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Elucidating the precise interaction of reduced and oxidized states of Neuroglobin with Ubc12 and Cop9 using molecular mechanics studies

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Abstract

BACKGROUD & OBJECTIVE: Neuroglobin is an oxygen binding globin protein highly expressed in neurons. It is an iron containing heme protein, that exist in both ferrous and ferric form. Recent studies have indicated its role as an endogenous neuroprotective molecule. It is proposed that neuroglobin undergoes post-translational modification by the process of neddylation and deneddylation. Hence in this study an attempt was made to investigate the mode and mechanism of interaction of ferrous and ferric forms of neuroglobin with Ubc12 and op9, proteins involved in regulation of neddylation and deneddylation respectively, utilizing molecular modelling calculations.

METHODOLOGY: In this study, the mode of interactions of Ubc12 and Cop9 with the reduced (Fe^{2+}) and oxidized state (Fe^{3+}) of neuroglobin was carried out using ZDock. The top scoring poses for each complex were subjected to energy minimization using CHARMM Polar H force field in RDock to obtain the binding affinities.

RESULTS: The binding affinities between of ferrous and ferric forms of neuroglobin with Ubc12 and Cop9 were calculated.

CONCLUSION: Results obtained strongly indicate that both Ubc12 and Cop9 interacts with neuroglobin in regulation of neddylation and deneddylation process. Furthermore these results guided us in precisely designing experiments for biological evaluations.

Keywords: Neuroglobin, Cop9, Ubc12, ZDock, RDock

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1. Introduction

Neuroglobin (Ngb) is an oxygen binding protein, which was discovered in the year 2000 as third member of the globin family[1]. This protein is highly expressed in central nervous system and the peripheral nervous system[1, 2]. It is a highly conserved protein, with an evolutionary rate observed about threefold slower than that of Myoglobin and Hemoglobin[3]. The Structural unit of Neuroglobin consists of monomer of 150 amino acids with a molecular mass of 16 kDa[1, 4, 5]. It is an iron containing heme protein, and can exist in both ferrous (reduced) and ferric (oxidized) form.

In general any intracellular globin is associated with either storing Oxygen for hypoxic phases (like in diving mammals) or facilitating Oxygen diffusion from the capillaries to the respiratory chain in the mitochondria[6]. Recent studies have clearly indicated that neuroglobin protect the cells from stroke damage, amyloid toxicity and injury due to lack of oxygen[7, 8, 9, 10]. Low levels of neuroglobin have been associated with increased risk of Alzheimer's disease[11, 12].

It has been proposed that neuroglobin undergoes post-translational modification by the process of Neddylation that leads to protein degradation and Deneddylation which reverses the process of neddylation. The key player involved in the neddylation process is Ubiquitin-conjugating protein (Ubc12) and in deneddylation process is an isopeptidase signalosome (Cop9)—both proteins possibly interact with neuroglobin for the regulation of protein degradation[13, 14]. Our extensive computational modelling efforts presented here elucidate the mode and mechanism of interaction of ferrous and ferric forms of Neuroglobin with Ubc12 and Cop9. The best way to understand the protein-protein interactions is to obtain a co-crystal structure. However, in the absent of co-crystal structures of Neuroglobin with Ubc12 and Cop9, molecular modeling calculations could provide precise interactions between them and guided us in designing experiments for further validation.

2. Material and Methods

2.1 Materials

The coordinates of neuroglobin (PDB_ID: 1OJ6), Cop9 (PDB_ID: 4E0Q) and Ubc12 (PDB_ID: 2NVU) were obtained from protein Data Bank. After visual inspection chain B of 1OJ6, chain A of 4E0Q and chain C of 2NVU were kept for molecular modeling studies.

2.1 Protein Preparation

Neuroglobin (PDB_ID: 1OJ6) obtained from PDB represents the reduced form of neuroglobin (Fe^{2+}). Therefore, the oxidized form of neuroglobin was generated by modifying the charge of iron to Fe^{3+} using Schrodinger package. All the four proteins - neuroglobin (Fe^{2+}), neuroglobin (Fe^{3+}), Cop9 and Ubc12) were prepared using the multistep protein-preparation wizard (Schrodinger package). Explicit all atom model was applied, missing hydrogen atoms were added leaving no lone pair, water molecules were removed and thereafter structure was optimized. The proteins obtained were then energy minimized

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using OPLS 2005 force field with Polak-Ribiere Conjugate Gradient (PRCG) algorithm. The minimization was stopped either after 5,000 steps or after the energy gradient converged below 0.001 kcal/mol.

