HOMOLOGY MODELING AND MOLECULAR DYNAMICS STUDIES OF EC1 DOMAIN OF VE-CADHERIN TO ELUCIDATE DOCKING INTERACTION WITH CADHERIN-DERIVED PEPTIDE

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ABSTRACT

VE-cadherin is a protein in the cadherin family that is found at the adherens junctions of the microvessel endothelial cells of the Blood-Brain Barrier (BBB). It is recognized as the homotypic cell adhesion molecules and there is limited structural information on how VE-cadherins mediate cell-cell adhesion. It has been shown that the EC1 domain of cadherins is important for the homophilic interactions for cell-cell adhesion. Therefore, the aims of this study are to model the structure of the EC1 domain of VE-cadherin, study its molecular dynamics properties and evaluate its interactions with cadherin peptides. In this study, the sequence alignment between EC1 domain of VE-cadherin and the template protein were conducted by CLUSTALW2 platform. The SWISS-MODEL platform performed the homology modeling of the EC1 domain of VE-cadherin structure. Structural refinement was done by using KOBAMIN. Some validation analysis platforms also were conducted included PROCHECK, VERIFY3D, ERRAT and MOLPROBITY to check the allowed residues region in Ramachandran Plot (RP) and the quality of the structure. The most favored region was found 95.5% in RP value and overall model structure quality is 71.34%. Molecular Dynamics (MD) was run under CABS-FLEX to determine the flexibility of the residue index. The RMSD value of MD is 1.5Å per residue index. Eventually, molecular docking by AUTODOCK VINA was conducted to investigate protein-ligand interaction. From docking, it is found that the affinity energy is -4.8 kcal/mol which has the most favorable binding of EC1 domain with the peptide.

Keywords: VE-Cadherin, Blood Brain Barrier, Homology Modeling, Molecular Dynamics, Molecular Docking

1. INTRODUCTION

The difficulty of delivering drugs to the brain is due to the presence of the Blood-Brain Barrier (BBB). The BBB consists of microvessel endothelial cells that deliver nutrients to the brain; however, BBB prevents most drug molecules from entering the brain to make it difficult to treat drug brain diseases. The drug molecules cannot readily partition into the cell membranes to cross the BBB. The presence of metabolic enzymes and efflux pumps (i.e., P-glycoprotein (Pgp) and Multidrug Resistance Proteins (MRPs)) inhibits drug molecules from crossing the BBB via transcellular pathway. Alternatively, drug molecules can penetrate through the paracellular pathways of the BBB; however, this pathway is limited by the presence of the tight intercellular juctions (Trivedi *et al*., 2012; Zheng *et al*., 2006). Thus, the paracellular permeation of drugs can be improved by

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increasing the porosity of the intercellular junctions. It is hoped that modulation of cadherin-cadherin interactions in the BBB can improve brain delivery of therapeutic agents across the BBB to treat brain diseases such as Alzheimer's, Parkinson's, brain tumors, multiple sclerosis (Zheng *et al*., 2006).

It has been shown that E-cadherin peptides can modulate the intercellular junctions and improve the brain delivery of drugs and paracellular marker molecules in *in vitro* and *in vivo* models of the BBB. For example, HAV6 peptide (Ac-SHAVSS-NH$_2$) derived from the EC1 domain sequence of E-cadherin enhances the permeation of ^{14}C-mannitol and ^{3}H-daunomycin molecules through the BBB using the *in-situ* rat brain perfusion model (Kiptoo *et al*., 2011). Furthermore, HAV6 peptide increases the *in vivo* brain delivery of Gd-DTPA, R800 Near-IR (NIR) dye and R800cw-PEG molecules (MW 25 KDa) into the brain of Balb/c mice as detected by magnetic resonance imaging and NIR imaging (On *et al*., 2014). These results suggested that modulation of cadherin-cadherin interactions in the adherens junction can increase the porosity of the paracellular pathway of the BBB (Prasasty *et al*., 2014).

Vascular Endothelial Cadherin (VE-Cadherin) is present on the BBB vascular endothelium, which is responsible for cell-cell adhesion in the intercellular adherens junctions (Brasch *et al*., 2011). VE-cadherin is a calcium-binding protein with five repeats at the Extracellular domain (EC1-EC5) (Nanes *et al*., 2012). The cytoplasmic domain of VE-cadherin interacts with catenins that are connected to the actin fiber proteins. Although the structural information of human VE-cadherin is not available, the homophilic interaction are found similar between VE-cadherin and E-cadherin (Nanes *et al*., 2012). The hypothesis is that HAV peptide derived form E-cadherin can bind to VE-cadherin to modulate the BBB porosity. To study the binding properties of HAV peptide to the EC1 domain of VE-cadherin, it is necessary to build the model structure.

