

Molecular Docking and Molecular Dynamics Studies of L-Glycyl-L-Glutamic Acid Dipeptide

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Abstract: The Gly-Glu (GE) dipeptide, which acts as a neurotransmitter, is made of glycine and glutamic amino acids that are used in the treatment of neurological diseases such as Schizophrenia, Parkinson and Alzheimer. Gly-Glu dipeptide is an important peptide structure that helps prevent neuronal degeneration, especially in Alzheimer's disease. Caspases which are cysteine proteases play a crucial role for apoptotic death of neurons in Alzheimer's disease. In patients with Alzheimer's disease, there was an increase in caspase-3 immunoreactivity in the death of pyramidal neurons, where the initial sites of neuronal loss were proven (Gervais et al. 1999). The molecular structure of the GE dipeptide having anti-apoptotic properties is very important for clarifying the activation mechanism with caspase-3 protein. Molecular dynamics and molecular docking calculations were applied to elucidate the most stable molecular conformation and to grasp the mechanism of activity of GE with caspase-3. Firstly, GROMACS program was used to reveal the conformation variations of the GE within the body. The stability of the peptide is ensured by confinement in 704 water molecules. Secondly, Glide SP (standard precision) module of the Maestro 11.4 version in the Schrodinger Software program was used to determine the linkages and activity of the peptide with the caspase-3 protein. In this study, the structure of this dipeptide, pharmacological properties and its mechanism of action with caspase-3 protein were investigated for the first time by molecular dynamics and docking calculations.

Keywords: Gly-Glu, dipeptide, Molecular Dynamics, Molecular Docking, ADME

1. Introduction

Alzheimer's disease (AD) is one of the neurodegenerative diseases associated with dementia as a clinical symptom. The disease is caused by the accumulation of amyloid β -peptide ($A\beta$) which consists of 39 to 43 amino acids in the brain. $A\beta$ causes neuronal degeneration but its neurotoxic effect is still unclear. $A\beta$ has been shown to induce apoptosis in the brain was proven by various in vivo and in vitro observations. In recent years, the studies based on the determination of compounds that can protect neurons are of paramount importance to eliminate degenerative effects caused by $A\beta$. For this reason, various proteins and peptides structures have been investigated and Gly-Glu (GE) dipeptide is one of

them (Ioudina et al. 2003). Gly-Glu is an important dipeptide that causes neuronal degeneration prevention by starting with accumulation of $A\beta$ and preventing its activation with Caspase 3. $A\beta$ accumulation causes changes in the structure of two proteins associated with apoptosis such as p53 and Caspase-3 (Brecht et al. 2001). In this study, for the first time, the molecular structure of GE was determined by molecular dynamics and the effect of GE on the changes induced by $A\beta$ to caspase-3 was investigated by molecular docking calculation method.

The activity of a dipeptide also varies according to the mechanism of action of the amino acids in its structure. Glycine amino acid which is the simplest form, has cell protection, immunomodulatory,

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cytoprotective agent and anti-inflammatory properties. It has a role as a neurotransmitter in the central nervous system and contains an antioxidant property (Zhong et al. 2003; Aprison et al. 1965). It is also used in the treatment of hepatic coma and anticancer drugs studies (Brandon et al. 1983; Li 2002; McDermott et al. 1955; Shadidi 1997) and required for synthesis of neurotransmitters such as GABA (Manto et al. 2007). Glutamic acid has also neuroprotective and neurotrophic effects (Koelle et al. 1985; Ioudina et al. 2003).

The aim of computer-aided drug design (CADD) is to help to research and discovery of drug candidate by reducing cost of drug design process. Technological advances in this field is effective in providing faster optimization and identification steps in silico methods (Taft and Da Silva, 2008). Structure based computational modeling of ligand-receptor interactions is a prominent part of modern drug discovery. Molecular dynamic and molecular docking calculations are often preferred methods that used in the structure-based drug design (SBDD) studies to determine conformational change that varies according to the environment and to define the interaction of the molecule with the protein in which the molecule interacts within the body, respectively. Molecular docking study is also able to predict the binding conformations and free energies of ligands that is drug candidate molecules within the appropriate target binding site (Ferreira et al. 2015). To define absorption, distribution, metabolism and excretion (ADME) properties in drug development is very important for the success rate of drug candidate compounds (Butina et al. 2002). In this work, we present molecular dynamics, molecular docking and ADME studies for Gly-Glu dipeptide for the first time by using GROMACS and Schrodinger Maestro software programs.

