RESEARCH ARTICLE

Preparation, antioxidant activity, inhibitory activity, and mechanism of action of pea tetrapeptide on dipeptidyl peptidase-4: Kinetic analysis and molecular docking

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Inhibition of dipeptidyl peptidase-4 activity by food-borne bioactive peptides is an effective treatment for type 2 diabetes and its complications. Pea tetrapeptide is a bioactive peptide, but there is no research on the inhibitory ability and mechanism of pea tetrapeptide on dipeptidyl peptidase-4. This research investigated the antioxidant capacity of pea tetrapeptide and its inhibitory ability and mechanism on dipeptidyl peptidase-4. The pea tetrapeptide was purified by the method of enzymatic hydrolysis to determine its antioxidant capacity. The mechanism of its antioxidant properties was explored by measuring its amino acid sequence. The conformational changes and bonding conditions of the composite system of dipeptidyl peptidase-4 and pea tetrapeptide were studied by using molecular docking technology. The results showed that pea tetrapeptide had good antioxidant properties and exhibited good inhibitory ability against dipeptidyl peptidase-4. It was a non-competitive inhibitor. The molecular docking results showed that there was no significant difference in energy change and convergence parameters, indicating that pea tetrapeptide and dipeptidyl peptidase-4 formed a stable system. Pea tetrapeptide bound to the inactive sites of dipeptidyl peptidase-4 enzyme to form three hydrogen bonds and eight hydrophobic bonds, which affected the enzymatic hydrolysis ability of dipeptidyl peptidase-4 enzyme and indirectly regulated blood sugar. This research provided some supports for pea tetrapeptide in the treatment of type 2 diabetes.

Keywords: pea tetrapeptide; antioxidant; antidiabetic; dipeptidyl peptidase-4; inhibitor; docking modeling.

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Introduction

Pea is an edible legume renowned for its high nutritional value, impressive yield, and efficient protein content. Consequently, pea protein serves as a vital source of high-quality plant-based proteins in the human diet [1]. Pea tetrapeptide (VEPQ) is the product derived from the proteolysis of pea protein. Unlike the complex enzymatic hydrolysis and digestion process that proteins undergo in the body, VEPQ can be directly absorbed and utilized by the intestinal mucosa. Moreover, its absorption rate surpasses that of amino acids [2]. This characteristic enables VEPQ to better preserve its structure and exhibits unique functions that are either absent or not as pronounced in the whole protein. In addition to these benefits, VEPQ

possesses favorable biological properties. It acts as an antioxidant and demonstrates emulsifying capabilities [3]. Furthermore, it can function as an intestinal nutrient to stimulate the growth of probiotics. Given these attributes, VEPQ shows great potential for use as a bioactive peptide in both the food and pharmaceutical industries.

Currently, several studies have demonstrated that the complications associated with diabetes are linked to an elevation in the levels of free radicals within the body along with the malfunctioning of the antioxidant defense system. These factors collectively give rise to pronounced oxidative stress phenomena [4]. In the context of Type 2 Diabetes Mellitus (T2DM) that is characterized by abnormal glucose metabolism and metabolic disturbances [5], most patients adopt a combined approach of pharmacological and dietary interventions to regulate their blood glucose levels [6]. Given this scenario, the quest for natural antioxidants assumes significant importance as they hold the potential to impede the pathways of oxidative stress induced injury, thereby offering promising avenues in the prevention and management of diabetes. Dipeptidyl peptidase-4 (DPP4) is a serine protease on the cells surface, which can rapidly hydrolyze Glucagon-like Peptide-1 (GLP-1) and subsequently influence the synthesis and distribution of glucagon. Numerous studies have demonstrated that inhibiting DPP4 activity represents an effective therapeutic approach for managing T2DM and its associated complications [7]. Many existing studies indicated that bioactive peptides derived from food sources exhibited superior DPP4 inhibitory activity. Luo *et al* indicated that purple *Prunus cerasifera* Ehrh contained peptides with potent DPP4 inhibitory effects [8]. Qi and Tian studied the inhibitory effect of asparagus aqueous extract on DPP4 and proved that asparagus aqueous extract had a good inhibitory effect on DPP4 [9]. Wang *et al*. demonstrated that peptides derived from Ginkgo biloba seeds exhibited the ability to effectively inhibit DPP4 activity [10]. These findings suggest that many kinds of food have DPP4 inhibitory food-borne peptides. However, there are a paucity of reports concerning the mechanism of DPP4 enzyme inhibition. Li *et al*. employed molecular docking technology to study the docking between DPP4 enzyme and five inhibitors [11]. This molecular docking approach offers a novel perspective and serves as a valuable source of inspiration for the present study. Further, Wang *et al*. provided evidence that pea oligopeptides could retain robust antioxidant activity after digestion with pepsin and trypsin [12]. Cui *et al*. also found that pea protein exerted a beneficial influence on the formation of sustained-release insulin [13]. However, the mechanism of intervention in diabetes and the inhibitory potential to DPP4 remain underexplored.

