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To study the mode and mechanism of interaction of Angiopoietin II with receptor tyrosine kinase Tie-2 using molecular mechanics and molecular dynamics approach

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Abstract

BACKGROUD & OBJECTIVE: Angiopoietins are protein growth factors which play key role in Angiogenesis. Angiogenesis is the process of forming blood vessels from pre-existing ones. Angiopoietin-1 (Ang-1) and Angiopoietin-2 (Ang-2) have been identified as ligands of the endothelial receptor tyrosine kinase Tie-2. ANG-2 is a key regulator of angiogenesis that exerts context-dependent effects on endothelial cell (ECs). ANG-2 binds the endothelial-specific receptor TIE2 and acts as a negative regulator of ANG-1/TIE2 signaling during angiogenesis, thereby controlling the responsiveness of ECs to exogenous cytokines. The transmembrane tyrosine kinase TIE-2 and the receptor for angiopoietins have been shown to be involved in angiogenic processes. They are also known to play a role in tumor angiogenesis. However, the mode of interactions between ANG-2 and TIE2 receptor is not known because of the absence of high resolution co-crystal structure. Therefore in this study attempts were made to investigate the mode and mechanism of molecular interactions between Tie2 with Ang2 using molecular modeling and molecular dynamics studies. **METHODOLOGY:** In the present study, both Tie2 (PDB Id: 2GY5) and Angiopoietins (PDB Id: 2GY7) were first prepared using protein preparation wizard (Schrodinger package). Protein-protein interaction between both the proteins was studied using ZDock followed by refinement using Rdock. The best docked pose was then subjected to Molecular dynamics (MD) simulations to study the precise interaction between TIE2 (Receptor) and Angiopoietin-2 (Ligand) over a specific time span using AMBER 11.0. The obtained MD trajectories were further used to estimate the binding free energy of the complex using the molecular mechanics/Poisson Boltzmann surface area (MM-PBSA) method. **RESULTS:** The binding energy ($\Delta G_{binding}$) between both the proteins, Tie2 and Ang2 was predicted to be -28.77 kcal/mol using Rdock. The other energy parameters between Tie2 and APC interactions such as electrostatic (E_{elec}), van der Waals (E_{vdw}) and desolvation (E_{sol}) energy are -44.68 kcal/mol, -99.83 kcal/mol and 6.10 kacal/mol respectively, demonstrating modest interactions between them. The interacting surface area between Tie2 and Ang2 is 842:858Å^2. **CONCLUSION:** Results obtained from this study revealed that both Ang2 and Tie2 bind with high affinity with modest interacting surface area. Further the results guided us in designing specific experiments for biological evaluations.

Keywords: MMPBSA, Molecular Dynamic simulation, Tie2, Angiopoietin

1. Introduction

Angiopoietins (ANGs) constitutes an important class of angiogenic molecules that regulates angiogenesis. Angiogenesis is the formation of blood vessels from pre-existing vessels which is controlled by a hierarchically structured signaling cascade of endothelial-cell-specifically expressed receptor tyrosine kinases. The growth of new blood vessels is essential during tissue repair, foetal development, and female reproductive cycle[1]. In contrast, uncontrolled angiogenesis promotes tumor and retinopathies, while inadequate angiogenesis can lead to coronary artery disease. A balance between pro-angiogenic and anti-angiogenic growth factors and cytokines tightly controls angiogenesis. Inhibition of angiogenesis can prevent diseases such as cancer, diabetic nephropathy, arthritis, psoriasis, whereas stimulation of angiogenesis is beneficial in the treatment of coronary artery disease, cardiac failure, tissue injury, etc.

Angiopoietins is the family of ligands that binds to receptor tyrosine kinase [2,5,6] and it has four members that is ANG-1 to ANG-4. The receptor tyrosine kinase TIE-2 is simulated by ANG-1 and ANG-4 whereas ANG-2 and ANG-3 inhibits ANG1–induced tyrosine phosphorylation of Tie-2 [3]. Ang-2 has been identified as a functional antagonist of Ang-1[4]. It binds to Tie-2 without inducing signal transduction in Tie-2-expressing endothelial cells. The opposing effects of Ang-1 and Ang-2 support a model of constitutive Ang-1/Tie-2 interaction controlling vascular homeostasis as the default pathway and with Ang-2 acting as a dynamically regulated antagonizing cytokine [7]. Angiopoietin–Tie2 signaling pathway is also involved in the reciprocal communication between endothelial cells and pericytes.

