MINISTRY OF EDUCATION AND SCIENCE OF UKRAINE
KYIV NATIONAL UNIVERSITY OF TECHNOLOGIES AND DESIGN
Faculty of Chemical and Biopharmaceutical Technologies
Department of Biotechnology, Leather and Fur

QUALIFICATION THESIS

on the topic **Simulated enzymatic cleavage and activity analysis of phycoerythrin-derived bioactive peptides**
First (Bachelor's) level of higher education
Specialty 162 "Biotechnology and Bioengineering"
Educational and professional program "Biotechnology"

Completed: student of group BEBT-21
Xu YIQUN

Scientific supervisor
Olga ANDREYEVA, Dr. Sc., Prof.

Reviewer
Tetiana HALENOVA, Ph.D., As. Prof.

Kyiv 2025

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Educational and professional program Biotechnology

APPROVE
Head of Biotechnology, Leather and Fur Department, Professor,
Dr. Sc., Prof.
_____Olena MOKROUSOVA
«___»_____2025

ASSIGNMENTS
FOR THE QUALIFICATION THESIS
Xu Yiqun

1. Thesis topic **Simulated enzymatic cleavage and activity analysis of phycoerythrin-derived bioactive peptides**
Scientific supervisor Dr.Sc., Prof. Olga Andreyeva

approved by the order of KNUTD "05" March 2025, № 50-уч

2. Initial data for work: assignments for qualification thesis, scientific literature on the topic of qualification thesis, materials of Pre-graduation practice

3. Content of the thesis (list of questions to be developed): literature review; object, purpose, and methods of the study; experimental part; conclusions

4. Date of issuance of the assignments 05.03.2025

WORK CALENDAR

№	The name of the stages of the qualification thesis	Terms of performance of stage	Note on performance
1	Introduction	until 11 April 2025	
2	Chapter 1. Literature review	until 20 April 2025	
3	Chapter 2. Object, purpose, and methods of the study	until 30 April 2025	
4	Chapter 3. Experimental part	until 11 May 2025	
5	Conclusions	until 15 May 2025	
6	Draw up a bachelor's thesis (final version)	until 25 May 2025	
7	Submission of qualification work to the supervisor for feedback	until 27 May 2025	
8	Submission of bachelor's thesis to the department for review (14 days before the defense)	28 May 2025	
9	Checking the bachelor's thesis for signs of plagiarism (10 days before the defense)	01 June 2025	Similarity coefficient ____% Citation rate ____%
10	Submission of bachelor's thesis for approval by the head of the department (from 7 days before the defense)	04 June 2025	

I am familiar with the task:

Student _____ Xu YIQUN

Scientific supervisor _____ Olga ANDREYEVA

SUMMARY

Xu YIQUN. Simulated enzymatic cleavage and activity analysis of phycoerythrin-derived bioactive peptides. – Manuscript.

Qualification thesis on the specialty 162 «Biotechnology and Bioengineering». – Kyiv National University of Technologies and Design, Kyiv, 2025.

Red algae are naturally growing algae, and phycoerythrin is the main bioactive substance in red algae, possessing rich amino acid species and various biological activities, which is a high-quality protein raw material for the preparation of bioactive peptides. With the improvement of science and technology, bioinformatics technology can now be used to study the preparation of bioactive peptides. Compared with the method of conducting traditional experiments, the use of bioinformatics tools to study the target object has the advantages of reducing experimental costs and improving experimental efficiency.

In this study, algal red protein was used as the research object. Firstly, bioinformatics tools were used to simulate enzymatic cleavage of seven algal red protein chains using pepsin (pH=1.3) and proteinase K together and to predict the bioactivity of the resulting peptides, and 65 biologically active peptides were found out of 302 peptides. Secondly, bioinformatics tools were used to predict the physicochemical properties and biological activities of the peptides, and 9 peptides with good non-toxic water solubility, immunomodulatory, antioxidant and antimicrobial properties with one or more biological activities were found. Finally, the peptides with high affinity to TLR2 receptor and KEAP1 receptor were screened by molecular docking software, and the molecular docking results of the peptides were visualized and analyzed using Pymol software. Through the above steps, nine active peptides of phycoerythrin such as SAADAAGRF, RDM, DAF, AGDP, KKF, SGDCSAL, SGDP, CRY and CAKGM were finally found to meet the expectation. Among these peptides, a total of 9 peptides had immunomodulatory properties, 6 peptides SAADAAGRF, DAF, AGDP, KKF, SGDCSAL and SGDP had antioxidant

properties, and 4 peptides RDM, DAF, KKF and CAKGM had antimicrobial properties. The peptides SAADAAGRF, AGDP and DAF can spontaneously generate interaction force with TLR2 receptor, and all 9 peptides can spontaneously generate interaction force with KEAP1 receptor. All 9 peptides can be used as novel phycoerythrin active peptides, which can provide biological value for future research.