The present study investigated the *in vitro* DPP4 inhibitory activity and antioxidant activity capacity of VEPQ obtained through enzymatic hydrolysis. The docking modeling and molecular dynamics (MD) simulation techniques were employed to delve into and elucidate the molecular mechanisms underlying the interaction between VEPQ and DPP4, which included analyzing the type of binding, the energy of binding, and the conformational changes of the DPP4-VEPQ complex in an aqueous environment. The results of this study not only brought foodborne antioxidant pea polypeptide into the field of diabetes management but also offered novel theoretical insights to support the future applications of pea tetrapeptide in food and pharmaceutical industries.

Materials and methods

Preparation of VEPQ

The pea protein was obtained from Yantai Shuangta Food Co., Ltd. (Yantai, Shandong, China) and was treated following the methods of Li et al. with slight modifications [14]. The sample was mainly composed of water (4.60 ± 0.02%), protein (73.20 ± 0.23%), crude fat (0.22 ± 0.01%), and ash content (4.62 ± 0.03%). VEPQ was synthesized and purified by China Peptide Co.,

Ltd. (Shanghai, China) using solid-phase synthesis and Agilent 1100 high-performance liquid chromatography (Agilent Technologies, Inc., in Palo Alto, California, USA) with a reversed-phase C18 column. The obtained enzymatically hydrolyzed liquid was dialyzed and then subjected to rotary evaporation. The concentrated liquid was frozen at -40℃ before was dried by freeze-drying using a freeze dryer (Jutong Electronics Co., Ltd, Hangzhou, Zhejiang, China) to obtain the enzymatic hydrolyzed product of pea protein.

Amino acid sequencing of VEPQ

The sample was dissolved and filtered. The amino acid sequencing was performed by using Hitachi L-8900 amino acid analyzer (Hitachi, Tokyo, Japan) following the manufacturer's instructions. The N-terminus amino acid sequence of VEPQ was analyzed using PPSQ-33A Protein Sequencer System (Shimadzu, Kyoto, Japan) with Edman degradation method.

Determination of antioxidant capacity of VEPQ

The antioxidant activity of VEPQ consisted of the scavenging ability of VEPQ on ultra-oxygen anion, 2,2-diphenyl-1-picrylhydrazyl (DPPH), and •OH free radical, which were determined using the experimental methods of Gu *et al*. [15]. Briefly, the superoxide anion clearance rate was determined by mixing 0.3 mL of sample solutions of different concentrations with 5.0 mL of pH 8.2 Tris-HCl buffer solution and incubating at 37℃ for 20 minutes before adding 0.3 mL of 7.5 mmol/L resorcinol solution. The absorbance at 320 nm was measured every 30 seconds using a microplate reader (Thermo Fisher Scientific, Waltham, MA, USA). DPPH scavenging rate was determined by taking 1.0 mL of sample solutions of different concentrations mixed with 4.0 mL of 0.1 mmol/L DPPH solution (1:1 mixture of 95% ethanol and distilled water), respectively. After being reacted in the dark for 30 minutes, the absorbance at 517 nm was measured. The hydroxyl radical scavenging rate was measured by mixing 2.0 mL of sample solutions of different concentrations with 1.0 mL of 1.8 mmol/L ferroin solution, 2.0 mL of 0.02 mol/L phosphate buffer, and 1.0 mL of 1.8 mmol/L ferrous sulfide aqueous solution. After adding 1.0 mL of hydrogen peroxide solution (0.02%, V/V), the reaction mixture was placed at 37℃ for 60 minutes before measuring the absorbance at 536 nm.

Determination of DPP4 inhibitory activity and enzymatic kinetics of DPP4

The reactions were performed in 96 well plates by mixing 100 ng/mL of DPP4 isolated from the porcine kidney (Sigma-Aldrich, St. Louis, MO, USA), 500 μM Gly-Pro-pNA (Sigma-Aldrich, St. Louis, MO, USA), and increasing amounts of hydrolysates in a 100 mM pH 8.0 Tris reaction buffer and incubating at 37℃ for 1 h. The readings were taken at 415 nm with sitagliptin as a positive control of inhibition. In the reaction system, DPP4 enzyme concentration and inhibitor concentration remained unchanged, while the substrate concentration was changed to measure the absorbance value of the enzyme reaction and calculate the corresponding reaction speed. According to the Line weaver-Burk plot, the type of inhibition, the K_m of the Michaelis-Menten equation, and the maximum reaction rate were determined.

Docking analysis of DPP4 and VEPQ

The initial structure for the molecular dynamics (MD) simulation was derived from the molecular docking results between DPP4 and VEPQ in the crystal structure of 2 BGR DPP4. MD simulation was conducted using the Amber 16 software package (https://ambermd.org/) with the Amber 14 SB all-atomic force field [16]. The force field parameters of proteins were calibrated based on experimental data with the simulated temperature of 300 K, the pH of 7.0, and utilizing the TIP3P water model as the solvent. Before the MD simulation, the system was optimized by two energy optimization steps. Initially, the solute was constrained. Subsequently, the constraints were removed, and the entire system was optimized using a combination of the steepest descent and conjugate gradient methods over a total of 5,000 steps. Moreover, the MD simulation process was also carried out in two steps including 100 ps constrained solute MD

