Probing the interaction between EC1-EC2 domain of E-cadherin with conformational structure of cyclic ADTC7 (Ac-CDTPDC-NH₂) peptide using molecular docking approach

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Abstract. Increasing significantly brain disease every year make difficult to help people who suffer disease in their brain. Drug delivery can be reached through a paracellular pathway. The use of the derivative cadherin peptides (ADT and HAV) to enhance the porosity in this pathway was investigated. The aim of this studied to determine the best conformation of cyclic ADTC7 peptide which interacts with EC1-EC2 domain of E-cadherin with energy binding and active sites parameters. The methods used in this study are: 1). MD simulation using GROMACS software, and 2). Molecular docking with AutoDock software. The variation used on MD simulation are atomic distances and constant restrains in atom S₁₄…S₇₈ for 20 ns. The result of MD simulation for 20 ns shows that the linear and cyclic ADTC7 peptide are -118,824.84 kJ.mol⁻¹ and -52,985.95 kJ.mol⁻¹, respectively. The best conformation of cyclic ADTC7 peptide with the EC1-EC2 domain of E-cadherin is C1 with the lowest binding energy of -24.56 kJmol⁻¹. The active site at residues such as Val3, Ile4, Pro5, Pro6, Ile7, Ser8, Leu21, Val22, Gln23, Lys25. It has RMSD value less than 2 Å, low energy binding and low inhibition constant, a large population and a stable pose when validation docking.

1. Introduction

Brain disease increases significantly every year in many countries such as Alzheimer’s, brain cancer, and Parkinson disease. Some researchers believe that it is too difficult to help people who suffer disease in their brain. However, there is the blood-brain barrier (BBB) which block potential toxic compounds that circulate in the blood [1]–[3]. While, at the same moments, the BBB admit several essential compounds for brain function to enter this organ by various mechanisms, including passive diffusion and active transporters. Furthermore, the approaching of invasive and non-invasive have been used to solve the BBB and send drugs to the brain. Then, some compounds such as peptides [4]–[6], nanoparticles [7]–[10], amino acids [11], [12], sugars [13]–[15] and antibodies, [16]–[18] are applied as shuttles to bring drugs (that cannot reach the brain unaided) across the BBB for vector-mediated strategies.
In recent years, Many researchers use peptides as drug carriers to treat brain disorders[4], [19]. Drug delivery can be reached through a paracellular and transcellular pathway [19][20]. Although some peptides can cross the BBB by passive diffusion through the transcellular pathway, many peptide and macromolecule cannot, because of their physicochemical properties such as hydrophilicity, charge, size, and hydrogen bonding [19][21]. Meanwhile, Macromolecule likes protein and peptide normally bypass through the paracellular pathway[19]. However, there are tight junctions as a wall in the paracellular pathway which inhibit the permeation process. In addition, the macromolecule which can pass through the membrane must have a size 11 Å and a molecular weight less than 500 daltons [19]. Hence, some inventors argue that increasing the porosity of the tight junction is one of the solutions to overcome this problem [19].

Currently, the use of cadherin peptides to enhance the porosity in the paracellular pathway was investigated by some scientists. Chaderin is transmembrane in the tight junction which consists of 5 extracellular domains from EC1 to EC5. Moreover, the derivative of cadherin such as ADT and HAV can open the tight junction. Whereas, these will block the binding site of adjacent cells especially chaderin molecule so the interaction between intercellular junction will be weaker and tight junction will be open. Laksitorini et al (2015), have investigated the ability of cyclic-ADT peptides (ADTC1, ADTC5, and ADTC6) to modulate the intercellular junctions in (Madin Darby Canine Kidney) MDCK cells and to improve permeation of marker molecules through the BBB in a paracellular pathway [19], [21].

