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Original Article

Evaluation of pH Effect on Conformation of Protein Interaction E-Cadherin...ADTC5 Complex: Molecular Dynamic Simulation

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Abstract

Blood Brain Barrier (BBB) is a barrier located in the brain that controls the delivery of peptide drug to the brain. The difficulties of delivering drugs through BBB is because of E-Cadherin…E-Cadherin interaction that prevent drugs to pass through. ADTC5 has shown positive results to improve drug delivery through the BBB by modulating E-Cadherin…E-Cadherin interaction. Conformation are one of the factors that can affect the modulation stability between E-Cadherin…ADTC5. To analyze the conformation and stability of E-Cadherin…ADTC5 complex throughout the simulation time with pH effect, Molecular Dynamic (MD) method was used to simulate the conformational changes. The results indicate that pH 7.4, E-Cadherin…ADTC5 is the most stable conformation, with the lowest maximum radius gyration value 28.906 Å and the lowest ΔG Binding -168.244 kJ/mol. In the other hand, the most unstable conformation can be seen at pH 2.4, indicated by the positive ΔG Binding values 51,802 kJ/mol, high RMSD average at 2.8 Å and high RMSF fluctuations on residues.

Keywords: *Blood Brain Barrier (BBB), E-Cadherin, ADTC5, Drug Delivery, Molecular Dynamic*

1. Introduction

The difficulty in drug delivery to brain is Blood-Brain Barrier (BBB) in the paracellular pathway [1]. BBB is a diffusion barrier essential for protecting normal brain by blocking or degrading most compounds or drugs from transiting from the blood to the brain [2] [1] [3]. Paracellular pathway is the possible route for peptide drugs. However, paracellular pathway only allow molecules <11 A and < 500 D to pass through [2] [4].

Cadherin is group of transmembrane glycoproteins cells that can interact with other cadherin (Homophilic Interaction) to form Adherent Junction [5]. The adherent junction (AJ) is a cell-cell junction component in which cadherin receptors bridge neighboring plasma membranes via homophilic interactions. Cadherins form complexes with cytoplasmic proteins known as catenin, which then bind to cytoskeletal components such as actin filaments and microtubules. These molecular complexes then interact with other proteins, including signaling molecules, transforming AJs into highly dynamic and controllable structures [6]. Epithelium Cadherin (E-Cadherin) is a transmembrane protein that made up from 5 extracellular domain (EC1-EC5). E-Cadherin (EC1-EC5) can form cis and trans-dimer by interacting homophilic with other E-Cadherins [3].

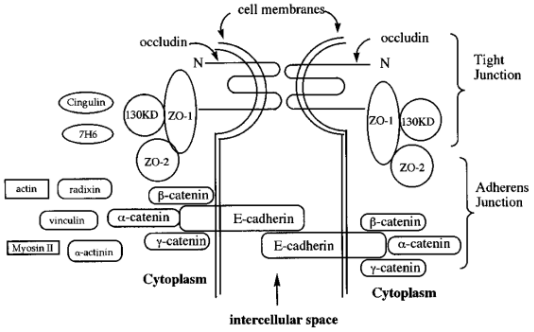

Figure 1. Intercellular Junction Structure [7] [8]

ADT Peptides can bind into E-Cadherin binding sites and interfere with the interaction between adherent junction to modulate tight junction. ADTC5 Cyclic (AC-CDTPPC-NH2) is known to have the ability to modulate the interaction of homodimer e-cadherin by inhibiting E-cadherin interactions that are important to improve paracellular porosity in delivering drug molecules to target cells [11].

