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Probing Potent Interfacial Residues in Microvirin before Complexation with Glycoproteins: Visualizing Anti-HIV Activity *in silico*

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Abstract

Microvirin, a mannose binding anti-HIV (Human Immunodeficiency virus) lectin has been proved as a potent agent in combating HIV. Ligand binding residues of Microvirin was taken in to account for analysis of interfacial residues and identify surface/Buried residues based on Accessible surface area and free energy based calculations. After categorization, Comparative B factors were assessed for prediction of Rigid/Non-Rigid residues among the Ligand binding residues. Furthermore docking microvirin with glycoproteins gp120 and gp41 revealed the conserved interfacial residues in complexes and affirms the computational dissection of microvirin's anti-HIV activity.

Keywords Anti-HIV activity; B factor analysis; Interfacial residues; Ligand binding residues; Microvirin

Introduction

Acquired immune deficiency syndrome (AIDS) is a fatal disease of the human immune system caused by the human immunodeficiency virus (HIV). Human Immunodeficiency virus (HIV) is still a significant risk of high morbidity and mortality with secondary infections and devastated over 25 million deaths globally [1]. The disease is characterized by opportunistic infections and tumors, which render the individual's immune system debilitating. HIV-1 entry in to host cell is mediated by viral envelope glycoproteins gp120 and gp41. Initially after binding to CD4 cells they bind on to CXCR4 and CCR5 [2,3]. Anti-HIV medication broadly fall in to three main categories namely nucleoside Reverse Transcriptase, Non- Nucleoside Reverse Transcriptase inhibitors and protease inhibitors. However, drugs presently in the pipeline are administered combinatorially for arresting the pathogen [4]. Nevertheless the genetic diversity of HIV virus still remains quizzical for several chemists and Biologists as Human Immunodeficiency virus evades the combating mechanism at ease. Vaccines for HIV have been still in testing stages which comprise of Subunit vaccines [5], Recombinant viral vaccines [6] and DNA vaccines [7]. But there has been reduced success in developing an anti-HIV vaccine. Cyanobacterial lectins like Cyanovirin-N, 11-kDa protein isolated from the cyanobacterium Nostoc ellipsosporum is effective against both laboratory strains and primary isolates of HIV-1 and HIV-2 at an order of nanomolar concentration. Cyanovirin-N aborts cell-to-cell fusion and transmission of HIV-1 infection [8]. Antiviral activity is attributed to the binding with the viral envelope glycoprotein gp120. Nevertheless the lectin microvirin (MVN) from the cyanobacterium Microcystis aeruginosa PCC7806 shows 33% identity at the amino acid level with CV-N. MVN has anti-HIV-1 activity comparable with that of CV-N but a much higher safety profile [9]. Microvirin (MVN) compared with cyanovirin-N (CVN), show potent HIV-1 neutralization with reduced toxicity profiles [10]. The present analysis deals with ligand binding properties of microvirin for categorizing surface and buried residues based upon solvent accessibility and free energy of ligand binding residues. Docked complex interfacial residues also affirm the fact that majority of interfacial properties in interacting residues.

Methods

Ligand binding site prediction

Ligand binding sites were predicted using Q site finder [11], surface topology and pocket information were analyzed by the castP server [12]. Pocket detection and occupancy was found using Q-SiteFinder. Clefts were identified in the protein-surface using Q-SiteFinder. The solvent accessible surface area (SASA) was found by GETAREA [13]. The atomic Solvent Accessible Surface Area (SASA) covered by each cleft was calculated by utilizing radius of water probe 1.4 A0 and the area/ energy per residue was calculated. Dielectric constant was set to 80.0, and Poisson-Boltzman method of computation for 20 cycles was used for calculating the electrostatic potential in SWISS-PDB viewer [14]. All the ligand binding residues were among hotspots as predicted by Meta-PPISP [15]. Furthermore, PIC [16] was made used to calculate the nature of interaction in the ligand binding residues.

Identification of surface/Buried residues

Two methods were adopted for presicting surface/buried residues among ligand binding residues. First, relative accessible surface area by Accessible solvent area calculations (ASAVIEW) [17] based calculations demarcated the residues based on five criteria namely positively charged, negatively charged, polar uncharged, cystein rich and hydrophobic residues. Among the predictions hydrophobic and positively charged residues were constituted as buried as amino acids are positively charged. Whereas the remaining criteria satisfied the surface residue pattern due to solvent accessibility. In total 18 residues were pooled as surface residues by this method. Secondly, GROMOS'96 version of SWISS-PDB viewer [14] was used to calculate G of individual ligand binding residue. Values of free energy in negative were designated as surface and positive values were regarded

as buried residues as energies in Kcal/mol provide binding energies. Moreover, reduced binding energies yield high affinity.

