Identification of C-phycoerythrin-derived Peptides as Angiotensin Converting Enzyme and Dipeptidyl Peptidase IV Inhibitors *via* Molecular Docking and Molecular Dynamic Simulation

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Abstract

The objective of this study was to screen angiotensin converting enzyme (ACE) and dipeptidyl peptidase IV (DPP-IV) inhibitory peptides from simulated gastrointestinal digestion of C-phycoerythrin using *in silico* methods. The molecular interaction and complex stability of C-phycoerythrin-derived peptides with two receptors (ACE and DPP-IV) were studied. After *in silico* enzyme digestion, 32 unreported peptides were screened by comparing with BIOPEP-UWM and AHTPDB database, and none of them exhibited toxicity to the body based on ADMET predicted results. Moreover, four unreported peptides (LSPSW, CAR, GEF and MAAC) were predicted to have good biological activity. Molecular docking showed that the four peptides all formed hydrogen bonds and hydrophobic interactions with the key groups in ACE and DPP-IV active pockets, of which LSPSW was strongest binding peptide and supposed to be the most potential inhibitor for ACE and DPP-IV. LSPSW – ACE and LSPSW – DPP-IV systems were found to reach equilibrium within 50 ns of MD simulation according to their RMSD and energy results. In addition, the binding of LSPSW to ACE and DPP-IV caused the structure of receptor to be more compact. This study can provide valuable information for the development of C-phycoerythrin-derived peptides as ACE and DPP-IV inhibitors.

Keywords: C-Phycoerythrin-Derived Peptides; Angiotensin Converting Enzyme; Dipeptidyl Peptidase IV; Molecular Docking; Molecular Dynamics Simulation

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1. Introduction

Hypertension and diabetes are among the ten major chronic risks of death in the world. According to incomplete statistics, the number of people worldwide suffered from diabetes and hypertension are approximately 425 million and 1.4 billion, respectively.^[1-3] Both of them belong to complex metabolic diseases and share common risk factors. They are also known as the complication of each other probably due to the

intertwined pathophysiological pathways, and associated with a higher risk of stroke and heart attack.^[4-5] More than half of patients worldwide die from cardiovascular complications caused by hypertension or diabetes.^[3] At present, there are a variety of commercially available drugs for the treatment and/or control of diabetes or hypertension, such as biguanide, thiazolidinedione, angiotensin converting enzyme (ACE) inhibitors and calcium channel blockers.^[5-6] Although these drugs show good therapeutic effects for a single disease, they can cause side effects to the body and fail to treat hypertension and diabetes at the same time.^[5] Thus, how to minimize side effects has become a challenge and prospect of current research. Additionally, ACE and dipeptidyl peptidase IV (DPP-IV) are considered as the important targeted membrane peptidases, which regulate blood pressure and blood sugar, respectively.^[7-8] Previous studies have shown that inhibiting the activity of ACE and DPP-IV may help control hypertension and diabetes at the same time, which provides

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new ideas for the development of new drugs.^[9-10] In recent years, biologically active ingredients obtained from food materials not only can prevent hypertension and diabetes, but also have fewer side effects and consider to be safe and healthy, which are worthy of further study.^[5]

Bioactive peptides are amino acid sequences derived from proteins by protease hydrolysis that play an important functional role in regulating human's health.^[10] Many studies have reported that bioactive peptides obtained from animal and plant protein hydrolysates have good inhibitory effects on ACE or DPP-IV.^[11-15] As we know, only a few studies have investigated the inhibitory effects of peptides on both ACE and DPP-IV.^[9-10] C-Phycocyanin, a marine natural blue photosynthetic pigment protein purified from *Spirulina*, can be used as a natural healthy ingredient for functional foods around the world.^[16] Current studies of C-phycocyanin mainly focus on anti-inflammatory,^[17] anticancer,^[18] and antioxidant activities.^[19] However, few reports have been conducted on the inhibitory effects of C-phycocyanin and its hydrolysates on ACE and DPP-IV.^[9,20] Moreover, the separation and purification of bioactive peptides is complicated, time-consuming and expensive.^[21] However, the process can be simplified and accelerated by multistep virtual screening method and *in silico* gastrointestinal (GI) digestion. With the growing popularity of bioinformatics, computer analysis methods was well used to predict the biological activity of peptides released after food proteolysis.^[10] Many studies have demonstrated the reliability of virtual screening, which can be considered as an effective substitute for classical enzymatic methods and activity prediction.^[22-24] Therefore, *in silico* enzyme digestion, virtual screening and computer simulation could be used in our study to predict and screen the potential ACE and DPP-IV inhibitory peptides derived from C-phycocyanin.