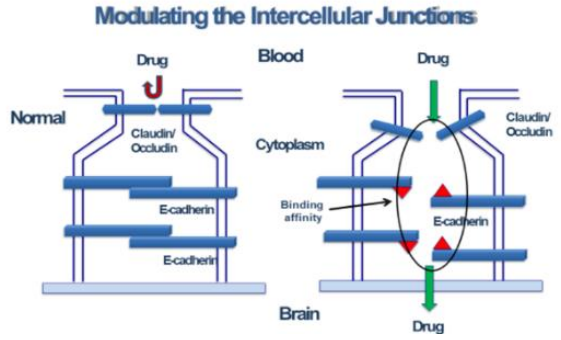

Figure 2. Intercellular Junction Modulation [7] [9] [10]

Bungaran has studied interaction between ADTC5 Cyclic peptide (AC-CDTPPC-NH2) and E-Cadherin protein using molecular docking method [5]. Molecular Docking is a computational method to simulate and predict the conformation of receptor-ligand complex [12].

Molecular Dynamic is a method for analyzing the movement of large molecules in a solvent that implements a derivative of Newton's equation of motion to produce a trajectory or series of atoms coordinates generated during simulation [13] [14].

Temperature, pH, and salt all have an impact on the stability of the Protein-peptide complex. In the previous research, the greatest interactions between ligands and protein residues occurred at 310 K, as evidenced by the shortest hydrogen bond distance and the most negative ΔG binding value of −164.552 kJ/mol [15]. The structure, function and dynamics of protein and peptides in solution are highly dependent on pH. The effects of pH occur through electrostatic interactions as one of the most powerful forces at the molecular level and can directly affect molecular structure [16]. The focus of this research is to determine effect of pH on conformational stability and interaction between E-Cadherin…ADTC5 modulation at 310K using molecular dynamics method.

2. Research Methods

The Molecular Dynamic simulation of E-Cadherin…ADTC5 complex was simulated using Yasara Software, by solvating in a 10 x 10 x 10 periodic boundary box at 310K. The TIP3P solvent system with a density of 0.997 g/L was used in the solvation process. To neutralize the system, NaCl ions at a physiological concentration of 0.9% are added. In addition, energy is minimized using a speed descent approach, and the system is equilibrated until the temperature and pressure are constant. The Van der Waals force has a cutoff distance of 8, whereas the electrostatic interaction employs the Ewald particle mesh method (PME) [17].

The molecular dynamics simulation took place six times at a pH 2.4, 5, 7.4, 9, 12. Each MD production process lasted for 20 ns with a timestep of 2.5 fs. A Berendsen thermostat is used to maintained pH and pressure stable. Every 50 ps, the trajectory results are saved. Complex conformational changes, residual interactions, RMSD (Root Mean Square Deviation), RMSF (Root Mean Square Fluctuation), Radius of Gyration (RG) and Binding Free Energy (ΔG).

3. Result and Discussion

MD Simulations is method carried out to determine the movement of atoms and molecules over a given period of time [18]. MD simulations of the E-cadherin…ADTC5 complex were completed in a 10 Å box using water solvent type T1P3P at temperatures of 2.4, 5, 7.4, 9 and 12. The results of the MD simulation analyzed were: Conformational changes during simulation, post-simulation protein-ligand interactions, potential energy, RMSD, radius of gyration, and RMSF, binding free energy.

