INTERNATIONAL JOURNAL OF SCIENCE AND NATURE

www.scienceandnature.org

DOCKING STUDY OF RHOC WITH EGCG OF GREEN TEA: AN INSILICO APPROACH

[1*]P Suresh Kumar, [2]Rajasekhar chikati, S. Roja, Vemula sai jahnavi
[1]Loyola Academy, Hyderabad, TS, INDIA-500010
[2]Yogi Vemana University, Ganganapalle, Andhra Pradesh, India -516216
*Corresponding Author email: sureshkp6@gmail.com

ABSTRACT
The aim of the present research was to study the anticancer effects of EGCG of green tea on human Rho-C. A solved protein structure for human Rho-C is available at the protein data bank (PDB). Therefore, we had taken it from RCSB PDB. Keeping the aim to determine molecular level interactions (molecular simulations and protein docking) of human Rho-C with EGCG of Green tea. We extended our work *in vitro* to *in silico* studies. To gain better relaxation and accurate arrangement of atoms, refinement was done on the human Rho-C by energy minimization (EM). The human Rho-C showed that known key residues playing important role in active site for ligand binding. The information thus discussed provides insight to the molecular understanding of human Rho-C together with enzymatic docking studies, to reveal key differences that could be useful for development of new anti-cancer drugs. These *in vitro* and *in silico* structural studies prove the effective inhibition of Rho-C of homo sapiens activity by EGCG of green tea in neoplastic cells and thereby provide new insights for the development of novel anti-cancer drugs.

KEYWORDS: Rho-C, Homo sapiens, EGCG, Green tea, Molecular Dynamics, Docking, Anticancer.

INTRODUCTION

Cancer is known to be associated with genetic instability in which c-myc serves as a major modifier of many targeted genes (Mai and Mushinski, 2003). Likewise, the mutation of proto-oncogene ras has been identified in many types of tumor (Rajalingam *et al*., 2007). The dysregulation of these oncogenes is well recognized as an initial step in the development of tumorigenesis.

Molecular docking is done to find the better orientations of the ligand interactions and overall minimal energies. Molecular docking studies are also used to determine the interactions between two molecules. Ligands are the small molecules that bind to the protein of interest to activate the active sites of the molecule. Docking score, calculation of interaction energy and 3D visualization of molecule are some components of molecular docking. Different visualization tools are employed to get the 3D Structure of molecule such as Pymol, rasmol, discovery studio etc. which help to predict the mode of ligand protein interactions and protein annotations. Major application of docking studies is drug designing and discovery.

A molecular docking study includes Dock Ligands (Ligand Fit), Dock ligands (CDocker) and Dock Ligands (LibDock).

Ligand fit - ligand conformations generated using Monte-Carlo techniques are initially docked into an active site based on shape, followed by further CHARMm minimization.

CDocker- random ligand conformations are generated using CHARMm based molecular dynamics. The positions of the ligands are optimized in the binding site using rigid body rotations followed by simulated annealing.

LibDock- a high-throughput docking algorithm that positions Catalyst generated ligand conformations in the protein active site based on polar and a polar interaction sites (hotspots). The drugs that are designed to cure or prevent malaria known as antimalarial drugs. Some compounds like chloroquine and hydroxychloroquine are commercial drugs used to treat malaria. These compound bind specifically to the active sites of the protein and inhibit its activity.

MATERIALS AND METHODS

Structural Refinement

Energy minimization of the human Rho-C is necessary in order to relieve short contacts and correct bad geometry. A solved protein structure for human Rho-C is available at the protein data bank (PDB). Therefore, we had taken it from RCSB PDB (01). The best initial model obtained from the template structure human Rho-C was solvated with solvent water molecule and were roughly energy-minimized.

Structural Validation of the Rho-C 2GCN

Stereo chemical quality and structure analysis (backbone and dihedral angle values) for the crystal structure of 2GCN was done with the PROCHECK (Laskowski et al., 1993), ERRAT (03), ProS A-Web (04), and VADAR (05) programs respectively. The Rho-C 2GCN PDB was taken directly from the RCSB PDB (Colovos C, Yeates, 1993)
.

