ALKIMIA : Jurnal Ilmu Kimia dan Terapan

ARTICLES

View Article Online
View Journal | View Issue

OPEN ACCESS

Study of Interaction Between ADT-C2 Cyclic Peptide (Ac-CADTPPC-NH_2) and E-Cadherin Protein Using Molecular Docking Method

Digna Renny Panduwati[a]*

Abstract. Research has been conducted on the study of the interaction between cyclic peptide ADTC2(Ac-CADTPPC-NH_2) and E-cadherin protein using the molecular docking method. The aim of this study is to determine the position of the binding site and the energy of interaction between the ADTC2 peptide and the EC1-EC2 domain of e-cadherin. This research was divided into two parts, (1) Preliminary test using molecular dynamics (DM) method with Gromacs v4.5.4 software, (2) interaction of the peptide ADTC2 with EC1-EC2 using the molecular docking method (MD) with Autodock 4.2 software. Docking was performed with the blind dock method on EC1 and EC2 position. In the second step, the gridbox position was reduced based on the binding activity between e-cadherin and peptides. The strongest interaction and Van der Walls bonds were obtained in boxes B, C and D. The results showed that the ADTC2 peptide had a biological activity to inhibit the interaction of e-cadherin...e-cadherin by forming a complex with the EC1-EC2 domain. This inhibition occurred by forming two binding sites in the EC1 domain (interaction energies are 23.309 kJ/mol and -26.234 kJ/mol, respectively) and one binding site in the EC2 domain (interaction energies are -22.677 kJ/mol). Based on preliminary tests, it can be proven that the native structure of ADTC2 is cyclic with optimization energy of -52504.78 kJ/mol and very stable from the beginning to the end of DM with an RMSD was <2 Å.

Keywords : ADTC2 peptide, E-cadherin, Molecular Dynamic, Molecular docking

[a]*Poltekkes Kemenkes Medan, Jalan Jamin Ginting km 13,5, Medan, Sumatra Utara, Indonesia.*

Correspondence and requests for materials should be addressed to Panduwati, D.R. (email: dignarenny@gmail.com)

Introduction

The methods developed in drug delivery to the brain tissue or the Central Nervous System (CNS) are growing rapidly and becoming a challenge for the medical field. This is due to a barrier, called Blood-Brain Barriers (BBB) [1]. Drugs such as peptides are only able to pass through the paracellular pathway [2] . The paracellular pathway is composed of barrier proteins at the zonular occludens (tight junctions), zonular adherens, gap junctions and desmosomes [3]. This protein becomes a selective control, only molecules with diameter <11 Å and molecular weight <500 Dalton are able to pass through. Adherent junction play a crucial role in network structure to form tight junctions. This junction consists of a complex of occluding proteins and claudins at the tight junction. Tight Junction as a part of the junctional apical complex. Tight junctions form continuous bonds, these bonds serve as gatekeepers on paracellular pathways [2]. Delivery of large drugs is carried out by paracellular pathways by modulating cadherin-cadherin bond at the adherence junction area. The stability of the bond will be disturbed if the cadherin bond is modulated, in the zonular adherens area of the BBB, thus making the pores of the paracellular pathway becomes bigger. E-cadherin has five the outside parts or called EC (extracellular) there are EC1, EC2, EC3, EC4, EC5 [3]. An ADTC2 (Ac-CADTPPC-NH_2) peptide has been synthesized that derived from the bulge region of the EC 1 domain. This peptide has biological activity to open the porosity of paracellular pathways, by modulating E-cadherin...E-cadherin interactions. At the EC1 section is located at the very end which will bind to EC1 at the other end. This bond between EC1 and EC1 makes it difficult for large molecules to enter because of the narrow porosity formed [4].