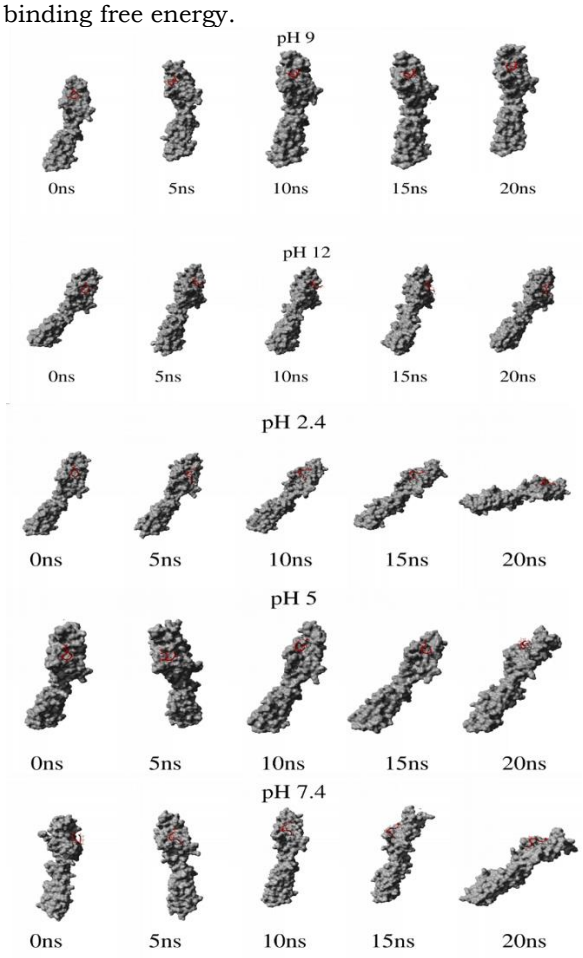

Figure 3. E-Cadherin…ADTC5 Conformational Changes During Simulations

Table 1. Residue contact with ligands during the MD simulation at pH of 2.4 – 12

pH	0ns	5ns	10ns	15ns	20ns
2.4	Hydrogen Bond Ile38:Cys1 Hydrophobic Ser37:Cys1 Val48:Val6 Ile53:Cys1 Arg55:Cys1	Hydrogen Bond Ala43:Cys7 Hydrophobic Phe17:Asp2 Asp44:Cys7 Val48:Pro5 Ile52:Cys1 Ile53:Cys1	Hydrogen Bond Ala43:Cys7 Hydrophobic Ile38:Cys7 Val48:Pro5 Ile53:Cys1	Hydrogen Bond Ala43:Cys7 Gly49:Asp2 Hydrophobic Ile38:Cys7 Ala43:Cys7 Val48:Pro5 Ile52:Cys1 Glu64:Asp2	Hydrogen Bond Ala43:Cys7 Gly49:Asp2 Hydrophobic Ile38:Cys7 Val48:Pro5 Ile52:Cys1 Ile53:Cys1 Glu64:Asp2
5	Hydrogen Bond Ala43:Cys7 Hydrophobic Ser37:Ace0 Ile38:Cys7 Asp44:Val6 Val48:Val6 Ile52:Cys7 Ile52:Pro4 Arg55:Cys1	Hydrogen Bond Arg55:Asp2 Arg55:Cys1 Hydrophobic Ser37:Ace0 Ser37:Cys1 Ile38:Cys1 Asp44:Val6 Val48:Val6 Ile52:Cys7 Ile53:Cys1	Hydrophobic Ser37:Ace0 Ser37:Cys1 Ile38:Cys1 Ala43:Cys7 Asp44:Pro4 Val48:Pro4 Val48:Thr3 Ile53:Cys1	Hydrogen Bond Ser37:Cys1 Ala43:Cys7 Hydrophobic Ser37:Ace0 Ser37:Cys1 Ile38:Cys1 Val48:Pro4 Gly49:Pro4 Ile52:Cys7 Ile53:Cys1	Hydrogen Bond Ile53:Cys1 Hydrophobic Ser37:Ace0 Ile38:Cys1 Ile52:Cys7 Ile52:Asp2 Ile53:Ace0 Ile53:Cys1 Arg55:Ace0