The mode of interactions between ANG-2 and TIE2 receptor is not known because of the absence of high resolution co-crystal structure. In this study we have made an attempt to investigate the mode and mechanism of molecular interactions between

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Tie2 with Ang2 using molecular modeling and molecular dynamics (MD) simulation. MD simulation was applied on the docked complex obtained from R-Dock to estimate the binding free energy of complex using the MM–PBSA method (Molecular Mechanics – Poisson Boltzmann Solvation Approach) using Amber 11.0. The components of the binding free energies were also estimated and used to explore the type of guest/host interactions responsible for complex formation, which may provide further insights into the mode of interaction between the two proteins.

2. Experimental Methodology

2.1Computational Details

Crystal structure of Receptor Tyrosine Kinase (Tie2, PDB Id: 2GY5) and Angiopoietins (PDB Id: 2GY7) were obtained from the protein Databank (PDB, *www.rcsb.org*). Structures were prepared using the protein preparation wizard in 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 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.

2.2 Molecular Docking

Receptor Tyrosine Kinase TIE2 and Angiopoietins (ANG2) were docked using the ZDOCK program followed by refinement using RDOCK. The ZDOCK/RDOCK method has been validated by multiple independent protein-protein docking studies and has been found to be a reliable method for the prediction of protein-protein interactions. The program ZDOCK [8, 9] initially identifies the docking positions within the receptor for the ligand(ANG2) based on shape, stearic, and electrostatic complementarities. Once docking positions are identified, they are ranked and refined by the second program, RDOCK [10,11] , which is based on the CHARMM force field and calculates the energetic between the protein and docked peptides and ranks the docked poses. RDOCK calculations were performed on the top 400 ZDOCK-docked structures. The top RDOCK result was identified and characterized as the top potential ligand binding site.

2.3 MD Simulation of the Complex

The protein-protein complex obtained from docking was used as initial complex conformations in MD simulations. The MD simulation was carried out in AMBER 11.0 package [14] using Amber force field (ff99SB) [15]. Topology prep files for ligand , receptor and the complex were built with the amber force field (ff99SB) . The system was then solvated using atomistic TIP3P [16] water in a cubic box with a distance of 15 Å between the wall of the box and the closest atom of the complex. Then eight Na+ ions were added as counter ions to neutralize the system. The complex was minimized in three consecutive rounds each of which consisted of 1000 steps (500 using steepest descent followed by 500 using conjugate gradient method), so as to remove the bad contacts in the crystal structure. Positional restraints were applied to the whole system in the first and second rounds, the force constants of 10 and 2kcal^{-1}Å^{-2} respectively. In the third round the whole system was minimized without restraint. After full relaxation the system was heated from 0 K to 300 K in 50 ps. Finally, a 2ns MD simulation was carried out following 400 ps of equilibration at 300K at 1 atm with the same force field constant (2 kcal^{-1}Å^{-2}). Hydrogen bond lengths were constrained using the SHAKE algorithm [17], and the equation of motion was integrated with a 2 fs time step. The non bonded cutoff distance was 10 Å, and the Particle Mesh Ewald (PME) method[18] was used to calculate long-range electrostatics interactions. The temperature of the system was regulated using the langevin thermostat. All equilibration and subsequent MD stages were carried out in an isothermal isobaric (NPT) ensemble using Berendsen barometer[19] with a target pressure of 1 bar and a pressure coupling constant of 2.0 ps, recording trajectories every 1 ps.