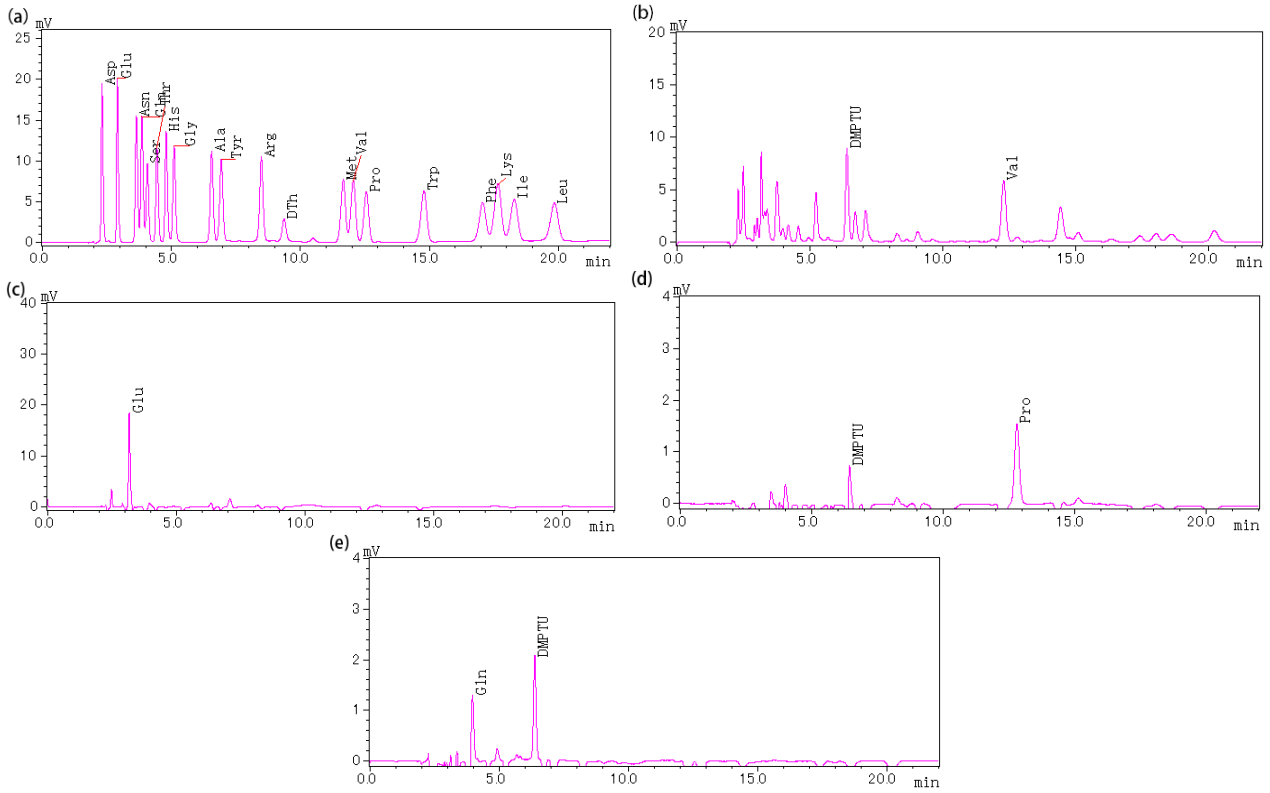

Figure 1. (**a**) Standard samples of 19 PTH amino acids. (**b**) The first amino acid sequencing of VEPQ. (**c**) The second amino acid sequencing of VEPQ. (**d**) The third amino acid sequencing of VEPQ. (**e**) The last amino acid sequencing of VEPQ.

simulation with the system temperature gradually increased from 0 K to 300 K and 50 ns unconstrained constant temperature MD simulation with the SHAKE algorithm to constrain the bond length and the integral step of two fs. The conformation was collected every 10 ps, and a total of 5,000 conformations were collected. The VMD software (https://www.ks.uiuc.edu/Research/vmd/) was employed to monitor the dynamic process of MD simulation.

Statistical analysis

All experiments were done in triplicate. SPSS version 19 (IBM, Armonk, New York, USA) was employed for statistical analysis. One-way analysis of variance (ANOVA) and Duncan's multiple range test were applied for group means comparisons. *P* value less than 0.05 was defined as statistically significant difference. Origin version 8.5 (OriginLab, Northampton, MA, USA) was used for graphic development.

Results and discussion

N-terminus sequencing of VEPQ

Protein synthesis initiates at the N-terminus, and the amino acid composition of this region exerts a profound influence on the protein's biological functions. The N-terminus sequence analysis helps understand the advanced structural features of VEPQ and uncover its biological roles. The phenylthiohydantoin (PTH) amino acid standard mixture was calibrated and tested to obtain the test atlas of the standard (Figure 1). VEPQ was then added to the liquid chromatography column to separate the N-terminus sequence of tetrapeptide amino acid Val-Glu-Pro-Gln, which laid the foundation for further analyzing the inhibition effect and binding mechanism of the DPP4-VEPQ composite system.

The capacity of antioxidant activity of VEPQ

The antioxidant activity of VEPQ was determined with Vc as a contrast. The •OH radical-scavenging capacity of VEPQ was the strongest one with IC_{50} as 12.81 mM, while the scavenging rate of $•O_2^-$ was relatively weak with IC_{50} as 30.43 mM (Figure 2). The scavenging ability of different free radicals was affected by many factors including the type, conformation, and sequence of amino acids of VEPQ.