According to the previous studies, porosity opening by the ADTC2 peptide was estimated by forming a complex with the EC1 domain and all five E-cadherin domains (EC1,…,EC5). How ADTC2 can unlock the porosity of the paracellular pathway is not known certainly. This can be determined by studying the interaction between the ADTC2 peptide and E-cadherin. Knowledge of molecular interactions will provide an understanding of the structure eventually will be able to predict the function and properties of biomolecules [5] [6]. Molecular dynamics is a form of computer simulation in which atoms and molecules interact over a period of time based on the laws of physics. The basic principle used in this molecular dynamics is classical mechanics. Molecular dynamics provides knowledge about the dynamics of large macromolecules, including biological systems such as proteins, nucleic acids (DNA and RNA), and membranes. Molecular docking (MD) is a method of modelling interactions between small molecules such as drugs (can be called ligands) with proteins (can be called receptors) or between proteins in silico [7]. MD can be used to study noncovalents, so that it is able to predict the orientation, position and conformation that is favoured by a molecule relative to other molecules, thus it can form a stable complex. MD has two main components, sampling and scoring [8]. The use of the MD method to determine the interaction of the ADTC2 cyclic peptide with the EC1-EC2 domain on E-cadherin has never been carried out. The aim of this study was to determine the site position and interaction energy between the ADTC2 peptide against the EC1-EC2 domain of E-cadherin [8].

Experimental

This research was conducted into two steps, a preliminary test and molecular docking. The peptides used were ADTC2 and EC1-EC2 domain E-cadherin crystals (code 2O72) as host.

Preliminary test

ADTC2 peptide was prepared in silico using PyMol software and optimized using the PDB Utility server and molecular dynamics (DM) with the Gromacs v4.5.5 software [9]. The native structure of ADTC2 peptide is cyclic (Figure 1), to obtain it, ADTC2 peptide optimization was performed using molecular dynamic. In this test the two sulfur atoms in the two amino acids cysteine (C) (S14...S89) with the distance and the holding force, according to Table 1 in order to form a cyclic stucture. If a cyclic is not formed, then manual cyclization is carried out and then DM is carried out with the treatment in Table 1.

Molecular dynamic were conducted in three steps: (1) System preparation step. The aim of this step is to prepare ADTC2 structure and solvation system using energy minimization and position restraint, respectively. This solvation system uses a simple cubical periodic box (the di-

mensions of the box based upon setting the box edge approximately 1.0 nm from the molecule periphery), TIP3P (Transferable Intermolecular Potential 3-Point) water as a solvent, with an ionic concentration of 0.15 M. (2) trajectory generation was conducted with 20 ns (3) Analyzing trajectory by using total energy and RMSD (Root Mean Square Deviation) of a Cα atom each frame (structure of each ps) during DM to the initial structure.

Molecular docking of the peptide ADTC2 with EC1-EC2 domain E-cadherin

Molecular docking was run by using Autodock v.4.2 software. This method involves two stages, run autogrid and run autodock.

Run autogrid. Using gridbox placed on (1) sequence Ala43, Asp44, Thr45 on EC1 for all cyclic ADTC2 variations (S1-S3).

Run autodock. Involved in two stages, sampling and scoring. Which used the Lamarckian genetic algorithm. All default parameters were used according to the Autodock software, using the amount of energy evaluation of 5 million and a number of runs of 150. All the hydrogen added to the polar atoms on the ligand and receptor, the charge type used was Gasteiger. After running the autodock, the data was grouped into one weight based on RMSD < 2 Å, redocking was done.

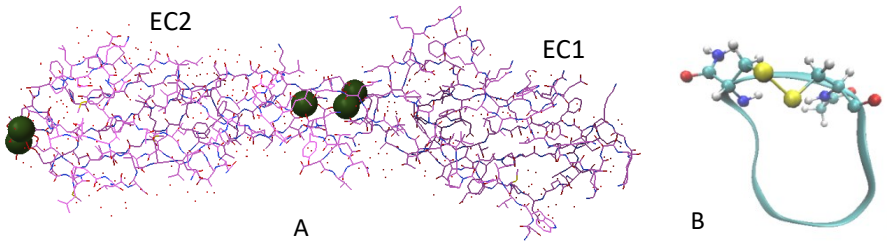

Figure 1. (A) Crystal structure of E-cadherin domain EC1- EC2 (code 2O72), (www.rcsb.org/pdb). (B) Peptide cyclic structure of ADTC2.