2.4 **Binding energy calculations:**

The binding free energy was calculated using MM-PBSA and MM-GBSA [21, 22] approaches. A total of 260 frames were generated. For each frame the free energy is calculated for each molecular species (complex, TIE2, ANG2), and the binding free energy is computed as the difference between the energy of complex with the combination energy of protein1 and protein2.

$$\Delta G_{bind} = Gc_{omplex} - (G_{protein1} + G_{protein2})$$

The free energy, G for each species can be calculated by the following scheme using the MM-PBSA and MM-GBSA methods.

$$G = E_{gas} + G_{sol} - TS$$
$$E_{gas} = E_{int} + E_{ele} + E_{vdw}$$
$$G_{ele,PB(GB)} = E_{ele} + G_{PB(GB)}$$
$$G_{sol} = G_{sol\text{-}np} + G_{PB(GB)}$$
$$G_{sol\text{-}np} = \gamma SAS$$

Here, E_{gas} is the gas –phase energy; E_{int} is the internal energy; E_{ele} and E_{vdw} are the coulomb and van der walls energies, respectively. E_{gas} was calculated using the ff99SB molecular mechanics force field. G_{sol} is the solvation free energy and can be decomposed into polar and non- polar contributions. $G_{PB(GB)}$ is the polar solvation contribution calculated by solving the PB and GB equations [21,22]. $G_{ele,PB(GB)}$ is the polar interaction contribution. $G_{sol\text{-}np}$ is the nonpolar solvation contribution and was estimated via the solvent-accessible surface area (SAS), which was determined using a water probe radius of 1.4 Å. The Surface tension constant γ[20] was set to 0.0072 kcal mol^{-1} Å^{-2} .T and S are the temperature and the total solute entropy, respectively. Using the GB model, it was possible to compute the binding free- energy contribution of each residue at the interface between two interacting proteins.

3. Results and discussion

3.1 ZDOCK and RDOCK:

Zdock is a rigid-body docking algorithm that uses a Fast Fourier Transform (FFT). These conformations were ranked using ZRank scoring function[8] that uses a combination of pairwise shape complementarity (PSC), electrostatics and desolvation parameters. ZDOCK[9] generated two thousand poses and out of which the best clusters were chosen and refined using RDOCK. [11, 12]. The binding energy ($\Delta G_{binding}$) of the best docked complex between Tie2 and Ang2 was predicted to be -28.77kcal/mol. The other energy parameters between Tie2 and Ang2 interactions such as electrostatic (E_{elec}) and van der Waals (E_{vdw}) energy are -31.97 kcal/mol and -115.24 kcal/mol respectively (Figure I), demonstrating modest interactions between them. The mode of interaction was analyzed using PDBSum and DIMPLOT and it was found that six hydrogen bonds were involved in the interactions between Tie2 and Ang2. The interacting surface area between Tie2 and Ang2 is 842:858Å^2 (Figure II).

3.2 MM-PBSA and MM-GBSA analysis:

The binding free energy between Tie2 and Ang2 was predicted using both MM-PBSA and MM-GBSA approaches. For the MM-PBSA calculations, we calculated the difference in free energy between the protein-ligand(TIE2-ANG2) complex and the unbound protein(TIE2) plus the unbound ligand(ANG2). The two major contributions of the MM-PB(GB)SA with a bonding character are the gas phase Coulombic energy, E_{elec}, and van der Waals energy, E_{vdW}, whose sum is labeled as G_{gas} which comes out to be -395.89 kcal/mol. The binding free energy predicted using MM-GBSA (E_{GB}) is -367.955kcal/mol, whereas using MM-PBSA technique (E_{PB}) is -376.26kcal/mol.