2. Material and Method

2.1. Molecular dynamic simulation

Initially, the 3D structure of Gly-Glu was taken from Gaussian 09 (Frisch et al. 2009) software where optimization was performed at DFT/B3LYP level of theory with the 6-31++G(d,p) basis set. GROMACS 5.2 simulation software package (Van der Spoel et al. 2005) with the SPC (simple point charge) water model (Smith & Van Gunsteren 1993) was used for MD calculations. Firstly, the Gly-Glu was put into a box that was filled with water molecules, and Na⁺ and Cl⁻ ions were added

to the box to provide a neutral simulation. After the system was neutralized, energy minimization was actualized using the steepest-descent algorithm. The energy of the system was minimized with a 2000-step.

50 ps NVT simulation was carried out before 500 ps NPT simulation. For 25,000 steps with a 2 fs time step, NVT was performed and temperature coupling was ensured using the Berendsen method (Berendsen 1991). For 500 ps, (250,000 steps with a 2 fs time step) NPT calculation was implemented to fix the pressure isotropically to a value of 1.0 bar, the Parrinello-Rahman method (Parrinello & Rahman 1981) is also used. NVT and NPT simulations using Leapfrog algorithm have been performed at 310 K and 1 bar pressure for the balance of system. GROMOS 54a7 force field has been utilized for the simulations (Van Gunsteren et al. 1987). A 5 ns (5000 ps) MD simulation was performed with a 2 fs time step. The linear constraint solver (LINCS) algorithm (Hess et al. 1997) was carried out to all bonds containing hydrogen bonds. All graphics of molecular dynamic calculation of Gly-Glu were plotted using XMGRACE (Turner 2005).

2.2. Molecular docking and ADME properties

The purpose of molecular docking is to present a prediction of the ligand and receptor complex. A protein-ligand docking program consists of sampling and scoring components. Sampling refers to the generation of putative binding orientations of ligand near a binding site of a protein. Scoring is the prediction of the binding tightness for individual ligand orientations with a physical or empirical energy function (Huang & Zou 2010). For molecular docking study, ligprep, prepwizard, grid generation and docking calculations were executed by Schrodinger Maestro software using the Glide SP (standard precision) module.

Firstly, the protein used for the docking study was selected from PDB databank (PDB code: 1RHK) (Becker et al. 2004). For better results, we were obtained our protein structure by the SWISS-MODEL server (Bienert et al. 2016). Our ligand molecule structure was taken from the result of MD simulation. The preparation and calculations of molecular docking were done in Schrödinger Maestro software using the Glide SP (standard precision) module. (Schrödinger Release 2017-4: Maestro, Schrödinger, LLC, New York, NY, 2017) (Friesner et al. 2006; Halgren et al. 2004; Friesner

et al. 2004). Firstly, Gly-Glu ligand molecule was prepared for docking calculations by the LigPrep tool in the Maestro 11.4 version of the Schrödinger Software program using the OPLS3 force field (Harder et al. 2015). A maximum of 32 stereoisomers were produced for the ligand after the ionization states at $\text{pH } 7.0 \pm 2.0$ were selected. The structure of selected receptor Caspase-3 having a solubility of 2.5 \AA was prepared by the Protein Preparation Wizard tool (Sastry et al. 2013). The polar hydrogens were added to the heavy atoms and all water and ions in the structure were removed. The bond orders were assigned, charges were defined at $\text{pH } 7.0$ and the selected receptor was optimized using PROPKA (Søndergaard et al. 2011). The heavy atoms in the receptor were converged by preferring 0.3 \AA RMSD and the OPLS3 force field. The Grid box were defined to the receptor by centering the existing co-crystallized ligand using grid generation tool in Maestro 11.4 version. The ligand was docked into the receptor based on the grid using standard precision (SP) docking algorithm to rank the ligand with specific conformation of the receptor molecule (Venkatesan et al. 2018). Drug candidate molecules display favorable absorption, distribution, metabolism and excretion (ADME) parameters. The Qik-Prop module (Schrödinger Release 2017-4: QikProp, Schrödinger, LLC, New York, NY, 2017.) was used to determine the ADME profile of the drug candidate molecule.