In this study, a homology model of the EC1 domain of human VE-cadherin was build because of the of its crystal or NMR structure was not available. The structure of chicken VE-cadherin was used as a template to build the structure of the EC1 domain of human VE-cadherin. Structural refinements and molecular dynamics simulations were conducted to find structural movement and flexibility. The resulting EC1 structure was used for docking studies with the HAV peptide using Autodock Vina program. The best interaction with the lowest affinity energy will be used to describe the potential binding properties of HAV peptide with the EC1 domain of VE-cadherin. The result will also be used to improve

our understanding the mechanisms of action of HAV peptide in modulating the BBB in vitro and in vivo. Furthermore, the results can be used to design better molecules for modulating the BBB.

2. MATERIALS AND METHODS

2.1. Homology Modeling of Human EC1 Domain of VE-Cadherin

The sequence of human EC1 domain of VE-cadherin was selected from the universal protein resource (Uniprot) (Consortium, 2014) (entry code: P33151). The template for sequence alignment was downloaded from PDB (Bernstein *et al*., 1977) and used the BLASTp (Altschul *et al*., 1990; Mount, 2007) program provided by Uniprot with default parameters. The 3D structure of chicken EC domain 1 and 2 of VE-cadherin (Uniprot ID: Q8AYDD) was downloaded from PDB (entry ID: 3PPE) (Bernstein *et al*., 1977; Sussman *et al*., 1999) as the template structure. Domain 2 of chicken cadherin as removed using Pymol (http://www.pymol.org/citing). The homology model of EC1 domain of human VE-cadherin was built with SWISS-MODEL (Arnold *et al*., 2006). The secondary structure of EC1 domain of human VE-cadherin was predicted using the Procheck program (Laskowski *et al*., 1993). The target (human EC1 domain) and template (chicken EC1 domain) sequences were aligned using the ClustalW (Goujon *et al*., 2010; Larkin *et al*., 2007; McWilliam *et al*., 2013) method. Subsequently, the Kobamin (Rodrigues *et al*., 2012) tool is employed for the 3D protein structure refinement.

2.2. Validation of Protein Structure and Its Molecular Dynamics

The assesment of EC1 domain structure quality is done by a number of tools to check the internal consistency and reliability of the model structure. PROCHECK (Laskowski *et al*., 1993) analysis, which quantifies the residues in available zones of Ramachandran plot, is used to assess the stereochemical quality of the model. ERRAT (Colovos and Yeates, 1993) tool, which finds the overall quality factor of the protein, was used to check the statistics of nonbonded interactions between different atom types. Similarly to determine the compatibility of the atomic model (3D) with its own amino acid sequence (1D), the VERIFY-3D (Eisenberg *et al*., 1997) program was used. The MolProbity web server (Chen *et al*., 2010; Davis *et al*., 2004) was used in the quality validation of the 3D model, which provides details of atomic contact analysis of any steric problems within the molecules.

2.3. Molecular Docking of Protein-Ligand Complex

The EC1 domain structure for the molecular docking experiments was built from the SWISS-MODEL (Arnold *et al.*, 2006) homology model The EC1 domain model was generated by maintaining the interface region and removing the EC2 domain and water molecules using Discovery Studio Visualizer 2.5. The 3D structures of HAV6 (Ac-SHAVSS-NH$_2$) were generated using InsightII Program (Accelrys Inc.) and their conformations were optimized by energy minimization using CVFF91 force field. The final receptor and ligand coordinates were saved as pdb files.

Molecular docking experiment is performed using Autodock Vina program (Vina, The Scripps Institute) (Trott and Olson, 2010). The AutodockTools is used to add partial charges using Gasteiger method and to arrange the polar hydrogens in the protein. The ligand is set to have flexible torsion angles at all rotatable bonds, while the protein is prepared as a rigid structure. Both protein and ligand are saved as output pdbqt files. For specific docking of HAV6 peptide onto the EC1 domain, the volume grid size was adjusted to 28×28×28 Å in the x, y and z axes, respectively, with grid-points have a space within 0.375-0.625 Å. The conformation search was done using the Lamarckian Genetic Algorithm (LGA) and nine conformers for docking were chosen for best hits. Using affinity energy (kcal/mol) and the Root-Mean-Square Deviation (RMSD) <2.0Å, score and rank are calculated while running the docking program.

