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IN SILICO TARGET DECONVOLUTION OF CURCUMIN (DIFERULOYLMETHANE) AGAINST HUMAN JOHN CUNNINGHAM VIRUS

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ABSTRACT

Studies on antiviral properties of Curcumin bioconjugates such as di-O-tryptophanylphenylalanine curcumin and di-O-decanoyl curcumin have shown good results with EC_{50} 0.011 mM and 0.029 mM against Vesicular stomatitis Indiana virus and Feline herpesvirus, respectively[1]. In relation to its antiviral properties, Curcumin has been docked against the proteome of the dsDNA Human John Cunningham Virus (JCV) to identify if any of these proteins may act as targets for curcumin. The docking studies show low binding energies of curcumin with Agnoprotein of JCV with K_i value as low as 4.84 µM. The study has identified the same protein to be the susceptible target in JCV for binding of curcumin or its bioconjugates to combat the virus.

KEYWORDS: **Autodock, I-TASSER, Progressive multifocal leukoencephalopathy, Viral Zone, ZINC**

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INTRODUCTION

Curcumin (diferuloylmethane) is the active component of the herb *Curcuma longa*, also known as turmeric. Studies on curcumin reveal that this compound has significant anti-inflammatory, antimicrobial, antioxidant, anticarcinogenic and more recently discovered antiviral properties[1,2,3,4]. It is reported that the antiviral activities of di-O-tryptophanylphenylalanine curcumin and di-O-decanoyl curcumin might be due to their higher lipophilicity, which enhances their cellular uptake. JC virus (JCV) is a polyomavirus infecting greater than 80% of the human population early in life. Replication of this virus in oligodendrocytes and astrocytes results in the fatal demyelinating disease progressive multifocal leukoencephalopathy (PML), a neurodegenerative disease of the human central nervous system resulting from the lytic infection of oligodendrocytes. This disease mostly develops in patients with underlying immunosuppressive conditions including Hodgkin's lymphoma and lymphoproliferative diseases, as well as those undergoing antineoplastic therapy and those with acquired immunodeficiency syndrome (AIDS)[4,5]. The genome of JCV consists of three functional regions, including the viral early and late coding regions and viral noncoding regulatory region. The viral early coding region encodes two regulatory proteins, small T and large T-antigens. Although little is known about the function of small T-antigen, the large T-antigen was shown to be a multifunctional phosphoprotein involved in both the initiation of viral DNA replication and T-antigen mediated activation of JCV late genes.

The viral late coding region encodes the structural proteins, VP1, VP2, and VP3. These three proteins form capsids for the viral genome and function in the attachment, adsorption, and penetration of the virus to the host cells. The leader sequences of the late transcripts also contain an open reading frame encoding a small protein called agnoprotein. This protein is known to have regulatory roles in replication & transcription[6]. The regulatory region of JCV genome consist of the origin of DNA replication, promoter elements for both early and late genes, and cis-acting enhancer elements[6]. In spite of these findings neither a drug nor a vaccine has yet been produced for the virus. In such a quandary, identifying the precise target of curcumin in JCV 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 Polλ[7]. For this reason JCV proteins such as Large T Antigen, Small T Antigen, Major Capsid Protein and VP1 were docked with curcumin and the mode of interactions were studied.

MATERIALS AND METHODS

The viral protein structures of Large T Antigen and Small T Antigen were obtained from MODBASE and the crystal structure of Major Capsid Protein was obtained from Protein Data Bank. Agnoprotein and VP1 structures were modeled using I-TASSER[8,9,10]. The tool employs eight different threading approaches such as MUSTER, HHSEARCH, PROSPECT2, PPA-I, FFASO3, SP3, SPARKS & SAMT99 to align the protein sequence on the different templates. Also, top 10 templates were obtained on the basis of Structure Alignment using program TM-Align. The structure with highest C – score (a confidence score for estimating the quality of predicted models by I-TASSER, calculated based on the significance of threading template alignments and the

convergence parameters of the structure assembly simulations) and TM score (Measure of global structural similarity between query and template) was selected as the best model for the docking studies. Any model with a TM score more than 0.5 indicates a model of correct topology. 3D structure of the ligand was obtained from the chemical database ZINC. 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 torsional free energy were used for separate docking runs. The scoring function included the electrostatic energy, vanderwaals 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

