International Journal of Advance Research in Science and Engineering
Vol. No.6, Issue No. 01 , January 2017
www.ijarse.com
IJARSE
ISSN (O) 2319 - 8354
ISSN (P) 2319 - 8346

Docking of novel RGD analogues with $\alpha_v\beta_3$ integrin

Vinay Kr. Singh[1], Anjani Kr. Tiwari [2]

[1]Department of Chemistry,Dr.Shakuntala Misra National Rehabilitation University, Mohaan Road Lucknow(India)

[2]Division of Cyclotron and Radiopharmaceutical Sciences, Institute of Nuclear Medicine an Applied Sciences, Brig. S. K. Mazumdar Road, Delhi (India)

ABSTRACT

Ligand -protein interactions have been recognized as central phenomena in most biological processes ranging from catalysis to signaling . Theoretical and computational methods are widely used to analyze, investigate, and predict ligand-macromolecule interactions. In order to better understand the structural requirements of Integrins-ligand binding a series of RGD analogues were examined using docking techniques. The G-score of the DOTA (RGD-Nitrophe) is -9.09 which is the highest G-score value,-8.17 being the next highest score of RGD among all the ligand molecules.G-score of RGD-Nitrophe and [DTPA (Nitrophe-)$_2$] is -7.35 and -7.49 simultaneously. Minimum energy is required for the more binding affinity of ligand and receptor complex. The E-value of DOTA (RGD-Nitrophe) is -114.83. DOTA (RGD-Nitrophe) required minimum energy for the formation of interaction complex which indicates DOTA (RGD-Nitrophe) have good binding affinity as compare to other RGD Analogs.

I. INTRODUCTION

Docking predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex [1-4]. Docking is frequently used to predict the binding orientation of small molecule drug candidates to their protein targets in order to in turn predict the affinity and activity of the small molecule. Hence docking plays an important role in the rational design of drugs [5].

Molecular docking can be thought of as a problem of *"*lock-and-key*"*, where one is interested in finding the correct relative orientation of the *"*key*"* which will open up the *"*lock*"* (where on the surface of the lock is the key hole, which direction to turn the key after it is inserted, etc.). Here, the protein can be thought of as the "lock" and the ligand can be thought of as a "key" [6]. Molecular docking may be defined as an optimization problem, which would describe the "best-fit" orientation of a ligand that binds to a particular protein of interest. During the course of the process, the ligand and the protein adjust their conformation to achieve an overall "best-fit" and this kind of conformational adjustments resulting in the overall binding is referred to as **"**induced-fit**"** [7].

Integrins being ubiquitously expressed as cell surface receptors and their contribution to important physiological processes such as development, immune responses and cancer, their defects have been implicated in many common diseases from, cancer to pathogen invasion. Thus to control certain pathological states by blocking the possible routes entertaining a particular integrin-ligand interaction, the integrins have now become an attractive targets for drug design [8].

IJARSE
ISSN (O) 2319 - 8354
ISSN (P) 2319 - 8346

Integrin-ligand interactions are determined by several factors. One major determinant of ligand binding specificity is the subunit composition of an integrin. In mammals, 18 α and 8 β subunits combine to form 24 integrins. These subunits are subject to alternative splicing [9] and post-translational modifications [10], enriching this structural diversity. A second major factor in determining integrin binding to ligands is the presence of integrin recognition sequences in ligands [11]. These include a large number of extracellular matrix proteins (e.g. various collagens, fibronectins, vitronectin, laminins, von Willebrand factor and thrombospondins), counter-receptors (e.g. VCAM-1, ICAMs, and generally members of the immunoglobulin superfamily) and plasma proteins [12]. Aspartic-acid-based sequences (e.g. RGD, LDV, KGD, RTD and KQAGD [single letter amino acid code]) bind to the majority of integrins[13].

RGD peptides are proving to be promising new tools for drug therapy and imaging of tumors, thrombosis, and inflammatory-related diseases [14-15]. Thus my current work is designed to analyze the binding affinity of RGD analogs derived from novel chelating vehicles by using the automated computational docking. Structural models for the interaction of ligands (RGD & RGD analogs) with the $\alpha_v\beta_3$ integrin receptor were generated to find the best binding ligand molecule on the basis of various parameters involved like G-score, H-bond and E-value. These models may provide new insights into the molecular basis for ligand binding specificity in integrins.

