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Interaction Studies Between Cyclic Peptide ADT-C3 (Ac-CADTPC-NH_2) with E-Cadherin Protein using the Molecular Docking Method Simulated on 120ns

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Abstract

The treatment of diseases that attack the brain is very difficult, because the delivery of drug molecules to the brain is often hindered by the blood-brain barrier (BBB). So that the drug delivery is not right on the target cell. Thus, was developed a method in modulation of intercellular junctions using ADTC3 cadherin peptide, Where the cadherin peptide is derived from the cadherin sequence itself. The method used in this research is molecular dynamics (DM) and molecular docking. In this study have been evaluated some peptide conformation in modulating intercellular junction. The results show that cyclic peptide ADT-C3 (Ac-CADTPC-NH2) was conducted DM for 120 ns (120000 ps), which has considerable activity in modulating intercellular junctions with binding energies of -33.10 kJ.mol^{-1} and K_i of 1.58 µM at the 79187 ps conformation. The binding site on residues Asp1, Trp2, Ile4, Lys25, Ser26, Asn27, Met92 in the adhesion *arm-acceptor pocket* region.

1. Introduction

Diseases that attack the brain is a disease that is difficult to treat because the delivery of drug molecules to the brain is very difficult [1]. This is due to a biological barrier that is blood-brain barrier (BBB)[2]. Drug transport through the BBB can be achieved through the transcellular and paracellular pathways. Transport pathways that may be bypassed by macromolecular compounds are paracellular pathways. However, in this path there is a tight junction which is the most apical component and is generally regarded as a barrier to paracelular permeability [3]. Molecules that can pass through this pathway have a diameter size of less than 11 Å or with molecular weight of less than 500 Dalton [4]. Thus, the presence of BBB becomes a separate challenge in the process of drug delivery to target cells. One way to improve drug delivery systems is by increasing the tight junction porosity by inhibiting cadherin-cadherin interactions [5].

Cadherin is a transmembrane protein consisting of 5 extracellular domains (EC1-EC5), Where in the Ca^{2+} ion attaches as a liaison between parts of E-Cadherin [6]. Cadherin is found in the adherens zonula [7]. Zonula adherens is one part of the junction between cells that are in zonula occluden (tight junction) and Demosom. In the formation of junctions between cells, the cadherin molecule in one cell interacts with the cadherin molecule in another cell that is nearby to form an adherent zonula [8]. Duration of BBB opening is also most important factor, so that for the future research is very important to develop the drug delivery system in chitosan polymer matrix [9, 10].

Porosity in the paracellular pathway may be enhanced by cadherin peptides i.e peptides sequentially derived from the cadherin molecule itself (eg, HAV and ADT peptides). Thus, the cadherin peptide can occupy the binding site of the cadherin molecule and block the interaction between the cadherin molecules in adjacent cells and the tightness of the junction between cells can be adjusted. Although the HAV and ADT peptide experimentally increase the porosity of cadherin, the HAV and ADT peptide mechanism binding to cadherin have not been understood comprehensively in molecular level. So that, both experimentally NMR and computationally docking method are interesting research.

Experimentally, Laksitorini [11] uses ADT6 cadherin peptide derivatives (eg, ADTC1, ADTC5, and ADTC6) to increase the delivery of drug molecules from the brain to the central nervous system and its results suggest that ADT6 cadherin peptide has the ability to inhibit inter-junction interactions of cells within the MDCK (Madin - Darby Canine Kidney) and the formation of cyclic peptides can enhance the ability of cadherin peptides to modulate inter-cell junctions [11]. Significant insight has already been gained by Alaofi *et.al.* [1] from the interplay of computationally docking method and experimentally NMR method in the study of supramolecular features such as interactions for probing the interaction between cHAVc3 peptide and E-cadherin domain EC1 and be able to explain that cHAVc3 peptides were able to increase the porosity of junction between cells [1]. Siahaan *et.al.* [12] have applied the docking method to probe the interaction between cyclic ADTC5 and E-cadherin, and the docked cyclic ADTC5 structure obtained by molecular dynamics simulations using the GROMACS with the trajectory generation was conducted with 120 ns (120,000 ps) running time [12]. ADTC3 peptides that have not been experimentally or computationally studied. So that, in this research will be studied the interaction between cyclic peptide ADTC3 with E-Cadherin domain EC1-EC2 by molecular docking. The study of ADTC3 begin by Manna *et.al.* [13] by molecular dynamics simulations conducted with 20 ns [13]. Molecular docking is a computational modeling performed to predict interactions and bonding sites [14]. Computational modeling can also explain the driving force that causes the interaction process between ADTC3 peptide and E-cadherin domain EC1-EC2, and with such interactions can increase the porosity of intercellular junction. In the molecular level the interaction between HAV or ADT with cadherin results in the hydrogen bonding between $-NH_2$ and $-C(=O)OH$ funtional groups of amino acids. To obtain the precise binding energy, the smaller system can be applied such as Ac-AD-NH_2 and Ac-PV-NH_2 and calculated by ab initio method [15].