1. Modelling of Agnoprotein

I-TASSER identifies template proteins of similar folds from PDB library by LOMETS, a meta-threading approach. The continuous fragments excised from the PDB templates are reassembled into full-length models by replica-exchange Monte Carlo simulations with the threading unaligned regions (mainly loops) built by ab initio modeling. The top 3 templates used to model Agnoprotein were 1agm, 2vn7, 4fus with TM Scores 0.629, 0.624, 0.622 Figure 1 shows structure of the modeled Agnoprotein with its predicted binding sites that was used for docking studies, estimated accuracy of the model with TM Score being 0.40 ± 0.13. TM-score weights the close matches stronger than the distant matches, a single score between 0 - 1 is assigned to each comparison. Based on statistics, if a template/model has a TM-score around or below 0.17, it means the prediction is nothing more than a random selection from PDB library. For predicting the binding site of a protein the I-TASSER server matches the predicted 3D models to the proteins in 3 independent libraries which consist of proteins of known enzyme classification (EC) number, gene ontology (GO) vocabulary, and ligand-binding sites. The final results of binding site predictions are deduced from the consensus of top structural matches with the function scores calculated based on the confidence score of the I-TASSER structural models, the structural similarity between model and templates as evaluated by TM-score, and the sequence identity in the structurally aligned regions. For Agnoprotein binding site residues were predicted by I-TASSER based on 5 templates of which the best local match score of 1.14 between agnoprotein and template was reported for the template 1o7dB, the predicted binding site residue numbers being 29, 33 and 36 as shown in Table 1.

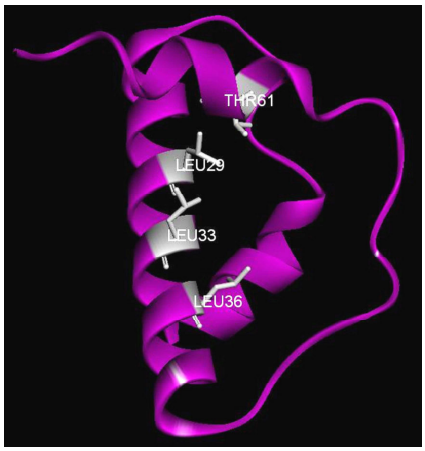


Figure 1
Structure of Modelled Agnoprotein with predicted binding sites

Table 1
Predicted Binding Sites of Agnoprotein

Rank	PDB Hit	Cscore[LB] (confidence score)	IDEN[a] (% sequence identity in the structurally aligned region)	Predicted binding site residue number
1	1dogA	0.01	0.029	21, 22, 25, 26, 27
2	3eqaA	0.01	0.029	20, 21, 64, 67, 69
3	1o7dB	0.01	0.021	29, 33, 36
4	2olkB	0.01	0.052	30, 33, 61
5	3d5b2	0.01	0.016	6, 32, 33, 36

2. Results of Docking

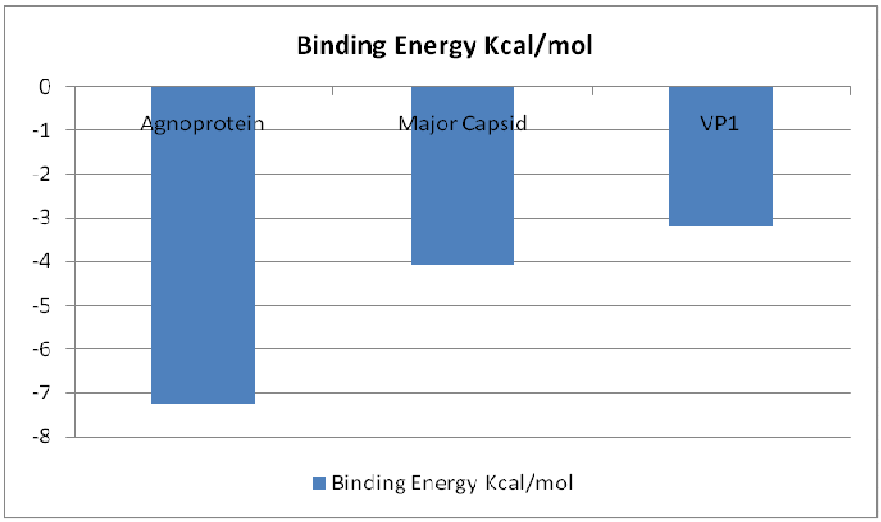
Table 2 shows the binding energy and inhibitory concentration of the JCV proteins that were docked with curcumin.

Table 2
Binding Energy and Inhibitory Concentration of the JCV Proteins Docked with Curcumin

Protein	Conformation	Binding Energy Kcal/mol	K_i
Agnoprotein	1	-7.25	4.84μM
	2	-6.75	11.32 μM
	3	-6.68	-12.63 μM
Major Capsid	1	-4.08	1.03 mM
	2	-3.55	2.51mM
	3	-3.49	2.75mM
Minor Capsid Protein VP1	1	-3.21	4.44mM
	2	-2.95	6.83mM
	3	-2.8	8.79mM
Large T – Antigen	1	146.16	--
	2	154.55	--
	3	166.06	--
Small T – Antigen	1	31.91	--
	2	36.02	--
	3	36.28	--

A graphical representation of the binding energy of JCV proteins is shown in Graph 1.