3. Results and Discussion

3.1. Molecular dynamic simulation results

The Gly-Glu dipeptide was generated by using an ideal geometry into a cubic box of SPC (simple point charge) water molecules with 704 water molecules. Counter ions ($\text{Na}^+(2)$) and ($\text{Cl}^-(1)$) are added to neutralize system.

Although minimization was planned for 2000 steps, the energy minimization was realized using steepest descent algorithm for 224 ps with -3.5298168×10^4 kJ/mol potential energy, as shown Fig 1.

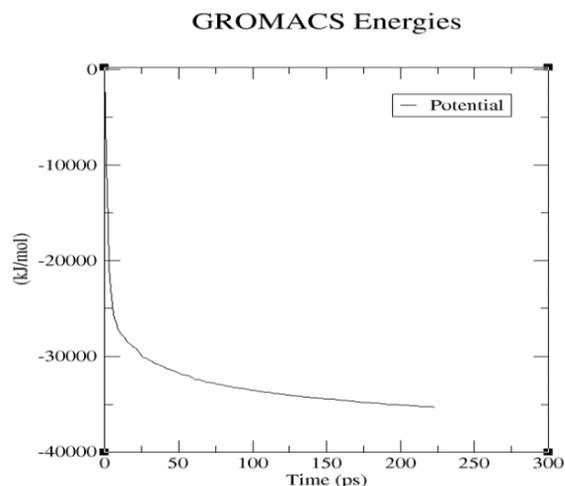


Figure 1. The potential energy of Gly-Glu dipeptide in aquatic box as a function of the minimization step using Steepest Descent algorithm.

After minimization step, the balance conditions for temperature and pressure for the system was provided with NVT and NPT ensembles using Leapfrog algorithm at 310 K and 1 bar. NVT was carried out for 25,000 steps with a 2 fs time step and temperature coupling was performed using the Berendsen method. NVT results are showed that system was well equilibrated around the target temperature at 310 K in Fig 2.

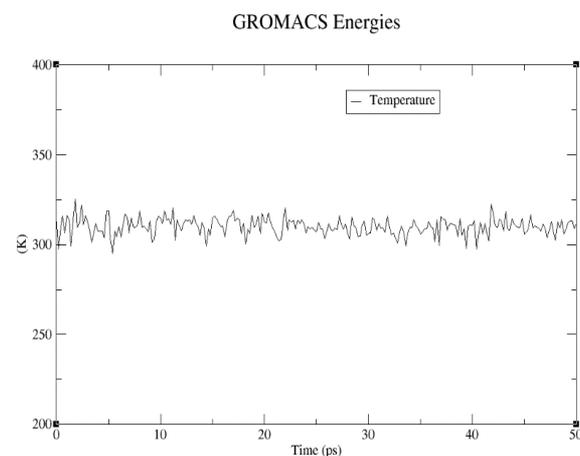


Figure 2. The equilibrated temperature of Gly-Glu dipeptide in aquatic box around 310 K.

NPT calculation was performed 250,000 steps with a 2 fs time step (a total of 500 ps). The Parrinello-Rahman method was also used to couple pressure isotropically to a value of 1.0 bar. The NPT Simulation reported a density of 982.219 kg/m³ as averaged over the last 500 ps of simulation in Fig 3.