2. Material and Methods

Protein and ADTC3 cyclic Peptide Preparation

In this study the model used is the crystal structure E-cadherin domain EC1-EC2 (code 2O72) as host and cyclic peptide ADTC3 as guest shown in Figure 1. The ADTC3 linear peptide is created using the PyMol program [16]. A cyclic peptide ADTC3 (cyclic (1.6) Ac-CADTPC-NH2) was formed by forming a disulfide bond on the thisol group of cysteine residues using the avogadro program.

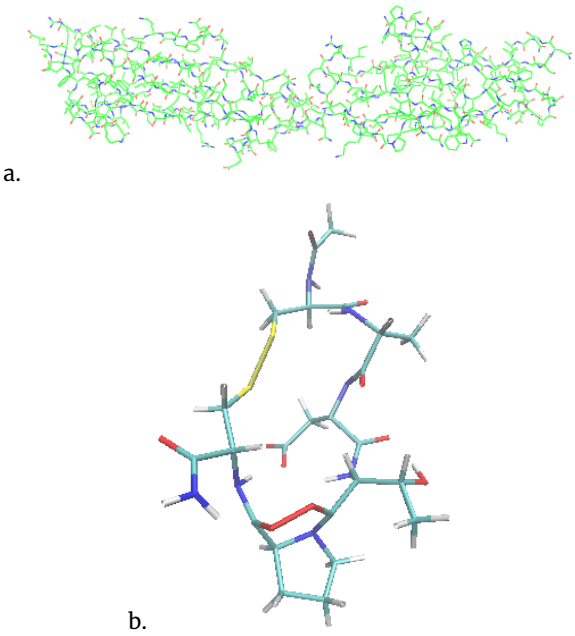


Figure 1. (a) The crystal structure of E-cadherin domain EC1-EC2. (B) The structure of the cyclic peptide ADTC3

Molecular Dynamics Simulation (DM)

Molecular dynamics simulations were performed using the GROMACS program v.4.6.5 [17]. The DM simulation was performed to find out the dynamics and optimization of cyclic peptide ADTC3 to obtain the lowest conformation and energy in the water solvent and added ion. In preparation of the system is done giving force field charmm27 and added tip3p water solvent on a box-shaped container measuring 1 nm [18]. In addition, on the system added 4Na^+ and 4Cl^- ions to obtain physiological concentration of 0.15 M. Minimation energy of system is done to relax the excess force on the system. Molecular dynamics (DM) is carried out for 120 ns with the peptide at restrain position. Then the system equilibrium between the peptide conformation with the solvent / ion becomes the expected representation at a temperature of 300K and a pressure of 1 atm by DM for 100 ps. After the equilibrium system, the income of trajectory with parameters is changed by doing DM 120 ns (120000 ps) at constant temperature 300 K and constant volume to obtain trajectory data. The resulting trajectory then analyzed the Cα's root-mean-square-deviation against the initial structure and total energy analysis during DM by taking 20 conformational structures having the lowest energy with time variation for 120 ns.