3. RESULTS

3.1. Homology Modeling of Human EC1 Domain of VE-Cadherin

The CLUSTALW2 program was performed to analyse the highest score of identity and similarity to the template acquired from PDB. Approximately 71% identity and 84.2% similarity showed sequence alignment of EC1 domain of VE-cadherin with the template (**Fig. 1**). Homology modeling was built by superimposing the desired protein with the template (**Fig. 2**). The crystal structure of EC1 domain of chicken VE-cadherin was recently determined at a resolution of 2.1 Å and was deposited in PDB (entry ID: 3PPE) (Brasch *et al.*, 2011). The amino acid sequence of EC1 domain is from chicken VE-cadherin is retrieved from 1 to 203 and covers the full length of EC1 domain of human VE-cadherin.

3.2. Structure Validation and Dynamics

In assessing 3D protein structure, PROCHECK was used to check the reliability of the backbone torsion angles and the built model, which quantified the residue clusters in the available zones of Ramachandran plot, as shown in **Fig. 3.** Ramachandran plot analysis for the modeled EC1 domain showed 95.1% residues clustered in the most favored regions, 3.3% residues were in additional allowed regions and 1.1% residue were in generously allowed regions and 0.5% residues in disallowed regions. The summary of overall assessments were performed in **Table 1.** The high quality of the structure was further assessed by the PROCHECK G-factor showed -0.22 for psi/phi only value and -0.14 for all dihedral angles value. The quality of EC1 domain model was further computed by a high ERRAT score (**Fig. 4**) of 71.134, which indicated acceptable protein environment (Colovos and Yeates, 1993). The VERIFY-3D results of EC1 domain model showed 0.36 of the amino acids (amino acids in an average 3D-1D score of >0.2 showed the good quality of 3D model) and the Molprobity score was 37.71 showed positive scores of the residues (cutoff score > 0), indicating the reliability of the proposed model. The structure dynamics was performed by using CABS-FLEX platform, showing the average cluster of Root Mean Square Deviation (RMSD) value was 1.5Å (**Fig. 5a**). The global distance test (GDT) or GDT Total Score (GDT TS) is calculated as the largest set of amino acid residues alpha carbon atoms in the model structure falling within a defined distance cutoff of their position in the experimental structure. The GDT TS score was 1.0. The fluctuation also known as Root Mean Square Fluctuation of per residue index is depicted in **Fig. 5b.** The average flexibility pattern of EC1 domain showed score below 20, which is acceptable value for structural dynamic stability.

3.3. Molecular Docking of Protein-Peptide Complex

Molecular docking of EC1 domain-HAV peptide had been performed with Autodock Vina (Trott and Olson, 2010). From docking result, nine conformers had been selected to have high score and rank. From the nine conformers, one conformer with the lowest affinity energy value -4.8 kcal/mol, had been selected for further analysis. The binding mode of selected HAV peptide conformer with the EC1 domain is depicted in **Fig. 6.**

In the interaction showed that two hydrogen bonds were involved as depicted in green dash line in **Fig. 7.** The hydrogen bonds are contributed first from hydrogen atom from serine 1 residue of HAV peptide and oxygen from carboxylic group from residue Cysteine 23 of EC1 domain.

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Chicken  DWIWNRMHIREEIDSPLPHHVGKLTSSVGNKNAMYIIEGESANTIFKVQGYDGDIYAFER 60
human    DWIWNQMHIDEEKNTSLPHHVGKIKSSVSRKNAKYLLKGEYVGKVFRVDAETGDVFAIER 60
         ****:*** ** ::.*******:.***..*** *:::** ...:*:*:.  **:*:**

Chicken  LDREKKAEYELTAHIIDRRNNRSLEPPSKFIIKVSDINDNAPIFVQKIFNGSVPEMSRLG 120
human    LDRENISEYHLTAVIVDKDTGENLETPSSFTIKVHDVNDNWPVFTHRLFNASVPESSAVG 120
         ****: :**.*** *:*: ....**.**.* *** *:*** *:*.::**.**** * :*