Docking with EGCG

After the download of the crystal structure of Human Rho-C (PDB ID: 2GCN) from RCSB PDB, the next step was to build the corresponding Human Rho-C by docking epigallocatechin gallate (EGCG) into the respective active sites. The atomic partial charge of epigallocatechin gallate (EGCG) was added by online PRODRG server (06). Autodock 4.2(07) was used for the docking study of

crystal structure of Human Rho-C combined exploits the Lamarckian genetic algorithm (08).The grids (one for each atom type in the ligand plus one for electrostatic interactions) were chosen to be sufficiently large to include not only active site but also significant portions of the surrounding surface. The docking grid size was prepared with the autogrid utility of Autodock setting to 60X60X60 points with a grid spacing of 0.375 A^0. After docking the ligand-receptor complexes were analyzed by Pymol program (09). The grid center was placed in the active site pocket center. The grid boxes included the entire binding site of the enzyme and provided enough space for the ligand translational and rotational walk. The consistencies of the maps were ascertained by checking the minimum and maximum values of the vanderwaal energies and electrostatic potentials for each calculated grid map. Docking was carried out using the empirical free energy function and the Lamarckian genetic algorithm, applying a standard protocol, the energy evaluations were 250,000, the maximum number of iterations 27,000 for an initial population of 150 randomly placed individuals. The number of docking runs was 100 and, after docking, the 100 solutions were clustered into groups with the RMS deviations lower than 0.5 A^0. The clusters were ranked by the lowest-energy representative of each binding mode. The study was performed on an AMD 64 bits dual processor with Linux operating system. Protein structure checks were conducted using the ADIT validation server (http://deposit.pdb.org/validate/); WHAT IF web interface (http://swift.cmbi.ru.nl) and ProSA-web (https://prosa. services.ca-me.sbg.ac.at). Figures were developed using Pymol (http://pymol.source-forge.net/).

RESULTS & DISCUSSION

RhoC is a member of the Rho family of Ras-related (small) GTPases and shares significant sequence similarity with the founding member of the family, RhoA (Sandra, 2007). However, despite their similarity, RhoA and RhoC exhibit different binding preferences for effectors proteins and give rise to distinct cellular outcomes, with RhoC being directly implicated in the invasiveness of cancer cells and the development of metastasis. A solved protein structure for human Rho-C is available at the protein data bank (PDB). Therefore, we had taken it from RCSB PDB, the 3D structure of Rho-C generated by using Pymol software in shown in Fig.1a, EGCG compound taken from green tea, epigallocatechin gallate (EGCG) pdb was developed from Dundee PRODRG2 server, the 3D structure of compound EGCG shown in fig.1b, it was docked with Rho-C of homosapiens (fig.1 c)it reveals that the residues involved in binding of various feedback inhibitors in template Isoleucine-10, Valine-11, Glycine-12, Proline-36, Threonine-60, Phenyl alanine-84, Isoleucine-95, Leucine-114, Glycine-144, Methionine-147 and Alanine-148 shown in fig.1d.

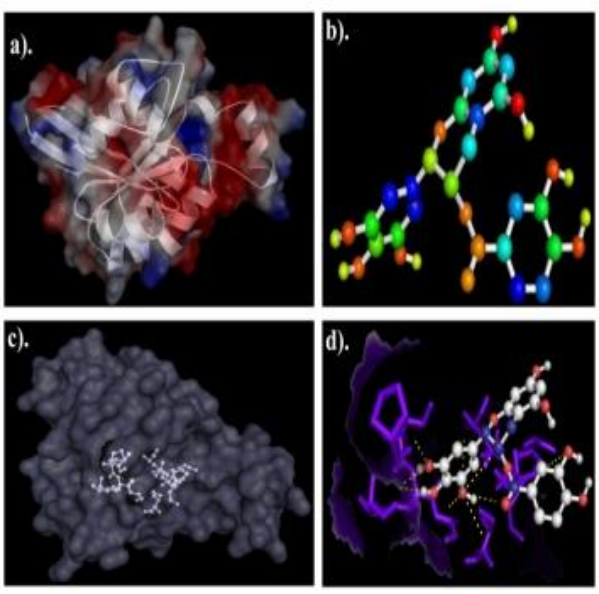

FIGURE 1(a)3D Structure of Human Rho-C in electrostatic representation, (b). 3D Structureof EGCG of Green tea in ball & stick model representation (c) Docking interaction ofhuman Rho-C (dark blue surface model) with EGCG of Green tea (white colored ball &stick). (d). Protein – ligand docking interacting amino acidresidues on the human Rho-C with EGCG. The interactingregion of human Rho-C is representedin Purple color sticks andthe regions of EGCG areshown by ball & stick model. Polar contacts represented in yellow colored doted lines. The 3D structure of the human Rho-C and EGCG complex waspredicted by Protein dockingusing Autodock software.