Molecular Docking

Molecular docking is done using the AutoDock v.4.2 program [19]. Autogrid is a rapidly binding pre-evaluation of energy between the atomic types of cyclic ADTC3 ligands (C, HD, N, OA, SA), electrostatic, and desolved with E-cadherin protein. Evaluation using a grid box with a grid spacing of 0.375 Å at the protein bond site and the evaluation of this study was conducted only on the EC1 domain of E-cadherin. Furthermore, the Autodock process is docking process starting from E-cadherin as rigid molecule and selecting cyclic peptide

ADTC3 as ligand. Conformation search using Lamarckian-Genetic algorithm with binding energy determination using semi-empirical free force energy field approach [20]. The number of algorithms executed and the number of evaluation processes each set at 150 and 10,000,000.

3. Results and Discussion

Simulation of Molecular Dynamics

The simulated results of cyclic DM peptide ADTC3 using Gromacs can be shown in Figure 2. In the simulation of cyclic peptide DM ADTC3, RMSD analysis was performed to determine the movement of soluble peptides in water and ions by comparing peptide chains in native structures at Cα which have residual amount (N) equal to alternative structure for 120 ns. Based on RMSD analysis on DM simulation 20 ns and 120 ns respectively obtained distance fluctuation of 1.13- 2.47 Å and 1.07-2.54 Å. The movement of peptide molecules is said to be stable if the RMSD does not exceed 3 Å.

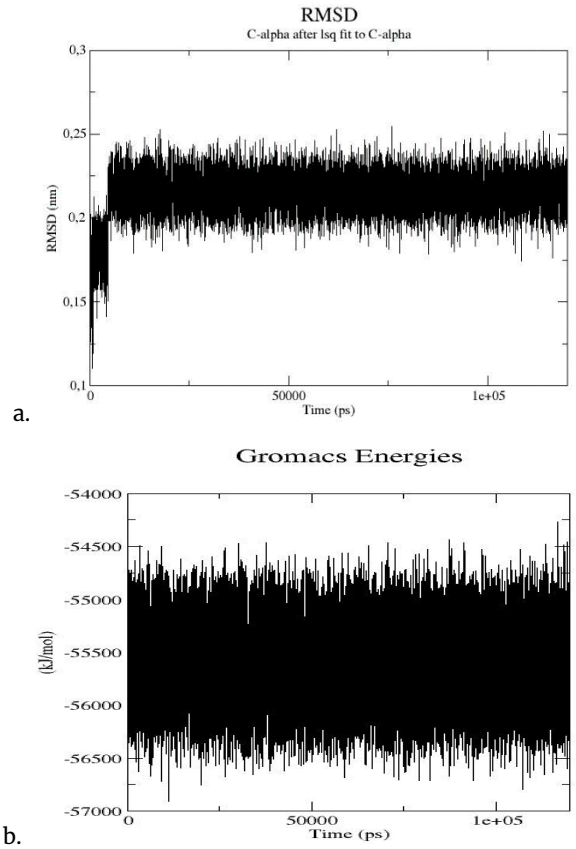


Figure 2. (a) Graph of RMSD Cα. (B) Total energy graph

From the analysis of RMSD shown in Figure 2. shows he movement of the cyclic peptide molecule ADTC3 tends to be stable and convergent. In addition, it can be proved by the change of distance between S_{14} ... S_{78} at the start and end terminus of the amino acid cysteine which tend to be stable is shown in Table 1. Furthermore, total energy analysis is conducted to find the most stable peptide structure in accordance with actual approximate conditions. Conformation of cyclic peptide conformation ADTC3 has folding / unfolding, peptide having folding structure is more stable because it has the lowest energy [21]. In this analysis, the selection of 20 conformations at the lowest energy to perform molecular docking can be seen in table 2.