Chicken  TSVTKVTAEDADDPTVAGHATVTYQIIKGNEYFTVDDSGVIFTARADLDRESQSAYEIIV 180
human    TSVISVTAVDADDPTVGDHASVMYQILKGKEYFAIDNSGRIITITKSLDREKQARYEIVV 180
         ***.***.******..**:* ***:**:***:*.** *:*  .****.*: ***.**

Chicken  KAKDALGLTGESSTATVIIRLTD 203
human    EARDAQGLRGDSGTATVLVTLQD 203
         :*:** ** *:*.****:: * *
```
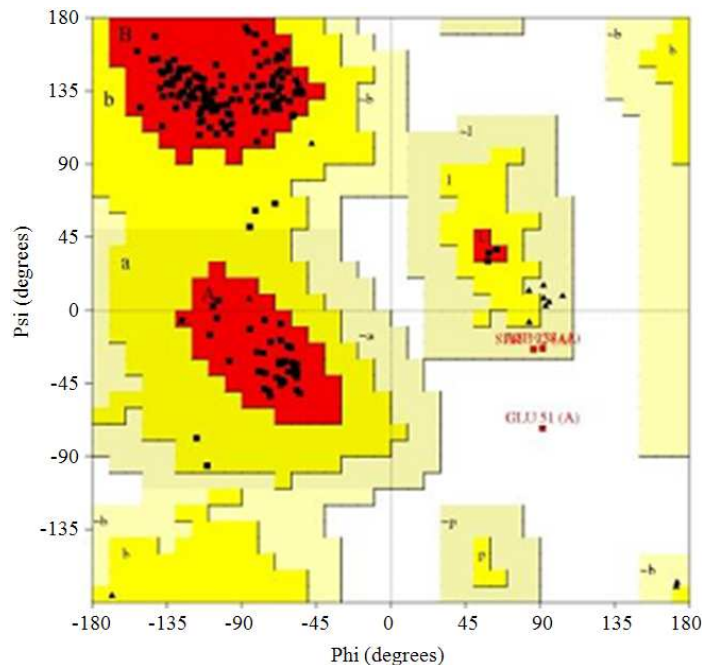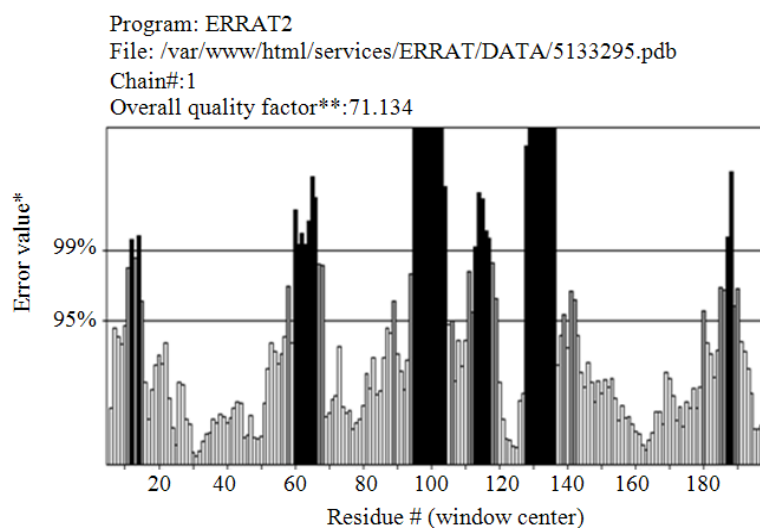
Fig. 1. Sequence alignment between chicken and human EC1 domain of VE-cadherin. The red, blue, magenta, green and grey colors indicate: hydrophobic (include aromatic), acidic, basic, hydroxyl+sulphydryl+amine and unusual amino or imino acids, respectively
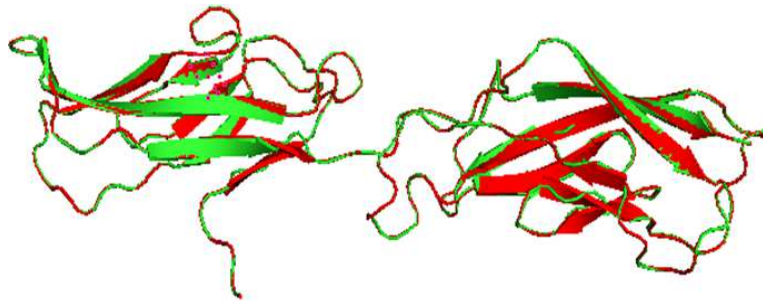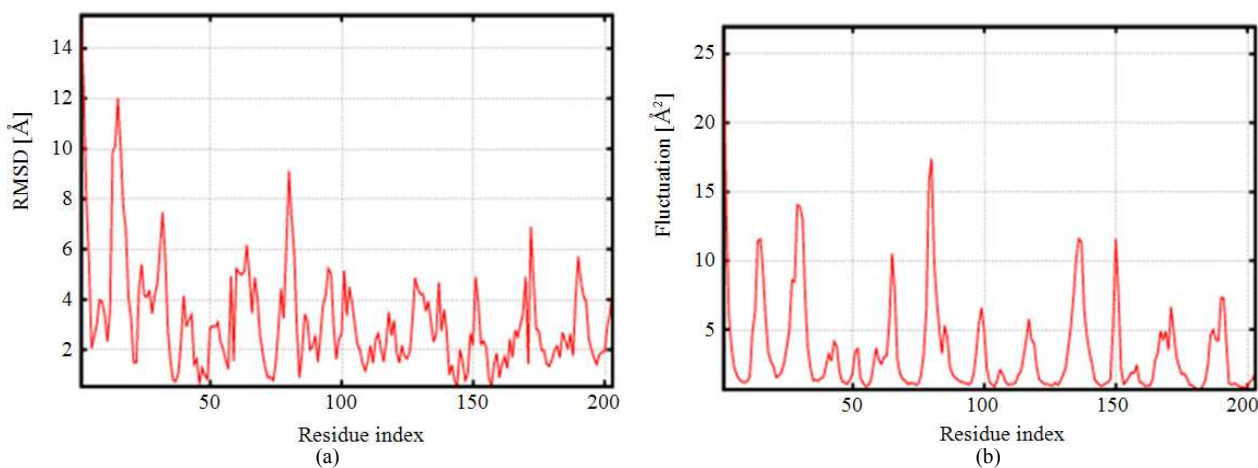

Fig. 2. The superimposed 3D EC1 domain structures. Green and red colors indicate chicken EC1 domain and human EC1 domain structure models, respectively
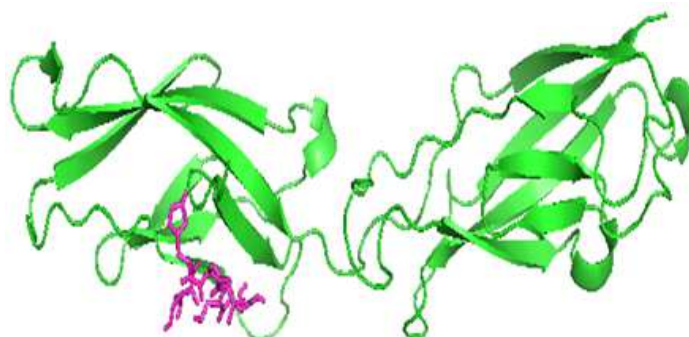

**Fig. 3.** Ramachandran Plot of EC1 domain of human VE-cadherin

Program: ERRAT2
File: /var/www/html/services/ERRAT/DATA/5133295.pdb
Chain#:1
Overall quality factor**:71.134

Fig. 4. The graph of error value in EC1 domain residues using ERRAT program
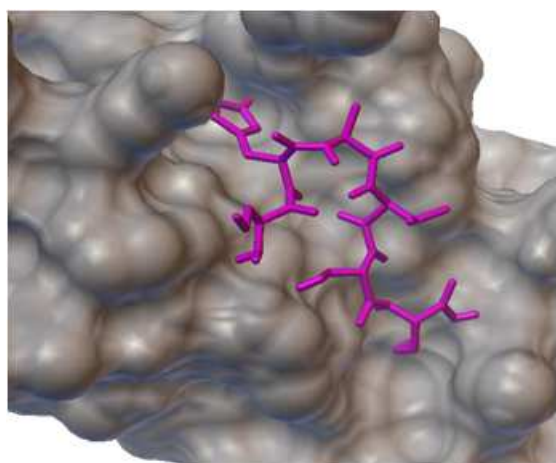

(a)

(b)

Fig. 5. (a). RMSD value of single structure mode. (b). Fluctuation value of total dynamics per residue

Fig. 6. Binding mode between EC1 (green) domain and HAV peptide (magenta)
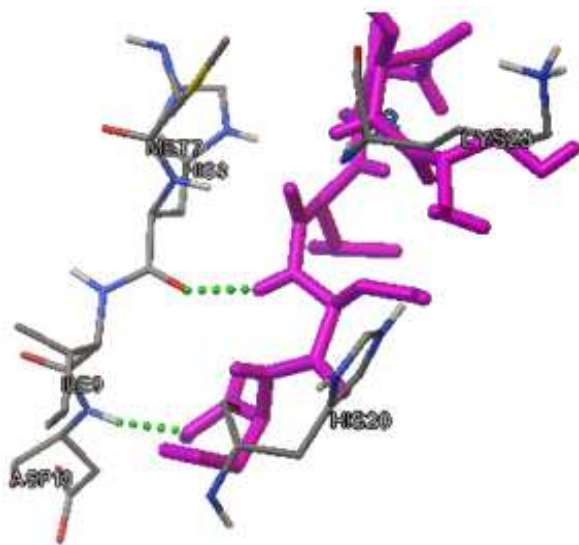
Fig. 7. Complex interaction showing hydrogen bond involved. Magenta color is HAV, multicolor is EC1 domain protein

Fig. 8. The EC1 domain (grey) with the HAV peptide (magenta) docked in bulk sphere showing binding pocket