The objective of this study was to screen ACE and DPP-IV inhibitory peptides from simulated gastrointestinal (GI) digestion of C-phycocyanin using *in silico* methods. Molecular docking and molecular dynamics (MD) simulation techniques were used to analyze the potential inhibitory mechanism of virtual screened peptides on ACE and DPP-IV and the stability of their complexes at the molecular level. The results of this study will provide valuable information for the development of C-phycocyanin-derived peptides as ACE and DPP-IV inhibitors.

2. Experimental and computational methods

2.1. *In silico* gastrointestinal (GI) digestion of C-phycocyanin

The amino acid sequence of C-phycocyanin chain (alpha subunit ID: P07121, beta subunit ID: P07120) were chosen from the UniProtKB database (<https://www.uniprot.org/>). In the present study, pepsin (EC 3.4.23.1), trypsin (EC 3.4.21.4), and chymotrypsin (EC 3.4.21.1) were chosen for simulation of proteolysis in the GI tract. The program ExPASy

PeptideCutter (https://web.expasy.org/peptide_cutter/) was used to hydrolyze C-phycocyanin, which could predict potential cleavage sites cleaved by proteases or chemicals in a given protein sequence.^[25]

2.2 *In silico* screening of peptide sequences

Peptides obtained from *in silico* GI digestion were compared with known ACE and DPP-IV inhibitory peptides in BIOPEPUWM database (http://www.uwm.edu.pl/biochemia/biopep/start_biopep.php) and AHTPDB database (<http://crdd.osdd.net/raghava/ahtpdb/pep.php>).^[21] Subsequently, unknown peptides with 2 to 5 amino acids in length supposed to have good bioavailability were selected,^[26] and their solubility, biological activity, and absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties were predicted (<http://lmmd.ecust.edu.cn/admetstar1/predict/?smiles=CAR&action=A>) (<http://www.swissadme.ch/>).^[27-28] PeptideRanker was used to predict biological activities of the peptides according to their calculated score (<http://distilldeep.ucd.ie/PeptideRanker/>).^[29]

2.3 Molecular docking and dynamic simulations

2.3.1 Structure preparation

The 3D crystal structures of ACE (ID: 1O86) and DPP-IV (ID: 5J3J) were obtained from Protein Data Bank,^[10,30] and the unnecessary ligands and water molecules were removed. Swiss-model was used to add the missing structure and atoms for ACE.^[31] Peptides for computational simulation were created using Chemdraw, and their 3D structures were obtained by energy minimization using the MM2 force field in Chem3D.

2.3.2 Molecular docking study

The AutoDock Vina package was used for docking simulation.^[32] Firstly, the polar hydrogen atoms and Gastieger charges were added to the peptides and the two receptors (ACE and DPP-IV) using AutoDock Tools software (ADT, version 1.5.6). Formal charges and van der Waals parameters were assigned to the zinc ion in ACE based on GROMOS 43a1 force field.^[23] The grid coordinates used for molecular docking research were as follows: ACE (x = 42.544, y = 38.322, z = 45.569), DPP-IV monomeric unit (x = 23.193, y = -0.736, z = 45.414) and a spacing of 1.0 Å between grid points. The semi-flexible docking was adopted, where the structure of protein was kept rigid, and all the torsional bonds of the peptides were set free, during the whole docking process. The conformations and interactions were visualized using PyMOL (version 2.3.1) (<https://pymol.org/>) and Ligplot+.^[33] Fosinopril (ACE inhibitor) and saxagliptin (DPP-IV inhibitor) were used as positive controls.