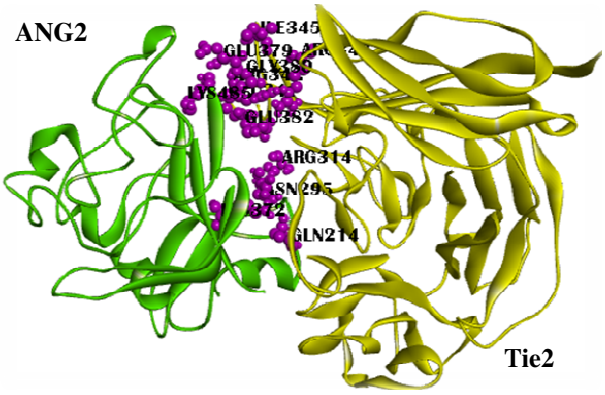

Figure I: Figure 1: Cartoon representation of the docked complex between TIE2 (Yellow) and Ang2(green). The binding energy ($\Delta G_{binding}$) between both the proteins, Tie2 and ANG2 was predicted to be -28.77kcal/mol using Rdock. The other energy parameters between Tie2 and Ang2 interactions such as electrostatic (E_{elec}) and van der Waals (E_{vdw}) energy are -31.97 kcal/mol and -115.24 kcal/mol respectively, demonstrating modest interactions between them. The residues participating in Hydrogen bonding are marked with spheres.

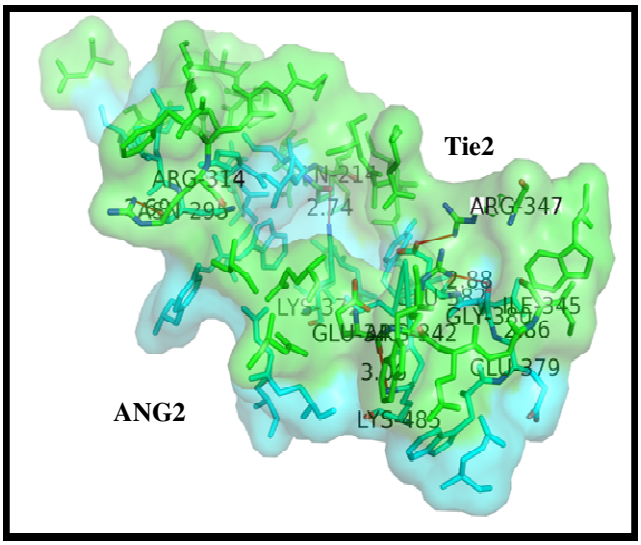

Figure II: The interface surface between Tie2 (Green transparent) and Angiopoitein chain B (Cyan transparent). We can clearly see 6 Hydrogen bonds along with the distance. The transparent surface represents atoms participating in Non–Bonded interactions. The interacting surface area between Tie2 and ANG2 is 842:858Å^2

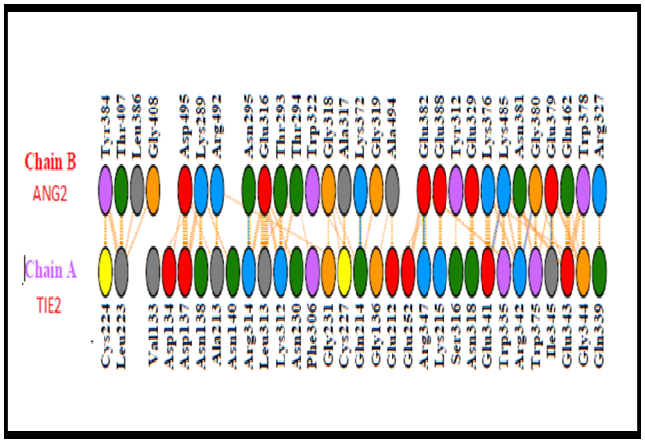

Figure IV: The Image was generated using PDBSum represents 6 Hydrogen bonds that is represented by a single green line and other non -bonded contacts that is shown by dotted yellow line.

((Figure III & IV) Both the methods demonstrate modest interactions between Tie2 and Ang2. The other energy parameters between Tie2 and Ang2 are included in Table I.

Conclusion

Results obtained from this study revealed that both ANG2 and Tie2 bind with high affinity with modest interacting surface area. Further the results guided us in designing specific experiments for biological evaluations.