Comparative B factor analysis and prediction of rigid/non rigid nature of ligand binding residues

Binding factor for ligand binding residues were predicted using BFPred [18] and individual B factors were statistically depicted for summarizing rigid/non rigid nature of the ligand binding residues. The normalized B-factor per residue (Bi,N) was calculated based on

Bi,N = Bi-<Bi>

σ Bi

where Bi - B-factor of residue

<Bi> - mean B factor

σ - Standard deviation

Bi,N ≤ 0.04 were considered as rigid whereas ≥ 0.04 were regarded non-rigid [19].

Results and Discussion

Ligand binding residues in a protein constitute a subset of hotspot residues in protein - protein interactions. Hotspot residues have been correlated to energy hot spots and structurally conserved residues. Hot spots in the interface of protein complexes identify unique binding sites from the rest of the surface [20]. Presently, microvirin a mannose binding lectin's ligand binding residues were assessed for conservation and properties which renders them to be potential binders with glycoproteins of HIV. Table 1 depicts the ligand binding residues with the potent interaction whether they conform hydrophobic, catioinic-pi, ioinic and disulfide linkages. Tyr 13, Arg37, Cys 60,61 are responsible for Cationic – pi, ioinic and disulphide linkages respectively. Hotspot residues as predicted by meta-PPISP also affirm that ligand binding residues confer interfacial properties. Fourier transform MAP (FTMAP) scans also confer the hydrophobic and hydrogen bond interactions among ligand binding residues of microvirin (Table 2). The present approach combinatorially includes Kortemme and Baker with hydrogen bond contribution to hotspots and pertaining to hydrophobic interactions [21,22].

Ligand binding residues	Type of interaction
Tyr 13	Cationic-pi
Asp 14	Hydrogen bond
Pro 15	Hydrogen bond
Asp 16	Hydrogen bond
Ser 17	Hydrogen bond
Thr 18	Hydrogen bond
Ile 19	Hydrogen bond
Leu 20	Hydrogen bond
Leu 36	Hydrogen bond
Arg 37	Ionic
Leu 38	Hydrogen bond
Ser 39	Hydrogen bond
Asp 40	Hydrogen bond
Ile 42	Hydrogen bond
Asn 55	Hydrogen bond
Phe 56	Hydrogen bond
Glu 57	Hydrogen bond
Glu 58	Hydrogen bond
Thr 59	Hydrogen bond
Cys 60	Disulfide bridge
Glu 61	Hydrogen bond
Asp 62	Hydrogen bond
Cys 63	Disulfide bridge
Cys78	Disulfide bridge

Table 1: Interaction characteristics of ligand binding site residues of Microvirin.

Ligand binding residues	Energy in Kcal/mol
Tyr 13	-87.719
Asp 14	11.07
Pro 15	-8.249
Asp 16	22.317
Ser 17	16.402
Thr 18	-21.202
Ile 19	-19.293
Leu 20	-36.652
Leu 36	-36.355
Arg 37	-227.821
Leu 38	-28.355
Ser 39	19.881
Asp 40	7.498
Ile 42	9.271
Asn 55	-173.702
Phe 56	-50.602
Glu 57	-173.616
Glu 58	11.874
Thr 59	-24.101
Cys 60	-16.145
Glu 61	-182.734

Asp 62	-2.57
Cys 63	1.46
Cys78	-46.419

Table 2: Energy values of binding residues.

Rigid/Non rigid residues

Figure 1 pictorially demonstrates individual nature of aminoacids in the lectin, microvirin. Free energy based calculations were done using GROMOS'96 version of SWISS-PDB Viewer. Negative delta G were regarded surface residues and positive values were considered buried in the present analaysis. Relative accessible surface area calculations revealed the presence of 18 surface residues (Figure 2) due to their polar uncharged and negative nature of aminoacids. As aminoacids are positively charged, hydrophobic and positively charged residues were regarded as buried.

Figure 1: Relative accesssible surface area of individual residues indictaed by Blue – Positively charged residues, Red – Negatively charged residues, Green – Polar uncharged residues, Yellow – Cystein, Gray – Hydrophobic residues.

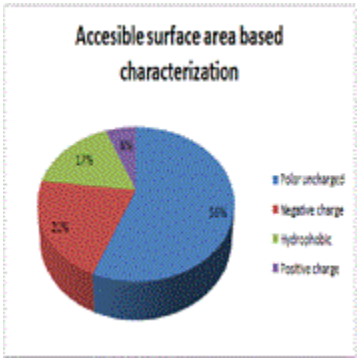

Figure 2: Pie chart depicting solvent accessible surface area.