Table 1. ADTC2 Peptide with Molecular Dynamic Treatment

Code	Treatment of linier ADTC2
Li1	All bond freely rotatable
Li2	Distance S14...S89 restrain 0.3-0.4 nm without force restraint
Li3	Distance S14...S89 restrain 0,3-0,4 nm with 4000 kJmol^{-1}nm^{-2} force restraint
Li4	Distance S14...S95 restrain 0,3-0,4 nm with 12000 kJmol^{-1}nm^{-2} force restraint
Li5	Distance S14...S89 restrain 0,2-0,3 withot force restraint
Li6	Distance S14...S89 restrain 0,2-0,3 nm with 4000 kJmol^{-1}nm^{-2} force restraint
Li7	Distance S14...S89 restrain 0,2-0,3 nm with 12000 kJmol^{-1}nm^{-2} force restraint
Code	Treatment of cyclic ADTC2
Si1	All bond freely rotatable
Si2	bond S14...S89 with force restraint 4000 kJmol^{-1}nm^{-2} for all atoms
Si3	bond S14...S89 with force restraint 12000 kJmol^{-1}nm^{-2} for all atoms

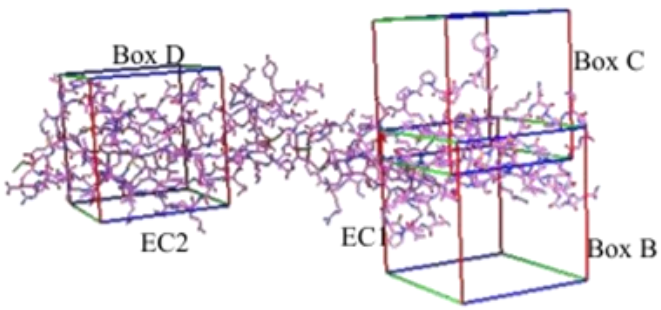

Figure 2. Gridbox position of the boxes (B to D)

Results and Discussion

The Preliminary Test

DM for 20 ns states that each ps will get one structure, so at the end of DM will get 20000 structures. Then a structure with the lowest energy level was selected, namely the Si_3 peptide, which was -52504.78125 kJ/mol. This indicates that the Si_3 peptide interacts less with the electrostatic force of the ion Na^+, Cl^- and the solvent. The stiffness of the cyclic peptide causes the torsional degrees of freedom to decrease, so the probability becomes smaller and the conformation tends to be more stable. In other words, if a molecule is not too s trongly interacting with the environment (ions and solvents), then the structure will be more stable than those that are easy to interact with. This is supported by the RMSD value indicated by Si_3 less than 2Å. The existence of hydrogen bonds that hydrogen bonds that occur between the amino acids in the peptide with water solvent molecules. The stability of the structure did not cause the cyclic ADTC2 peptide to remain unchanged. Peptides continue to undergo folding and unfolding so that they produce very varied energy. The cyclic ADTC2 peptide has a rigid ring structure (Figure 3), which does not cause changes during DM. The cyclic ADTC2 peptide with As1 treatment was chosen as the structure closest to the native structure of ADTC2 peptide.

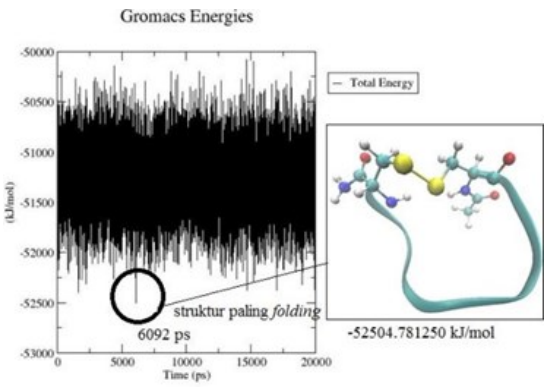

Figure 3. Energy graph of ADTC2 Si_3 coded peptide during MD simulation

Molecular docking of ADTC2 with EC1-EC2

The cyclic ADTC2 peptide is estimated to be closest to the native ADTC2 structure in nature, so the cyclic ADTC2 interaction can represent the native ADTC2 interaction actual (in vivo test). Molecular docking is one of the most frequently used methods for designing drug molecules or often called structure-based drug design (SBDD) because of its ability to predict with a high degree of accuracy [10]. The most commonly used parameter in molecular dynamic is the RMSD value with the native E-cadherin ligand, because there is no native ligand, the more reliable parameter is the lower interaction energy. In the EC1 domain, there are two active sites which are the groove region containing HAV residues (His-Ala-Val) and the bulge region is the bulge region containing ADT residues (Ala-Asp-Trp). The groove region have homofilic reaction with bulge region, so we can called groove…bulge region interaction occur frequently. Besides groove…bulge region there are other areas that are also trans dimer interaction occur, namely adhesion arm…acceptor pocket area which can see in Figure 4 [5].

