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In silico Target Deconvolution of Curcumin (Diferuloylmethane) Against Respiratory Syncytial Virus

Meha Shikhi, Ashwini Geraldine R.K., Diana Scindia V. and Roopa L*

Department of Biotechnology, Mount Carmel College, Bangalore.

***Email:** roopasarahpaul@gmail.com

Abstract

In relation to its antiviral properties, curcumin at higher concentrations is found to lower the growth of Respiratory syncytial virus (RSV) in non-tumorigenic human bronchial epithelial cells (BEAS-2B) infected with RSV. However the mechanism of action curcumin in RSV remains unknown. This analysis unlocks the ways to increase the killing of RSV even at lower concentrations of curcumin, also preventing apoptosis of the host cells when concentration of curcumin is increased (if a higher concentration is needed). The study has identified the viral proteins RNA polymerase L and Ribonucleoprotein N to be susceptible targets in RSV for binding of curcumin or curcumin bioconjugates to combat the virus.

Keywords: RNA polymerase L, Ribonucleoprotein N, AutoDock, PsiThread.

Introduction

Curcumin (diferuloylmethane) is the active component of the herb *Curcuma longa*, also known as turmeric. Studies on curcumin reveals that this compound has significant anti-inflammatory, antimicrobial, antioxidant, anticarcinogenic and more recently discovered antiviral properties. RSV the causative agent of bronchiolitis in infants, is an enveloped virus, with 10 genes encoding 11 proteins namely **Matrix M2-2, Small hydrophobic protein, Major** surface glycoprotein G, RNA-directed RNA polymerase L, Matrix M2-1, Nucleoprotein, Nucleoprotein (Protein N) (Nucleocapsid protein) Non-structural protein 1, Non-structural protein 1 (Non-structural protein 1C) Non-structural protein 2, Non-structural protein 2 (NS2) (Non-structural protein 1B) Phosphoprotein Phosphoprotein (Protein P), Fusion glycoprotein F0. The virus can infect all age groups, causing upper and lower respiratory tract infections, ranging in severity from subclinical infection to pneumonia and death. Though curcumin is known to slightly lower the growth of RSV at higher concentrations in non-tumorigenic human bronchial epithelial cells (BEAS-2B) infected with RSV, it was noted that there were many more dead cells in the wells receiving the highest dose of curcumin. Also the study revealed pre treatment of cells with curcumin at doses below 7.5 µM does not significantly reduce RSV growth in Beas2B cells. In such a quandary, identifying the precise target of curcumin in RSV shall help in designing curcumin bio-conjugates which has greater possibilities to inhibit the viral replication at lower concentrations.

Curcumin is proven to bind directly to diverse proteins owing

to its hydrophobic structure with the phenolic and the carbonyl functional groups located in the ends and the center of the molecule respectively. The structure enables curcumin not only to participate in hydrogen bonding with the targets, but also provides a strong electrostatic interaction to increase favorable free energies of association. According to a study on multitargeting by curcumin it is shown that curcumin binds selectively to the N-terminal domain of DNA Polë (Subash *et al.*, 2011). One of the RSV proteins being RNA directed RNA polymerase L (RNA Pol), it was suspected that this protein could be a susceptible target for curcumin. Yet not only RNA Pol but the other RSV proteins such as Ribonucleoprotein N, Glycoprotein G, Fusion Protein F0, Matrix protein M2-1 were also docked with curcumin and the mode of interactions were studied.

Materials and Methods

All the above mentioned viral protein structures except RNA Pol were obtained from PDB and the 3D structure of the ligand was obtained from ZINC. The structure for RNA Pol was modeled using *Ab initio* model prediction method. Three different threading approaches namely Gen Threader, pGen Threader and pDom Threader were employed. The most appropriate template was selected with respect to its function (as a polymerase), score, query coverage and the RSV RNA Pol query sequence was modeled using this. One hundred models were generated in this process which were screened using DOPE energy. It is to be noted that only the N terminal domain of the query (37 - 429) which is expected to contain the curcumin binding site (51, 57 – 75) (Subash *et al.*, 2011) was modeled. It is also reported that in both Influenza A H5N1 and Influenza A H1N1 RNA Polymerase, it is the N terminal domain that contains the polymerase activity (Xiaojing *et al.*, 2008), (Eiji *et al.*, 2008). For this reason it was decided that the N terminal domain shall suffice for the docking studies.