2.3 Protein interaction studies

The mode of interactions of both the proteins, Ubc12 and Cop9 with the reduced (Fe^{2+}) and oxidized state (Fe^{3+}) of neuroglobin was studied using a two step process that involved initial protein-protein docking using Zdock (version 2.3) followed by refinement of docked complex using Rdock. Zdock is a rigid-body docking algorithm based on the principle of Fast Fourier Transform (FFT) [15]. In Step 1 the putative binding poses were predicted using Zdock with 6° rotational sampling and randomized start position to obtain 54000 poses each for Ubc12 with the reduced (Fe^{2+}) and oxidized state (Fe^{3+}) of neuroglobin and Cop9 with the reduced (Fe^{2+}) and oxidized state (Fe^{3+}) of neuroglobin. These conformations were ranked using ZRank scoring function that uses a combination of pair wise shape complementarity (PSC), electrostatics and desolvation parameters[16]. In step 2, the top scoring poses obtained from step 1 for each complex was subjected to 130 steps of Adopted Newton-Raphson (ABNR) energy minimization process using CHARMM force field[17]. Rdock refined the docked complex by removing clashes, optimizing polar interaction and by optimizing charge interaction and calculated the electrostatic (E_{elec}) and desolvation (ΔG_{ACE}) energies of the refined structure[18]. The desolvation energy of the protein complex was estimated, which is the sum of the ACE (atomic contact energy) scores of all receptor-ligand atom pairs within a distance cutoff of 6 Å. Furthermore, the binding affinity ($\Delta G_{binding}$) between the protein complexes was predicted using Rdock scoring function which is the sum of desolvation and electrostatics contribution.

$$\Delta G_{binding} = \Delta G_{ACE} + \beta \text{ X } \Delta G_{elec}$$

where β is a scaling factor, a value of 0.9 was used in this study.

3 Results and Discussion

3.1 Protein- Protein Docking

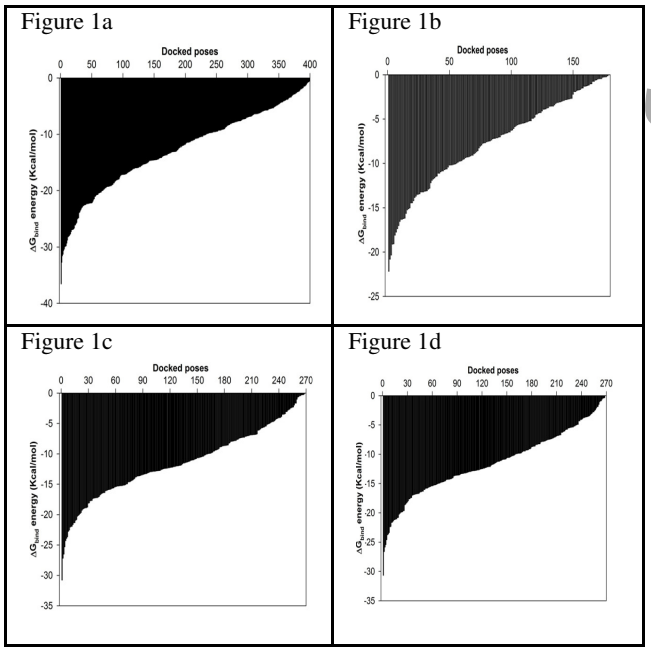
Initial docking of Ubc12 and Cop9 was performed with oxidized and reduced forms of neuroglobin using Zdock to obtain 54000 poses with allowed conformations. A total of 399 top scoring poses for Ubc12- neuroglobin (Fe^{2+}) complex and 179 top scoring poses for Ubc12- neuroglobin(Fe^{3+}) were selected for further refinement (Figure 1a and 1b).