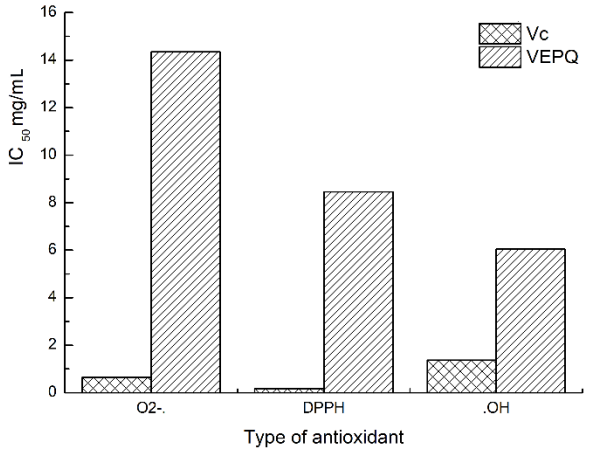

Figure 2. The antioxidant properties of VEPQ.

After comparing with the standard samples, the results showed that the N-terminus sequence of VEPQ was Val-Glu-Pro-Gln. Among them, Glu was a polar amino acid with a negative charge. Carboxylic acid radicals have a good ability to adsorb electrons and can improve the water solubility of tetrapeptide after binding to electrons, so that electrons could be easily removed. Kim *et al*. indicated that Pro residue had the nature of sub-amino acid, and prolyl polypeptide had good antioxidant activity owing to easy to oxidize and, as a hydrophobic amino acid, it could enhance the interaction between VEPQ and groups and improve the ability to capture •OH free radicals [17]. Thus, VEPQ showed an excellent •OH radical-scavenging capacity. The amino acid sequence of VEPQ imparted its acidic nature. When the sample concentration increased, the negatively charged acidic amino acid Glu in the solution gradually accumulated. Due to the mutual repulsion between negative charged electrons, the VEPQ molecules tended to embed within the sample matrix, hindering the close contact between DPPH and VEPQ, which, in turn, imposed constraints on the radical-scavenging capacity of DPPH. On the other hand, Chibuike and Rotimi demonstrated that the $•O_2^-$ scavenging ability was closely related to the amino acid composition [18]. Amino acids such as Lys and Leu were positively correlated with it, while sulfur-containing amino acids showed an inverse relationship. Furthermore, Suetsuna *et al*. found that the deletion of Tyr at the C-terminal reduced the quenching ability of superoxide anions, which suggested that the Glu-Leu structure played a pivota role in antioxidant activity [19]. In addition, the amino acid composition of VEPQ was relatively simple, and the singlet oxygen quenching ability was limited. As a result, VEPQ was unable to effectively combine with $•O_2^-$, which had a notable impact on its scavenging efficiency.

Analysis of DPP4 inhibition type

The DPP4 inhibitory activity of VEPQ exhibited a concentration-dependent enhancement. The IC_{50} value of Sitagliptin (STP) was determined to be 19 nM, whereas VEPQ demonstrated an IC_{50} of 3.43 mM. Additionally, based on the Lineweaver-Burk plot analysis, the apparent Michaelis-Menten constant (K_m) for VEPQ was calculated to be 1.156 mg/mL with a maximum reaction velocity (V_{max}) of 0.561 ΔA/min. After adding VEPQ, the K_m remained unchanged, and the maximum reaction rate decreased to 0.489 ΔA/min (Figure 3). Michaelis-Menten equation demonstrated that VEPQ functioned as a noncompetitive inhibitor, which was because DPP4 enzymes could specifically recognize the penultimate proline residue at the N-terminus of VEPQ, and VEPQ bound to DPP4 in the form of non-covalent bonds at inactive sites, while other molecules remained capable of participating in the catalytic reaction of the DPP4-substrate triplet [15]. However, within the DPP4-VEPQ complex system, the intermolecular interactions and bonding forces could influence the conformation of the enzyme, which, in turn, affected the