2.3.3 Molecular dynamic (MD) simulations

Table 1 Peptide Ranker score, predicted solubility, and ADMET properties of selected peptides using *in silico* hydrolysis with pepsin, trypsin and chymotrypsin.

C-phycocyanin	Peptide	Peptide mass	PeptideRanker score	ADMET/Toxicity	Water solubility
C-phycocyanin alpha subunit	LSPSW	588.661	0.729126	Non-toxin	Soluble
	CAR	348.42	0.648416	Non-toxin	Soluble
	IAG	259.305	0.353186	Non-toxin	Soluble
	ANHG	397.391	0.267712	Non-toxin	Soluble
	DVGHY	589.605	0.235698	Non-toxin	Soluble
	IDY	409.439	0.235455	Non-toxin	Soluble
	IITY	508.615	0.187729	Non-toxin	Soluble
	QSAR	460.49	0.184146	Non-toxin	Soluble
	AADSR	518.527	0.170427	Non-toxin	Soluble
	MVK	376.515	0.151909	Non-toxin	Soluble
	AINA	387.436	0.14882	Non-toxin	Soluble
	HIK	396.49	0.131799	Non-toxin	Soluble
	AAAS	318.33	0.122854	Non-toxin	Soluble
	LDEY	538.555	0.121625	Non-toxin	Soluble
	EAAR	445.476	0.0932543	Non-toxin	Soluble
	ER	303.318	0.0704548	Non-toxin	Soluble
	LGNTE	532.551	0.0703335	Non-toxin	Soluble
	VEA	317.342	0.0315941	Non-toxin	Soluble
C-phycocyanin beta subunit	GEF	351.359	0.719129	Non-toxin	Soluble
	MAAC	394.504	0.619389	Non-toxin	Soluble
	MT	250.313	0.378539	Non-toxin	Soluble
	LNG	302.33	0.362654	Non-toxin	Soluble
	GDCSQ	508.503	0.259158	Non-toxin	Soluble
	DDR	404.38	0.239945	Non-toxin	Soluble
	DMEII	619.731	0.19119	Non-toxin	Soluble
	TNR	389.412	0.096177	Non-toxin	Soluble
	EGNK	446.461	0.072893	Non-toxin	Soluble
	ANVVK	529.637	0.0593585	Non-toxin	Soluble
	SNEQ	476.443	0.0574877	Non-toxin	Soluble
	VTY	381.429	0.0562376	Non-toxin	Soluble
	ETY	411.412	0.047448	Non-toxin	Soluble
	DV	232.236	0.0461662	Non-toxin	Soluble

MD simulations were performed for 50 ns using the GROMACS 19.5 package with GROMOS 43a1 force field and explicit solvation.^[34] The topology of selected peptide was built using the PRODRG2 Server^[35] and its geometric structure and charge property were modified according to the method proposed by Lemkul, Allen & Bevan.^[36] All the complexes were placed in a rectangular box of SPC water molecules with a minimum distance of 10 Å between any solute atom and the edges of the periodic box. Counter ions

were added to neutralize the total charge of the system. Energy minimization was performed using the steepest descent method, and the maximum was set to 1000.0 kJ/mol/nm. After energy minimization, the system was balanced by two steps: (1) canonical ensemble (NVT, 0.2 ns); (2) isothermal-isobaric (NPT, 0.5 ns). Afterwards, the MD simulation started running. All bonds containing hydrogen atoms were constrained using the default linear constraint (LINCS) solver algorithm.^[37] The V-rescale thermostat^[38] and Parrinello-rahman barostat^[39] were

used control the simulation temperature (300.0 K) and pressure (1.0 bar) with time constants of 0.1 and 2 ps, respectively. The Particle-Mesh Ewald (PME) method was employed to deal with long-range interactions, and a 10 Å cutoff was used for Van der Waals interactions.^[40] In addition, the constraint model was used to treat the zinc ion of ACE to make sure it still remained in the active pocket during MD simulation. The time step was 2 fs, and a snapshot was collected every 1.0 ps. After MD simulation, the GROMACS package (version 19.5) was used to analyze the trajectories.