7.4	Hydrogen Bond Ala43:Val6 Ile53:Cys1 Hydrophobic Phe35:Ace0 Val48:Val6 Arg55:Cys1 Ile52:Pro4	Hydrogen Bond Ala43:Val6 Arg55:Asp2 Arg55:Asp2 Hydrophobic Val48:Pro5 Ile52:Ace0 Ile53:Cys1	Hydrogen Bond Ala43:Val6 Ala43:Cys7 Hydrophobic Ile38:Cys1 Val48:Pro5	Hydrogen Bond Ala43:Val6 Ala43:Cys7 Arg55:Asp2 Hydrophobic Gly49:Pro5 Ile52:Ace0 Ile53:Cys1 Ile38:Cys1 Ionic Interaction Arg55:Asp2	Hydrogen Bond Arg55:Asp2 Ala43:Val6 Hydrophobic Val48:Pro5 Gly49:Pro5 Ile52:Ace0 Ile53:Cys1 Ionic Asp44:Val6 Arg55:Asp2
9	Hydrogen Bond Ala43:Cys7 Hydrophobic Ser37:Ace0 Ser37:Cys1 Ile38:Cys7 Val48:Val6 Ile52:Cys7	Hydrogen Bond Ile53:Cys1 Arg55:Ace0 Arg55:Asp2 Hydrophobic Asp44:Val6 Ile53:Cys1 Arg55:Ace0 Arg55:Cys1 Ionic Arg55:Asp2	Hydrogen Bond Ile53:Cys1 Hydrophobic Val48:Pro4 Gly49:Pro4 Ile52:Ace0 Ile53:Cys1	Hydrogen Bond Ile38:Cys7 Ile53:Cys1 Hydrophobic Ser37:Cys1 Ile38:Cys7 Asp44:Cys7 Asp44:Val6 Val48:Val6 Ile53:Cys1 Arg55:Ace0	Hydrogen Bond Ile53:Cys1 Arg55:Asp2 Hydrophobic Ile38:Cys7 Asp44:Val6 Ile53:Cys1 Arg55:Ace0 Arg55:Cys1 Ionic Arg55:Asp2
12	Hydrogen Bond Ala43:Cys7 Hydrophobic Ser37:Ace0 Ser37:Cys1 Ile38:Cys7 Val48:Val6 Gly49:Pro5 Ile52:Cys7 Ile52:Pro4 Arg55:Ace0 Arg55:Cys1	Hydrogen Bond Arg55:Asp2 Hydrophobic Ser37:Cys1 Asp44:Val6 Val48:Pro4 Arg55:Cys1 Ionic Arg55:Asp2	Hydrophobic Ser37:Ace0 Ser37:Cys1 Ile38:Cys7 Asp44:Val6 Val48:Pro4 Gly49:Pro4 Ile52:Cys7 Ile53:Cys1	Hydrogen Bond Ala43:Cys7 Hydrophobic Ile38:Cys7 Asp44:Val6 Val48:Val6 Gly49:Pro4 Ile53:Cys1 Arg55:Ace0	Hydrogen Bond Ala43:Cys7 Hydrophobic Bond Ile38:Cys7 Ala43:Cys7 Val48:Pro4 Gly49:Pro4 Gly49:Thr63 Ile52:Cys1 Ile53:Cys1 Thr63:Thr3