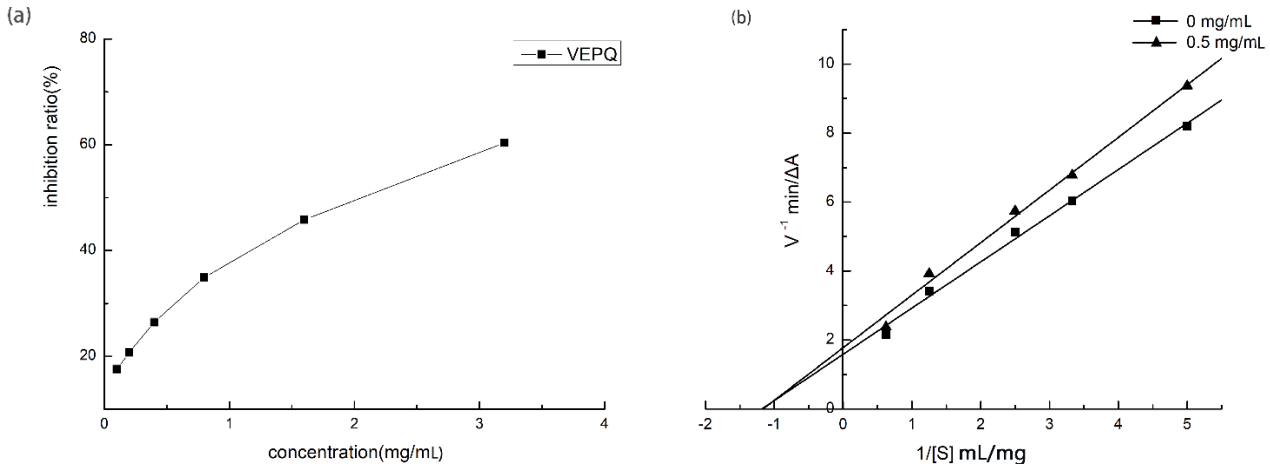

Figure 3. (**a**) Inhibition rate curve of DPP4 enzyme by VEPQ. (**b**) The enzymatic kinetics of DPP4 with VEPQ.

affinity of other molecules to DPP4 and the active state of the enzyme. To elucidate the specific binding sites and the mechanism underlying the conformational changes of the enzyme, molecular simulation was required.

Molecular docking studies

(1) Simulation convergence parameter

Root Mean Square Deviation (RMSD) serves as an indicator of the extent of changes in the molecular structure, while Root Mean Square Fluctuation (RMSF) reflects the degree of freedom of atomic motion within the molecule. The Cα atoms in the two systems were selected for analysis to investigate the associated alterations in the properties and physical conformation of the whole molecule in the systems. The results demonstrated that the fluctuation range of DPP4 increased slightly after being combined with VEPQ and stabilized after 20 ns. The average RMSD values of DPP4 and DPP4-VEPQ were 0.157 ± 0.011 nm and 0.190 ± 0.012 nm, respectively, while the RMSF values ranged from Phe95 to His100, Lys190 to Tyr195, Phe240 to Lys250, Ser275 to Ile285, Tyr330 to Leu340, and Pro676 to Leu680, which indicated that the free-running Cα atoms were active in two glycosylation regions at N-terminus, three cysteine rich regions, and one catalytic region at C-terminal (Figure 4a and 4b). It could be concluded that Cα atoms, as a constituent atom of the backbone of the main chain in the DPP4-VEPQ composite system, remained stable after being bonded. It's degree of freedom of molecular movement in the active region had not been weakened. So, it generally had the role of connecting molecules. However, RMSD values were changed by protein folding and conformation changes. As DPP4 and VEPQ combined with a hybrid system, the interaction and bonding forces between molecules changed the conformation of the DPP4 enzyme. RMSD values also showed a slightly increasing trend, which reflected that the inhibition of DPP4 had a special relationship with its conformation changes. The total radius and the surface area of the DPP4 enzyme did not change significantly (Figure 4c and 4d). The average Rg values of DPP4 and DPP4-VEPQ were 2.731 ± 0.006 nm and 2.738 ± 0.007 nm, which indicated that, after the combination of DPP4 and VEPQ, the Rg values increased slowly and the protein chain length decreased, but the compactness of the whole DPP4 protein system did not change significantly. The surface areas of DPP4 and DPP4-VEPQ systems were 339.764 ± 2.753 nm^2 and 341.909 ± 2.453 nm^2, respectively, which was because DPP4 and VEPQ formed non-covalent bonds, which changed the original folding state of the DPP4 enzyme and made the surface area slightly more extensive. On the other hand, the electrostatic force and binding between