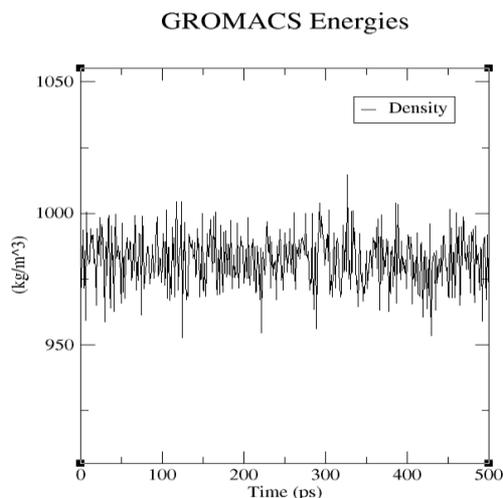


Figure 3. The density of Gly-Glu dipeptide in aquatic box.

MD simulations were performed for 2,500,000 MD steps with a 2 fs time step, totaling 5000 ps (or 5 ns). According to MD simulation results, total, kinetic and potential energy were obtained as - 2.57064x10⁴ kJ/mol, 5.49438x10³ kJ/mol and - 3.12008x10⁴ kJ/mol, respectively.

After MD simulation, Root Mean Square Deviation (RMSD) and Radius of Gyration (Rg) were calculated. The range of RMSD was seen under 0.1 nm, when looking at the graph. Gyrate shows the compactness of each molecule. Rg value changed from 0.272485 to 0.291 for 5000 ps. The Root Mean Square Deviation (RMSD) and Gyration (Rg) graphics were shown in Fig 4.

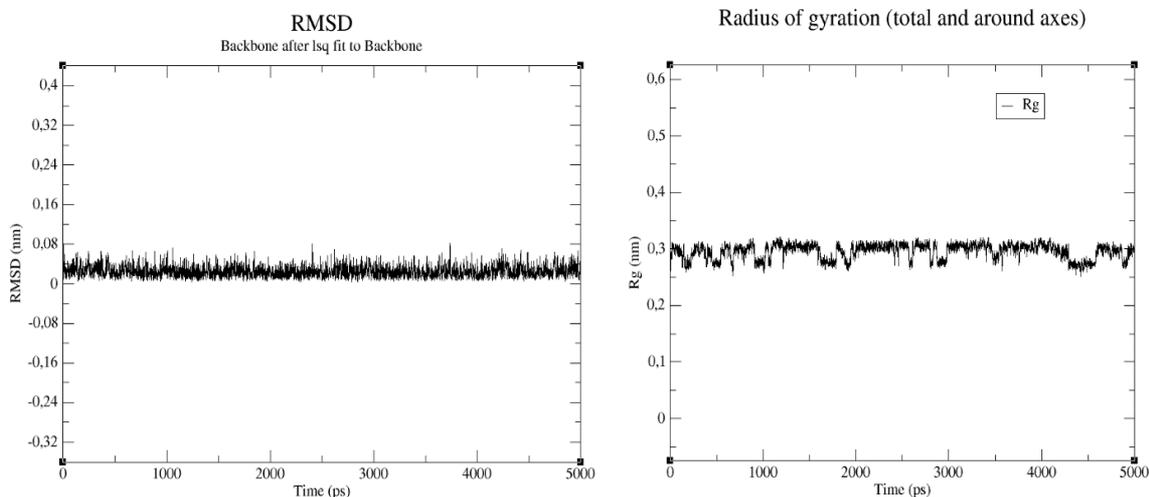


Figure 4. The RMSD values and radius of gyration of Gly-Glu dipeptide in aquatic box

3.2. Molecular docking results

Molecular interaction and docking studies are very important for computer aided drug design. The

docking score energy which generated by the binding of the Gly-Glu to the Caspase-3 with 147 sequence length, was obtained as -5.374 kcal/mol (Table-1).