The results of full docking on the EC1 domain obtained several clusters, with various active sites, namely the bulge…groove area and the adhesion arm…acceptor pocket area. Docking was carried out again on the two areas of the adhesion arm…acceptor pocket, namely the Asp1, Trp2, Val3 (adhesion arm) residues and the acceptor pocket on the Lys25, Asn27, Glu89, Asp90, Met92 residues. This area is an area where trans dimer interactions occur (Figure 4).

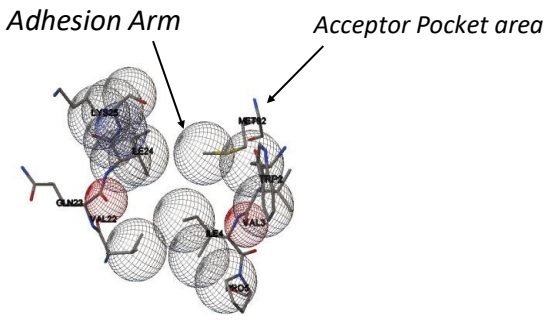

Figure 4. The active site of the interaction between ADTC2 peptides with e-cadherin on the EC domain

The calculation results (Table 2) show that the cyclic ADTC2 peptide has a lower binding energy, this is due to the rigidity of the conformational conformation of the cyclic peptide itself with a low degree of torsion, which is 7 out of 32. The small torque value will make the population of each torsion large. This is due to its rigid conformation. The active site of e-cadherin tends to interact hydrophobicly because the amino acids involved are non-polar amino acids. The ADTC2 peptide interacts with e-cadherin in the Acceptor

Pocket region in box B and interacts with the Adhesion Arm region in box A.

Box B shows the highest population at pose 146 and the lowest energy at pose 46 (Figure 5). At pose 146, the residue of E-cadherin Lys25 hydrophilically binds to the Cys1 residue of the ADT-C2 peptide, so that the peptide will be locked in the acceptor pocket area.

The hydrophobic interaction of the Val3 residue with the ADT-C2 peptide Pro5 residue, which makes it locked in the adhesion arm region (Figure 6). Reinforced by the presence of hydrogen bonds between polar atoms in Glu89 and Cys7 along 1.811 , with the type of SH bond on the Cys1 residue with O atoms on the Glu89 residue. Overall, the three boxes are the active sites of E-cadherin which binds to the cyclic ADTC2 peptide, which causes the interaction at the adheren junction to weaken so that the paracellular pathway will be more wide open because the porosity of E-cadherin will increase, so that in the medical field, (drug delivery system) ADTC2 peptide will increase its porosity and allow drug molecules to pass through. However, this will not last long, because the weak level of inhibition of cyclic ADTC2 peptide and the bond that occurs is a van der Waals bond, will not make the porosity of E-cadherin open for a long time, only for a certain time. In box C, the lowest energy shown in pose 120. In box C the lowest energy is shown in pose 120 (Figure 6), with the bond that occurs is between Arg55…Asp3. Asp3 in ADTC2 binds to Arg55 in the EC1 domain by forming hydrogen bonds between NH…O atoms and the bond distance shown in the bond is 2,986 Å. In box C shown that pose 45 (Figure 5) have the lowest energy and its supported by a lower constant of inhibition compared to the other poses. This shows that position 45 is the most likely position for in silico interactions to occur, so it is possible if laboratory tests are carried out.

