

Interaction of ribosome inactivating proteins with growth factor receptors - a cheminformatic approach

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ABSTRACT

Protein interactions are the basis of life processes at the nano level. Most of the protein interactions are with other proteins. Thus efforts to recreate the network of protein-protein interactions are important for interpretation. Our study has such protein-protein interactions with a computational approach directly targeting ligand-receptor interactions through docking method and simulating the binding. *In silico* studies on interaction of Ribosome Inactivating Proteins (RIP) with various growth factor receptors suggests "First approximation" for *in vitro* studies. Docking of Ricin and Abrin polypeptide chains with Epidermal growth factor receptor and Fibroblast growth receptor was done by using Hex programme. This high throughput docking studies indicate results in terms of energy values. The interaction between the ligand and the receptor with minimal energy value is the best pair. Accordingly, the best docking results were obtained for interaction of the pairs, Ricin B chain - Epidermal growth factor receptor, Ricin B chain - Fibroblast growth factor receptor, Abrin - Epidermal growth factor receptor, Abrin - Fibroblast growth factor receptor with an E_{total} values of -562.43, -427.77, -511.13 and -490.48 respectively.

Key words. Epidermal Growth Factor Receptor (EGFR), Fibroblast Growth Factor Receptor (FGFR), Docking, Ricin, Abrin, Hex programme.

INTRODUCTION

Many plants contain proteins that are capable of inactivating eukaryotic ribosomes, these are termed as "Ribosome-Inactivating Proteins" (RIPs)¹. Ricin from *Ricinus communis* and Abrin from *Abrus precatorius* belong to type II RIPs, both have got molecular similarity and similar mechanism of action^{2,3}. They are composed of 2 chains A and B. The B chain helps in binding of proteins to cell surface receptors which are terminated with galactose containing oligosaccharides. The A chain is taken up into the cell and has N-glycosidase activity bringing about depurination of a specific adenine residue from the GAGA loop of 28S rRNA

from 60S ribosome, thereby halting protein synthesis and leading to cell death. .

Type-2 RIPs have a possible implication in therapeutics since these are more toxic to tumor cells than normal cells⁴. Hence these molecules were chosen for cheminformatic studies to understand protein-protein interactions, which offer a theoretical opportunity to develop antitumor drugs.

Cheminformatic study is based on computer guided system which is capable of varying parameters. Modern drug designing efforts are exploiting at least three core technologies aimed at increasing the efficiency of finding drug leads

viz., Genomics, High throughput Screening and Combinatorial Chemistry. Computational modeling has been developed and widely applied in better understanding of protein-protein interactions^{5,6}. Computer simulations are done by studying macromolecular dynamics and free energy calculation methods⁷. Detailed energetics and structural knowledge of interactions between biomolecules is fundamental to understand the complex interactive mechanism that takes place in living organism and also to design drugs for blocking or modifying these interactions using molecular docking^{8,9}. In our study, we have used Rapid prototyping computational simulations using software tool Hex4.2. Using molecular dynamics and free energy calculations, the docking of ricin, and abrin was done with EGFR and FGFR. Growth factor receptors were selected for this *insilico* study because, in cancer, aberrant signaling can cause constitutive activation of growth factor receptors which may in turn influence key steps in the process of tumor invasion and metastasis. Involvement of EGFR and FGFR in tumor spread has indicated them as potential target receptors for antimetastatic studies.

EXPERIMENTAL

For the present study, Hex4.2 program was used which is an interactive molecular graphics program used to calculate the feasible docking pairs such as protein-protein (of our choice). In Hex docking calculations, 3D parametric functions were used to encode both surface shape and electrostatic charge and their potential distribution. Each property was represented by a vector of coefficients. From Hex's surface skin model of protein topology, an expression for a docking score as a function of six degrees of freedom in a rigid body docking search with suitable scaling factors was derived. This docking score was interpreted as interaction energy. This is a spherical polar approach where the molecules were relatively rotated to generate and evaluate good docking orientations which were effectively a six dimensional Fourier Correlations.

Hex program was started by downloading and displaying the PDB structure of Ricin, Abrin and Epidermal growth factor receptor and fibroblast growth factor receptor. Docking was separately done

with each RIP with EGFR and FGFR respectively. This procedure was repeated three times to ensure reproducible results.

There were four stages or controls in the program. The first control was the 'orientation'. Here the two molecules were oriented with their favorable positions for docking. This was followed by the second control, 'clustering' where the program uses a simple clustering algorithm to group spatially similar docking orientations. The third control of this program was 'matching' in which there was superposition of pair of proteins ie, RIP and the receptor and a search for maximum similarity. The final stage was 'docking' which was much similar to matching. At this stage, the surface skin coefficients followed by docking correlation scores at each of the specified angular and intermolecular increment were calculated.

