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Computer Simulation Evaluation of Angiotensin-Converting Enzyme Inhibitory Peptides from *Gadus morhua* Myoglobin

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Abstract:

The primary objective of this study is to assess the likelihood of occurrence and abundance of bioactive peptides in *Gadus morhua* myoglobin through computer simulations of gastrointestinal digestion. The amino acid sequence of myoglobin was retrieved from the NCBI database, imported into Peptide Cutter, and subjected to enzymatic hydrolysis simulations using various enzymes. The resulting short peptides were then analyzed using BIOPEP-UWM's "enzymatic tool." Additionally, their physicochemical properties and toxicity characteristics were evaluated using Toxin Pred. Furthermore, the findings were imported into Peptide Ranker to prioritize peptide activities, leading to the identification of two angiotensin-converting enzyme (ACE) inhibitory peptides, CW and LFPK. The peptides exhibiting higher activity were subjected to molecular docking with ACE to investigate the inhibitory mechanisms of CW and LFPK, providing valuable insights for future research on discovering ACE inhibitory peptides from food sources.

Keywords:

Gadus morhua myoglobin
Angiotensin-converting enzyme
Computer simulation
Molecular docking

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1. Introduction

Hypertension is a common cardiovascular disease. In the regulation of human blood pressure, the Renin-Angiotensin System (RAS) and the Kallikrein-Kinin System (KKS) play crucial roles [1]. In the RAS system, angiotensinogen is hydrolyzed to produce angiotensin I (Ang I), which is further hydrolyzed by angiotensin-converting enzyme (ACE) to generate angiotensin II. This peptide promotes smooth muscle contraction in blood vessels, increases vascular pressure, and induces a pressor

response [2]. ACE inhibitory peptides can hinder ACE from participating in the biochemical reactions mentioned above, thereby lowering blood pressure.

Currently, the main synthetic antihypertensive drugs available on the market and used in clinical practice are angiotensin-converting enzyme inhibitors (ACEI), angiotensin receptor blockers (ARB), and calcium channel blockers (CCB) [3]. However, long-term use of these drugs can have certain side effects. Therefore, there is increasing attention on finding nutritional components in food that can lower blood pressure.

Bioactive peptides possess biological functions such as lowering blood pressure, hormone regulation, immune regulation, and anti-thrombotic effects [4]. However, these peptides can be structurally altered by enzymatic hydrolysis when passing through the gastrointestinal tract, affecting the activity of ACE inhibitory peptides. Therefore, computer simulation is essential to evaluate peptide activity. Computer simulation assessment methods are fast and efficient with high throughput. Traditional *in vitro* simulation experiments are time-consuming and labor-intensive, and the experimental results are greatly affected by external factors. Computer simulation studies enable low-cost primary screening of peptides in a shorter time [5].

In this study, computer simulation assessment methods will be used to extract bioactive peptides from food and perform molecular docking to analyze the activity of ACE inhibitory peptides. Atlantic cod (*Gadus morhua*) is widely consumed by humans due to its high economic value. The hydrolysates of fish protein possess various biological activities [6] and exhibit certain antihypertensive effects after being digested by the human body. Research has shown that endogenous bioactive peptides present in bones and muscles [7] play an important role in reducing hypertension [8].

Therefore, this study focuses on the biological activity of *Gadus morhua* myoglobin and analyzes the potential of this myoglobin to produce ACE inhibitory peptides through computer simulation. It proposes an alternative method to ACE inhibitory peptide drugs, providing a reference for finding ACE inhibitory peptides in food.

2. Materials and methods

2.1. Protein amino acid sequence search

The amino acid sequence of *Gadus morhua* (Atlantic cod) myoglobin was retrieved from the NCBI database (https://www.ncbi.nlm.nih.gov/). By entering the Latin name "*Gadus morhua*" and selecting "protein," all protein sequences of the cod were searched, and the myoglobin sequence consisting of 145 amino acids was identified and downloaded.

2.2. Computer simulation of gastrointestinal digestion

The amino acid sequence of myoglobin obtained from the NCBI database was subjected to simulated enzymatic hydrolysis using Peptide Cutter (https://web.expasy.org/peptide_cutter/). Pepsin (EC 3.4.23.1) was selected to simulate gastric digestion, while trypsin (EC 3.4.21.4) and chymotrypsin (EC 3.4.21.1) were used to simulate gastrointestinal digestion. The myoglobin sequence was cleaved into short peptide chains consisting of two to 13 peptides, which were then ranked based on their predicted biological activity probabilities using Peptide Rank.