Nowadays, the use of theoretical approach can help to study the interaction and mechanism in the molecular world. Because of that, Computational chemistry is used to help the researcher who studies the interactions among molecules deeply. Molecular docking is a branch of molecular modeling which predict the interactions and bonding sites especially for macromolecule such us protein [22]. In this case, molecular docking was also applied to determine the driving force of interaction among E-cadherin and ADTC peptide. So, it can be used to predict the enhancement of porosity in the intercellular junction. Siahaan et al (2013) have evaluated the interaction between cyclic ADTC5 and E-cadherin by docking method [23].

In this paper focus on the molecular docking simulation between EC1-EC2 with the both of cyclic and linear ADTC7. The objective of this studied is to determine the best conformation of cyclic ADTC7 peptide which interacts with E-cadherin protein with energy binding and active site parameters.

2. Methodology

The EC-EC2 domain of E-cadherin was obtained from Protein Databank (PDB) code 2O72. The structure of linear ADTC7 peptide was constructed using PyMol program. The MD simulation was carried out to optimize the linear ADTC7 peptide using GROMACS v.4.6.5 software with variation distance constraint of S14…S78 and force constraint for 20 ns as shown in the Table I.

The optimized linear ADTC7 peptide with minimum total energy is cyclized by pulling the disulfide atomic bond on the cysteine amino acid between S14…S78 using Avogadro program. The cyclic ADTC7 peptide was re-optimized to obtain a stable peptide structure. The EC1-EC2 domain of E-cadherin, the linear and cyclic ADTC7 structure can be shown by Figure 1.

The optimized cyclic ADTC7 peptide selected 10 structures with a minimum total energy. The cyclic ADTC7 peptide as guest and the EC1-EC2 domain of E-cadherin as host. Molecular docking was carried out to interact 10 structures of the cyclic ADTC7 peptide with the EC1-EC2 domain of E-cadherin using Autodock 4.2. The strong interaction between 10 structures of the cyclic ADTC7 peptide and the EC1-EC2 domain of E-cadherin is seen from the lowest binding energy and best position of the binding site.
Table 1. The MD simulation with variation for 20 ns

| Code | Distance constraint of $S_{14}...S_{78}$ (nm) | Force restraint (kJmol$^{-1}$nm$^{-2}$) |
|------|------------------------------------------|-------------------------------------|
| L1   | All bond freely rotatable                | None                                |
| L2   | 0.2-0.3                                  | None                                |
| L3   | 0.3-0.4                                  | None                                |
| L4   | All bond freely rotatable                | 4000                                |
| L5   | 0.2-0.3                                  | 4000                                |
| L6   | 0.3-0.4                                  | 4000                                |
| L7   | All bond freely rotatable                | 8000                                |
| L8   | 0.2-0.3                                  | 8000                                |
| L9   | 0.3-0.4                                  | 8000                                |

3. Result and discussion

3.1 Optimize the ADTC7 peptide

There were 3 stages in the MD simulation including system preparation, trajectory generation, and trajectory analysis. In the system preparation stage for the linear ADTC7 peptide is done by adding 2451 water solvents, 7Na$^+$ and 6Cl$^-$ ions in a 1 nm cube. Energy minimization is obtained by potential energy of $-1.08 \times 10^5$ kJmol$^{-1}$ and potential force of $1.88 \times 10^3$ kJmol$^{-1}$nm$^{-1}$. MD carried out for 20 ps in restraint position. The final equilibrium of the unconstrained system is produced for 100 ps. The trajectory generation stage is done for 20 ns with parameters changing. The trajectory analysis stage has produced RMSD (Root Mean Square Deviation) and total of energy analysis. The result of deviation standard RMSD and total energy analysis as shown in Table 2.