The receptors and ligand were prepared using MGLtools 1.5.1 and grids were prepared for all the proteins, the grid size and the grid points varied from protein to protein owing to the different sizes of each. The grid was set to encompass the complete protein which is a procedure for blind docking analysis. Docking studies were performed using AutoDock4.2.3. The Lamarckian Genetic Algorithm (LGA) search engine with empirical free energy function for estimation of binding energy, docking energy, inhibitory constant, intermolecular energy and energy were used for separate docking runs. The scoring function included the electrostatic energy, vanderwaal energy, hydrogen bonding energy and the desolvation effect based on which the binding free energy were calculated.

The docking results of each protein was tabulated and each docked conformation was studied and compared based on binding energy, electrostatic energy and inhibitory constant to identify the possible target(s) of the ligand.

Results and Discussion

Results of Threading Analysis

Eighty template structures were reported by the three methods used for threading. The top three templates selected by the server for each method ranked based on *p* –value and score is shown in the Table:1.

Of the 80 templates only three were related to RNA binding and polymerase activity, which were the viral proteins Sigma 70 subunit of E.coli RNA Polymerase (1SIG), RNA Polymerase of Influenza A H5N1(3CM8) and Polymerase acidic protein of Influenza A H1N1(2ZNL). The RSV RNA Pol was modeled using H5N1 RNA polymerase and one hundred structures were produced of which the best was selected based on DOPE, (DOPE=-35733.07813). Per residue DOPE of the model and the template is shown in the Graph 1.

Table 1: Top three PSIPRED Server Results

Sl. No.	Threading Algorithms	Name of the protein with PDB ID	Score	P-Value
1.	Gen	Gibberella zeae Endonuclease (4EFJ)	28.374	0.069
2.		Staphylococcus Exotoxin (3R2I)	27.997	0.076
3.		Streptococcus pyogenes Csn2 (3TOC)	27.226	0.090
1.	pGen	Rattus norvegicus Clathrin (1HF8)	1.171	0.003
2.		Pseudomonas Lectin (1L7L)	1.084	0.004
3.		Sulfolobus ABC Transporter (1OXX)	1.000	0.004
1.	pDom	Bacillus flhF (2PX0)	32.869	0.024
2.		Influenza A RNA Polymerase (3CM8)	32.641	0.026
3.		Salmonella sifA (3CXB)	29.417	0.054

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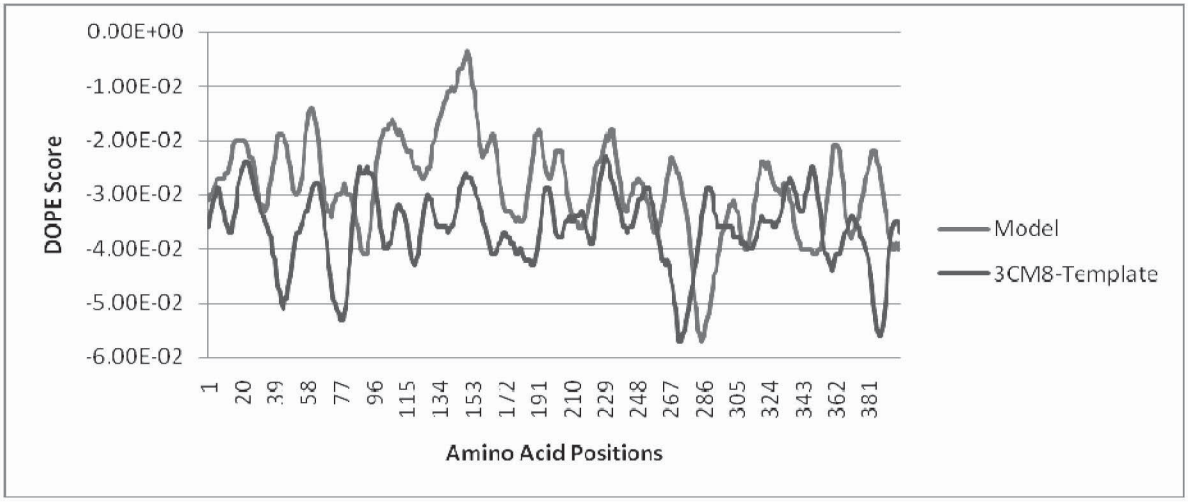