Table 1. The movement of cyclic peptide ADTC3 during DM 120 ns

Time (ns)	Total Energy (kJ/mol)	$Rs_{14}...s_{75}$ (Å)
0	-55423.32	2.02893
1	-55619.96	2.02931
5	-56395.01	2.02927
10	-55950.31	2.02909
15	-55482.96	2.02888
20	-56000.62	2.02887
25	-55003.70	2.02869
30	-55680.39	2.02958
35	-55385.38	2.02890
40	-56267.23	2.02891
45	-55475.67	2.02888
50	-55417.34	2.02857
55	-55484.88	2.02887
60	-55800.77	2.02927
65	-55210.07	2.02916
70	-55967.90	2.02959
75	-55183.36	2.02882
80	-55691.77	2.02919
85	-55542.82	2.02864
90	-55521.95	2.02890
95	-55386.18	2.02848
100	-55681.81	2.02892
105	-55624.86	2.02826
110	-55766.13	2.02911
115	-55654.79	2.02911
120	-56000.62	2.02832

Table 2. Twenty B code conformations at the lowest energy

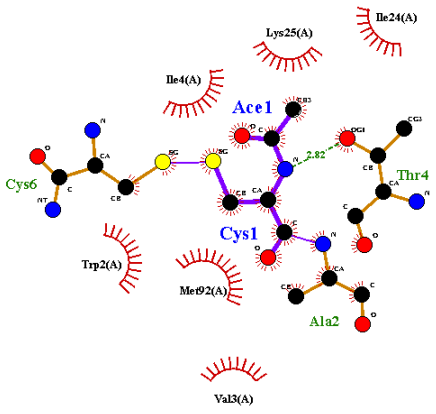
Code	Lowest Total Energy (kJ/mol)	Time (ps)	RMSD (Å)
B1	-56910.86	11139	2.12
B2	-56797.31	107325	2.02
B3	-56756.62	20001	2.17
B4	-56720.14	9270	2.24
B5	-56714.69	93956	2.17
B6	-56713.12	82003	2.10
B7	-56712.86	79187	2.04
B8	-56704.23	73586	2.15
B9	-56702.36	107321	2.15
B10	-56685.10	59008	2.12
B11	-56681.76	109365	2.32
B12	-56677.22	72273	2.17
B13	-56673.41	26439	2.13
B14	-56671.74	6155	2.12
B15	-56671.06	98475	2.18
B16	-56668.51	87228	2.24
B17	-56657.55	113125	2.31
B18	-56655.01	33979	2.26
B19	-56640.10	69541	2.29
B20	-56637.58	23380	2.18

Molecular Docking

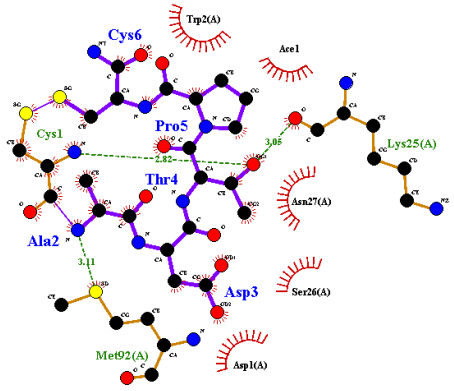
Molecular docking is one of the most commonly used methods of determining structure-based drug design (SBDD) because of its ability to predict with substantial accuracy [22]. Molecular docking is performed to determine the conformation and binding energy between cyclic peptides ADTC3 with E-cadherin domain EC1-EC1. The cyclic peptide ADTC3 has a gasteiger load of 28 non-polar hydrogens, 9 rotable bonds, and degrees of torque free as much as 7 from 32. Docking stage there are 2 that is Autogrid and Autodock. In the Autogrid stage gridbox positioning is done blind docking with gridbox size 62x62x62, this method is done because not yet known active side on E-cadherin domain EC1 with cyclic peptide ADTC3. And the AutoDock stage uses Genetic Algorithm or GA parameters of 150 and population of 150 and number of eval of 10.000.000. From the result of blind docking done on cyclic peptide ADTC3 by DM 120 ns (code B) to E-cadherin domain EC1 (figure 3), In code B1-B20 which has the lowest binding energy in the seventh conformation (code B7) with energy of -29.79 kJ/mol and amplified by the presence of hydrogen bonds between residues Trp2 ... Asp3 and Trp2 ... Ala2 type hydrogen bonds are O ... NH and O ... NH with respective spacing of 2.115 and 1.893 Å.

The residual bond site in code B is Asp1, Trp2, Ile4, Lys25, Ser26, Asn27, Met92 with the amount of residue 7 in the adhesion arm-acceptor pocket region. In the Parisini study (2007) showed that residues that act in the adhesion arm region are D1, W2, E89, D90, M92 and W2, V3, P5Q23, K25 [23].