Similarly, top scoring 269 poses were selected for both Cop9-neuroglobin(Fe^{2+}) complex and Cop9- neuroglobin Fe^{3+} complex for further refinement (Figure 1c and 1d).

3.2 Refinement of Protein-Protein Complex

The protein-protein interactions calculated using both ZDock and RDock revealed that Ubc12 binds at high affinity (-36.57 kcal/mol) with reduced state of neuroglobin (Fe^{2+}). The other energy parameters between Ubc12 and Neuroglobin (Fe^{2+}) interactions such as electrostatic (E_{elec}) and van der Waals (E_{vdw}) energy are -40.63 kcal/mol and -84.22 kcal/mol respectively. The protein-protein interactions calculations also reviled that Ubc12 binds at lower affinity (-22.17 kcal/mol) with the oxidized state of neuroglobin (Fe^{3+}). Cop9 binds with the reduced state of neuroglobin with the binding affinity of -30.78 kcal/mol. The other energy parameters between Cop9 and Neuroglobin(Fe^{2+}) interactions such as electrostatic (E_{elec}) and van der Waals (E_{vdw}) are -34.20 kcal/mol and -86.78 kcal/mol respectively. Also the binding energy ($\Delta G_{binding}$) between Cop9 and the oxidized state of neuroglobin(Fe^{3+}) was predicted to be -30.68 kcal/mol using Rdock. The other energy parameters between Cop9 and Neuroglobin(Fe^{3+}) interactions such as electrostatic (E_{elec}) and van der Waals (E_{vdw}) are -34.09 kcal/mol and -89.20 kcal/mol respectively, demonstrating modest interactions between them.

Figure 1: 1a. Comparison of $\Delta G_{binding}$ energy of different docking complexes between Neuroglobin (Fe^{2+}) and Ubc12 calculated using Rdock. Out of 2000 docked poses, only 399 docked complexes having $\Delta G_{binding}$ energy in the range of 0 to -36.57 kcal/mol were selected for the study. Figure 1b Comparison of $\Delta G_{binding}$ energy of different docking complexes between Neuroglobin (Fe^{3+}) and Ubc12 calculated using Rdock. Out of 2000 docked poses, only 178 docked complexes having $\Delta G_{binding}$ energy in the range of 0 to -22.17 kcal/mol were selected. Figure 1c Comparison of $\Delta G_{binding}$ energy of different docking complexes between Neuroglobin(Fe^{2+}) and Cop9 calculated using Rdock. Out of 2000 docked poses, only 269 docked complexes having $\Delta G_{binding}$ energy in the range of 0 to -30.78 kcal/mol were selected. Figure 1d: Comparison of $\Delta G_{binding}$ energy of different docking complexes between neuroglobin (Fe^{3+}) and Cop9 calculated using Rdock. Out of 2000 docked poses, only 269 docking complexes having $\Delta G_{binding}$ energy in the range of 0 to -30.68 kcal/mol were selected.



3.3 Analysis of Protein – Protein Complexes

The final Complex of Ubc12- neuroglobin(Fe^{2+}), Ubc12-neuroglobin (Fe^{3+}), Cop9-neuroglobin (Fe^{2+}) and Cop9-neuroglobin (Fe^{3+}) were analyzed using LigPlot and PdbSum. Numbers of interface residues calculated between Neuroglobin (Fe^{2+}) and Ubc12 within a distance of 5Å were found to be 21:21 respectively. A total of 6 H-bonds and 153 of non-bonded contacts (within a distance of 5Å) were found to be involved in the interactions between Neuroglobin (Fe^{2+}) and Ubc12 demonstrating significant interaction. The interacting surface area between Neuroglobin (Fe^{2+}) and Ubc12 was 982:1048Å^2 (Figure 2a, 3a and 4a).