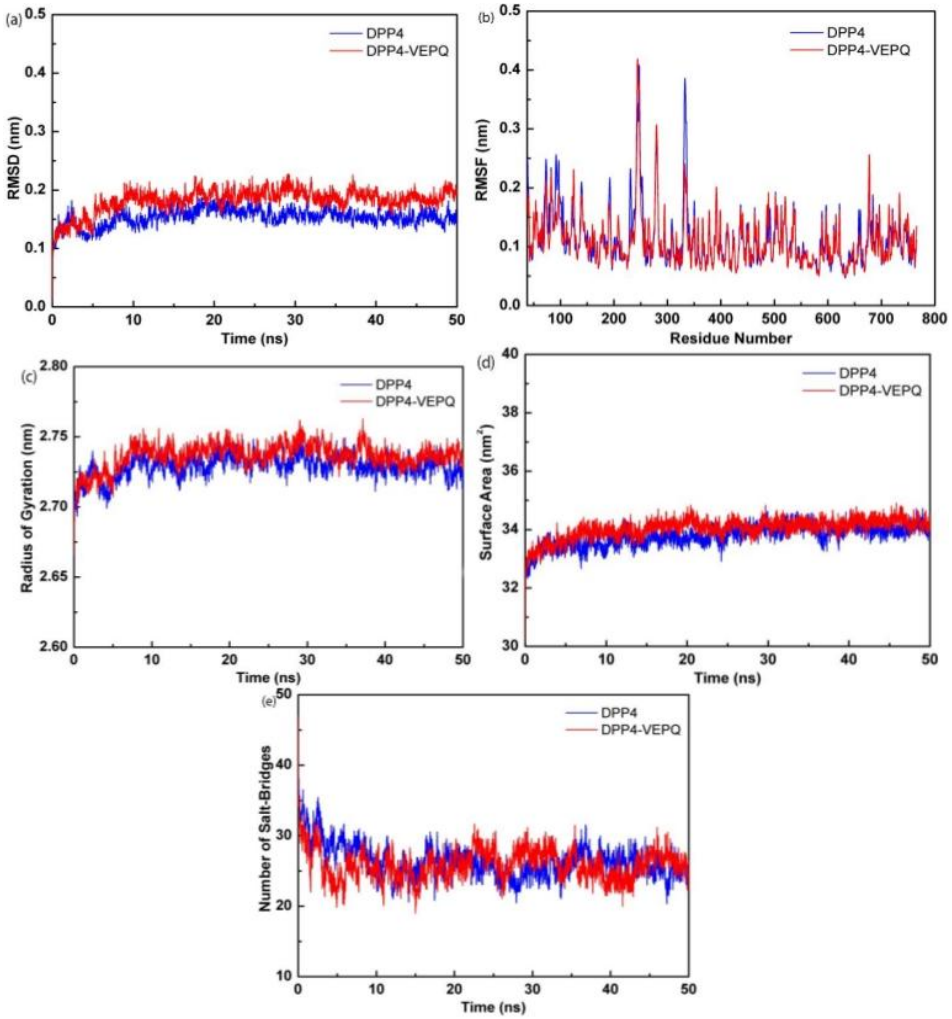

Figure 4. (**a**) The RMSD of VEPQ and DPP4. (**b**)The RMSF of VEPQ and DPP4. (**c**) The radius of gyration of VEPQ and DPP4. (**d**) The surface area of VEPQ and DPP4. (**e**) The number of salt-bridges of VEPQ and DPP4.

molecules could be reflected by measuring the number of salt bridges in the DPP4 structure with the change of simulation time (Figure 4e). The average number of salt bridges in DPP4 and DPP4-VEPQ systems were 25.600 ± 1.800 and 26.100 ± 2.000, respectively. The formation of this stable composite system was closely related to Glu amino acid in VEPQ, which played an essential role in docking conventional drugs with DPP4 [11]. For example, Alogliptin inhibitors and DPP4 could form salt bridges at Glu205, while Sitagliptin and DPP4 could form salt bridges at Glu206 and π-π bond at Tyr666, Vildagliptin and DPP4 formed a salt bridge at Glu206. The inhibitory mechanism of VEPQ on DPP4 was similar to them. Liu *et al*. found that Glu residue in VEPQ could form salt bridge or hydrogen bond with DPP4, which could enhance the electrostatic force between molecules and make the residues closely bound together, so as to obtain a stable composite structure system [20]. By analyzing the variations in the convergence parameters of the two systems during the molecular dynamics (MD) simulation, the results showed that the structural fluctuations of DPP4 protein increased slightly following its binding to VEPQ, but the structural parameters of the whole system remained relatively stable without experiencing drastic changes. The non-covalent binding between VEPQ and DPP4 mainly depended on

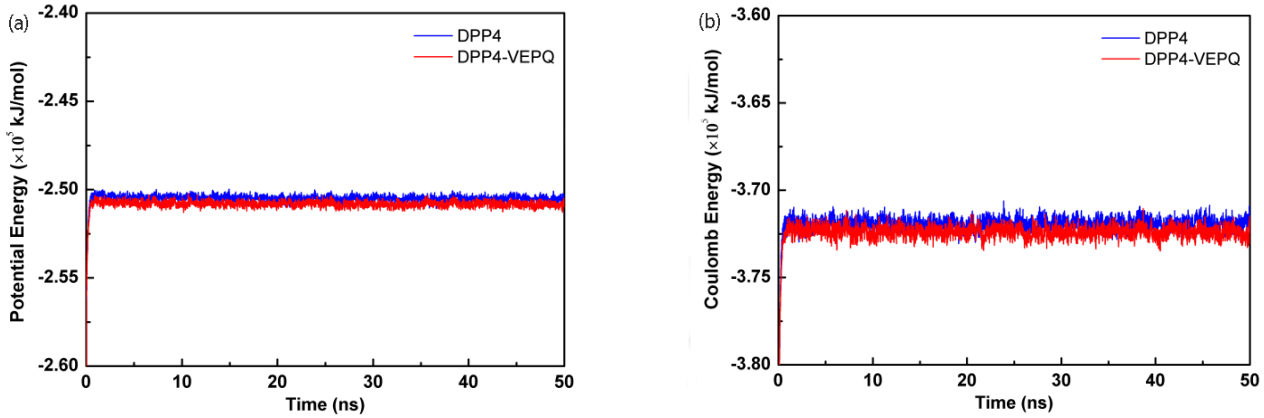

Figure 5. (**a**) The change in potential energy of VEPQ and DPP4. (**b**)The change in Coulombic energy of VEPQ and DPP4.

salt bridges and electrostatic forces. The RMSD value of VEPQ increased slightly. The central carbon atom played a connecting role with active, and the radius of rotation and the surface area of protein did not change notably after being bonded to VEPQ. VEPQ and DPP4 formed a stable composite system, which could inhibit the ability of DPP4 to hydrolysis bioactive peptides, GLP-1, and GIP, and indirectly play a role regulating blood glucose.