Based on the results of docking analysis there are several clusters for each structure, the election starts from the structure with the minimum binding energy. If two or more structures are produced with the minimum energy, then selected structure with the most frequencies. And the results will be validated docking.



a.

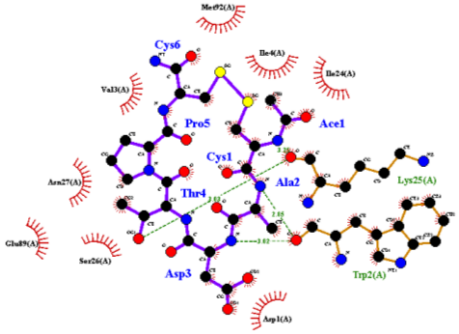


b.

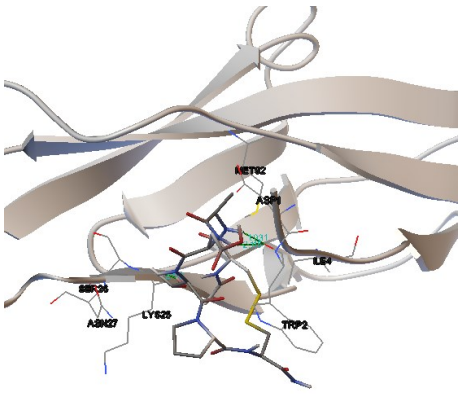
Figure 3. Molecular docking structure of E-cadherin domain EC1-EC2 with cyclic peptide ADTC3 using ligplus rogram.

Docking validation is done by Re-docking

After blind docking, we can know the binding site between the cyclic peptides ADTC3 premises E-cadherin domain EC1-EC2. Then re-docked to validate the docking result and RMSD should be <2 Å [24]. Re-docking is done on protein bonding sites with the same parameters. Each conformation has a different protein bonding site, so it has different gridbox sizes but the same grid spacing is 0.375 Å. Based on the re-docking result, the lowest binding energy obtained in the same conformation as the docking result (figure 4) with binding energy is -33.10 kJ / mol, Site bonding on residues Asp1, Trp2, Ile4, Lys25, Ser26, Asn27, Met92 (code B). The following site data interaction against E-cadherin domain EC1 can be shown in table 3. For data docking results can be shown in table 4.



a.



b.

Figure 4. Molecular docking structure of E-cadherin domain EC1-EC2 with cyclic peptide ADTC3 using (a) ligplus and (b) Autodock

