Molecular Docking Study of Auto Inhibitory Domain Fragments to Calcineurin A

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Abstract—Cyclosporine (CsA) and FK506 are used to inhibit the activity of Calcineurin (CN), a well known protein phosphatase involved in the suppressing immunity during organ transplantation. Since AID, a C-terminal fragment of CN A inhibits CN activity in in vivo, hence there is a need to explore the possibility of AID and its fragments for their inhibitory properties. In the current study, the CN A was modeled using I-TASSER. Modeled CN A has been used to generate peptide fragments from its AID domain and these fragments were docked back with CN A using Hex docking software. Further CsA and FK506 known inhibitor of CN were also docked with CN A. From docking study we have found that fragments AIDP1 derived from AID has lowest docking Energy-total (E-total) than AID. Another peptide AIDP2 was also found to have similar docking E-total to that of AID. In conclusion the study predicts that the possibility of using AIDP1 as a potent inhibitor of CN either in vitro or in vivo.

Index Terms— Calcineurin; AID; Binding affinity; Immunophilins

1 INTRODUCTION

CN is a Ca++/calmodulin dependent serine/threonine protein phosphatase [1]. It is a heterodimer composed of 61 KDa protein-calcineurin A (CN A) and 19 KDa protein-calcineurin B (CN B). CN A possess catalytic domain, CN B binding region and calmodulin binding region present in position 71-325, 350-370 and 391-415 respectively and also Auto Inhibitory Domain (AID) from the 467-491 (ITSFEEAKGLDRINERMPPRRDAMP) regions present at C-terminal [2].

CN mainly present in neurons, T-cells other than brain and activation of T-lymphocyte signal is inhibited by drugs like CsA and FK506 with the help of immunophilins [3] or may directly inhibition of CN [4] [5]. Crystal structure of CN showed that drug-immunophilin complexes i.e., CyP-CsA and FKBP12-FK506 inhibit CN activity by interacting with common binding surface, but recognizing the various residues [1]. CsA and FK506 form complex with immunophilins and then binds to the LXVP binding site of CN to inhibit CN phosphatase activity [6][7][8]. Earlier reports suggest that CN and NFAT signaling is mainly involved in cardiac hypertrophy [9]. Due to discover this molecules and its role, organ transplantation is increased gradually by using the first CN inhibitor CsA in mid 1980 and later with FK506.

CN A subunit has different regions having autoinhibitory action which in turn inhibit CN activity [10][11]. The AID is one among different regions involved in inhibitory action of CN mainly by binding to active site [12]. It is also known that CsA and FK506 disrupt the interaction of catalytic subunit A and AID of CN [13]. 1995) there by bringing down its activity. Despite the development of CN:CsA and CN:FK506, complete structure of CN still not available. But the direct inhibition of CN is still under the purview of

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innovation. Hence CN A was modeled completely and generated peptide fragments from modeled AID and binding affinity with CN was determined. This study may help to design a potential peptide which can inhibit phosphatase activity during immunosuppression and to determine the CN activity in vitro.

2 MATERIAL AND METHODOLOGY

2.1 Molecule retrieval

Primary sequence of CN A including AID, protein sequence was retrieved from the SwissProt database (ID:Q08209) [14]. Retrieved sequences were queried in BLAST search tool to confirm homology of CN A and AID protein sequence with CN in protein data bank (PDB). BLAST results showed maximum identity with human CN, PDB ID 1AUI and its partial sequence of AID. 1AUI was retrieved from PDB to be used as receptor and subjected to further process before docking. Drugs CsA and FK506 were retrieved from PDB IDs are 1MF8 and 1YAT respectively.