2.3. BIOPEP analysis

The BIOPEP database (https://biochemia.uwm.edu.pl/biopep-uwm/) was utilized to predict potential ACE inhibitory peptides within the *Gadus morhua* myoglobin. These peptides were matched with known ACE inhibitory peptides in the database to identify the most active short peptides for further analysis. The cleaved hemoglobin peptides were sequentially imported into the BIOPEP-UWM "enzymatic tool" analysis, and the results interface displayed whether they had potential biological activities. Peptides with potential ACE inhibitory activity were selected based on A and B values for further analysis.

2.4. Physicochemical properties of short peptides released after computer-simulated hydrolysis

The physicochemical properties of the short peptides obtained from simulated gastrointestinal digestion of myoglobin were analyzed using Expasy (https://web.expasy.org/) and ToxinPred (https://webs.iiitd.edu.in/raghava/toxinpred/design.php). These properties

included molecular weight (MW), isoelectric point (PI), physicochemical properties, Peptide Ranker value, net charge, and toxicity characteristics, preparing for subsequent analyses.

2.5. Molecular docking

The crystal structure of ACE (PDB ID: 1O86) was downloaded from the PDB database (https://www.rcsb.org/) and pretreated in Pymol to prepare the receptor structure. The original crystallization coordinates were used as the center of the docking box for ACE. The three-dimensional structure of myoglobin was drawn using ChemDraw 19.0. Molecular docking was performed using AutoDock Tools 1.5.7, and the best docking site was selected based on energy. The docking results were visualized in Discovery Studio 2017.

3. Results and analysis

3.1. Physicochemical properties analysis of myoglobin

ACE inhibitory peptides were generated from *Gadus morhua* myoglobin after simulated gastrointestinal digestion. Software was used to analyze these ACE inhibitory peptides, and the results are presented in **Table 1.**

Based on **Table 1**, among the 20 peptides obtained from *Gadus morhua* myoglobin, there are dipeptides, tripeptides, and a few polypeptides. All peptides appear only one to two times, with molecular weights ranging from 188.25 to 1414.83 Da. The PI values of the five peptides CW, LG, AGL, IPINN, and ITEVIAK are between 5.85 and 6.35, and their net charge values are all 0. The PI values of the three peptides HMAEK, NTHGGL, and AASVAVASHGATV are all 7.10, with

Table 1. Identified ACE inhibitory peptides from *Gadus morhua* myoglobin with their peptide sequences, physicochemical properties, and toxicity predictions

Peptide sequence	Frequency of occurrence	MW/Da	PI	Peptide ranker value	Toxicity prediction	Net charge
CW	1	307.10	5.85	0.99581	non-toxic	0
LG	1	188.25	5.88	0.71728	non-toxic	0
TR	2	275.32	10.11	0.12779	non-toxic	1
AGL	1	259.34	5.88	0.59290	non-toxic	0
LKP	1	356.50	9.11	0.34964	non-toxic	1
LGE	1	317.38	4.00	0.15131	non-toxic	-1
LFPK	1	503.69	9.11	0.82371	non-toxic	1
MADY	1	498.59	3.80	0.42783	non-toxic	-1
IPINN	1	596.73	5.88	0.29364	non-toxic	0
AGVGE	1	431.51	4.00	0.11021	non-toxic	-1
HMAEK	1	614.78	7.10	0.11810	non-toxic	0.5
GDHAAL	1	582.69	5.09	0.42322	non-toxic	-0.5
NTHGGL	1	597.72	7.10	0.32483	non-toxic	0.5
ITEVIAK	1	773.04	6.35	0.04821	non-toxic	0
GPVEADY	1	749.86	3.67	0.14423	non-toxic	-2
DAAGQEA	1	660.72	3.67	0.10469	non-toxic	-2
TEHPDTQK	1	955.10	5.33	0.06187	non-toxic	-0.5
LATSHANVHK	1	1077.35	9.11	0.11043	non-toxic	2
VMSVVIADMDATY	1	1414.83	3.57	0.10440	non-toxic	-2
AASVAVASHGATV	1	1140.43	7.10	0.21306	non-toxic	0.5

a net charge value of 0.5. The PI values of the three peptides TR, LKP, and LFPK are greater than 9.0, with a net charge of 1. The PI values of the three peptides LGE, MADY, and AGVGE are less than or equal to 4, with a net charge of -1. Analysis of **Table 1** shows that the short peptides of this *Gadus morhua* myoglobin are partially negatively charged and slightly acidic, while a few are positively charged and slightly basic.