II. MATERIALS AND METHODS

Protein Setup: The crystal structure of the extracellular domain of the $\alpha_v\beta_3$ integrin receptor in the presence of the ion Mn^{2+} (PDB entry code =1L5G) (Xiong JP et al., 2002). Since in the X-ray structure the head group of $\alpha_v\beta_3$ integrin, which comprises the β propeller domain of α_v and the βA domain of β3, has been identified as the ligand binding region, only on the globular head is considered for docking study . A typical PDB structure file consists only of heavy atoms, can contain waters, cofactors, and metal ions, and can be multimeric. The raw state of protein which may be missing hydrogen atoms and having incorrect bond order assignments, charge states, or orientations of various groups can be changed to a state in which it is properly prepared for calculations is done by using the protein preparation panel in Glide 5.5 (Schrödinger, LLC).

Receptor Grid Generation: The grids were chosen to be large enough to include a significant part of the protein around the binding site. Ligand docking jobs cannot be performed until the receptor grids have been generated. Receptor grid generation requires a "prepared" structure: an all atom structure with appropriate bond orders and formal charges.

Ligand structure preparation: The structures of the ligands were constructed using ChemDraw Ultra 7.0 shown in **figure1.**The structures are processed using LigPrep panel that perform conversions, apply corrections to the structures, generate variations on the structures, eliminate unwanted structures, and optimize the structures.

Docking: Docking calculations were performed using the advanced version 5.5 of the docking program Glide using Maestro9.0 GUI (Schrödinger, LLC). X-ray crystal structure of $\alpha_v\beta_3$ integrin was taken from PDB entry 1L5G, having resolution of 3.20 A°. Solvent molecules were deleted and bond order for crystal ligand and protein were adjusted and minimized up to 0.30 A° RMSD.Glide searches for favorable interactions between

IJARSE
ISSN (O) 2319 - 8354
ISSN (P) 2319 - 8346

ligand molecules and a receptor molecule, usually a protein. Glide can be run in rigid or flexible docking modes. The combination of position and orientation of a ligand relative to the receptor, along with its conformation in flexible docking, is referred to as a *ligand pose*. For each core conformation, an exhaustive search of possible locations and orientations is performed over the active site of the protein.

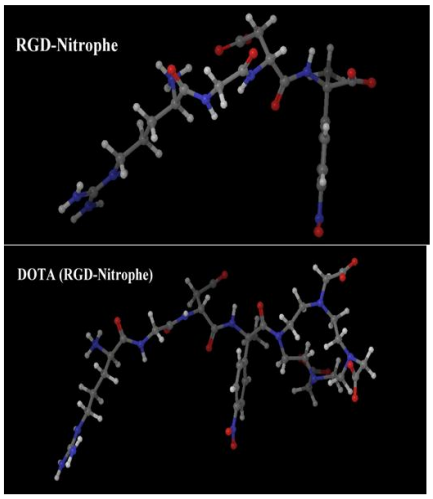

Fig 1 Optimized Ligand structure

The second stage of the hierarchy begins by examining the placement of atoms that lie within a specified distance of the line drawn between the most widely separated atoms (the *ligand diameter*). This is done by selecting possible orientations of the ligand diameter .

Next step is rotation about the ligand diameter is considered, and the interactions of a subset consisting of all atoms capable of making hydrogen bonds or ligand-metal interactions with the receptor are scored (*subset test*). If this score is good enough, all interactions with the receptor are scored.

The scoring in these three tests is carried out using discretized version of the chemScore empirical scoring function. Much as for ChemScore itself, this algorithm recognizes favorable hydrophobic, hydrogen-bonding, and metal-ligation interactions, and penalizes steric clashes. This stage is called "greedy scoring," because the actual score for each atom depends not only on its position relative to the receptor but also on the best possible score it could get by moving ±1 Å in *x*, *y*, or *z*. This is done to mute the sting of the large 2 Å jumps in the site-point/ligand-center positions. The final step in Stage 2 is to re-score the top greedy scoring poses via a "refinement" procedure (Step 2d), in which the ligand as a whole is allowed to move rigidly by ±1 Å in the Cartesian directions.