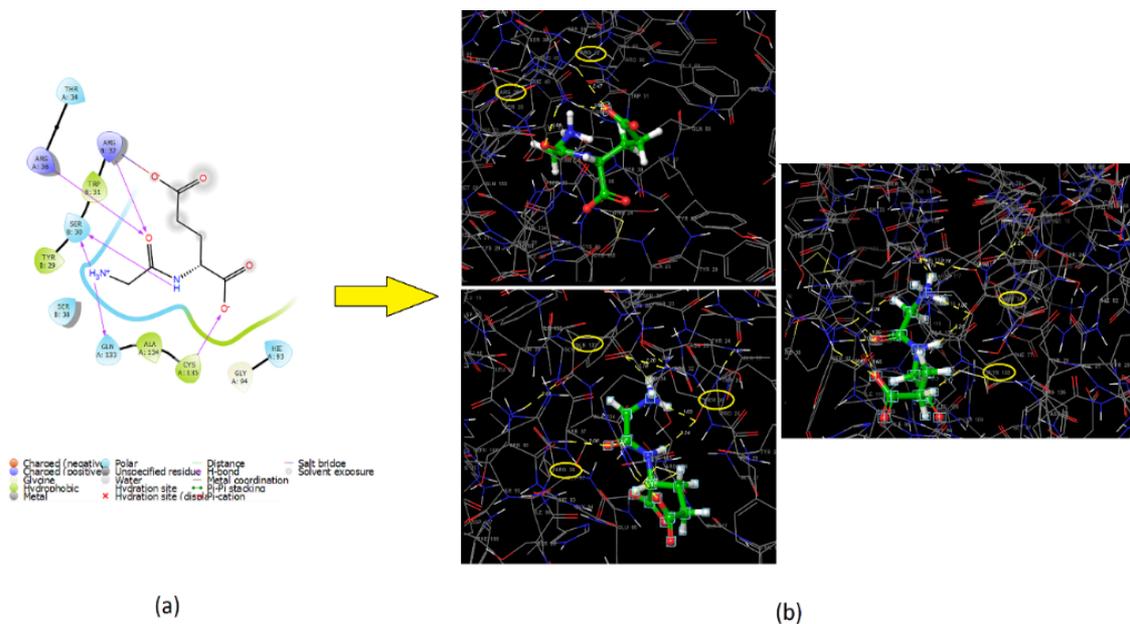


Figure 5. The 2D (a) and 3D (b) diagrams of the Gly-Glu interactions in the active side of the Caspase-3

Table 1. The conformation and docking score energies of Gly-Glu dipeptide

| Ligand | Energies of The Ligand (kcal/mol) | Docking Score (kcal/mol) |
|--------|-----------------------------------|--------------------------|
| 1 | 15.727 | -5.374 |
| 2 | 16.492 | -4.866 |
| 3 | 20.774 | -4.370 |
| 4 | 20.849 | -4.322 |

In the active region of protein, hydrophobic (green), polar (blue) and positive charged (dark blue) residues were located. The dipeptide, which binds in a stable conformation with the amino acids located in the active region of the Caspase-3, was attached to the protein by hydrogen bonds; ARG36 (2.06Å), SER30 (1.65Å and 2.24Å), ARG32 (1.65Å, 1.95Å and 2.47Å), GLN133 (1.78Å) and CYS135 (2.17Å) residues (Fig-5).

The O atom in the peptide group of Gly-Glu dipeptide makes H-bonds with positive charged amino acids ARG32 and ARG36, while the amino group (NH₃⁺) of the dipeptide creates H-bonds with polar amino acids SER30 and GLN133. The O atom in the carboxyl group of dipeptide was attached to hydrophobic amino acid CYS135 with H-bond as shown in Fig-5(b).

Additionally, a salt bridge between the O atom in the side chain of the glutamic acid and ARG32 in the caspase-3 was shown, in Fig. 5(a) with red and blue line. Salt bridges in the protein structures are created by close residues that are opposite charged to try electrostatic attraction. (Bosshard et al. 2004). The electrostatic potential map surfaces of the ligand and receptor protein were also constituted to define the regions that were electron-rich and electron-poor (Fig-6).