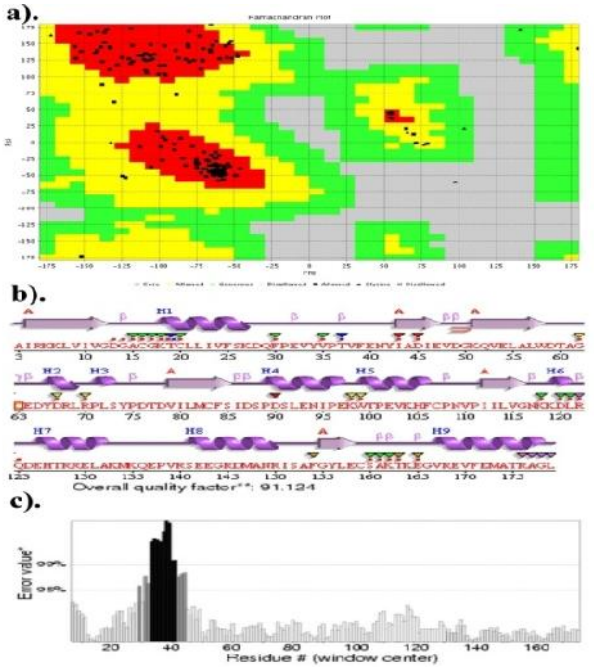

FIGURE 2 (a). Ramachandran plot of developed human Rho-C, in which 143 residues in most favored region (92.3%), 11 residues areadditionally allowed regions (7.1%), and reaming residuesgenerously allowed and disallowed regions (0.6%).(b). Secondary structure of of human Rho-C taken from pdbsum. (c). The ERRAT score for the human Rho-C (2GCN) is 91.124, the backbone conformation and non-bonded interactions of human Rho-C was all reasonable within a normal range.

Validation of the model

In general, the evaluation parameters of the crystallo graphic structure of 2GCN obtained RCSBPDB by WHATIF PROCHECK and ProsA-Web are within the interval of values derived for their homologs and for highly refined structures RMSD Z-score values for bonds and angle parameters for the 2GCN are within values typical of highly refined structures. The fact that the RMS Z-score values of bonding distances and angles for the crystal structures are small might indicate that too-strong constrains for 2GCN. From the analysis of backbone conformations, the 2GCN interface presents only two residues located in a generous region and the remaining interface residues are in the favorable region of the Ramachandran Plot shown in Figure 2a (10). One indication that our 2GCN is a well-refined structure is the fact that its evaluation criterion of stereo chemical and structural parameters is better.

The low overall RMS values for backbone superposition reflect the high structural conservation of this complex through evolution, making it a good system for molecular dynamics and docking studies as a drug target for cancer. From ProSA-Web analysis of a 2GCN protein structure shows the energy graphs having negative values correspond to stable parts of the structure. The ProSA web analysis of 2GCN showed the energy graphs within limits of results. The output graphs showed the Z scores,–9.69 for 2GCN, from this data we can consider the structure of the 2GCN interface as a good representation of the actual system. The detailed secondary structural investigation of the human Rho-C with PDB sum, a secondary structure prediction server revealed 40 (22.6%) residues were in strands, 61 (34.5%) residues were in α-helices, 06 (3.4%) residues that were in 3–10 helix and 70 (39.5%)residues were in other conformations (Fig. 2b). In the current case, the ERRAT score for the human Rho-C well within the range of a high quality model that is 91.124(fig.2c).