Toxicity prediction using Toxin Pred software revealed that the short peptides obtained from the simulated hydrolysis of *Gadus morhua* myoglobin do not have potential toxicity and can be further developed as food or medicine.

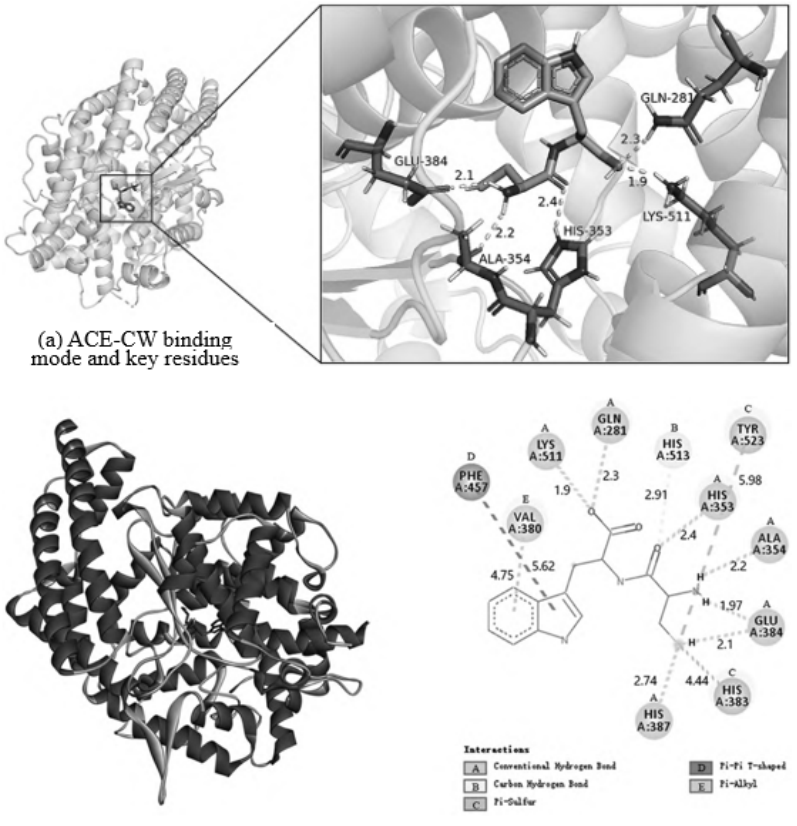
Using Peptide Ranker to rank the probability of biological activity of the 20 peptides, the score ranges from 0 to 1. The threshold for the Peptide Ranker score is set at 0.5, and a higher score indicates a greater likelihood of the peptide having biological activity [9]. According to **Table 1**, among the computer-simulated hydrolysis peptides of *Gadus morhua* myoglobin, four peptides have scores greater than 0.5. The CW peptide has the highest score of 0.99581, and the LFPK peptide has a score of 0.82371. Therefore, the analysis of *Gadus morhua* myoglobin is of research significance.

3.2. Visualization of molecular docking results

After computer-simulated hydrolysis of *Gadus morhua* myoglobin, the dipeptide CW and the tetrapeptide LFPK, which have the highest probability of activity, were selected for further molecular simulation and molecular docking. The results are shown in **Figures 1** and **2**.

Molecular docking is based on the "lock-and-key principle" of ligands and receptors. This process involves placing small molecule ligands individually at active sites of the receptor protein and continuously adjusting their positions to find the best conformation for binding [10]. This approach elucidates the mechanism of action between CW, LFPK, and ACE from a molecular perspective. The active center of ACE has three essential binding sites: S1, S2, and S3. This study investigated the interactions between small molecule ligands and the active sites of ACE, selecting S1 and S2 as the