Only a small number of the best refined poses (typically 100-400) are passed on to the third stage in the hierarchy—energy minimization on the pre-computed OPLS-AA van der Waals and electrostatic grids for the receptor. Finally, the minimized poses are re-scored using Schrödinger's proprietary Glide Score scoring function based on ChemScore. The choice of best-docked structure for each ligand is made using a model energy score (E_{model}) that combines the energy grid score, the binding affinity predicted by Glide Score and also calculates the Coulomb-van der waals interaction-energy score (C_{vdW}).

IJARSE
ISSN (O) 2319 - 8354
ISSN (P) 2319 - 8346

III. RESULT & DISCUSSION

To investigate the detailed intermolecular interactions between the ligand and the target protein, an automated docking program Glide 5.5 was used. Three-dimensional structure information on the target protein was taken from the PDB entry 1L5G; having resolution of 3.20 A°. Processing of the protein included the deletion of the ligand and the solvent molecules as well as the addition of hydrogen atoms. Extra precision (XP) mode of Glide 5.5 software was used for the docking studies. The RGD & RGD Analogues were docked into the active site of 1L5G. A correlation was calculated by Glide score.Docking of the compounds revealed a consistent set of recurring interactions.

RGD being the most prototypic example among those bioactive amino acid sequences which have been teased out of large extra cellular matrix proteins. Based on RGD tripeptide sequences some new analouges have been designed and are tested for their binding affinity to the integrin by analyzing the docking score. DOTA (RGD-Nitrophe) and [DTPA (Nitrophe-DOTA) $_2$] are are taken as docking ligands. The open acetic acid arm seems to play an important role in the interaction.

For the prediction of results mainly three parameters are considered, G-score, Glide energy and H-bonds. On the basis of these parameters the binding affinity of ligand towards receptor are discussed. The more negative value of G-score indicates good binding affinity of the ligand with receptor. The minimum energy (ΔE) for the formation of complex between ligand and receptor indicates a good stability pattern of ligand receptor complex. More H-bonds in the structure shows ligand having good binding as well as interactive arm with target sites in receptor. Most interactive interactions found at hydrogen bond from the guanidinium group of (β)-Arg214 to the carboxylate oxygen of the ligand **(Fig 2)**, Carboxylate oxygen of (β)-Asn215 forming hydrogen bond with the backbone amide hydrogen, β)-Asp119 **(Fig 3)** and (β)-Thr250 are involved in forming a bidentate salt bridge while at the same time carboxylate group of (β)-Ser123 side chain is involved in H-bonding.Besides this hydrogen bond from the guanidinium group of (α)-Arg248 to the backbone CO of the Asp in the ligand is observed and also the Arg side chain forming a bidentate salt bridge to the (β)- Lys 253 is found in both the ligand molecules RGD-Nitrophe and DTPA analogue of RGD **(Fig 4)**.

The docking results are shown in the **Table 1&2**. The G-score, H-Bond, and E-value shows binding affinity of ligand (RGD and RGD Analogs) towards protein ($\alpha_v\beta_3$ integrin). The G-score of the DOTA (RGD-Nitrophe) is -9.09 which is the highest G-score value,-8.17 being the next highest score of RGD among all the ligand molecules.G-score of RGD-Nitrophe and [DTPA (Nitrophe-)$_2$] is -7.35 and -7.49 simultaneously. Minimum energy is required for the more binding affinity of ligand and receptor complex. The E-value of DOTA (RGD-Nitrophe) is -114.83. DOTA (RGD-Nitrophe) required minimum energy for the formation of interaction complex which indicates DOTA (RGD-Nitrophe) have good binding affinity as compare to other RGD Analogs. If hydrogen bond is more, the binding affinity of the ligand is higher and it has been found that RGD has shown an extensive network of hydrogen bond and similar hydrogen bond interaction is found for DOTA (RGD-Nitrophe) thus suggesting quite good binding affinity towards $\alpha_v\beta_3$ integrin other than [DTPA (Nitrophe-)$_2$] and RGD-Nitrophe

.