3. Results and discussion

3.1 *In silico* GI digestion of C-phycoyanin

In the human GI tract, endoproteases such as pepsin (EC 3.4.23.1), trypsin (EC 3.4.21.4) and chymotrypsin (EC 3.4.21.1) are the main proteases that specifically cleave dietary proteins into smaller beneficial oligopeptides.^[41] In our study, a series of hydrolyzed peptides were obtained by *in silico* GI digestion of C-phycoyanin, of which, a total of 48 peptides with 2 to 5 amino acids in length were obtained (Data not shown). These peptides were compared with the known ACE and DPP-IV inhibitory peptides in BIOPEP-UWM and AHTPDB.^[42] Thirty-two unknown peptides were subsequently selected for further screening (Table 1). PeptideRanker (<http://distilldeep.ucd.ie/PeptideRanker/>) is a server for the prediction of bioactive peptides based on a novel N-to-1 neural network, which was trained at a threshold of 0.5. Any peptide predicted over a 0.5 threshold is labelled as bioactive.^[24] In the current study, four peptides LSPSW, CAR, GEF and MAAC showing PeptideRanker scores higher than 0.5 were therefore predicted to have good biological activity. Moreover, all of them are non-toxic and water-soluble to the body through the ADMET/Toxicity and SwissADME database prediction (Table 1 and Table S1).

3.2 Prediction of ACE inhibitory activity of C-phycoyanin-derived peptides

In order to investigate the biological activity of these C-phycoyanin derived peptides, Autodock Vina was used to dock the selected peptides with ACE receptor. Fosinoprilis, an angiotensin-converting enzyme inhibitor, was selected as the positive control according to the previous reports by animal models and human clinical trials.^[43-44] Previous studies have reported that ACE had three active pockets: S1 (Ala354, Glu384 and Tyr523), S2 (Gln281, His353, Lys511, His513 and Tyr520) and S1' (Glu162).^[45] Moreover, ACE had a zinc ion (Zn^{2+}) in its active sites that interacted with His383, His387, and Glu411 residues of ACE.^[13]

The four peptides (LSPSW, CAR, GEF and MAAC) screened from *in silico* GI digestion of C-phycoyanin could all interact with the active pockets of ACE which were basically consistent with the positive control (Fosinoprilis). Ligplot+ was used to further analyze the interaction between peptides and ACE residues, and the results were shown in Fig. 1A-D and Fig. S1. LSPSW could form hydrophobic

Fig. 1 The docking poses and interactions of peptides and ACE. (A) LSPSW – ACE, (B) CAR – ACE, (C) GEF – ACE, and (D) MAAC – ACE. The colors of the ligand are Blue (LSPSW), Red (CAR), Green (GEF), Yellow (MAAC) and Purple (Fosinoprilis, as positive control), respectively. Ligplot+ version v.1.4.5 software and PyMOL version v.2.3.1 were used to create the image.

interactions with Ala354 and Tyr523 (S1 Pocket) and His353 (S2 Pocket), and hydrogen bonds with Ser284 (3.25 Å), Thr282 (3.28 Å) and Asp415 (3.03 Å) in active pockets of ACE (Fig. 1A and Table 2). Compared with LSPSW, the results of the interactions between CAR, GEF and MAAC with ACE were similar since that they interacted with all the active residues in S1 and S2 pockets, of which Glu384 (S1 Pocket) formed hydrogen bonds and the others formed hydrophobic interactions (Fig. 1B-D). In addition to the active pockets, LSPSW, CAR, GEF and MAAC could also generate hydrogen bond and hydrophobic interactions with inactive pocket residues (Fig. 1). Furthermore, Zn^{2+} was involved in the interactions between CAR, GEF and MAAC with ACE. In terms of affinity, fosinoprilis (-9.2 kJ/mol) had the strongest combination with ACE, followed by LSPSW (-9.1 kJ/mol), GEF (-8.1 kJ/mol), CAR (-7.3 kJ/mol) and MAAC (-7.1 kJ/mol) (Table 2). LSPSW formed hydrophobic interaction with 14 residues in ACE, which was only less than that of fosinoprilis (18 residues). These results indicated that LSPSW may has the strongest inhibitory effect on ACE among the four selected peptides and further study is still needed to reveal the stability of the combination of LSPSW and ACE. Previous

Table 2 The affinities of peptides to ACE and DPP-IV receptors and the amino acid residues that form hydrophobic interactions and hydrogen bonds with peptides.