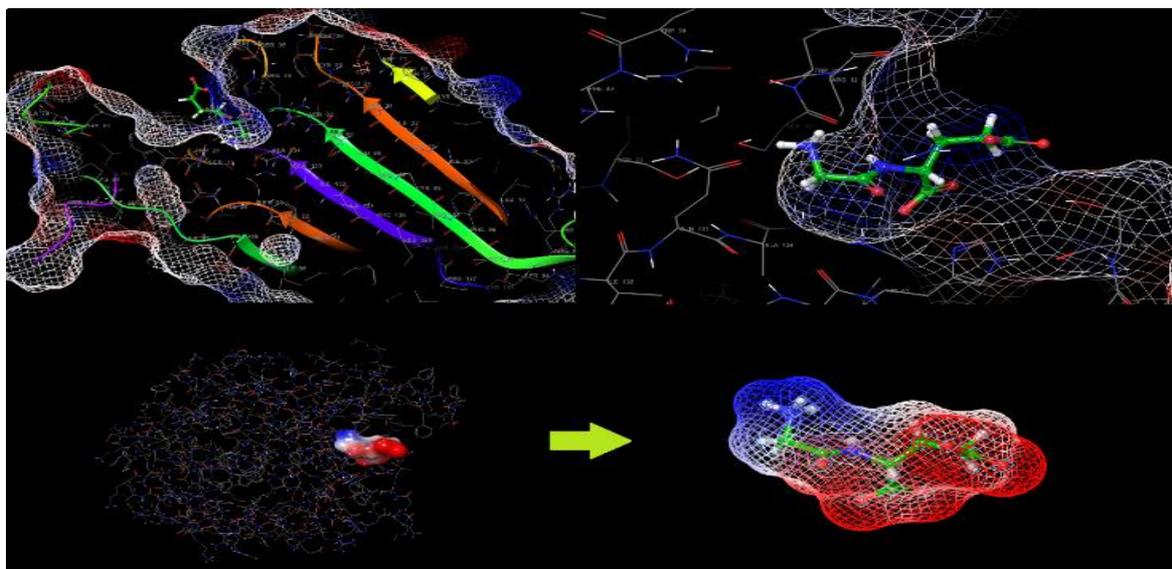


Figure 6. The electrostatic potentials of Gly-Glu and Caspase-3.

The surfaces are shown with red as the lowest electrostatic potential energy value (electrophilic region) and dark blue as the highest (nucleophilic region). White shows a potential halfway between the two extremes (red/dark blue).

Favorable absorption, distribution, metabolism and excretion (ADME) profile obtained using Qikprop tool of the Maestro software package for drug candidate molecule were tabulated in Table-2. QikProp provide a prediction that important descriptors required for the drug like properties of molecules.

Table 2. Docking score and calculated ADME properties

| Property | Value | Recommended (Schrödinger Release 2017-4: Maestro, Schrödinger) |
|--|---------|---|
| Docking score (kcal/mol) | -5.374 | |
| Polar surface area | 159.549 | 7.0 / 200.0 |
| PSA (Å ²) | | |
| Molecular weight | 204.182 | 130.0 / 725.0 |
| MW (g/mol) | | |
| Solute as Donor-Hydrogen Bonds | 4.250 | (0.0/ 6.0) |
| Solute as Acceptor-Hydrogen Bonds | 6.750 | (2.0/ 20.0) |
| Solute Ionization Potential (eV) | 9.772 | (7.9/ 10.5) |
| Solute Electron Affinity (eV) | -0.287 | (-0.9/ 1.7) |
| Polarizability (Angstroms ³) | 15.364M | (13.0 / 70.0) |
| QP log P for hexadecane/gas | 7.460M | (4.0 / 18.0) |
| QP log P for octanol/gas | 15.691 | (8.0 / 35.0) |
| QP log P for water/gas | 16.547 | (4.0 / 45.0) |
| QP log P for octanol/water | -3.461 | (-2.0 / 6.5) |
| QP log S for aqueous solubility | 0.863 | (-6.5 / 0.5) |
| QP log S - conformation independent | 0.614 | (-6.5 / 0.5) |