3.1. Total Potential Energy Analysis

The total potential energy includes bonding and nonbonding energies because the potential energy function is expressed in vibration, bending, torsion, van der walls, and electrostatics [19]. The total potential energy is formulated as follows: $E_{total} = E_{bonding} + E_{non\ bonding}$ (1)

$$E_{total} = E_{stretching} + E_{bending} + E_{torsion} + E_{vdw} + E_{electrostatics} \quad (2)$$

$$E_{total} = \frac{1}{2}k\,(R\text{-}Ro)^2 + \frac{1}{2}k(Ø\text{-}Øo)^2 + \frac{1}{2}k(Ø\text{-}Øo)^2 + \left(\frac{o}{r}\right)^{12} - \left(\frac{o}{r}\right)^{6} + \frac{q_1 q_2}{4\epsilon\pi_0 . r} \quad (3)$$

The total potential energy can be used to study conformational changes during simulation, the lower potential energy means that the complex has higher conformational stability [20].

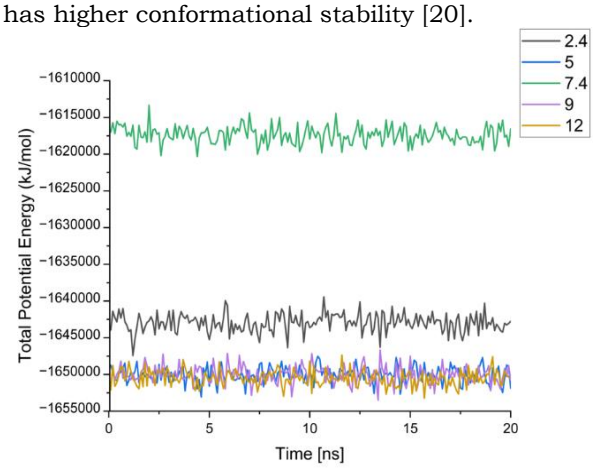

Figure 4. E-Cadherin…ADTC5 Total Potential Energy at Given pH

Table 2. Average Total Potential Energy of the E-Cadherin-ADTC5 complex with pH variations

pH	Average Total Potential Energy (kJ/mol)
2.4	-1,643,863.248
5	-1,651,128.007
7.4	-1,619,386.621
9	-1,650,943.917
12	-1,651,534.752

From the Table 1, pH 12 has the highest average total potential energy (-1.651.534,752 kJ/mol) and pH 7.4 has the lowest average total potential energy (-1.619.386,621 kJ/mol). It showed that at pH 12 the E-Cadherin…ADTC5 complex is unstable, because it undergoes significant conformational changes during simulation.

3.2. RMSD Analysis

Root Mean Square Derivatives (RMSD) is the most common quantitative measure that was used to measure the difference between protein backbones from its initial structure conformation to its final position during MD simulation [18].

$$RMSD = \sqrt{\frac{\sum_{i=1}^{N} m_1 (r_i - r_{ref})^2}{\sum_{i=1}^{N} m_i}} \quad (4)$$

Where mi is the atomic mass of i, r_i is the coordinate of atom i at a certain distance and r_{ref} is the coordinate of
atomic i at a standard distance. The RMSD graph of E-Cadherin…ADTC5 at given pH were illustrated below.

