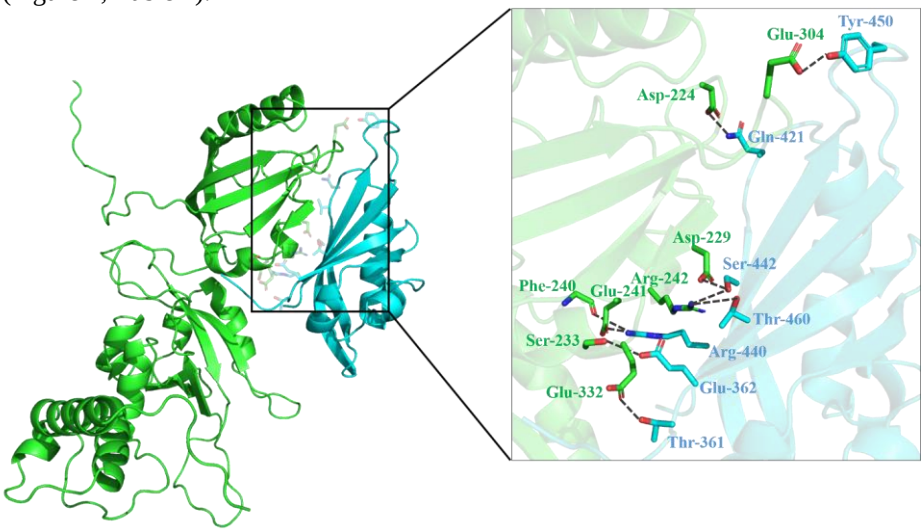

Figure 2: Top *ranking docked complex of NPAS4-ARNT. NPAS4 is shown in green while ARNT is in cyan.*

Table 2: Interacting residues of NPAS4 and ARNT.

NPAS4				ARNT		
S.No	**Atom Name**	**Residue Name**	**Type of interactions**	**Atom Name**	**Res Name**	**Distance**
1.	2110 OD2	ASP 224	H-bond	3800 NE2	GLN 421	2.74
2.	2151 OD1	ASP 229	H-bond	4039 OG	SER 442	2.61
3.	2151 OD1	ASP 229	H-bond	4218 OG1	THR 460	2.69
4.	2178 OE1	GLU 232	H-bond	3198 OG1	THR 361	2.76
5.	2186 OG	SER 233	H-bond	3210 OE2	GLU 362	3.23
6.	2255 O	PHE 240	H-bond	4021 NH2	ARG 440	3.33
7.	2262 OE1	GLU 241	H-bond	4018 NH1	ARG 440	2.61
8.	2262 OE1	GLU 241	Salt bridge	4021 NH2	ARG 440	3.24
9.	2275 NH1	ARG 242	H-bond	4025 O	ARG 440	2.89
10.	2275 NH1	ARG 242	H-bond	4039 OG	SER 442	2.89
11.	2275 NH1	ARG 242	H-bond	4222 O	THR 460	2.90
12.	2872 OE1	GLU 304	H-bond	4124 OH	TYR 450	2.80

Discussion:

NPAS4 is a transcription factor that encodes immediate early gene (IEG) which upon activation expresses a set of genes involved in cognition and plasticity of brain. Human Npas4 is mapped at chromosome 11q13.2 and has 11 exons encoding a 2406 bp transcript which translates into 802 amino acid long protein having 87.1 KDa molecular weight [7]. Although expression of this protein is highly specific to neuron, recent data also reports NPAS4 presence in pancreatic cells [8]. In neurons along with development and maintenance of inhibitory synapse NPAS4 also participates in dendritic growth. The primary function of NPAS4 protein is to regulate the gene involved in limbic patterning function and modulation by interacting with numerous other transcription factors [9]. Structurally, NPAS4 belongs to basic helix-loop-helix (bHLH) -PAS family of transcription factors. NPAS4 along with other members of this family, including NPAS1 and NPAS3, are associated with cognition-related disorders such as autism, bipolar disorders, schizophrenia and depression [10, 11]. The potential role of these proteins in the pathophysiology of said conditions can be understood by evaluating the interaction of NPAS4 with other bHLH-PAS proteins like ARNT.

Currently no structural data is available to characterize the interaction of NPAS4 with ARNT. The current study uses in-silico approaches to illustrate the first NPAS4-ARNT interaction and predict mechanistic links between NPAS4 and ARNT in order to evaluate the architecture of complete dimerized protein. HADDOCK was used for prediction of interaction pattern between NPAS4 and ARNT. Docking revealed interacting residues of both proteins in the heterodimer. The 20 residues of NPAS4 and 18 residues of ARNT are present at the heterodimer interface. The residues of NPAS4 at the interface are hydrophobic (Leu, Ser, Pro, Cys, Ala) and charged residues (Arg, Glu, Asp, and Lys) while the residues of ARNT at the interface are also hydrophobic (Ser, Thr, Val, Phe, Pro) and charged (Gln, Glu, Arg). It has been reported previously in case of HIF-1α, NPAS1 and NPAS3 that a hydrophobic cavity is present at the dimer interface of PASB domain [12], [13]. The same hydrophobic cavity is also observed in case of NPAS4 at the dimer interface formed by hydrophobic residues. The involvement of charged residues in the interactions showed the formation of stable NPAS4-ARNT complex. It is the property of all the bHLH-PAS domain containing protein that they form heterodimer with their partner ARNT and starts the transcription of downstream genes [14]. Same is the case with NPAS4, the stable interactions at the dimer interface resulted in stable activation of downstream genes and hence help in synaptic plasticity. The interactions identified in the study will help to understand the mechanism how NPAS4 interact with ARNT and it also providing a clue about the exact residues involved in interactions. This study will help in future to design some agonists or antagonists of NPAS4.

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