The second hydrogen bond is contributed from nitrogen atom of amide group from Aspartic acid 10 of EC1 domain with hydrogen atom of carboxylic group from residue Valine 23 of EC1 domain. The EC1 domain-HAV peptide docked in bulk sphere showing binding pocket as depicted in **Fig. 8.** Residue Histidine 2 of HAV peptide showed buried inside the EC1 pocket. It might induce hydrophobic interaction between the imizadole ring that is belonged to Histidine 2 of HAV peptide.

Table 1. Summary of 3D model structure assessment

Number of structures used:	1
Structure quality factors-overall statistics:	Mean score
Procheck G-factor e (phi/psi only)	-0.22
Procheck G-factor e (all dihedral angles)	-0.14
Verify3D	0.36
MolProbity clashscore	37.71
Ramachandran plot summary (Procheck)	
Most favoured regions (red)	95.1%
Additionally allowed regions (yellow)	3.3%
Generously allowed regions (light yellow)	1.1%
Disallowed regions (white)	0.5%
Ramachandran plot statistics (Richardson's lab)	
Most favoured regions	96.5%
Allowed regions	3.0%
Disallowed regions	0.5%

4. DISCUSSION

Homology model of human EC1 domain of VE-cadherin has extensively used to develop protein structure and function analysis. By modeled protein development, it is necessary to validate the EC1 domain model to ensure the good quality of its structure. The EC1 domain of VE-cadherin showed the high similarity with the template structure and high score in determining good quality of protein model after conducting energy minimization. Molecular dynamics simulation was used to find how much is the residue flexibility in terms of finding the structure stability. Eventually, this high quality structure further utilized for investigating the protein binding interaction with HAV peptide.