Table 2. Molecular binding energy results of ADTC2 peptide docking with e-cadherin in each box

Box	Pose	ΔG / kJmol^{-1}	Ki / M(1×10^{-6})	Population	Hydrogen bond		
					Interaction	type	Bonding Distance (Å)
B_EC1	146	-23,305	82.70	80	Glu89…Cys7	O…SH	1,811
	46	-29,539	6.72	30			
C_EC1	35	-20,836	223,56	45	Arg55…Asp3	NH…O	2,986
	120	-26,234	25,16	25			
D_EC2	103	-22,677	106.89	40	Thr164…Cys7	NH…O	2,046
	45	-25,733	30,34	36			

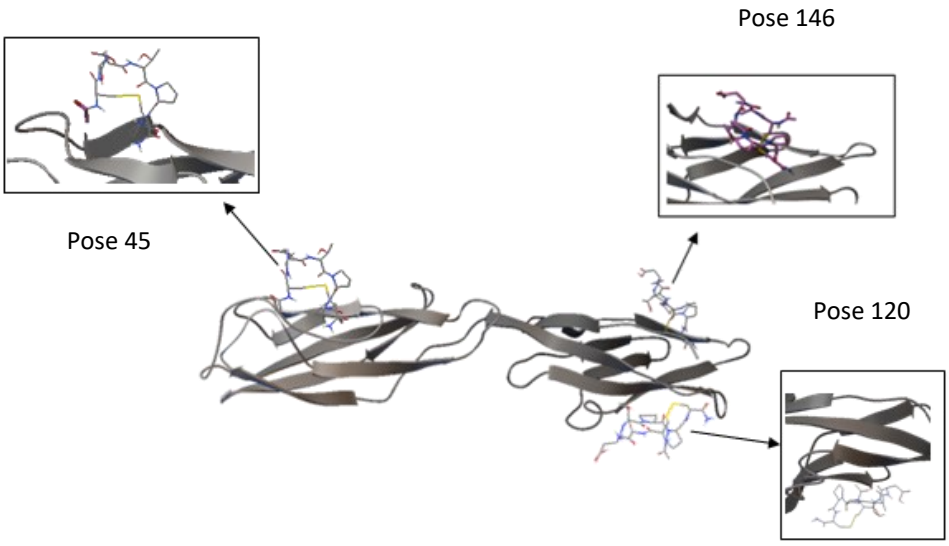

Figure 5. The conformational position of the ADTC2 peptide on the E-cadherin domains EC and EC2 at various poses

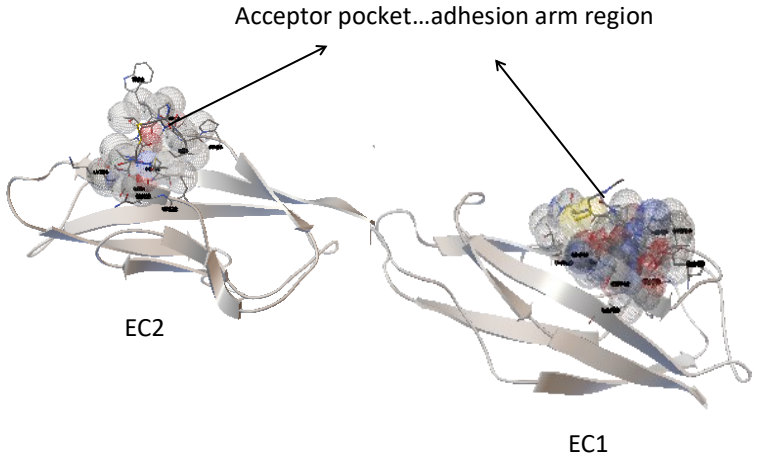

Figure 6. The interaction of the ADTC2 peptide with the E-cadherin EC1-EC2 domain

The inhibitory strength of the ADTC2 peptide could also be predicted through the interaction data obtained. Knowledge of the interaction energy can provide an understanding of the inhibition constant (Ki). The inhibitory power of a peptide is classified into three, namely: very strong (Ki<1M), strong (Ki between 1-100 M) and weak (Ki>100 M) [11]. ADTC2 peptide is a strong inhibitor of EC1-EC2 on E-cadherin, this is shown in table 2. Each box shows different inhibitory strengths. In the drug delivery system to the body and to the brain, the ADCT2 peptide will increase the porosity of the paracellular pathway and allow drug molecules to pass through. After some time, the ADTC2 peptide is released and the paracellular pathway is normal again, according to its inhibition constant.

Conclusion

The results have shown that the ADTC2 peptide has the lowest energy, in other words, the ADTC2 peptide owns a fairly stable structure. The molecular docking results show that the ADTC2 peptide is able to enlarge the paracellular pathway axis, thus allowing drugs with a sufficiently large molecular weight to penetrate the central nervous system (CNS). The inhibitory power shown is also quite strong, which is 6.72 µM in box B. This indicates that box B is an area that allows drugs to enter.