| | | |
|--|--------|----------------------------------|
| QP log K hsa Serum Protein Binding | -1.500 | (-1.5 / 1.5) |
| QP log BB for brain/blood | -1.747 | (-3.0 / 1.2) |
| No. of Primary Metabolites | 5 | (1.0 / 8.0) |
| Predicted CNS Activity (-- to ++) | -- | |
| HERG K+ Channel Blockage: log IC50 | 1.402 | (concern below -5) |
| Apparent Caco-2 Permeability (nm/sec) | 0 | (<25 poor. >500 great) |
| Apparent MDCK Permeability (nm/sec) | 0 | (<25 poor. >500 great) |
| QP log Kp for skin permeability | -8.237 | (Kp in cm/hr) |
| Jm. max transdermal transport rate | 0.009 | (micrograms/cm ² -hr) |
| Lipinski Rule of 5 Violations | 0 | (maximum is 4) |
| Jorgensen Rule of 3 Violations | 1 | (maximum is 3) |
| % Human Oral Absorption in GI (+- 20%) | 0 | (<25% is poor) |

Molecular weight (mol_MW), octanol-water partition coefficient (logPo/w), hydrogen bond donors and acceptors are important for the molecule to show its drug properties. These four properties are based on Lipinski 5 rules.

Lipinski's five rules are used to determine whether a molecule with a specific pharmacological or biological activity can be used as an active drug (Lipinski et al. 1997; Lipinski 2004). Molecular weight (mol_MW), octanol-water partition coefficient (logPo/w), acceptor hydrogen bonds (acptHB) and donor hydrogen bonds (donorHB) were obtained as 204.182 g/mol, -3.461, 6.750 (standard limits from 2.0 to 20.0), and 4.250 (standard limits from 0.0 to 6.0) using Qikprop tool, respectively. The ability of the drug to pass through the blood-brain barrier was given with QPlogBB parameter, QPlogBB (Brain/blood partition coefficient) parameter was obtained as -1.747 (standard limits from -3.0 to 1.2) for GE dipeptide. On the other hand, Human oral absorption was calculated poor property, as a result of work with Qikprop tool.

Additionally; Human serum albumin (HSA) plays an important role as it is effective for passive permeability and blood-brain barrier. Interactions of HSA and small molecules affect the ADME properties which calculated for small molecules (Benet et al. 1996; Lexa et al. 2014). The standard limit of QP log K hsa Serum Protein Binding value is between -1.5 and 1.5. This value was determined as -1.5. for GE dipeptide. The QP log Kp for skin permeability parameter is also significant for drugs

administered through the skin. Computed skin permeability value was -8.237.

Moreover, solute electron affinity, solute ionization potential (IP(eV)), and solubility (QPlogS) were also taken from the result of Qikprop analysis as -0.287 eV (standard limits from -0.9 to 1.7), 9.772 eV (standard limits from 7.9 to 10.5) and 0.863, respectively.

4. Conclusions

Gly-Glu dipeptide is a dipeptide that helps prevent neuronal degeneration, especially in Alzheimer's disease. CADD technology is important for novel and effective drug development studies for the Alzheimer's disease (Ece 2019). Firstly, we investigated the conformational variation of GE which has anti-apoptotic effect by using molecular dynamics calculations. Conformational changes of the peptide in an environment close to the body environment were examined and the most stable geometric structure was determined. Then, the interaction between GE dipeptide and caspase 3 was explained by the method of molecular docking. The GE dipeptide was linked to the active site of caspase 3 and revealed the locations and elongations of the hydrogen bonds that provided stable binding. According to molecular docking results, the docking score was determined as -5.374 kcal/mol. To predict the drug ability of GE dipeptide, ADME calculations were performed. The substantial pharmaceutical properties of GE were presented by Qikprop tool. Besides, Lipinski's five rule was used to evaluate druglikeness of GE dipeptide, which

would make it a likely active drug for Alzheimer's disease.

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