Number of interface residues between Neuroglobin (Fe^{3+}) and Ubc12 within a distance of 5Å were found to be 19:22 respectively. There was 1 H-bonds and 169 of non-bonded contacts (within a distance of 5Å) involved in the interactions between Ubc12 and Neuroglobin (Fe^{3+}).The interacting surface area between Ubc12 and Neuroglobin (Fe^{3+}) is 1024:1078 Å^2 (Figure 2a, 3a and 4a). The interacting surface area between Neuroglobin (Fe^{2+}) and Cop9 was calculated to be 1077:1080 Å^2 and the numbers of interface residues between neuroglobin (Fe^{2+}) and Cop9 within a distance of 5Å were found to be 20:19 respectively. There were 5 H-bonds and 130 non-bonded contacts (within a distance of 5Å) involved in the interactions between neuroglobin (Fe^{2+}) and Cop9 (Figure 2c,3c and 4c). Similarly, the interacting surface area between Cop9 and

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Figure 2: 2a. Cartoon representation of the docked complex between UBC12 (Cyan) and Neuroglobin (Fe^{2+}) colored Yellow. Figure 2b. Cartoon representation of the docked complex between Ubc12 (Cyan) and Neuroglobin (Fe^{3+}) colored Yellow. Figure 2c. Cartoon representation of the docked complex between COP9 (Cyan) and Neuroglobin (Fe^{2+}) colored Yellow. Fig2d: Cartoon representation of the docked complex between Cop9 (Cyan) and Neuroglobin (Fe^{3+}) colored Yellow. The residues participating in Hydrogen bonding are marked with spheres.

Figure: 2a

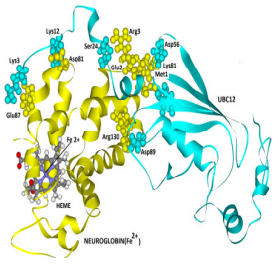


Figure: 2b

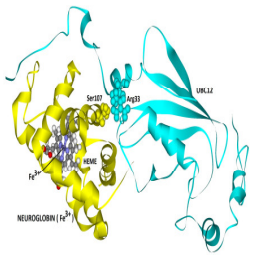


Figure: 2c

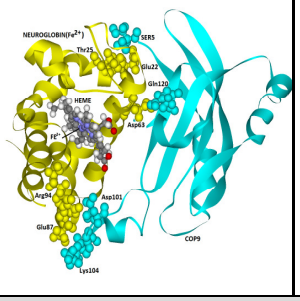


Figure: 2d

Figure 3: 3a: The interface surface between Ubc12 (Green transparent) and Neuroglobin (Fe^{2+}) (Cyan transparent). Figure 3b: The interface surface between Ubc12 (Green transparent) and Neuroglobin (Fe^{3+}) (Cyan transparent). Figure 3c: The interface surface between Cop9 (Green transparent) and Neuroglobin (Fe^{2+}) chain (Cyan transparent). Figure 3d: The interface surface between Cop9 (Green transparent) and neuroglobin (Fe^{3+}) (Cyan transparent). The transparent surface in all figures represents atoms participating in Non–Bonded interactions. These figures were generated using Pymol.