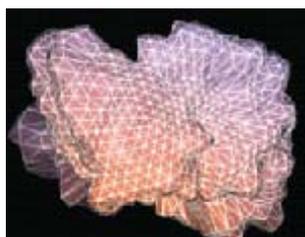
RESULTS AND DISCUSSION

The interaction studies reveal that the best docking results were for interaction of the pairs, Ricin B chain - Epidermal growth factor receptor, Ricin B chain - Fibroblast growth factor receptor, Abrin - Epidermal growth factor receptor, Abrin - Fibroblast growth factor receptor with an E_{total} values of -562.43 -427.77, -511.13 and -490.48 respectively as shown in the Table1.

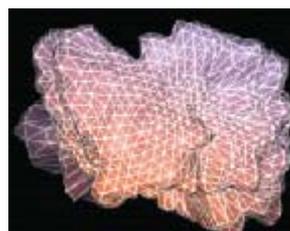
A large number of protein structures have been deposited into PDB, however only a small fraction of protein-protein complexes has been experimentally characterized so far. In this context theoretical prediction of protein-protein interactions is becoming critically important in structural biology. Using Hex4.2, we have used the 2 different RIPs as lead compounds which are prototype compounds having biological and pharmacological activities.

Table 1: Docking results

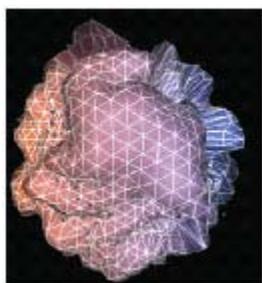
RIP	Receptor	Energy values	Bumps
Ricin	EGFR	-562.43	-1
Ricin	FGFR	-427.77	-1
Abrin	EGFR	-511.13	-1
Abrin	FGFR	-490.48	-1



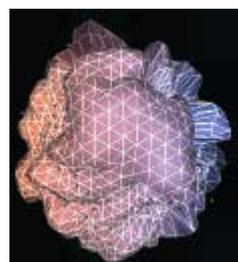
Harmonic surface of EGFR



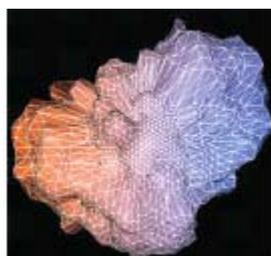
Harmonic surface of EGFR



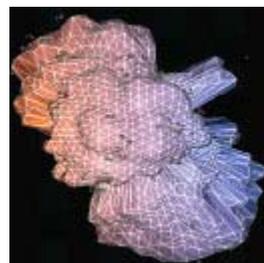
Harmonic surface of FGFR



Harmonic surface of FGFR



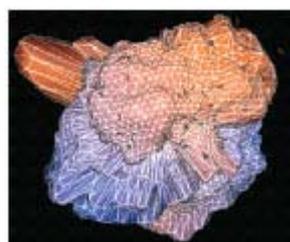
Harmonic surface of Ricin



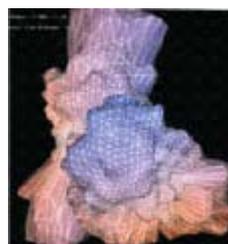
Harmonic surface of Arbin



Docking of Ricin with EGFR



Docking of Arbin with EGFR



Docking of Ricin with FGFR



Docking of Arbin with FGFR

Fig. 1. Docking of ricin with EGFR and FGFR

Fig. 2. Docking of Arbin with EGFR and FGFR

It is evident that the best interactive pattern is shown by ricin with EGFR with a very minimal energy value. The bumps of all the 4 interactions are -1 indicating that the steric clashes are very minimal and thereby better interaction. All these results indicate that both the growth factor receptor can be targeted by these lead compounds in treatment of cancer.

CONCLUSION

The computational docking techniques are promising to be an essential tool in predicting the interactions of ligand-receptor complexes since it is cumbersome and very laborious to obtain crystal

structures of protein complexes. Ricin and Abrin can be powerful lead molecules which interact strongly with Epidermal and Fibroblast growth factor receptors as evidenced by the docking results. The newly emerging field of cheminformatics with variety of software tools is addressing the needs of a broad community of scientists in First Approximation studies before venturing into *invitro* and *invivo* studies. Subsequently, these *insilico* data can be further extended into invitro studies and drug designing by targeting these lead molecules to the specific receptors which may have a positive implication in cancer therapy.

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