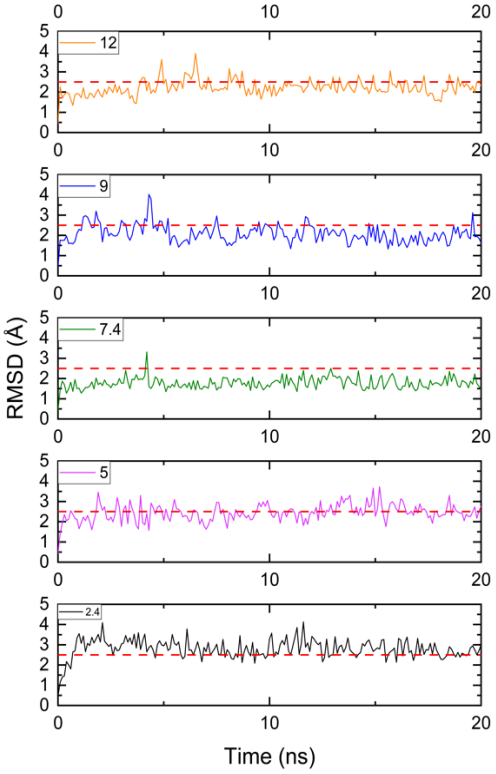

Figure 5. E-Cadherin…ADTC5 RMSD Ca at Given pH

The deviations generated during the Molecular Dynamic Simulation can be used to evaluate the stability of E-Cadherin…ADTC5. The lower the deviation occurred means that the protein initial backbone configuration is minor or the complex has high stability [18]. At pH 2.4, E-Cadherin…ADTC5 indicate deviations at 0 – 2.1 ns (0.466 – 4.093 Å) and start to increase again at 9.9 – 11.6 ns (2.472– 4.131 Å). At pH 5, E-Cadherin…ADTC5 has several high deviations point at 1.9 ns (3.45 Å), 5.5 ns (3.57 Å), 7.8 ns (3.29 Å), 15 ns (3.19 Å) and 19.9 ns (3.6 Å). At pH 7.4, the highest deviation value occurred at 4.2 ns (3.315 Å) and tend to stabilize afterwards. At pH 9, the increase of deviations occurred at 0.1 – 4.3 ns (1.75 – 4.02 Å), also has other high deviation point at 7.5 ns (2.96 Å), 11.7 ns (2.93 Å) and 19.6 ns (3.12 Å). At pH 12, E-Cadherin…ADTC5 has stabile deviation at 0.1 – 3 ns (2.27 – 2.2 Å) and increase in deviation occurred from 3.6 – 6.5 ns (1.43 – 3.9 Å).

The average RMSD value of E-Cadherin…ADTC5 at given pH 2.4, 5, 7.4, 9, 12 obtained are 2.8 Å, 2.436 Å, 1.773 Å, 2.08 Å, 2.21 Å. The highest average RMSD values are 2.8 Å, 2.436 Å, which indicate that at pH 2.4 and 5 the

stability of E-Cadherin…ADTC5 are low and has high mobility. The lowest average RMSD value is 1.773 Å at pH 7.4, which showed that E-Cadherin…ADTC5 has high stability and low mobility [21].

3.3. RSMF Analysis

Root Mean Square Derivatives (RMSF) is a measurement of a specific atoms or group of atoms displacement relative to the reference structure, averaged over the number of atoms [22]. The RMSF calculation is used to determine the residue's flexibility or how significantly a specific residue fluctuates during the simulation [23].

$$\text{RMSF} = \sqrt{\frac{1}{N_s}\sum_{i=1}^{N_s}\|\vec{r}_{ik}-\langle\vec{r}\rangle_k\|^2} \quad (5)$$

Where $\vec{r}_{ik}$ is vector position i against k and $\langle\vec{r}\rangle_k$ is the average position of the Atom K through the NS structure. The RMSF Figure of E-Cadherin…ADTC5 complex at given pH can be seen below.

Figure 5. illustrate that at pH 2.4 experienced high fluctuation on E-Cadherin residue Asn84, Glu31, Arg70 with RMSF 5.58, 5.18, 4.6 Å. The comparison of highest residue fluctuations of all pH is listed in the table below. High fluctuation level indicate that residues have high mobility [23]. From the RMSF fluctuation value protein we can conclude that pH 2.4 is the most unstable because the residues have lot of high fluctuations.

Comparison of Residual RMSF values bound to ADTC5 ligand for pH variation is essential because it is in the ligand binding region, hence the interaction at these residues will have significant effect on the conformational changes. At pH 5, 7.4, 9 Arg55 showed high fluctuations than the other residues with RMSF values of 4.34, 4.11, 4.04 Å. In the other hand, at pH 2.4 and 12 Val48 has the highest fluctuations with RMSF values of 2.56, 2.81 Å.

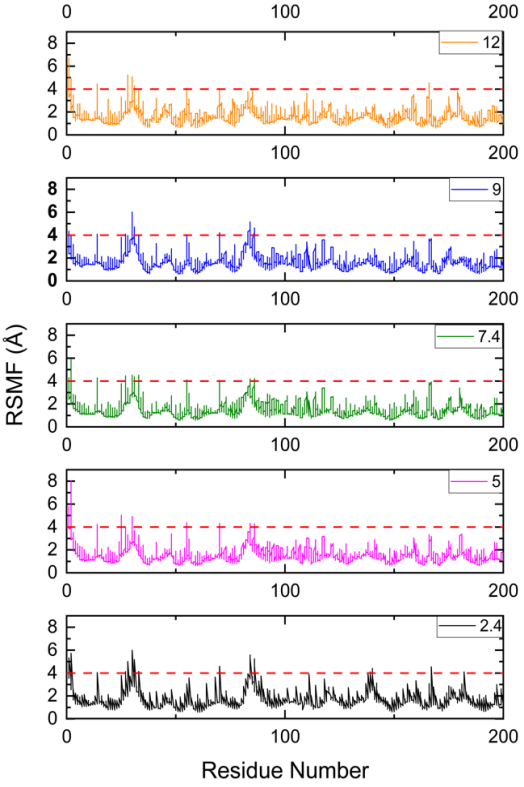

Figure 6. Detailed Analysis of RMSF per Plotted Residue Versus Residue Numbers of The E-Cadherin-ADTC5 Complex at 2.4, 5, 7.4, 9, 12