2.2 Molecular modeling of AID

To obtain complete 3D structure of AID, which was missing in x–ray structured CN, 1AUI, we used automated model building server I-TASSER. CN A sequence was retrieved from SwissProt containing AID was submitted to I-TASSER to predict the 3D structure of AID. It uses the following molecule 1AUI, 1WAO A and 1LL8 A as templates to build structure of AID contain CN A. Templates selected based on Template Modeling Score was used to predict 3D structure. Based on these templates I-TASSER predicted five models computationally by using C-score algorithm value -5 to 2, C-score represent the confidence score of model and if C-score increases, confidence of the model too increases. We have chosen best CN A model having C-score -1.87 among the 5 suggested models. (Based on C-score the generated models as follows as, model-2 C-score=-2.24, model-3 C-score=-2.09, model-4 C-score=-3.19 and model-5 C-score=-3.21) [15]. Then high C-score model structure of CN A was analyzed by RAMPAGE for geometrical study and then was submitted to Protein Model Database PMDB (ID.PM0079283). From modeled CN A, 3D structure of AID was extracted and peptide fragments were generated and used as inhibitor peptides.

2.3 Molecules preparation

CN molecule retrieved from PDB was subjected to preparation by removing water molecules and added missing hydrogen molecules using MGL tools 1.5.4 [16]. Using Pymol V 1.3 [17]. CN A chain was separated from CN B and truncated c–terminal contain partial AID region. Same procedure was followed for the CsA and FK506 preparation.

2.4 Validation of CN A and modeled AID

CN A and modeled AID were subjected to its quality, phi and psi angle checked by RAMPAGE [18]. Structure Analysis Verification Server (SAVS) through PROCHECK [19] analysis the errors and warning of the 3D structure were verified and using 3D-Verify program analysis 3D structure stability was checked by comparing with the 1D model [20]. ERRAT program was used to validate the overall quality of steriochemical structure of the CN A and AID through graphical representation [21].

2.5 Designing of peptide fragments and Binding pocket prediction

Peptide fragments were generated from the amino acids sequence of modeled AID using Pymol. Q-site finder is used to predict the active site of CN [22].

2.6 Docking

Hex V 6.12 is the only docking program to use spherical polar Fourier (SPF) correlations to accelerate the calculations of docking process and 3D structure superposition. Hence docking was carried using Hex [23].

CN A was used as receptor, and AID, AIDP1 (SFEEAKGLDRINERMP), AIDP2 (SFEEAKGLDRINERMPPR) peptide fragments were used as ligand in Hex protein-protein docking method. Parameter used in docking protocol to analyze lowest docking E-total evaluation is based on correlation type "shape only". First Fourier Transform (FFT) mode used in our docking process was 3D and with grid dimension 0.75, remaining all parameters was used as default. Docking process develop lowest E-total clusters for possible conformers at every interaction site. Finally interaction of peptides analyzed was based on cluster conformation formed at active site. Highest ranking score built at interaction of receptor and ligand complex.

Docking results generated 500 lowest energy cluster confirmation models for every docking complex. Post Processing applied 1000 solution to carry out filter and refine the confirmed clusters. Steric clashing clusters are removed in post process by using Clustering control panel by bump counter. Post processing applied to minimize each docking solution by calculating molecular mechanic energy or Newton like energy by using 'soft' Lennard-Jones and H-bond potential implemented from the OPLS force field parameters. Root Mean Squares Deviation (RMSD) value -1 is used. Including this mainly good docking solution is selected based on lowest docked E-total [24] [25]. The same procedure was followed to drugs CsA and FK506 with CN A.

2.7 Analysis of docking complex interaction

Docking complex of CN A- AID, CN A-AIDP1, CN A-AIDP2 and CN A-drugs were analyzed carefully and explored for residue interaction at active site and drug binding site using Molegro molecular viewer 20112.2 [26].