Graph 1
Binding Energy and Inhibitory Concentration of Agnoprotein, Major Capsid protein and VP1 Docked with Curcumin



As the binding energies of Large T Antigen and Small T antigen are too high, it is considered insignificant and not shown in graph 1. Best results were observed for Agnoprotein follwed by Major Capsid Protein and Minor Capsid Protein VP1with K_i value of 4.84µM, 1.03 mM and 4.44mM respectively. The other proteins showed a relatively positive binding energy with curcumin and so K_i values were not computed for these proteins. This is also indicative of the fact that these conformations were not stable and unfavorable on binding curcumin. The surface model of the protein interacting with curcumin reveals that curcumin binds to the active site of protein. As it is known that agnoprotein has replicating and regulatory functions in JCV, this interaction can inhibit viral replication[6].Figure 2 shows the Surface Model of Agnoprotein Interacting with Curcumin.

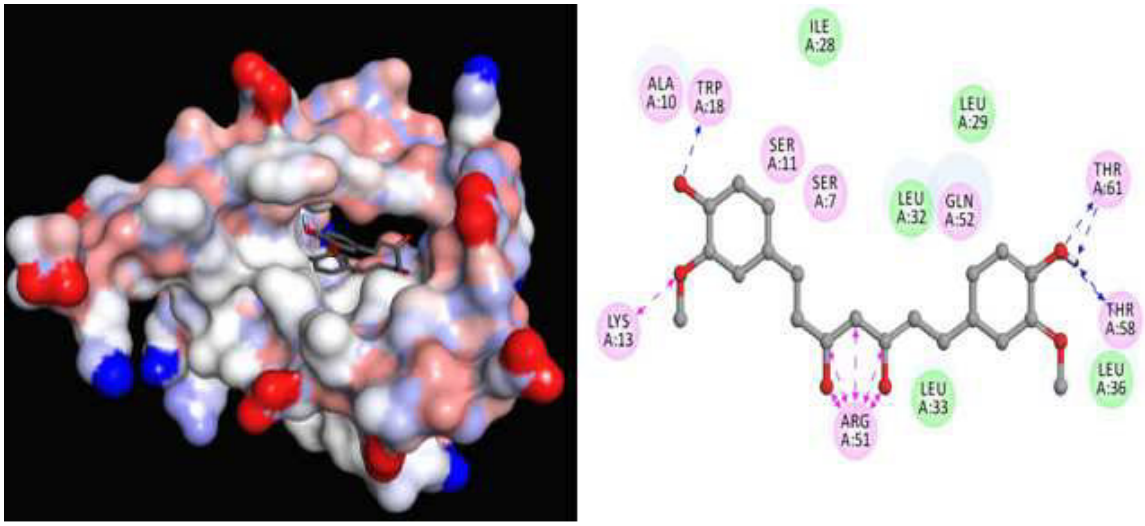


Figure 2
(Right) Surface Model of Agnoprotein Interacting with Curcumin and Figure 3 (Left) Interactions of Curcumin with Agnoprotein

Figure 3 shows the 2D diagram of interactions of curcumin with Agnoprotein; 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 and pink dotted lines indicate hydrogen bond donor and acceptor interactions respectively. Blue surface indicates aromatic ring edges.

DISCUSSION

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 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' ,Asp 30' of chain A and B respectively. These interactions suggest that extensive hydrogen bonding promoted by the O-hydroxyl structure and electrostatic interactions are important for both HIV -1 integrase and HIV-1 Protease interaction[7]. In our analysis curcumin binds to Agnoprotein in a similar manner as that in case of HIV -1 integrase and HIV-1 Protease mentioned above to a pocket that has charged residues as could be seen in Figure 2. It was observed that the Michael Acceptors in curcumin showed strong electrostatic interaction with Arg 51. One of the phenyl hydroxyl groups of curcumin showed hydrogen bond interaction Trp 18 and the same showed electrostatic interaction with Lys 13. The O-hydroxyl of the other phenyl ring showed hydrogen bond interaction with Thr 61 and Thr 58. Interestingly Thr 61 is one of the predicted binding site residues of Agnoprotein, also are Leu 29, Leu 33 and Leu 36 seen to interact by vander waals force of attraction with curcumin, all of which are predicted by I-TASSER as binding site residues.

CONCLUSION

Analyzing all the docked conformations of curcumin in Agnoprotein it was observed that curcumin binds to a pocket that is chiefly made of amino acids like Gln, Ser, Thr, Trp, Lys, Ala, Arg and Leu which is more of charged and hydrophilic residues. Moreover, the overall interaction of curcumin with Anoprotein showing a lot of electrostatic interactions and hydrogen bonds seem to align with the interactions reported for HIV 1 integrase and HIV 1 protease[7], indicating that the possible target of curcumin in JCV is Agnoprotein. Having known the mode of interaction of curcumin with agnoprotein curcumin bioconjugates with better bioavailability and lower inhibitory concentrations may be designed to interact and fight the complications of JCV. This may be achieved using Ligand-based drug design by deriving a pharmacophore model from the above mentioned curcumin agnoprotein interaction pattern, that defines the minimum necessary structural characteristics a molecule must possess in order to bind to the target. Alternatively, a quantitative structure-activity relationship (QSAR), in which a correlation between calculated properties of modified curcumin molecules and their experimentally determined biological activity, may be derived. These QSAR relationships in turn may be used to predict the activity of new analogs.

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