Key words: Phycoerythrin-Activated Peptide, Bioinformatics, Simulated Enzyme Digestion, Molecular Docking, Biological Activity

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INTRODUCTION

Red algae are mostly distributed in the ocean, which can obtain nutrients through photosynthesis and have rich biological value. Phycoerythrin is an important light-trapping pigment protein in red algae, containing a wide variety of amino acids, possessing a variety of biological activities such as antioxidant, anticancer, etc. It has a wide range of sources and is easy to be extracted, so it can be used as a source for exploring new bioactive peptides. It can be used as a source of new bioactive peptides. The active peptide of phycoerythrin is extracted from phycoerythrin, which also has the effects of antioxidant and blood pressure lowering, etc. However, compared with phycoerythrin, the active peptide of phycoerythrin has a small molecular structure and a clear target, so that it can be absorbed and utilised better. Bioactive peptides can be prepared by enzymolysis, chemical synthesis and microbial fermentation, which require high economic and time costs and high experimental uncontrollability. Therefore, the preparation of bioactive peptides by bioinformatics software has become a hot topic nowadays. Nowadays, most of the reports on the discovery of novel phycoerythrin active peptides are based on the use of physical experiments, and the use of bioinformatics for the discovery of phycoerythrin active peptides is not common. For this reason, the present study mainly focuses on algal red protein as the research object, and uses bioinformatics software to simulate enzymatic cleavage of algal red protein, to screen out peptides that are non-toxic after enzymatic cleavage and have antimicrobial, antioxidant and immunomodulatory activities, and to find out novel algal red protein active peptides that have a good affinity with receptor proteins through molecular docking test. Through the research of this topic, the peptide spectrum can be obtained quickly and accurately by simulating enzyme digestion to precisely reveal the enzyme cutting site and release specific peptide fragments; after toxicity and activity analysis, the sequence characteristics

of the active peptides are systematically analyzed, and peptide fragments with antioxidant and antimicrobial activities can be predicted, which will provide support for the development of novel products of phycoerythrin active peptides in the fields of food and medicine, etc.; finally, the molecular docking can visualize the relationship between the peptides and the protein receptor. Finally, molecular docking can intuitively present the conformational relationship between peptides and protein receptors, clarify the interaction mechanism between phycoerythrin active peptides and specific targeting molecules, and promote the design and development of food and medicine, so as to contribute to the development of China's society.

The relevance of this study lies in the use of bioinformatics tools for simulating enzyme digestion and activity analysis.

The purpose of this study is to use bioinformatics tools to simulate enzyme digestion and activity analysis to identify potential phycoerythrin active peptides, providing insights for related research.

The object of the study is bioactive peptides obtained from phycoerythrin.

The subject of the study is modeling the process of enzymatic cleavage and analysis of the activity of bioactive peptides obtained from phycoerythrin.

Scientific novelty and research methods. First, this study uses simulated enzymatic digestion to search for new active peptides of phycoerythrin. The amino acid sequence of phycoerythrin was simulated by using BIOPEP tool to optimize the enzymatic conditions on the basis of economic feasibility, to guide the experimental design and to improve the experimental efficiency. Next, in this study, bioinformatics tools were used to analyze the bioactivity and physicochemical properties of the active peptides of phycoerythrin. By using Toxin red, Innovagen and other bioinformatics software to predict the potential biotoxicity and water solubility of the enzymatically digested phycoerythrin peptides, the active phycoerythrin peptides were obtained and ensured to be safe for use; the antibacterial, immunomodulatory and antioxidant properties of the

peptides were predicted by using the four software, namely, CAMPR3, IL2pred, IL4pred and AnOxPP, to find peptides with antimicrobial, immunomodulatory and antioxidant properties. The four software, CAMPR3, IL2pred, IL4pred and AnOxPP, were used to predict the antibacterial, immunomodulatory and antioxidant properties of the peptides to find the peptides with certain biological activities; PepDraw was used to analyse the physicochemical properties of the peptides, such as isoelectric point, hydrophobicity, etc., to comprehensively evaluate the active peptides of the algal red protein. Finally, in this study, the molecular docking test was used to screen the active peptides of phycoerythrin. The molecular docking test using AutoDock Vina software can predict the binding sites, hydrogen bonding interactions and other binding modes between the active peptides of phycoerythrin and receptor proteins, and analyse the binding affinity of the active peptides and receptor proteins, so as to clarify the mechanism of interaction between the active peptides of phycoerythrin and receptor proteins, and to screen out peptide segments with strong binding ability and high activity.