3 RESULT

3.1 Structure and active site prediction

Due to lack of complete X-ray structure of CN we construct complete AID domain. We modeled entire CN A chain with AID domain since short amino acid sequence is less sufficient to carry out modeling in I-TASER. It predicted 5 structure model based on C-score. Among all constructed models, model with highest C-score -1.87 was selected for our studies. AID domain extracted from that modeled CN A and peptides were designed. Q-site finder predict the following amino acid residues present at active site and may involved in binding interaction in the CN are Phe95, Asp97, Arg122, Gly123, try124, Phe125, Pro309, Asn310, Try311, Leu 312, Asp 313, Cys336, Ser337, His339, Pro340, Try341, Trp342, Leu343.

2.2 Molecular quality assessment

Modeled structure of AID and X-ray crystal structure of CN A (1AUI) were subjected to quality and error check. Ramachandran plot analysis is shown in figure 1 indicate number of residues in

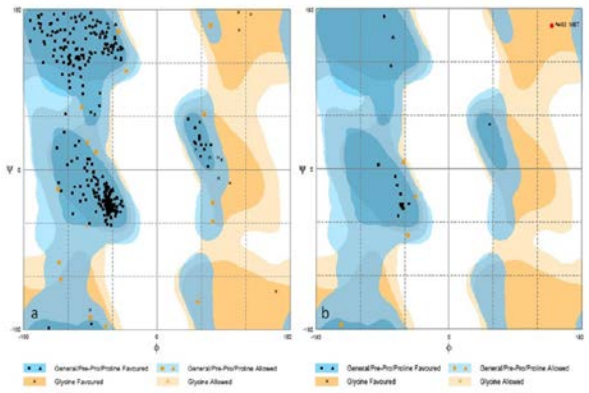

Fig 1. Ramachandran plot analysis of (a) CN A and (b) modeled AID by RAMPAGE.

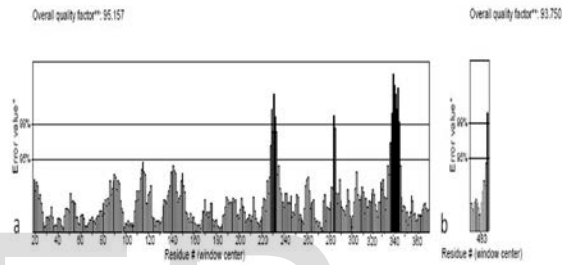

Fig 2. (a) Shows overall quality of CN A and (b) AID of CN quality factor predicted by ERRAT server.

favored region are 95.5%, and 78.3% in CN A and AID respectively. ERRAT was used for non bonded atomic interactions, to analyze quality of protein. Results shows that modeled AID is better than accepted value i.e., >5.0 [27] and overall quality was 94.872% suggesting that AID is good model and CN A showed 95.143% indicating quality is too good as showed in figure 2.

3.3 Docking of designed peptides with CN A

Molecular mechanism of protein-peptides and protein-drugs interactions were performed using 'electrostatic and shape only' parameters using Hex. Docking study of AID, AIDP1 and AIDP2 peptides with CN A were done and each peptide generated 500 clusters of possible confirmation. We considered the lowest docking E-total confirmation cluster as the most favorable cluster and are listed in table 1. Lowest docking E-total value refers to the highest binding affinity of the docking complex [28]. Docked complex of CN A and AID, AIDP1, AIDP2 are shows in Figure 3. Important residues involved in van der Waals force and H-bond interaction are shown in figures 4-6.

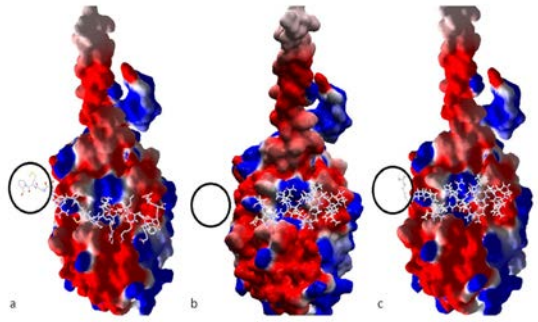

Fig 3. Electrostatic surface of docked model of (a) CN A-AID, c-terminal portion of ligand is present outside of active site, (b) CN A-AIDP1, ligand present exactly within the active site and (c) CN A-AIDP2 small portion of c-terminal ligand present outside active site. Ligand showing in white colour stick model interacting at active site location. Circled area is showing the peptide grows outside of the active site in AID, AIDP2, but no outer peptide grows in AIDP1.