(2) System energy change

The changes of potential energy and Coulombic energy with time demonstrated that the potential energy and Coulombic energy had no apparent fluctuation in the simulation process, which ensured the MD simulation trajectory's rationality and reliability under this condition. The potential energies of DPP4 and DPP4-VEPQ were (-2.505 ± 0.002) × 10^5 kJ/mol and (-2.508 ± 0.002) × 10^5 kJ/mol, while the Coulombic energies were (-3.719 ± 0.004) ×10^5 kJ/mol and (-3.723 ± 0.004) × 10^5 kJ/mol, respectively (Figure 5). The potential energy did not change a lot after the combination of VEPQ, which proved that when DPP4 and VEPQ were combined, the bond stretching energy, bond angle bending energy, and dihedral angle rotating energy of their molecules were relatively stable and no conformational changed. The Coulombic energy value increased lightly, which certified that the interaction energy of the DPP4-VEPQ system increased. The main contribution came from the newly formed hydrogen bonds and electrostatic forces between DPP4 and VEPQ molecules. It indicated that there were new non-covalent bonds in the binding process of DPP4-VEPQ, which made the binding of the DPP4-VEPQ system more stable, so that provided a guarantee for the VEPQ to have a good inhibitory effect.

(3) Bonding and interaction

Based on the amino acid composition of the VEPQ molecule, alterations in hydrogen bonding and hydrophobic interactions were selected as indicators to reflect the changes in the intermolecular interaction forces between VEPQ and DPP4. The results showed that the number of hydrogen bonds in the simulation process between VEPQ and DPP4 were about 3, in which Glu and Gln of VEPQ played an essential part in the formation of hydrogen bonds. As an acidic amino acid, Glu was negatively charged and easily interacted with positively charged amino acids in the main chain of DPP4 to form hydrogen bonds and could combine with hydroxyl oxygen on the DPP4 main chain and play a crucial role in maintaining the secondary structure of proteins. Meanwhile, the hydrophobic interaction between VEPQ and DPP4 was about 8, and the formation of hydrophobic bonds was related to Val and Pro in VEPQ (Figure 6). VEPQ could follow the hydrophobic amino acid residues such as Val and Pro to drive DPP4 protein into the interior due to the hydrophobic effect. The initial regular arrangement of water molecules was disrupted,

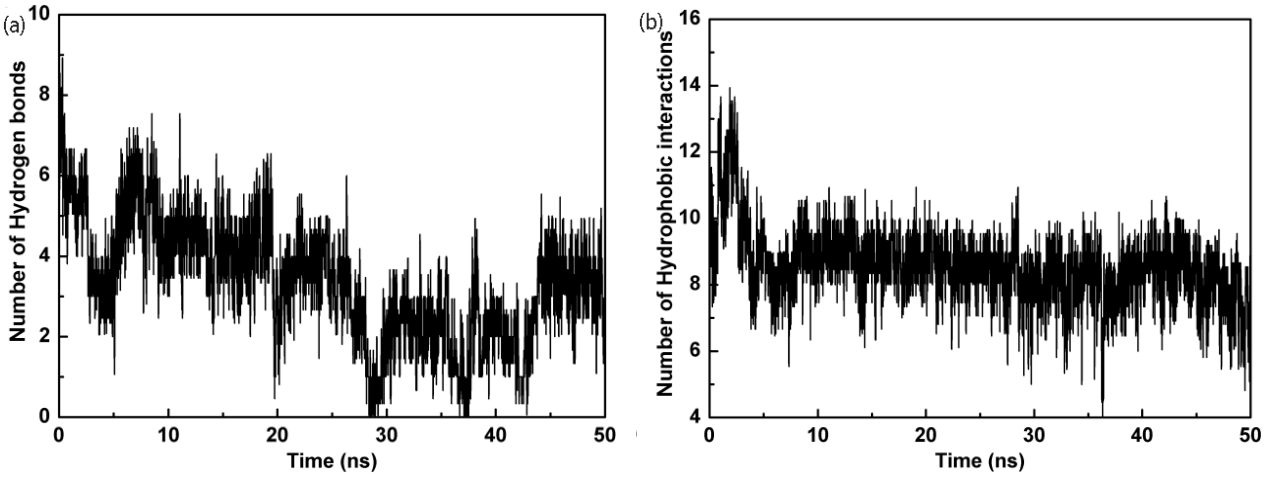

Figure 6. (**a**) The number of hydrogen bonds. (**b**)The number of hydrophobic interactions.