HAV peptide was first discover from the groove region of EC1 domain of E-cadherin (Sinaga *et al.*, 2002), however the binding location might be found in multiple sites. HAV peptide containing His-Ala-Val sequence is very important to improve the paracellular porosity of the cadherin-cadherin interaction (Sinaga *et al.*, 2002). Even though HAV peptide was derived from EC1 domain of E-cadherin, this peptide also can inhibit the cadherin interaction of Neural cadherin (Yu, 2011), other classical cadherin superfamily. Therefore, extensive study to determine HAV interaction with other type of classical peptide such as VE-cadherin is strongly suggested. From docking study, the binding mode of the complex EC1 domain-HAV peptide was found in binding pocket of EC1 domain. Furthermore, this binding interaction could be a starting point and complementary information for further binding studies of VE cadherin with the HAV peptide and other potential cadherin-derived peptides using CD, NMR and Fluorescence spectroscopies. In

the future, these conformational models will be used to design potent modulator of the tight junctions to deliver drug to the brain.

5. CONCLUSION

The homology modeling of human EC1 domain of VE cadherin was successfully built with high quality score. The calculated validation of the EC1 model has suggested acceptable model for further structure and function analysis such as molecular dynamics and docking interaction. From docking result, we also had successfully examined the most favorable binding mode of EC1-HAV interaction with the lowest energy affinity. This finding would give an advantage to investigate HAV peptide improvement in modulating cadherin-cadherin interaction of VE-cadherin in paracellular pathway of the BBB.

6. ACKNOWLEDGEMENT

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