Based on Table 2 shows that peptide code L9 is the best structure of the linear ADTC7 peptide. Peptide code L9 with distance constraint of $S_{14}...S_{78}$ of 0.3-0.4 nm and force constraint of 8000 kJmol$^{-1}$nm$^{-2}$ has a minimum total energy of $-118,824.84$ kJmol$^{-1}$. This result is supported by relatively stable RMSD data with a value of less than 2Å. The linear ADTC7 peptide (code L1) without variation distance constraint of $S_{14}...S_{78}$ and force constraint has fluctuative graph that indicates their movements are unstable. In general, the ADTC7 peptide without variation force constraint have a large range of distance $S_{14}...S_{78}$ compared with the ADTC7 peptide with variation force constraint. The linear ADTC7 peptide (code L7) with variation force constraint has movement toward stable. The linear ADTC7 peptide (code L8 and L9) with variation distance constraint of $S_{14}...S_{78}$ and force constraint has not fluctuative graph. This indicates that their movement is more stable. The peptide code L9 has a more stable movement.
compared to the peptide code L8 which shows the compatibility of the RMSD profile with total energy. Thus, the linear ADTC7 peptide code L9 was taken for the next step in the formation of the cyclic ADTC7 peptide to increase its biology activity in modulating the tight junction. The RMSD profile as shown in Figure 2.

Table 2. Deviation standard RMSD and total energy in the MD simulation

| Code | Deviation standard RMSD (nm) | Total energy (kJ/mol) |
|------|-------------------------------|-----------------------|
| L1   | ±0.030385                     | -86,551.78            |
| L2   | ±0.030385                     | -86,551.78            |
| L3   | ±0.030385                     | -86,551.78            |
| L4   | ±0.028973                     | -86,530.23            |
| L5   | ±0.010347                     | -86,556.08            |
| L6   | ±0.009421                     | -86,910.27            |
| L7   | ±0.028973                     | -86,530.23            |
| L8   | ±0.007912                     | -118,694.20           |
| L9   | ±0.011803                     | -118,824.84           |

Figure 2. The RMSD profile of the linear ADTC7 peptide (a) black: code L1, (b) blue: code L7, red: code L8, and green: code L9.

The cyclic formation is made from the linear ADTC7 peptide code L9 using Avogadro program. The cyclic ADTC7 peptide are re-optimized using GROMACS software to make their structure stable. The optimized cyclic ADTC7 peptide was analyzed RMSD and total energy. The search for stable peptide structures was carried out by selecting 10 structural models out of 20,000 structures through the total energy approach. The stable peptide structures with minimum total energy will be interacted with the EC1-EC2 domain of E-cadherin using molecular docking method as shown Table 3.
3.2 Docking interaction between peptide with E-cadherin

Molecular docking method can be used to determine the conformation and binding energy between peptide and protein using Autodock 4.2 software. This method has the ability to predict with a high degree of accuracy so it is often used as a method for designing drugs (structure-based drug design). The stages of autodock are done with autogrid and autodock. Autogrid stage is done by blind docking to determine the size of the gridbox 60x60x60 Å and grid spacing in the EC1 region of E-cadherin. At this stage the active side of the E-cadherin protein with the cyclic ADTC7 peptide is unknown. Autodock stage is a docking process between 10 structures of the cyclic ADTC7 peptide as a ligand with the EC1-EC2 domain of E-cadherin as a rigid molecule. Lamarckian Genetic Algorithm (LGA) parameter to determine energy binding with the number of algorithm being run by 150, population at 150 and evaluation at 10,000,000. This results of 10 conformations of the cyclic ADTC7 peptide docking with EC1 region of E-cadherin to get the lowest binding energy is C1 conformation of -19.37 kJmol\(^{-1}\) and the largest population is 73. This result is strengthened by the presence of hydrogen bonds between peptide and E-cadherin through residues Leu21…Cys6 and Gln23…Cys1, with the type of bonds O…NH and the distance of each is 1.966Å and 1.984Å. The active site of bonding between peptide and E-cadherin is found in residues Ile4, Pro5, Pro6, Ile7, Ser8, Leu21, Val22, Gln23, Trp59. The results of 10 conformations of the cyclic ADTC7 peptide docking with the EC1 domain of E-cadherin as shown Table 4. The result of docking analysis, there are several clusters for each structure. The structure with the lowest energy and lowest frequency population is selected for the next process, docking validation (re-docking).