Figure: 3a

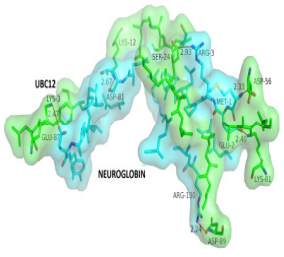


Figure: 3b

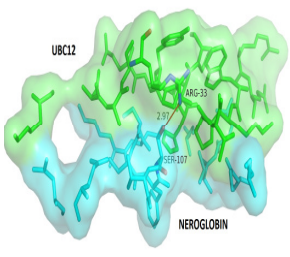


Figure: 3c

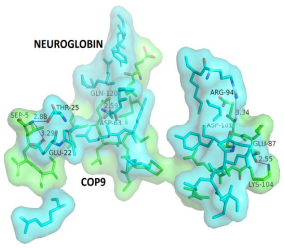


Figure: 3d

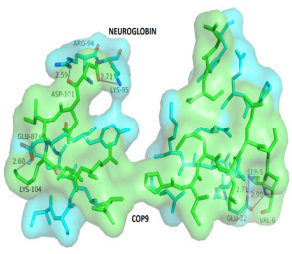


Figure 4: 4a: Two-dimensional representation of the interactions observed between the amino acids (orange) of Neuroglobin (Fe^{2+}) and the amino acids (pink) of Ubc12. Figure 4b: Two-dimensional representation of the interactions observed between the amino acids (Pink) of Ubc12 and the amino acids (Red) of Neuroglobin (Fe^{3+}) chain. Figure 4c: Two-dimensional representation of the interactions observed between the amino acids (orange) of Neuroglobin (Fe^{2+}) and the amino acids (pink) of Cop9. Figure 4d: Two-dimensional representation of the interactions observed between the amino acids (Pink) of Cop9 and the amino acids (Red) of Neuroglobin(Fe^{3+}) chain. The Dashed lines denote hydrogen bonds, and numbers indicate hydrogen bond lengths in Å. Hydrophobic interactions are shown as arcs with radial spokes. The figures were made using LIGPLOT [19].

Figure 4a

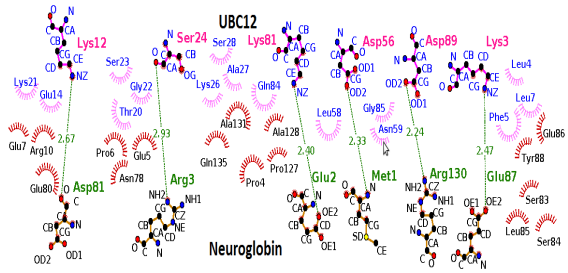


Figure 4b

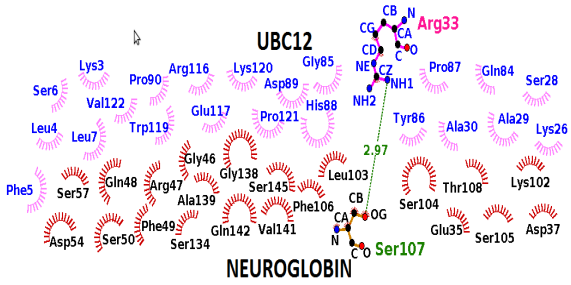


Figure 4c

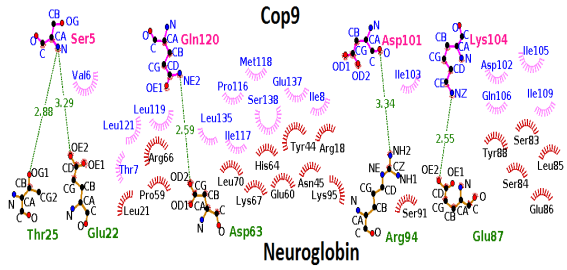
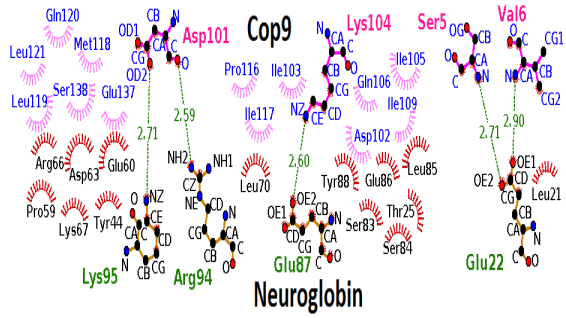


Figure 4d



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Neuroglobin (Fe^{3+}) was 1069:1007 $Å^2$ and the numbers of interface residues between neuroglobin (Fe^{3+}) and Cop9 within a distance of 5Å were 19:22(neuroglobin (Fe^{3+} & Cop9). Also, there were 5 H-bonds and 139 number of non-bonded contacts (within a distance of 5Å) involved in the interactions between Cop9 and neuroglobin (Fe^{3+}) (Figure 2d, 3d and 4d).

Conclusion

The protein-protein interactions calculated using both ZDock and RDock revealed that Ubc12 binds at high affinity (-36.57 kcal/mol) with reduced state of neuroglobin (Fe2+) and at low affinity (-22.17 kcal/mol) with the oxidized state of neuroglobin (Fe3+). Similarly Cop9 binds with both the reduced and oxidized neuroglobin at comparable binding affinity (-30.78 kcal/mol and -30.68 kcal/mol respectively). Results obtained from this study provide useful information that both Ubc12 and Cop9 interacts with neuroglobin in regulation of neddylation and deneddylation process. Furthermore these results guided us in precisely designing experiments for biological evaluations.

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