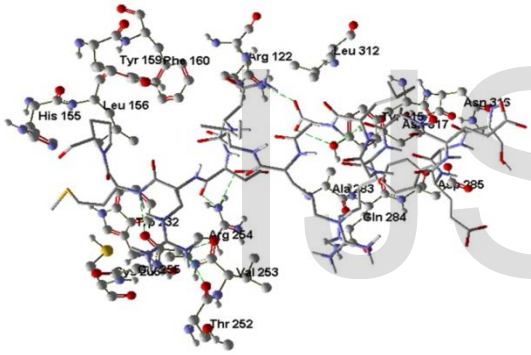

Fig 4. CN A:AIDP1 complex shows H-bond interaction between residues. Ball and stick model represents protein residues and stick model represent peptide fragment.

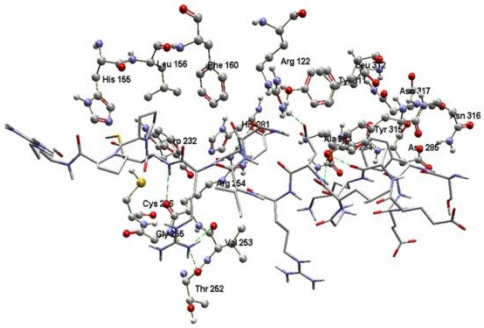

Fig 5. CN A:AIDP2 complex shows H-bond interaction between residues. Ball and stick model represents protein residues and stick model represent peptide fragment.

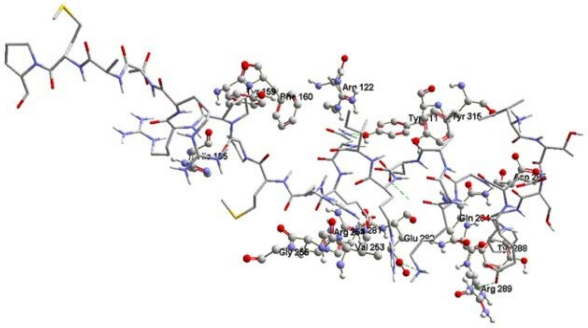

Fig 6. CN A:AID shows H-bond interaction between residues. Ball and stick model represents protein residues and stick model represent peptide fragment.

TABLE 1. SHOWING HEX DOCKING E-TOTAL VALUES OF AID AND ITS DERIVED PEPTIDES

S.N.	Peptide fragments	E-total value at active site
1	AIDP1	531.6
2	AIDP2	-530.5
3	AID	-530.4

TABLE 2. DOCKING E-TOTAL VALUE OF IMMUNOSUPPRESSANTS WITH CN A WITHOUT IMMUNOPHILINS AT ACTIVE SITE AND DRUG BINDING SITE OF CN A

S.N.	Drug molecules	E-total value at active site	E-total value at drug binding site
1	CsA	-284	-400
2	FK506	-227	-350

3.4 Docking of CN A with known drugs

Two known immunosuppressant drugs CsA and FK506 were docked with CN A and docking energies were compared with AID generated peptide fragments. Docking of immunosuppressant drugs directly without immunophilins were carried out and docking E-total was analyzed at both drug binding site and active site of CN A. Obtained docking E-total values are listed in table 2 which indicates that CsA has lowest docking E-total than FK506. These results corroborates with our earlier findings [4].