Receptor	Peptide	Affinity (kJ/mol)	Number of hydrophobic interactions	Amino acid residues involved in hydrophobic interactions	Number of hydrogen bonds	Amino acid residues and bond length (Å) involved in hydrogen bonds
ACE	LSPSW	-9.1	14	Trp279, Gln281, His353, Ala354, Leu375, Glu376, Val379, Val380, His383, Asp453, Lys454, Phe457, Tyr523, Phe527	3	Thr282 (3.25), Ser284 (3.28), Asp415 (3.03)
	CAR	-7.3	6	Thr282, Ser355, Glu384, Phe457, Phe512, Phe527	8	Gln281 (3.12), His353 (3.23), Ala354 (3.10), His383 (3.34), Lys511 (2.85), His513 (2.86), Tyr520 (2.90), Tyr523(3.05)
	GEF	-8.1	6	Ser355, Val380, Glu384, Asp415, Phe457, Phe527	7	Gln281 (2.80), His353(3.12), Ala354 (3.24), Lys511 (2.80), His513 (2.89), Tyr520 (2.65), Tyr523(2.85)
	MAAC	-7.1	5	Trp279, Ser355, Val380, Glu384, Phe457	8	Gln281 (2.95), His353(3.24), Ala354 (3.16), Lys511 (2.90, 3.10), His513 (2.80), Tyr520 (3.07), Tyr523 (2.97)
	Fosinopril (positive control)	-9.2	18	Glu162, Thr166, Trp279, Gln281, Thr282, Asn285, His353, Asn374, Glu376, Asp377, Val379, Val380, His383, Asp415, Asp453, Phe457, Tyr523, Phe527	2	Ala354(3.07),Asn277(2.95)
DPP-IV	LSPSW	-7.7	13	Arg125, Ser209, Phe357, Tyr547, Ser552, Gln553, Lys554, Tyr631, Val656, Trp659, Tyr662, Tyr666, Val711	4	Glu205 (2.98), Tyr585 (3.26, 3.13), Ser630 (2.97)
	CAR	-6.3	10	Arg125, Glu206, Val207, Phe357, Arg358, Ser630, Val656, Trp659, Tyr662, Tyr666	4	Glu205 (2.76, 3.03), Ser209 (2.76), Tyr547 (3.07)
	GEF	-6.9	7	Glu206, Phe357, Tyr631, Val656, Trp659, Tyr666, Arg669	6	Arg125 (3.14, 3.02), Tyr547 (3.07), Ser630 (3.16), Tyr662 (3.14), His740 (3.29)
	MAAC	-5.8	11	Arg125, Glu206, Ser209, Phe357, Tyr547, Ser630, Tyr631, Val656, Tyr662, Tyr666, Val711	2	His126 (3.18), Glu205 (3.10)
	Saxagliptin (positive control)	-6.7	7	Arg125, Phe357, Tyr547, Ser630, Tyr662, Tyr666, His740	3	Glu205 (3.06), Glu206 (2.99), Ser209 (2.92)

LSPSW, Leu-Ser-Pro-Ser-Trp; CAR, Cys-Ala-Arg; GEF, Gly-Glu-Phe; MAAC, Met-Ala-Ala-Cys.

studies also reported that peptides (ADF, MIR, FGR, FIV, WAR and WQR) screened from hen egg, *Larimichthys crocea* titin and smooth-hound viscera protein had good ACE inhibitory activity by *in silico* analysis and molecular docking simulation.^[10, 46-47]

3.3 Prediction of DPP-IV inhibitory activity of C-phycocyanin-derived peptides

Similar to ACE, DPP-IV also has three active pockets, namely S1 (Tyr547, Ser630, Tyr631, Val656, Trp659, Tyr662, Tyr666, Asn710, Val711 and His740), S2 (Glu205, Glu206, Tyr662) and S3 (Ser209, Arg358 and Phe357).^[10, 48] Saxagliptin was selected as a positive control in this study, which was reported to selectively inhibit DPP-IV to increase glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP) levels, thereby regulating blood glucose.^[49-50]