Table 3. simulation MD of the cyclic ADTC7 peptide

| Code | Time (ps) | Total Energy (kJ/mol) | Time (ps) | r S\(_{14}…S_{78}\) (Å) |
|------|----------|-----------------------|----------|--------------------------|
| C1   | 3732     | -52985.95             |          | 2.02975                  |
| C2   | 12385    | -52916.57             |          | 2.02862                  |
| C3   | 14239    | -52832.16             |          | 2.02877                  |
| C4   | 1864     | -52812.66             |          | 2.02884                  |
| C5   | 2270     | -52806.77             |          | 2.02962                  |
| C6   | 16882    | -52777.79             |          | 2.02921                  |
| C7   | 8841     | -52763.64             |          | 2.02859                  |
| C8   | 3112     | -52763.23             |          | 2.02901                  |
| C9   | 17207    | -52762.79             |          | 2.02896                  |
| C10  | 16810    | -52744.38             |          | 2.02923                  |

Table 4. The result of docking interaction between cyclic ADTC7…E-cadherin

| Code | Pose | ∆G (kJ/mol) | K\(_i\) (μM) | Population |
|------|------|-------------|--------------|------------|
| C1   | 25   | -19.37      | 400.85 μM    | 73         |
| C2   | 66   | -18.62      | 547.17 μM    | 34         |
| C3   | 130  | -17.45      | 882.48 μM    | 29         |
| C4   | 60   | -15.65      | 1.81 mM      | 17         |
| C5   | 131  | -15.61      | 1.86 mM      | 23         |
| C6   | 82   | -15.77      | 1.71 mM      | 27         |
| C7   | 79   | -19.12      | 448.66 μM    | 56         |
3.3 Re-docking interaction between peptide with E-cadherin

The docking validation is done to validate docking results with RMSD ≤2Å. The docking and re-docking processes are carried out with the same parameters by adjusting the gridbox on the active site of docking results. Based on the result of re-docking obtained the lowest binding energy is conformation C1 of -24.56 kJmol⁻¹. The lowest $K_i$ value is owned by conformation C1 of 50.10 μM. The strength of the inhibition constants can be classified into 3 groups, namely very strong inhibition with the value of $K_i$ ≤ 1 μM, strong inhibition with the value of $K_i$ between 1-100 μM, and weak inhibition with the value of $K_i$ ≥ μM. In general when viewed from the relationship of $ΔG$ and $K_i$, the more minimum energy binding, the ability of ADTC7 peptide to inhibit cadherin-cadherin interaction is getting stronger, so that the porosity of the junction between cells can be modulated. The results of docking interactions between 10 conformation cyclic ADTC7 peptide with EC1-EC2 domain of E-cadherin as shown in Table 5.

| Code | Pose | $ΔG$ (kJ/mol) | $K_i$ (μM) | Population |
|------|------|---------------|------------|------------|
| C1   | 56   | -24.56        | 50.10      | 146        |
| C2   | 132  | -22.84        | 100.20     | 80         |
| C3   | 78   | -19.83        | 334.31     | 128        |
| C4   | 114  | -18.54        | 568.96     | 53         |
| C5   | 122  | -20.21        | 289.99     | 77         |
| C6   | 85   | -21.09        | 202.53     | 113        |
| C7   | 9    | -22.80        | 101.42     | 105        |
| C8   | 54   | -21.63        | 162.92     | 103        |
| C9   | 13   | -19.54        | 377.75     | 117        |
| C10  | 45   | -16.90        | 1.09       | 109        |

The active site of bonding between peptide and E-cadherin is found in residues Val3, Ile4, Pro5, Pro6, Ile7, Ser8, Leu21, Val22, Gln23, Lys25. In the conformation of structure C1 hydrogen bond occur between cyclic ADTC7 peptide with EC1-EC2 domain of E-cadherin through residues Gln23…Cys1 and Leu21…Cys6. The type of bonds that occur are O…NH and O…NH with distance each is 1.842 and 2.156Å. The existence of hydrogen bond confirmed using the Ligplus program there are 2 hydrogen bonds. The active site of bonding and hydrogen interactions between conformation C1 and EC1-EC2 domain of E-cadherin can be seen in Figure 3.
Figure 3. The interaction between conformation C1 of the cyclic ADTC7 peptide with EC1-EC2 domain of E-cadherin using (a) Autodock 4.2 software, (b) Ligplus program