International Journal of Advance Research in Science and Engineering
Vol. No.6, Issue No. 01 , January 2017
www.ijarse.com
IJARSE
ISSN (O) 2319 - 8354
ISSN (P) 2319 - 8346

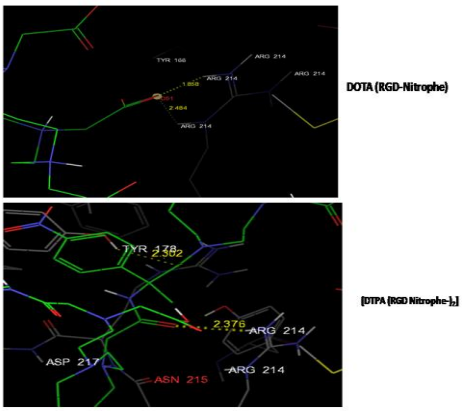

Fig 2 Hydrogen bond from the guanidinium group of (β)-Arg214 to the carboxylate oxygen of the ligand

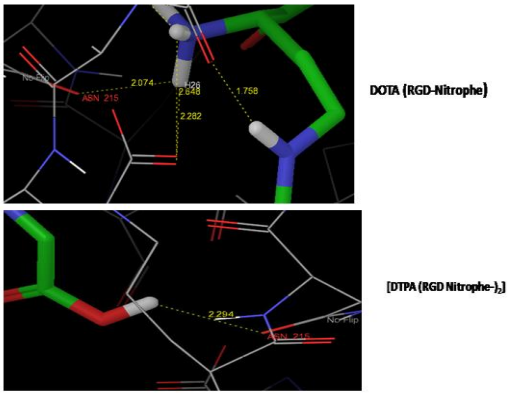

Fig 3 :Carboxylate oxygen of (β)-Asn215 forming hydrogen bond with the backbone amide hydrogen

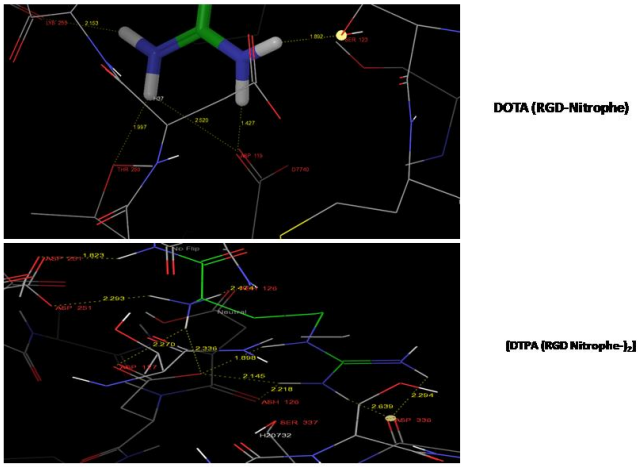

Fig 4 :- A network of bidentate salt brigde is found to be associated with Asp to the backbone of ligands

Table 1. Docking results of RGD & RGD Analogues with $\alpha_v\beta_3$ integrin receptor

LIGAND	CONFORMATIONS	POSES	G_{SCORE}	E-Value (ΔE)
RGD	LIG-1	3	-5.1	-59.04
	LIG-2	4	-8.14	-65.43
	LIG-3	5	-8.17	-76.3
	LIG-4	10	-7.86	-88.22
RGD-Nitrophe	LIG-5	3	-7.35	-77.02
	LIG-6	6	-7.24	-67.4
	LIG-7	8	-6.63	-69.2
	LIG-8	4	-6.5	-75.67
DOTA (RGD-Nitrophe)	LIG-9	4	-9.09	-114.83
	LIG-10	5	-7.82	-112.25
	LIG-11	7	-7.26	-113
	LIG-12	9	-6.57	-97.39
[DTPA (RGD Nitrophe-)$_2$]	LIG-13	1	-5.47	-56.67
	LIG-14	1	-2.62	-78.54
	LIG-15	1	-7.49	-176.54
	LIG-16	3	2.03	-67.87