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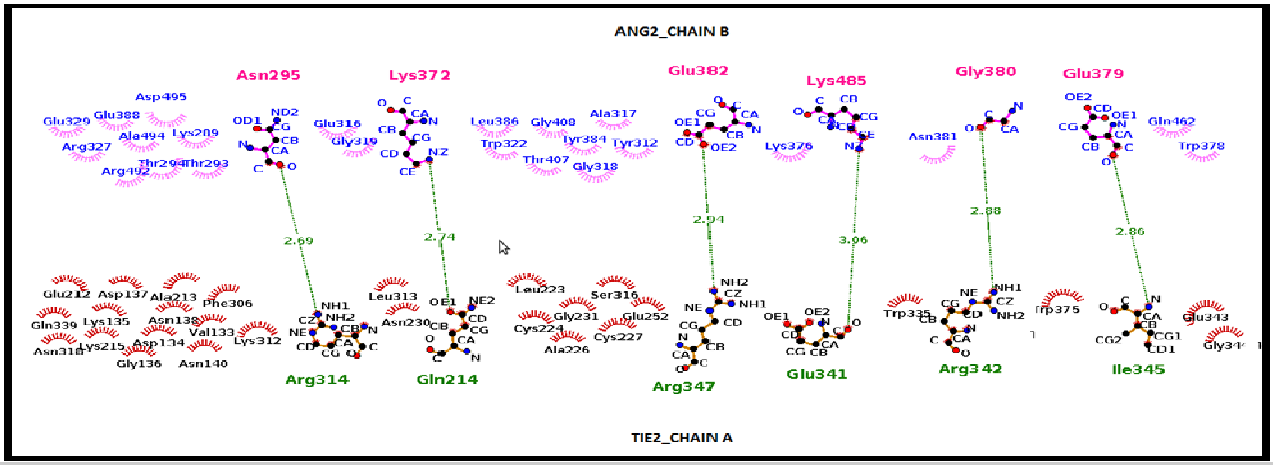

Figure III: Two-dimensional representation of the interactions observed between the amino acids (orange) of Tie2 and the amino acids (pink) of Ang2. Dashed lines denote hydrogen bonds, and numbers indicate hydrogen bond lengths in Å. Hydrophobic interactions are shown as arcs with radial spokes. Numbers of interface residues between Tie2 and Ang2 within a distance of 5Å are 26:23. Similarly, there are 6 H-bonds (within a distance of 5Å) involved in the interactions between Tie2 and Ang2. The figure was made using LIGPLOT.

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Table I: Binding free energy and its components (Kcal mol-1) for the TIE2/ANG2 complex.

Contribuition	Complex(Ang2/Tie2)	Receptor(Tie2)	Ligand(Ang2)	Delta
E_{BOND}	1910.2552	1253.5891	656.666	0
E_{ANGLE}	5109.1215	3447.529	1661.5925	0
E_{DIHED}	6618.9642	4382.5954	2236.3687	0
$E_{VDWAALS}$	-5065.7843	-3179.8099	-1750.0954	-135.879
E_{EEL}	-46327.8566	-29852.2464	-16215.5944	-260.0158
$E_{VDW\ GB}$	2196.7036	1421.5523	775.1512	0
$E_{EEL\ GB}$	27626.9764	17804.9896	9821.9868	0
E_{GB}	-7186.8209	-4967.1732	-2587.6032	-367.9555
E_{SURF}	194.0882	148.1982	65.5348	-19.6449
$G_{GAS,GB}$	-7931.6201	-4721.8008	-2813.9245	-395.8949
$G_{SOLV,GB}$	-6992.7327	-4818.975	-2522.0684	348.3107
$H_{TOTAL,\ GB}$	-14924.3529	-9540.7757	-5335.9929	-47.5842
$E_{VDW,PB}$	2196.7036	1421.5523	775.1512	0
$E_{EEL,PB}$	27626.9764	17804.9896	9821.9868	0
E_{PB}	-6611.5477	-4610.4258	-2377.3862	-376.2643
E_{CAVITY}	154.3016	115.227	55.5511	-16.4766
$G_{GAS,PB}$	-7931.6201	-4721.8008	-2813.9245	-395.8949
$G_{SOLV,PB}$	-6457.2461	-4495.1988	-2321.8351	359.7878
$H_{TOTAL,PB}$	-14388.8662	-9216.9996	-5135.7596	-36.1071