A total of 16 surface residues (Figure 3) were significant from the study. Although the energy based and surface based approaches revealed specific properties for ligand binding residues, Comparative B factor analysis (Figure 4) after B factor prediction by BFPRED server showed that rigid residues dominate non rigid residues in both relative accessible surface area and energy based calculations. In this context, self-protein stabilizing residues are alone taken into account for assessing their rigidity as the same method was used to analyze transient protein complexes [19] in which some interfacial residues stabilize the self-protein. Here a similar approach reveals that ligand binding residues apart from forming complexes do self-stabilize the protein. Hence the rigid/non rigid analysis yields predominant stability of ligand binding Residues.

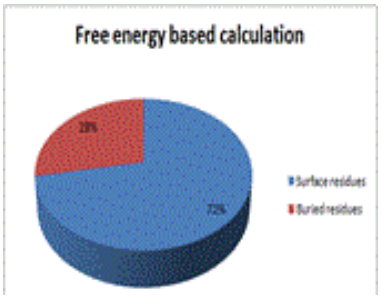

Figure 3: Free energy based categorization of surface and buried residues.

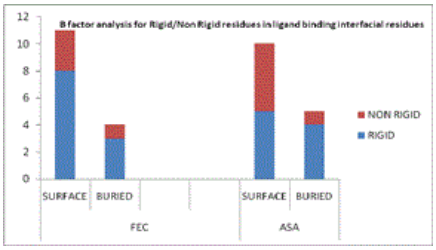

Figure 4: B factor analysis of ligand binding residues for rigid/non rigid residues where FEC- Free Energy calculation and ASA – Accessible Surface Area.

Conservation of ligand binding residues in docked complexes of microvirin with gp120 and gp41.

NFSHTFQE (Asn, Phe, Ser, His, Thr, Phe, Gln, Glu) were conserved in both docked complexes of glycoproteins with high binding affinities. Amongst which, His alone is not enlsited in ligand binding residue. However it may be a surface interacting residue (Figure 5). Structural and physicochemical signatures at Protein-protein interaction interfaces are enough to detect optimal ligand binding of unliganded protein [23]. Fourier transform mapping revealed 12 clusters of small molecule binding sites for gp120 interaction compared to 10 clusters with gp41 (Figure 6). Hence from the present study it is evident that ligand binding residues can act both as self-stabilizing and binding attributes. Furthermore it can be categorized as structural conservation of lectins like microvirin is effcieint in glycan binding with conservatory aminoacids.

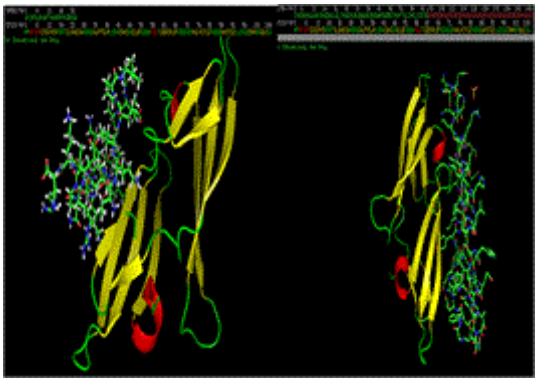

Figure 5: Docked complexes of microvirin with gp120 and gp41 indicating residue conservation. Ball and stick models refer to microvirin.

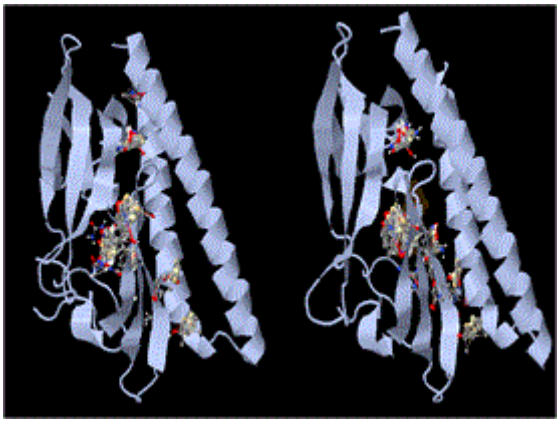

Figure 6: FTMAP of docked complexes showing 12 and 10 clusters of small molecule binding in gp120 and gp41 respectively. Ball and stick configuration refers to microvirin clusters.

Conclusion

Ligand binding residues between gp120 and gp41 show potent interaction consisting hydrophobic, catioinic-pi, ioinic and disulfide linkages. Tyr 13, Arg37, Cys 60,61 are responsible for Cationic – pi, ioinic and disulphide linkages. Rigid/non rigid analysis yields predominant stability of ligand binding Residues. NFSHTFQE (Asn, Phe, Ser, His, Thr, Phe, Gln, Glu) were conserved in both docked complexes of glycoproteins with high binding affinities. Fourier transform mapping revealed 12 clusters of small molecule binding sites for gp120 interaction compared to 10 clusters with gp41. Structural conservation observed from the present study of lectins like microvirin prove glycan binding is conserved in protein-protein interaction. Experimental data to prove the above phenomenon can be foreseen in the near future.

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