International Journal of Advance Research in Science and Engineering
Vol. No.6, Issue No. 01 , January 2017
www.ijarse.com
IJARSE
ISSN (O) 2319 - 8354
ISSN (P) 2319 - 8346

Table 2 Docking results of most appropriate RGD & RGD Analogues with surrounding residues

Ligand	G_{Score}	Surrounding Residues
RGD	**-8.17**	(α)-T116, (α)-K119, (α)-R122, (α)-G153, (α)-W179,(α)-Y166, (α)-F154, (α)-A215, (α)-D218, **(β)-Y122,(α)-Y178**, **(β)-A218**, (β)-E220, (β)-M180, **(β)-N313,**(β)-N215, (β)-D251,**(β)-T250**,**(β)-D119**,(β)-R216,**(β)-D217**,(β)-P219,**(β)-E220,(β)-S123**
RGD-Nitrophe	**-7.35**	**(α)-D148,** (α)-A149,(α)-D150, (α)-G153, (α)-F154, (α) P-174, (α)-G175, (α)-S176, (α)-F177, (α)-Y178, (α)-W179, (α)-Q180, (α)-G181, (α)-T212, (α)-A213, (α)-Q214, (α)-A215, (α)-I216, (α)-F217, (α)-D218, (α)-D219, (α)-S220, (α)-Y221, (α)-A247, (α)-**248**, (α)-Y254**, (β)-D127**, (β)-Y116, (β)-L120, (β)-S121, (β)-Y122, (β)-S123, (β)-M124, (β)-K125, (β)-D126, (β)-D127, (β)-G222, (β)-F248, (β)-T249, (β)-T250, **(β)-D251**, (β)-A252**, (**β**)-K253**
DOTA (RGD-Nitrophe)	**-9.09**	(α)-H113,(α)-W114,(α)-T116,(α)-M118,(α)-K119, (α)-Q120,(α)-S176,(α)-F177,**(α)-Y178**,(α)-A246, (α)-A247, **(α)-R248**, (α)-T249, (α)-L250, (α)-Y254, (α)-M272, (β)-M118, (β)-D119, (β)-L120, (β)-S121, (β)-Y122,(β)-P163,(β)-Y164,(β)-M165, **(β)-Y166**, (β)-R214,(β)-N215,(β)-R216,**(β)-D217**,(β)-A218, (β)-T250,**(β)-D251,** (β)-A252,(β)-K253,(β)-T254, (β)-T311, (β)-E312, **(β)-N313**, (β)-V314.
[DTPA (RGD Nitrophe-)$_2$]	**-7.49**	α)-H113,(α)-W114,(α)-T116,(α)-M118,(α)-K119, (α)-Q120,(α)-S176,(α)-F177,**(α)-Y178**,(α)-A246, (α)-A247, **(α)-R248**, (α)-T249, (α)-L250, (α)-Y254, (α)-M272, (β)-M118, (β)-D119, (β)-L120, (β)-S121, (β)-Y122,(β)-P163,(β)-Y164,(β)-M165, **(β)-Y166,** (β)-R214,(β)-N215,(β)-R216,**(β)-D217**,(β)-A218, (β)-T250,**(β)-D251,**(β)-A252,(β)-K253,(β)-T254, (β)-T311, (β)-E312, **(β)-N313**, (β)-V314.

IV. CONCLUSION

The recent availability of the X-ray structure of the extra cellular segment of integrin $\alpha_v\beta_3$ in complex with an RGD ligand has allowed us to determine the receptor-bound conformations of the most representative $\alpha_v\beta_3$ ligands.Taken together these docking results suggest that DOTA (RGD-Nitrophe) has shown the best G-Score and is thought to have maximum binding affinity with the $\alpha_v\beta_3$ integrin receptor among all other ligand molecules.