Acknowledgements

The author Thanks to Prof. Teruna J. Siahaan, Ph.D. (Department of Pharmaceutical Chemistry, University of Kansas, USA) who has provided input and discussion on research in the field of drug delivery and drug targeting in cells and drug delivery systems. Thanks also to Prof. Krzysztof Kuczera, Ph.D. (Department of Chemistry, University of Kansas, USA) who has provided input and discussion on molecular computational modeling research.

References

[1] N. H. On, P. Kiptoo, T. J. Siahaan, and D. W. Miller, “Modulation of Blood–Brain Barrier Permeability in Mice Using Synthetic E-Cadherin Peptide,” Mol. Pharm., vol. 11, no. 3, 2014, doi: 10.1021/mp400624v.

[2] M. D. Laksitorini, P. K. Kiptoo, N. H. On, J. A. Thliveris, D. W. Miller, and T. J. Siahaan, “Modulation of Intercellular Junctions by Cyclic-ADT Peptides as a Method to Reversibly Increase Blood–Brain Barrier Permeability,” J. Pharm. Sci., vol. 104, no. 3, Mar. 2015, doi: 10.1002/jps.24309.

[3] Prasasty, “Homology Modeling And Molecular Dynamics Studies Of Ec1 Domain Of Ve-Cadherin To Elucidate Docking Interaction With Cadherin-Derived Peptide,” Online J. Biol. Sci., vol. 14, no. 2, Feb. 2014, doi: 10.3844/ojbsci.2014.155.162.

[4] A. Alaofi, E. Farokhi, V. D. Prasasty, A. Anbanandam, K. Kuczera, and T. J. Siahaan, “Probing the interaction between cHAVc3 peptide and the EC1 domain of E-cadherin using NMR and molecular dynamics simulations,” J. Biomol. Struct. Dyn., vol. 35, no. 1, Jan. 2017, doi:

10.1080/07391102.2015.1133321.

[5] A. Manna, M. D. Laksitorini, D. Hudiyanti, and P. Siahaan, "Molecular Docking of Interaction between E-Cadherin Protein and Conformational Structure of Cyclic Peptide ADTC3 (Ac-CADTPC-NH_2) Simulated on 20 ns," J. Kim. Sains dan Apl., vol. 20, no. 1, Apr. 2017, doi: 10.14710/jksa.20.1.30-36.

[6] T.-Y. Na, L. Schecterson, A. M. Mendonsa, and B. M. Gumbiner, "The functional activity of E-cadherin controls tumor cell metastasis at multiple steps," Proc. Natl. Acad. Sci., vol. 117, no. 11, Mar. 2020, doi: 10.1073/pnas.1918167117.

[7] V. Salmaso and S. Moro, "Bridging Molecular Docking to Molecular Dynamics in Exploring Ligand-Protein Recognition Process: An Overview," Front. Pharmacol., vol. 9, Aug. 2018, doi: 10.3389/fphar.2018.00923.

[8] A. Sethi, K. Joshi, K. Sasikala, and M. Alvala, "Molecular Docking in Modern Drug Discovery: Principles and Recent Applications," in Drug Discovery and Development - New Advances, IntechOpen, 2020.

[9] T. Makarewicz and R. Kaźmierkiewicz, "Molecular Dynamics Simulation by GROMACS Using GUI Plugin for PyMOL," J. Chem. Inf. Model., vol. 53, no. 5, May 2013, doi: 10.1021/ci400071x.

[10] L. Ferreira, R. dos Santos, G. Oliva, and A. Andricopulo, "Molecular Docking and Structure-Based Drug Design Strategies," Molecules, vol. 20, no. 7, Jul. 2015, doi: 10.3390/molecules200713384.

[11] F. Z. Muttaqin, "Studi Molecular Docking, Molecular Dynamic, dan Prediksi Toksisitas Senyawa Turunan Alkaloid Naftiridin sebagai Inhibitor Protein Kasein Kinase 2-α pada Kanker Leukemia," Pharmacoscript, vol. 2, no. 1, Aug. 2019, doi: 10.36423/pharmacoscript.v2i1.241.