Graph 1: Per residue DOPE of the model and the template

The per residue amino acid energy profile reveal that the energy of the model is closely getting aligned with the template which support the threading reported by Gen threader. This says that the model share the same fold as that of the template.

Results of Docking

Table 2 and Graph 2 shows that the best results for the binding energy and inhibitory concentration were observed for RNA polymerase L follwed by Ribonucleoprotein N. Moreover the Ki value for RNA polymerase L was just 860.59 nM and slightly increased to 3.09uM for Ribonucleoprotein N. Beyond this we see a huge increase when compared to the base value 860.59 nM for the other proteins i.e. 216 fold increase for the fusion protein F0, 379.3 folds for major surface glycoprotein G and 862.2 folds for matrix protein M2-1. From this it is clear that RNA polymerase L and Ribonucleoprotein N could be the targets of curcumin.

In case of HIV-1 integrase curcumin binds to the catalytic core comprising of Asp 64, Thr 66, His 67, Glu 92, Thr 93, Asp 116, Ser 119, and Asn 120and Lys 159. In the HIV-1 Protease the curcumin structure fits well into active site interacting with amino acids Asp 25, Gly 27, Asp 29, Asp 30 and Asp 29' and Asp 30'. These interactions suggested that extensive hydrogen bonding promoted by

Table 2: Binding energy and Ki for the top three conformations of RSV proteins docked with curcumin

Protein	Conformation	Lowest binding energy Kcal/mol	Ki	Energy Electrostatic Energy Kcal/mol
Fusion Protein F0	1	-5.09	186.03uM	-0.56
	2	-5.09	187.27uM	-.21
	3	-4.77	318.93uM	-0.29
Major surface Glycoprotein G	1	-4.76	326.44uM	-0.25
	2	-4.76	759.21uM	-0.06
	3	-3.97	1.22mM	-0.07
Matrix Protein M2-1	1	-4.27	742.02uM	-0.01
	2	-3.93	1.31mM	-0.12
	3	-3.89	1.4mM	-0.03
Ribonucleoprotein N	1	-7.52	3.09uM	-0.19
	2	6.36	21.73 uM	-0.24
	3	-6.27	25.31 uM	-9.25
RNA Polymerase L	1	-8.27	860.59 nM	0.00
	2	-7.21	5.20 uM	0.00
	3	-6.55	15.76 uM	0.00

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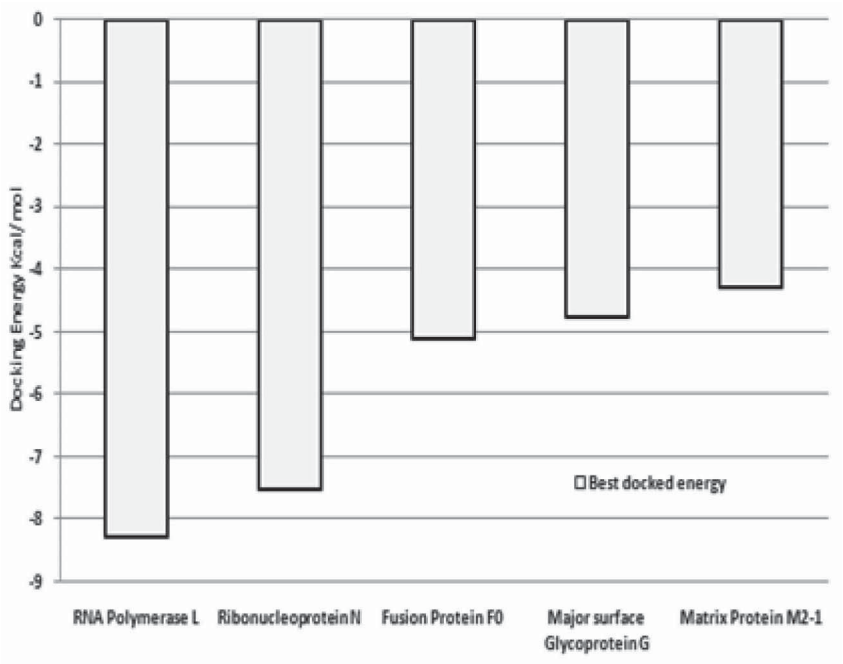