The obtained complexes need to be evaluated for their consistency with structure-activity relationships and site-directed mutagenesis data. Then a comparison between the calculated interaction free energies and the experimental biological activities need to be made. All the possible interactions of the investigated compounds at the active site and the probable ligand binding conformations then will help in providing an improved basis for structure-based rational ligand design.

IJARSE
ISSN (O) 2319 - 8354
ISSN (P) 2319 - 8346

Acknowledgement: - The authors like to thank VC DSMNRU, Mohaan road Lucknow for constant encouragement to do research work. The authors are also thankful to Director, INMAS, for providing all the facilities and for his deep interest during the course of the study.

REFERENCES

[1.] Lengauer T, Rarey M . Computational methods for biomolecular docking. *Curr. Opin. Struct. Biol.* 1996 **6**: 402–406.

[2.] Kubinyi, H: Structure-based design of enzyme inhibitors and receptor ligands. *Curr. Opin. Drug. Discovery Dev*. 1998, **1**: 4-15

[3.] McCarthy Joseph D: Computational approaches to structure based ligand design. *Pharmacol. Ther*. 1999, **84**: 179-191

[4.] Jorgensen WL. Rusting of the lock and key model for protein-ligand binding. *Science* 1991, **254**: 954–955.

[5.] Kitchen DB, Decornez H, Furr JR, Bajorath J: Docking and scoring in virtual screening for drug discovery: methods and applications. *Nature reviews. Drug discovery* 2004.**3**:935–49.

[6.] Jorgensen W L, Maxwell D S, Tirado-Rives J: Development and testing of the OPLS all-atom force field on conformational energetics and properties of organic liquids. *J. Am. Chem. Soc*. 1996, **118**: 11225-11236.

[7.] Wei BQ, Weaver LH, Ferrari AM, Matthews BW, Shoichet BK: Testing a flexible-receptor docking algorithm in a model binding site. *J. Mol. Biol.* 2004, **337**: 1161–82.

[8.] Hynes RO: A reevaluation of integrins as regulators of angiogenesis. *NatureMedicine* 2002, **8**: 918-921

[9.] Ziober BL, Vu MP, Waleh N, Crawford J, Lin CS, Kramer RH: Alternative extracellular and cytoplasmic domains of the integrin alpha 7 subunit are differentially expressed during development. *J Biol Chem* 1993, **268**:26773-26783.

[10.] Fagerholm S, Morrice N, Gahmberg CG, Cohen P: Phosphorylation of the cytoplasmic domain of the integrin CD18 chain by protein kinase C isoforms in leukocytes. *J Biol Chem* 2002, **277**:1728-1738.

[11.] Peishoff CE, Ali FE, Bean JW, Calvo R, D'Ambrosio CA, Eggleston DS, Hwang SM, Kline TP, Koster PF, Nichols A, Powers D, Romoff T, Samanen JM, Stadel J, Vasko J, Kopple KD: Investigation of conformational specificity at GPIIb/IIIa: evaluation of conformationally constrained RGD peptides. *J. Med.Chem.* 1992, **35**: 3962-3969.M Amin Arnaout et al., 2002

[12.] M Amin Arnaout, Simon L Goodman, Jian-Ping Xiong : Coming to grips with integrin binding to ligands Opinion *Current Opinion in Cell Biology* 2002, **14**:641–651

[13.] Ruoslahti E: RGD and other recognition sequences for integrins**.** *Annu Rev Cell Dev Biol* 1996, **12**:697-715.

[14.] Mathieu Galibert , Zhao-Hui Jin, Takako Furukawa , Toshimitsu Fukumura , Tsuneo Saga Yasuhisa Fujibayashi , Pascal Dumya, Didier Boturyn , RGD–cyclam conjugate: Synthesis and potential application for positron emission tomography, Bioorganic & Medicinal Chemistry Letters 20 (2010) 5422–5425

[15.] Masahiro Miyashita, Miki Akamatsu, Yoshio Hayashi and Tamio Ueno, Three-Dimensional Quantitative Structure Activity Relationship Analyses of RGD Mimetics as Fibrinogen Receptor Antagonists, Bioorganic & Medicinal Chemistry Letters 10 (2000) 859-863