Fig. 2 The docking poses and interactions of peptides and DPP-IV. (A) LSPSW – DPP-IV, (B) CAR – DPP-IV, (C) GEF – DPP-IV, and (D) MAAC – DPP-IV. The colors of the ligand are Blue (LSPSW), Red (CAR), Green (GEF), Yellow (MAAC) and Purple (Saxagliptin, as positive control), respectively. Ligplot+ version v.1.4.5 software and PyMOL version v.2.3.1 were used to create the image.

The combination conformation and interaction results of saxagliptin and DPP-IV were shown in Fig. S1 and Table 2. In the internal cavity of DPP-IV, Arg125, Phe357, Tyr547 (S1),

Ser630 (S1), Tyr662 (S1), Tyr666 (S1) and His740 had hydrophobic interactions with saxagliptin, while Glu205, Glu206 (S2 pockets) and Ser209 (S2 pockets) formed hydrogen bonds with saxagliptin showing H-bond lengths between 2.92~3.06 Å. The results of molecular model showed that saxagliptin could act simultaneously with the three pockets (S1, S2 and S3) of DPP-IV, which was consistent with previous reports and indicated the reliability of the model.^[10]

Fig. 2A-D shows the combination conformation and interaction of LSPSW, CAR, GEF, MAAC and DPP-IV, respectively. These peptides were able to interact with residues in three pockets (S1, S2 and S3) of DPP-IV by hydrogen bond and hydrophobic interactions, which were similar to that of saxagliptin. The four peptides all formed hydrophobic interactions with Tyr631, Val656, Trp659, Tyr666 in S1 pocket of DPP-IV. Moreover, these residues were also found in the interaction between alogliptin (another DPP-IV selective inhibitor) and DPP-IV.^[51]

In addition, the affinity and interaction between the four peptides and DPP-IV were also evaluated. As shown in Table 2, the affinity of the four peptides to DPP-IV decreased in the order: LSPSW (-7.7 kJ/mol) > GEF (-6.9 kJ/mol) > Saxagliptin (-6.7 kJ/mol) > CAR (-6.3 kJ/mol) > MAAC (-5.8 kJ/mol), indicating that the interaction strength of LSPSW and DPP-IV were stronger than the other three peptides. Nongonierma et al. reported that DPP-IV inhibitory peptides generally had a hydrophobic or aromatic amino acid at the N-terminus.^[52] Thus, Leu at N-terminus of peptide chain may play an important role in the binding of LSPSW and DPP-IV, which was also confirmed by the number of hydrophobic interactions of Table 2. Moreover, the affinity between GEF and DPP-IV was lower than that of saxagliptin, which may be because the Phe at C-terminal of peptide chain may enhance the binding affinity of peptides to DPP-IV.^[10, 53]

Fig. 3 The molecular dynamics (50 ns) results of LSPSW and the two receptors (ACE and DPP-IV) complexes. (A) Root mean square deviations (RMSD, nm), (B) solvent accessible surface area (SASA) values, (C) Radius of gyration (Rg), (D) H-bond number, (E) Radial distribution function (RDF) of LSPSW to the surface of two receptors (ACE and DPP-IV) and (F) The fluctuation of binding free energy of LSPSW — ACE and LSPSW — DPP-IV.