Molecular Docking

The computer simulated automated docking studies were performed using the widely distributed molecular docking software, Autodock 4.2(07). Energy minimized human Rho-C were docked with epigallocatechin gallate (EGCG) it was developed from Dundee PRODRG2 server (06). The epigallocatechin gallate (EGCG) specifically bind at active site amino acids of Isoleucine-10, Valine-11, Glycine-12, Proline-36,Threonine-60, Phenyl alanine-84, Isoleucine-95, Leucine-114, Glycine-144, Methionine-147 and Alanine-148, give different docked energies AutoDock binding affinities of the epigallocatechin gallate (EGCG), showing binding affinity evaluated bythe binding free energies (Gb, kcal/ mol), inhibition constants (Ki), hydrogen bonds, and RMSD values. The obtained success rates of Autodock is highly excellent shown in (Table. 1), where the docked EGCG binding free energies -28.43 kcal/mol (fig. 3) From the results it will reveal that EGCG give lowest docked energy, this analysis reveal that the EGCG able to bind tightly with human Rho-C then the epigallocatechin gallate (EGCG) and showing greater binding energies. Fig.4 Active groove of human Rho-C with epigallocatechin gallate (EGCG) involve in binding of active site amino acids and the binding pocket shown in transparent solid surface with labeled amino acids (Pink) and the ligand EGCG shown as a ball & stick mode.

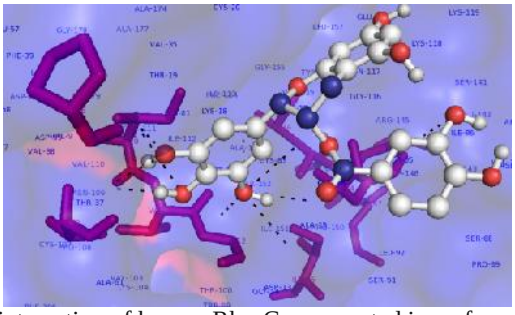

FIGURE 3: Protein-ligand dockinginteraction of human Rho-C represented in surface alongwith catalytic residues in stick form, and EGCG of green tea represented in ball & stick form. The image wasgenerated using Pymol

CONCLUSION

In spite of the availability of effective chemotherapy towards caner, still it remains a leading infectious killer world-wide. Many factors such as, human immunodeficiency virus (HIV) co-infection, drug resistance, lack of patient compliance with chemotherapy, delay in diagnosis, variable efficacy of drugs, various other factors contribute to the mortality due to cancer, for this there is need to development of a new anticancer drug. In this work we choose human Rho-C, which plays a role in directly implicated in the invasiveness of cancer cells and the development of metastasis, the enzyme deemed necessary for survival of cancer cells. It seems to be good target to develop a new anti-chemotherapy against cancer. In this work the 3D structure Human Rho-C taken from PDB RCSB. The structural orientations of the epigallocatechin gallate (EGCG) clearly indicates distinctive affinities of Human Rho-C, this distinctive feature helps to may be inhibition of Human Rho-C and the supporting experimental studies on this data have been conducting in our lab.

ACKNOWLEDGEMENTS

RSC, gratefully acknowledges University Grant Commission (UGC), New Delhi (F.No.33- 222/2007 (SR) 13-3-08 & UGC Lr.No.F.4-1/2008 (BSR), dated 12-02-2010) for financial support. The encouragement given by Sri Krishnadevaraya University, AP, India to our research work is gratefully acknowledged

REFERENCES

Schüttelkopf, A.W. and van Aalten, D.M.F. (2004) "PRODRG: a tool for high-throughput crystallography of protein-ligand complexes", *Acta Crystallogr* D60, 1355–1363.

Colovos, C., Yeates T.O. (1993) Verification of protein structures: patterns of non-bonded atomic interactions. Protein Sci 2:1511–151

Laskowski, R.A., MacArthur, M.W., Moss, D.S., Thornton, J.M. (1993) PROCHECK a program to check the stereo chemical quality of protein structure. J Appl Cryst 26:283–291

Mai, S. & Mushinski, J.F. (2003) c-Myc-induced genomic instability. Journal of Environmental Pathology, Toxicology and Oncology, Vol. 22, No. 3, pp. 179-199, ISSN 0731-8898

Rajalingam, K., Schreck, R., Rapp, U.R., & Albert, S. (2007) Ras oncogenes and their downstream targets. Biochimica et Biophysica Acta, Vol. 1773, No. 8, (January 2007), pp. 1177-1195, ISSN 0006-3002

Sandra, M.G. Diasand Richard, A. Cerione X-ray crystal structures reveal two activated states for RhoC. , 2007 Jun 5; 46(22):6547-58. Epub 2007

Sippl, M.J. (1993) Recognition of Errors in Three-Dimensional Structures of Proteins. *Proteins* 17, 355-362.