then the hydrophobic surface area was reduced, and the transformed water molecules became disordered. Therefore, entropy increased, and enthalpy change decreased, which lowered the system energy and allowed VEPQ to bind stably to DPP4. The hydrogen bonding and hydrophobic interaction played an essential role in maintaining the hybrid system's stability, which was also a significant driving force for VEPQ to recognize DPP4 enzyme. DPP4 enzyme had the activity of serine protease whose catalytic active site was a catalytic triplet of serine, histidine, and aspartic acid. An alpha helix was inserted into four beta-helix region layers. Eight glycosylation sites were observed in the beta-helix layer, and another site was found in the alpha or beta hydrolytic enzyme folding region. Among them, the region where DPP4 enzymes were more flexible than the others was mainly distributed in the loop region on the surface of proteins, which was greatly influenced by the interaction of surrounding water solutes. The loop region was flexible and played an essential role in the solubility of proteins, which could encapsulate hydrophobic residues through conformational changes, exposed hydrophilic residues, and increased the hydrophilicity of proteins. DPP4 formed “molecular cuff” at N85 and N219 sites, exposing the active sites that interacted with substrate molecules, and completing the interfacial activation of DPP4 enzymes to degrade specific proteins [21] (Figure 7(a)). However, the overall flexibility of the DPP4 protein remained unchanged after VEPQ binding. The conformation of the 50 ns MD simulated complex was analyzed, and the results showed that the C-terminal carboxyl group of VEPQ, the carboxyl group and N-terminus of Glu formed hydrogen bonds with Arg125, Tyr547, and Glu205 with their distances as 1.745, 1.609, and 1.817, respectively, including VEPQ the C-terminal and N-terminus Arg125 with opposite charges and strong electrostatic interaction between Glu205. More hydrophobic amino acids around the binding sites such as Phe357, Tyr547, and Tyr666, could further enhance the affinity between VEPQ and DPP4 (Figure 7(b)). Ser630 and His740 were also the common active site residues of serine proteinases. Although VEPQ did not interact directly with them, it was bound with strong hydrogen bond, electrostatic and hydrophobic interaction near the active sites, which had a significantly negative impact on the enzymatic hydrolysis of GLP-1 polypeptide by DPP4.

Conclusion

Antioxidants play an increasingly pivotal role in the treatment of diabetes by obstructing the pathways of oxidative stress-induced damage. In

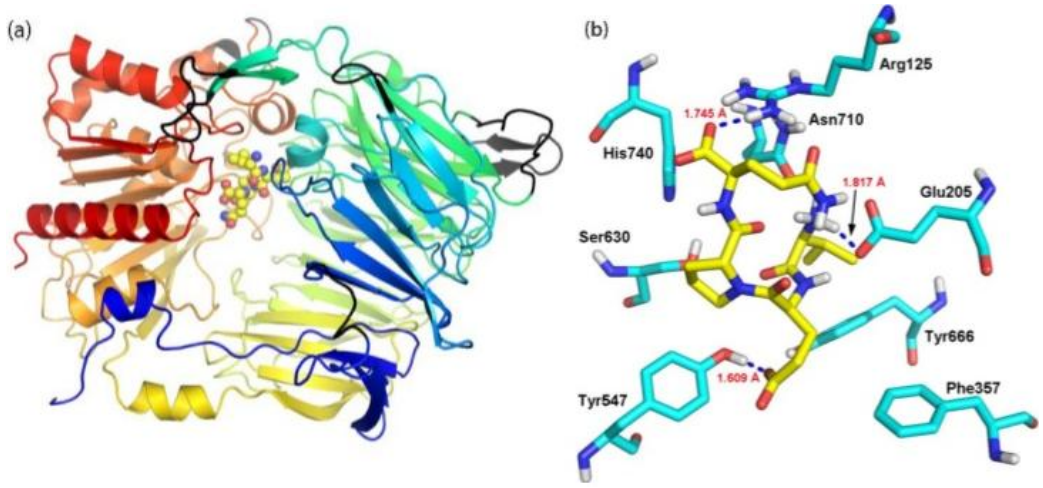

Figure 7. (**a**) The VEPQ and DPP4 simulated combination diagram. The black part represented the region where DPP4 enzymes were more flexible than the others. (**b**) The binding sites of VEPQ and DPP4 at 50 ns MD simulated.

the present study, VEPQ exhibited remarkable antioxidant and DPP4-inhibitory activities, which were closely intertwined with its amino acid composition and intermolecular interactions. The type, conformation, and sequence of amino acid in VEPQ were the key determinants of its potent antioxidant ability. Notably, VEPQ demonstrated the strongest •OH radical-scavenging ability with an IC_{50} value of 6.04 mg/mL. This exceptional performance could be attributed to the presence of hydrophobic amino acids, which enhanced the molecule's capacity to capture •OH free radicals. Molecular dynamics (MD) simulation results revealed that the convergence parameters, energy, and non-covalent bonds of the composite system remained relatively stable throughout the simulation. The hybrid system was stabilized through the formation of non-covalent bonds. VEPQ was bound to the non-active site of DPP4 enzyme to form a composite system. This interaction was characterized by the formation of three hydrogen bonds and eight hydrophobic forces. The hydrogen-bonding sites were located at the carboxyl group of the C-terminus, the carboxyl group of Glu, and the N-terminus of Glu of the VEPQ. These sites were bound to Arg125, Tyr547, and Glu205 of DPP4 enzyme with bond distances of 1.745 Å, 1.609 Å, and 1.817 Å, respectively. In addition, a robust electrostatic interaction existed between the C and N termini of VEPQ and Arg125 and Glu205 with opposite charges, which was one of the more stable factors of the composite system. As a strong oxidant and non-competitive inhibitor, VEPQ could block the oxidative stress injury pathway, which offered valuable theoretical insights for the molecular design of diabetes drugs.

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