Table 6. Hydrogen bond and binding site of cyclic ADTC7 peptide

| Code | Interaction     | Hydrogen bond | Jenis | Amount of residues | RMSD (Å) |
|------|----------------|---------------|-------|--------------------|----------|
| C1   | Gln23…Cys1     | 1.842         | O…NH | 10                 | 1.338    |
|      | Leu21…Cys6     | 2.156         | O…NH |                    |          |
| C2   | Gln23…Thr3     | 2.104         | O…HG1| 10                 | 0.575    |
| C3   | Gln23…Cys1     | 2.000         | O…NH | 9                  | 0.685    |
|      | Asn20…Cys6     | 2.148         | O…HT2|                    |          |
| C4   | Leu21…Asp2     | 1.844         | O…NH | 7                  | 2.604    |
|      | Leu21…Thr3     | 2.197         | O…HG1|                    |          |
| C5   | Gln23…Cys1     | 2.063         | O…NH | 9                  | 1.009    |
|      | Leu21…Cys6     | 2.175         | O…NH |                    |          |
| C6   | Gln23…Cys1     | 1.846         | O…NH | 10                 | 0.875    |
|      | Asn20…Cys6     | 2.019         | O…HT2|                    |          |
| C7   | Gln23…Cys1     | 2.043         | O…NH | 9                  | 1.245    |
|      | Leu21…Cys6     | 2.193         | O…NH |                    |          |
| C8   | Asn20…Cys1     | 1.898         | O…NH | 9                  | 1.503    |
|      | Ser8…Cys6      | 1.982         | O…HT1|                    |          |
| C9   | Gln23…Cys1     | 2.224         | O…NH | 9                  | 0.604    |
|      | Leu21…Cys6     | 2.235         | O…HT1|                    |          |
| C10  | Gln23…Cys6     | 1.917         | O…HT2| 7                  | 0.899    |
The molecular docking of 10 conformation cyclic ADTC7 peptide with EC1-EC2 domain of E-cadherin there are 2 hydrogen bonds except conformation C2. The existence of hydrogen bonds will add to the cooperativity effect so that the formation of molecular recognition between the host and the guest molecule will produce molecules that are more stable and more negative energy.

In addition, only one conformation has an RMSD value above 2Å. RMSD value aims to determine the changes that occur between ligand and receptor. The greater RMSD value indicates that the prediction deviation of ligand and protein is greater the changes that occur between ligand and receptor. The greater RMSD value indicates that the conformation C1 of the cyclic ADTC7 peptide is able to interact strongly with the EC1-EC2 domain of E-cadherin. The conformation C1 not only has the minimum total energy and the lowest binding energy as well.

4. Conclusion
The docking interaction between the cyclic ADTC7 peptide and EC1-EC2 domain of E-cadherin shows that conformation C1 is able to strongly inhibit cadherin-cadherin interaction with the lowest binding energy, low inhibition constant, and large population. The active site of binding at residues Val3, Ile4, Pro5, Pro6, Ile7, Ser8, Leu21, Val22, Glu23, Lys 25 and the lowest binding energy of -24.56 kJmol⁻¹.

Acknowledgements
Thank you to Prof. Teruna J. Siahaan, Ph.D. (Department of Pharmaceutical Chemistry the University of Kansas, US) for valuable knowledge, direction, and discussion of cells and drug delivery systems. Thank to Prof Krzysztof Kuczera, Ph.D. (Department of Chemistry the University of Kansas, US) for depth discussion of computational modeling. Last but not least, thanks to the Indonesian Directorate General of Higher Education, which has funded this research and technology Funding scheme 2018.

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