Table 3 Highest RMSF Fluctuation Value Protein

pH	Residue												
	Asp1	**Trp2**	**Lys14**	**Lys25**	**Asn27**	**Lys30**	**Glu31**	**Lys33**	**Arg55**	**Arg70**	**Asn84**	**Glu111**	**Arg167**
2.4	5.33	5.74	4.05	3.79	4.13	6	**5.18**	4.15	3.07	**4.6**	**5.58**	**3.91**	**4.54**
5	5.79	**8.05**	4.24	**5.04**	4.01	4.92	3.71	3.29	4.38	4.32	4.33	3.06	3.32
7.4	4.34	5.88	4.28	3.78	**4.47**	4.52	4.38	**4.52**	**4.11**	4.07	4.19	3.07	3.92
9	4.36	6.02	3.54	3.82	4.09	**6.02**	4.72	3.92	4.04	4.22	5.16	3.41	3.69
12	**6.73**	4.83	**4.47**	3.32	2.94	5.08	4.29	3.89	4.10	4.03	3.78	3.62	4.54

Table 4. Comparison of Residual RMSF values bound to ADTC5 ligand

pH	Residue – Ligand Interactions	RMSF (A)
2.4	Ala 43	1,38
	Gly49	1,23
	Ile 38	2,31
	Val 48	**2,56**
	Ile 52	1,92
	Ile 53	1,40
	Glu 64	3,10
5	Ile 53	1,37
	Ser 37	1,25
	Ile 38	1,33
	Ile 53	1,37
	Arg 55	**4,38**
7.4	Ile53	1,42
	Ala43	1,51
	Val48	2,73
	Gly49	1,26
	Ile52	2,06
	Arg55	**4,11**
9	Ile 53	1,37
	Arg 55	**4,04**
	Ile 38	1,28
	Asp 44	1,9
12	Ala 43	1,57
	Ile 52	1,63
	Ile 53	1,37
	Ile 38	1,28
	Val 48	**2,81**
	Gly 49	1,65
	Thr 63	1,67

3.4. Radius Gyration Analysis

The radius of gyration (Rg) is the mass-weighted root mean square distance between two atom clusters. In other words, the radius of gyration of a protein is a measure of its compactness [23]. Radius Gyration formula [24]:

$$Rg=\sqrt{\frac{\sum_{i=1}^{N} m_i \, r_i^2}{\sum_{i}^{N} m_i}} \qquad (6)$$

where mi is atomic mass i, ri is atomic distance i from the center of mass of protein.

Table 5. Average and Maximum radius of gyration complex with pH variations.

pH	Radius of Gyration (Å)	
	Average	**Maximum**
2.4	**28,264**	**28,986**
5	**28,502**	**29,02**
7.4	**28,434**	**28,906**
9	**28,667**	**29,106**
12	**28,594**	**29,103**