Graph 2: Best docking energies of curcumin docked aganist the five proteins

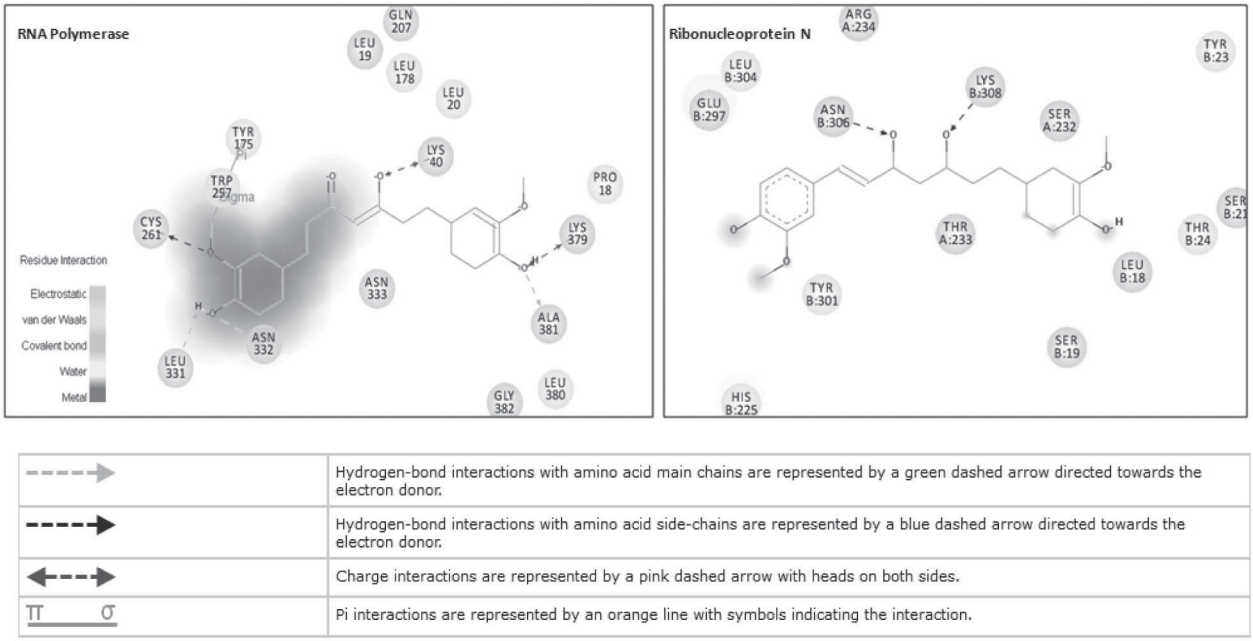

Fig 1: Interacting Amino acids of RNA polymerase and Ribonucleoprotein with curcumin

the O-hydroxyl structure and electrostatic interactions are important for both HIV -1 integrase and HIV-1 Protease interaction. In case of DNA Polymerase curcumin binds selectively to the N terminal residues Thr 51, and residues between 55- 75. (Subash *et al.,* 2011).