Table 3. Site interaction code B with E-Cadherin domain EC1-EC2

Code	Hydrogen bond	Center Gridbox X	Y	Z	Binding site Residues of ADTC3 Cyclic Peptide with EC1 domain	RMSD (Å)
B1	1	45.866	8.692	57.358	Trp2, Val3, Ile4, Ile24, Lys25, Ser26, Asn27, Ser78, Glu89, Met92	0.42
B2	2	47.668	8.015	57.447	Asp1, Trp2, Ile4, Lys25, Asn27, Met92	0.54
B3	1	46.529	9.821	57.358	Trp2, Val3, Ile4, Lys25, Ser26, Asn27, Glu89, Met92	2.31
B4	-	47.074	6.598	57.441	Trp2, Val3, Ile4, Gln23, Ile24, Lys25, Met92	1.13
B5	2	43.836	4.322	56.991	Trp2, Val3, Ile4, Val22, Gln23, Ile24, Lys25	2.84
B6	1	45.818	8.987	57.457	Trp2, Val3, Ile4, Ile24, Lys25, Ser26, Asn27, Ser78, Glu89, Met92	0.34
B7	3	47.196	9.015	57.653	Asp1, Trp2, Ile4, Lys25, Ser26, Asn27, Met92	0.28
B8	2	47.196	10.487	57.44	Asp1, Trp2, Ile4, Ile24, Lys25, Ser26, Asn27, Met92	0.49
B9	2	47.962	6.765	57.441	Trp2, Val3, Ile4, Lys25, Asn27	0.65
B10	-	41.668	9.626	57.414	Trp2, Val3, Ile4, Lys25, Ser26, Asn27, Glu89, Met92	2.78
B11	1	44.418	8.015	57.441	Trp2, Val3, Ile4, Lys25, Ser26, Asn27, Glu89, Met92	0.53
B12	1	46.585	9.848	57.441	Trp2, Val3, Ile4, Ile24, Lys25, Ser26, Asn27, Ser78, Glu89, Met92	0.38
B13	-	46.446	9.626	57.375	Trp2, Ile4, Lys25, Ser26, Asn27, Ser78, Glu89, Met92	0.54
B14	1	46.457	9.126	57.208	Trp2, Val3, Ile4, Lys25, Ser26, Asn27, Glu89, Met92	1.08
B15	2	46.64	7.065	57.525	Trp2, Val3, Ile4, Val22, Gln23, Ile24, Lys25, Ser26, Asn27, Glu89, Met92	0.86
B16	-	46.518	9.459	57.441	Trp2, Val3, Ile4, Ile24, Lys25, Ser26, Asn27, Glu89, Met92	0.35
B17	1	48.418	7.015	57.467	Trp2, Val3, Ile4, Gln23, Lys25, Asn27	2.64
B18	2	48.418	7.015	57.467	Trp2, Val3, Ile4, Ile24, Lys25, Ser26, Asn27, Glu89, Met92	3.12
B19	1	44.64	9.487	57.871	Trp2, Val3, Ile4, Lys25, Ser26, Asn27, Glu89, Met92	0.48
B20	1	44.529	9.876	57.832	Trp2, Val3, Ile4, Lys25, Ser26, Asn27, Glu89, Met92	0.36

Based on table 3 and table 4 shows the existence of driving force which causes interaction between cyclic peptide ADTC3 and E-cadherin domain EC1 that is binding energy and hydrogen bond [19]. The best interaction occurs in conformations that have the least energy of code B7. Binding energy that is increasingly minimum or more negatively influenced by the effect of cooperativity. Cooperativity effect is influenced by non-covalent interaction [25]. Cooperative interaction consists of three or more molecules and is an important component of the interaction between molecules that is the presence of hydrogen bonds [26]. The more interaction that is formed in the formation of molecular recognition between the guest and the host complex, the more stable the molecule becomes and the negative energy is called negative cooperativity. In the code docking results B7 has the highest number of hydrogen bonds than any other conformation, with the three hydrogen bonds increasingly complex interactions. In general, if viewed from the relationship ΔG and Ki the minimum binding energy the ability of ADTC3 in inhibiting EC1 stronger, so the porosity of junctions between cells can be adjusted.

Table 4. Energy binding and K_i code B results Re-docking

Code	pose	Lowest Binding Energy (kJ/mol)	K_i
B1	138	-27.99	12.41 μM
B2	5	-29.16	7.77 μM
B3	34	-27.11	17.84 μM
B4	97	-20.71	236.96 μM
B5	48	-27.91	12.86 μM
B6	78	-28.58	9.84 μM
B7	89	-33.10	1.58 μM
B8	19	-29.79	6.02 μM
B9	69	-24.43	52.12 μM
B10	126	-28.12	11.77 μM
B11	31	-26.94	19.14 μM
B12	55	-28.66	9.54 μM
B13	60	-27.57	14.7 μM
B14	6	-28.07	12.02 μM
B15	40	-24.06	60.82 μM
B16	132	-26.53	22.62 μM
B17	32	-21.67	159.89 μM
B18	74	-25.06	40.92 μM
B19	82	-26.69	21.19 μM
B20	6	-27.82	13.32 μM

4. Conclusion

The results show that the lowest energy have the binding energy of -33.10 kJ/mol (conformation B7). The binding site are on residues Asp1, Trp2, Ile4, Lys25 , Ser26, Asn27, Met92. This binding energy differ with simulation 20 ns which is -31.55 kJ/mol with the binding site are on residues Asp1, Trp2, Val3, Ile4, Lys25, Met92. Based on these results, the ADTC3 is also expected be able to modulate the intercellular junction and the tight junction porosity can be adjusted.

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