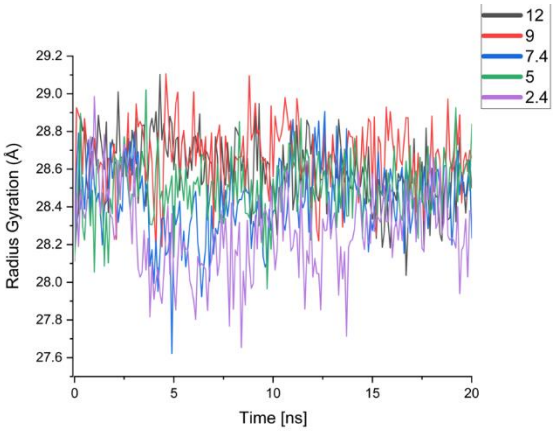

Figure 7. E-Cadherin…ADTC5 Radius Of Gyration Fluctuations at Given pH

Figure 6 illustrate Radius Gyration at each given pH from 0ns – 20ns. If a protein is stably folded, its Rg value will remain constant, but it will fluctuate if the protein unfolds [23]. From the maximum Radius Gyration value, we can conclude that at pH 9 has the highest Radius Gyration values (29.106 Å), it indicates that at this time the structure unfolds and became unstable.

3.5. MM/PBSA Analysis

The binding free energy is used to calculate the strength of the receptor-ligand interaction. Instead of using numerous MD snapshots, the binding free energy can be estimated using MM/PBSA using a single minimized structure [25]. The following formula is used to compute binding free energy:

Where ΔG_{PB} and ΔG_{SA} are the polar and non-polar solvation energies, and ΔG_{MM} is the molecular mechanics interaction (the sum of electrostatic and van der wall interactions). $T_{\Delta S}$ is the entropy contribution, but because to the poor forecast accuracy, the entropy contribution is not taken into account in this calculation [26] [15].

Binding Free Energy of E-Cadherin…ADTC5 Complex and ΔG Bind at given pH was illustrated in the Figure and Table above. As shown in the Figure and Table, we can see that at pH 2.4 E-Cadherin…ADTC5 has high Binding Free energy and hence required lot of energy for the interaction to occur. The more negative binding

free energy value means that protein-ligand binding will occur spontaneously and has better binding [15]. The most negative binding free energy can be spotted at pH 7.4

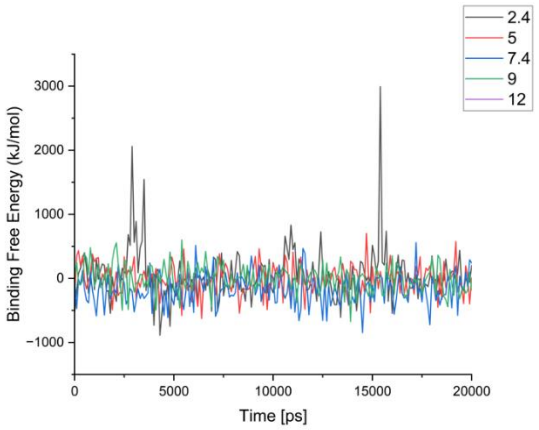

Figure 8. Binding Free Energy Fluctuation During The Simulation

Table 6. The value of binding free energy of the E-Cadherin...ADTC5 complex with pH variations using the MM-PBSA method

pH	ΔG Bind (kJ/mol)
2.4	**51,802**
5	**-12,483**
7.4	**-168.244**
9	**15,337**
12	**-22,636**

4. Conclusion

Based on the data obtained, pH has significant effect on the conformational changes during MD simulations that affect the stability of E-Cadherin...ADTC5 interaction. At pH 2.4, based on RMSD value the interaction between E-Cadherin...ADTC5 stabilized at 6.8 ns – 9.3 ns (2.162 – 2.093 Å). Also, pH 2.4 is the weakest conformational stability indicated by the high average RMSD value of 2.8 Å. RMSF also showed that pH 2.4 has the weakest stability, indicated by high fluctuation points occurred and has the highest amount of residues fluctuation. The positive ΔG Binding values of pH 2.4 indicated that the reaction is not spontaneous and required a lot of energy.

In the other hand, the best conformation of E-Cadherin...ADTC5 occurred at pH 7.4, indicated by the lowest Maximum value Radius Gyration at 28.906 Å, it also can be seen from most negative ΔG Binding with value -168.244 kJ/mol and lowest total potential energy at -1,619,386.621 kJ/mol.

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