Figure 1. Molecular docking interaction diagram of ACE-CW

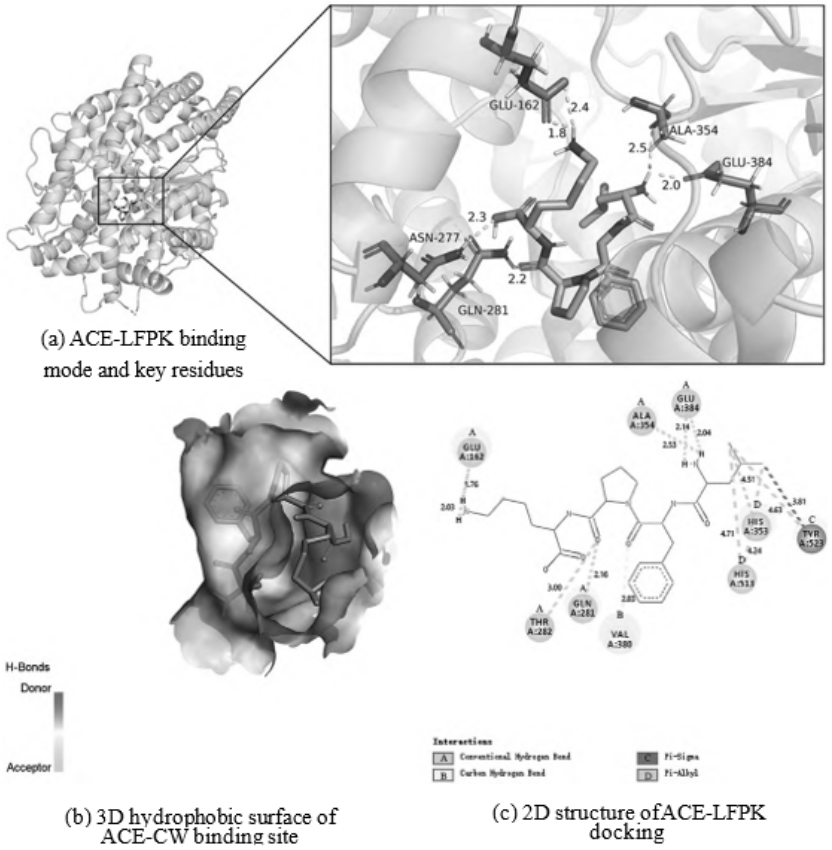


binding sites for the dipeptide CW, and S1, S2, and S3 as the binding sites for LFPK. Molecular docking was performed separately for CW and LFPK.

As shown in **Figures 1(a)** and **1(c)**, in the ACE-CW complex, the two primarily bind through hydrogen bonding, hydrophobic interactions, and pi-pi conjugation. For instance, CW forms seven hydrogen bonds of varying distances with the amino acid residues of ACE: Gln281 (2.3 Å), Lys511 (1.9 Å), His353 (2.4 Å), Ala354 (2.2 Å), Glu384 (2.10 Å/1.97 Å), and His387 (2.74 Å). Among these, the hydrogen bond formed with the Lys511 residue has the shortest distance and the tightest binding. Additionally, a carbon-hydrogen bond is formed with His513 (2.91 Å). CW also forms a pi-alkyl bond with the amino acid residue Val380 (4.75 Å), a pi-pi conjugation bond with Phe457 (4.75 Å), and pi-sulfur interactions with His383 (4.44 Å) and Tyr523 (5.98 Å). Notably, CW interacts with Tyr523 of the ACE active site S1 through a pi-sulfur interaction and forms three hydrogen bonds with Ala354 and Glu384. It also binds to the active site S2, forming hydrogen bonds with His353, Gln281, and Lys511 residues, and a carbon-hydrogen bond with His513. By binding to these amino acid residues in the active center of ACE, CW occupies the active center and inhibits ACE activity.

4. Conclusion

In this study, bioactive peptides from *Gadus morhua* myoglobin were initially screened using a computer, and they were found to be non-toxic. CW and LFPK, which have the highest probability of ACE inhibitory activity, were selected for further analysis of their ACE inhibitory mechanisms using molecular docking techniques. The results showed that the binding sites of CW in the active center of ACE are S1 and S2, while the binding sites of LFPK are S1, S2, and S3. These findings provide a theoretical basis for the development of *Gadus morhua* myoglobin as a precursor of ACE inhibitory peptides in the food industry.



(a) ACE-LFPK binding mode and key residues

(b) 3D hydrophobic surface of ACE-CW binding site

(c) 2D structure of ACE-LFPK docking

Figure 2. Molecular docking interaction diagram of ACE-LFPK

Disclosure statement

The authors declare no conflict of interest.

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