In our analysis (Fig: 1 and 1a) curcumin binds to RNA Pol in a pocket that has many charged residues. It was observed that the Michael Acceptors showed a strong electrostatic interaction with Lys 40. One of the phenyl hydroxyl groups of curcumin showed electrostatic interaction with Lys 379

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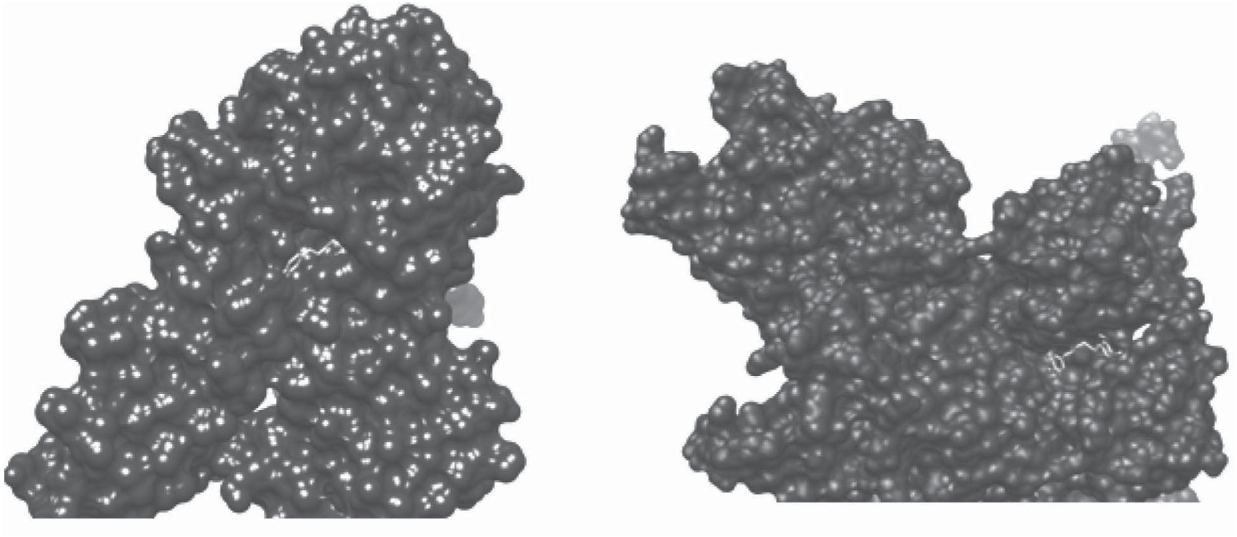

Graph 2: Molecular surface image of curcumin Interacking with RNA Polymerose L (right) and Ribonu cleoprotion N (L)

and the same showed a strong hydrogen bond interaction with Ala 381. The O-hydroxyl of the other phenyl ring showed hydrogen bond interaction with Asn 332 and Leu 331. The methoxy group of this phenyl ring showed a sigma bond with Trp 257 and pi bond with Tyr 175. The same showed a hydrogen bond interaction with Cys 261.

In the Ribonucleoprotein both Michael Acceptors showed hydrogen bond interaction with Lys 308 and Asn 306 (Fig: 1). Even in this curcumin was bound in a pocket composed of charged and hydrophobic residues.

In the figure: 1 the residues circled pink are those involved in hydrogen bond, charge or polar interactions and those circled green are involved in van der Waals interactions. Blue surface indicates aromatic ring edges.

Analyzing all the docked conformations of curcumin in RNA polymerase L and Ribonucleoprotein N it was observed that curcumin binds to a pocket that is chiefly made of aminoacids like Asp, Glu, Ser, Thr, Lys, His, Arg, Asn, Gln, Gly, Leu and Cys which is more of charged and hydrophilic residues. Moreover, the overall interaction of curcumin with both the proteins showing a lot of electrostatic and hydrogen bonding seems to align with the interactions reported for HIV 1 integrase and HIV 1 protease. (Subash *et al.,* 2011).

Comparing the mode of interaction of curcumin with RNA Polymerase and Ribonucleoprotein the former showed better results with respect to the above mentioned factors. Also, it was observed that curcumin binds to the RNA binding region of the ribonucleoprotein indicating that it can act as a competitive inhibitor preventing the binding of RNA, thus inhibiting viral assembly and replication.

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