4 DISCUSSION AND CONCLUSION

Docking complex of CN A:AID, CN A:AIDP1, CN A:AIDP2 and its spatial correlation at active site of CN A were analyzed. Spatial alignment and considerable interaction of peptide residues at active site were observed. Analyzing the binding affinity based on docking E-total of peptides and known drugs CsA and FK506 with CN A found that AID and its derived peptides have different binding affinity than existing drugs. Interestingly, docked complex of drug-CN A shows drugs binding at both drug binding site (LXVP binding site) [6][29] and active site too. But obtained docking E-total analysis indicates that both drugs have strong affinity at drug binding site than the active site of the CN A. Comparing the docked E-total value of AID, its derived peptides and drugs suggest that peptide AIDP1 shows strong binding affinity with CN A. Graphical representation shown in figure 3 clearly indicates that some amino acids are located outside active site of CN A in AID and AIDP2 docking complex. But AIDP1 docking complex shows that all peptide residues are located within the active site of CN A.

It has been experimentally proved that AID directly inhibits CN by interacting with amino acids of active site and brings down its dephospharylation activity [12]. Our docking model also shows that AID is docked on active site. Conserved Asp 477 of AID is a key molecule in decreasing dephospharylation of RII substrate [2]. These docking complexes propose significant interaction of residues present in this region. In AIDP1 docking complex, Asp 477 form H-bond with conserved Tyr 315, Arg 122 and in AIDP2 Asp 477 form H-bond contact with Arg 122 of CN A. In Docked model CN A-AID shows Asp 477 didn't form H-bond with active site residues of CN A. This could be the one of the reason where AID could exhibit the least binding affinity with CN A which makes docking complex less stable during interaction.

Further mechanism of binding interaction of docking complex examine the involvement of conserved AID fragment contain Asp 477 residue [2] interact with another conserved non ligand Arg 122 and another Met 483 interact with another active site conserved Arg 254 residue. Thus both the conserved amino acid Arg 122 and Arg 254 interaction with AID may disrupt the positive electro static sphere in the active site of CN [30]. Docking results did not show involvement of conserved Arg 122 and Arg 254 interaction in CN A:AID docking complex. Further we observed no interaction of Asp 477 and Arg 122 not involved in H-bond formation at active site. This may be the one key phenomenon of AID has less binding affinity to CN compared with other derived peptides. But analyzing highest binding affinity peptides found that interaction of H-bonds formed between Asp 477:Arg 122, Asp 477:Tyr 315 in AIDP1 and Asp 477:Arg 122 in AIDP2. This concludes sixteen amino acid residue peptide AIDP1 has more binding affinity than AID may play important role in CN inhibition. But another eighteen residue peptide AIDP2 has been observed similar binding affinity with AID. Spatial location of docked peptides shows c-terminal loop of AID is present outside the active site. This may indicates outer located residues are lacking the contact of active site residues. This shows c-terminal of AID is not participating for H-bond interaction with active site. It indicates fullength of AID domain is not involved in the CN inhibition. Hence, there is a scope of using our small predicted highest binding affinity peptide AIDP1 than AID for CN inhibition.

Successful use of computational tools in *insilico* method to study binding affinity between CN A and AID plays an important role. Aim of this work was to find out high affinity domain present within the AID of CN A and key amino acid interaction which may replace AID as regular inhibitor. Due to missing of complete 3D structure coordinates of AID, we modeled the AID for 3D structural coordinates since it is the key step to analyzes the binding affinity and generating its derivative peptides to explore specific residues at specific region in AID which may play important role in CN regulation. AID regulates CN in immunosuppression signaling pathway by bringing down partial immunity [2]. We carried out docking which predict the docking score of CN A with its AID and its derived peptides and compared with existing drugs to validate our study. Our results conclude that AID and its derived peptides have strong binding affinity with CN A than drugs CsA and FK506. This concludes highest binding affinity peptide AIDP1 of AID which is shorter peptide can be use as a better inhibitor of CN. Hence present work suggests AIDP1 peptides can be alternative for effective potent inhibitor than AID and existing drugs. However further in vitro study is required to validate our findings.

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