3.4. Molecular dynamic simulation studies

Among the four peptides, LSPSW showed the strongest interaction with key groups in the active pockets of ACE and DPP-IV. However, their binding stability cannot be determined by molecular docking results alone. Therefore, conformational fluctuation, compactness, H-bond number and energy changes were chosen to evaluate the structural stabilities of LSPSW – ACE and LSPSW – DPP-IV systems via a 50 ns MD simulation,^[54] and the results are shown in Fig. 3. Root mean square deviations (RMSD) was used to determine the mean deviation of complex conformation at a specific time from the original receptor, and it can evaluate whether the complex system reaches a stable state.^[54-55] The RMSD values for LSPSW – ACE and LSPSW – DPP-IV showed that the structures of the two complexes equilibrated well after 6.5 ns and 7 ns of MD simulations, respectively (Fig. 3A). In addition, the combination of LSPSW also affected the solvent accessible surface area (SASA) of ACE and DPP-IV. In the whole 50 ns MD simulation, the SASA values first decreased and then reached equilibrium at 293 nm² and 235 nm², respectively (Fig. 3B). This indicated that the combination of LSPSW made the structure of ACE and DPP-IV more compact, and the results from Rg also confirmed this (Fig. 3C).^[56] Similarly, peptide EEAGGATAAQIEM has been reported to increase the structural densification of ACE2 and SARS-CoV-2.^[24] Furthermore, hydrogen bond has been shown to be the main force between LSPSW and two receptor proteins (ACE and DPP-IV) that maintained the stability of the whole complexes. As shown in Fig. 3D, the H-bond number between LSPSW and ACE fluctuated within 4~6, while the H-bond number between LSPSW and DPP-IV decreased from about 5~7 to between 1 and 4 during the whole 50 ns MD simulation. This may be due to the strong hydrophobicity of Leu at the N-terminal of peptide chain, which is mainly combined with DPP-IV through hydrophobic interaction.^[52] Radial distribution function (RDF) can characterize the distance relationship between two types of molecules (atom to atom, ligand and receptor). The average density of ligand to protein surface in the stable period was obtained by analyzing the RDF of ligand to protein surface, and the results are shown in Fig. 3E. RDF for the LSPSW - receptor proteins (ACE and DPP-IV) showed distinct peaks at 0.25~0.35 nm, indicating that there were strong hydrogen bond interactions between LSPSW and the receptors. Moreover, the peak strength of LSPSW-ACE was higher than LSPSW-DPP-IV, indicating that the distribution density of LSPSW to ACE was better than that of DPP-IV, which is consistent with the corresponding H-bond numbers presented in Fig. 3D.^[57] Furthermore, Coulomb interaction (Coul) and Lennard-Jone (LJ) also participated in the binding of LSPSW to the receptors (ACE and DPP-IV). In terms of total energy, both LSPSW – ACE and LSPSW – DPP-IV complexes reached a stable state with the lowest energy (Fig. 3F). At the same time, we also compared the difference of the binding conformation before and after MD simulation, and the results

showed that LSPSW was kept in the active pockets of ACE and DPP-IV (Fig. 4). Thus, peptide LSPSW might be an effective inhibitor for ACE and DPP-IV.

Fig. 4 The result of combining conformation of LSPSW and the two receptors in before (green) and after (blue) molecular dynamics simulation (50 ns). (A) LSPSW – ACE and (B) LSPSW – DPP-IV

4. Conclusion

C-phycoyanin, a natural functional food pigment protein, has great potential and broad market prospects for food industry. However, few studies have reported the biological activity of the C-phycoyanin-derived peptides. Therefore, *in silico* enzyme digestion, molecular docking and MD simulation methods were used to predict the interaction and binding stability between C-phycoyanin-derived peptides and ACE and DPP-IV. Four unreported peptides were screened out by virtual screening, namely LSPSW, CAR, GEF and MAAC, and none of them exhibited toxicity to the body based on ADMET predicted results. Molecular docking showed that the four peptides formed hydrogen bonds and hydrophobic interactions with the key groups in ACE and DPP-IV active pockets, and the binding affinities between LSPSW and ACE and DPP-IV were stronger than the other three peptides. MD simulation indicated that the structural and energy of the LSPSW-receptor systems all were reach equilibrium stable within 50 ns of simulation. Electrostatic and van der Waal interactions were found to play important roles in the binding of LSPSW with ACE and DPP-IV, and the binding of LSPSW to ACE and DPP-IV caused the receptor structure to be more compact. The results indicated that LSPSW might be an effective inhibitor for ACE and DPP-IV. In summary, our study provides theoretical guidance for the development and utilization of C-phycoyanin-derived peptides. However, further experiments are still needed to confirm this hypothesis.

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Supporting information

Applicable.

Conflicts of interest

All contributing authors declare no conflicts of interest.

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