The practical significance of the obtained results is that they can help us quickly and efficiently obtain potential phycoerythrin active peptides, providing support for future research.

Chapter I
LITERATURE REVIEW

1.1 Overview of phycoerythrin

Phycoerythrin (PE) is a light-trapping pigment protein of algae that participates in photosynthesis and facilitates energy transfer, which is classified according to its spectral properties and belongs to the group of phycobiliproteins (PBPs) together with phycocyanin (PEC), phycocyanin (PC), and allophycocyanin (APC)[2] Phycoerythrin is widely distributed, mainly found in red algae, cyanobacteria and cryptophytes, and according to the source of phycoerythrin, it can be divided into three types: R-, C-, and B-, and it is highly representative in research and application because of its optical properties such as high absorption coefficient[3].

Phaeoglobin is a complex formed by the covalent binding of oligomeric proteins to open-chain tetrabilin chromophores through thioether bonds. The molecular weight of phycoerythrin is 240,000 daltons, and it has three subunits, α, β and γ. The molecular weight of each α and β subunit is about 20,000 daltons, and that of the γ subunit is about 30,000 daltons. The protein structure composed of these three subunits freely has the following two main forms, the most widely used form is assembled and folded by α and β subunits into a stable $\alpha\beta$ dimer or hexamer structure, the structure is simple making the mechanism of action more clear, and it can be efficient to complete the work; the other form is that, because of the small portion of phaeohydroglobin containing γ subunits, the three subunits of α, β, and γ are composed of a common ($\alpha\beta$)6-γ hexamer [4], which ensures a solid and stable scaffold for phaeohysterin.

On the luminescent moiety that constitutes phycoerythrin, the pigment molecules are mainly two major groups, phycoerythrin and phytouril[5], which can improve the ability of phycoerythrin to absorb light and increase the absorption range, for example, phycoerythrin's maximum absorbed light range is 540-570 nm[6]. In order to obtain alginate with high purity quickly and in large quantities, scholars at home

and abroad have continuously proposed and improved the separation and purification methods of alginate. For example, Song et al[7] purified algal red protein by using altar purslane as raw material, applying churning method and ultrafiltration-hydrogen bonding adsorption to separate and purify algal red protein, and finally obtained a purity of 7.98 and a recovery of 74.8%, which was simpler and quicker than that of the traditional method of salting out-gel filtration chromatography separation; Cui et al[8] used the tissue crushing method to break up the cells of Loblastus edulis and used the method of hydrophobic dwelling-molecule screening chromatography to obtain the algal red protein with a purity of 3.8; Dong et al[9] used red cytosol as raw material, took repeated freeze-thaw method to break red cytosol cells, and separated and purified phycoerythrin by ammonium sulfate graded precipitation and hydrophobic chromatography, and the purity of the final product could be up to 6.63; Ulagesan et al[10] used the red alga Chlorophyceae as raw material, and obtained the purity of 5.4 phycoerythrin by using ammonium sulfate precipitation method with fast protein liquid chromatography; Silva-Núñez et al[11] used repeated freeze-thawing and membrane filtration to isolate and purify phycoerythrin from the red alga Chlorophyceae, obtaining a purity of 6.06.

Most of these methods of isolation and purification of phycoerythrin use one or a combination of one or more of repeated freeze-thawing, ammonium sulphate graded precipitation and chromatographic purification, etc., to break up the cells and purify the products in an all-round, multistep process to obtain phycoerythrin with a high degree of purity.

Phaeoglobin itself possesses a variety of properties, so it has a variety of uses. According to current scientific research, phaeoglobin has properties such as fluorescence and immunomodulation[12], which can be applied in different fields. For example, alginin contains pigment molecules such as phycoerythrin and other pigment molecules with distinctive colors, so it can be used as a natural food pigment additive[13], which is green and healthy and safer for the human body; since alginin is a light-trapping protein that can absorb light energy at specific wavelengths and transfer

energy, alginin can also be used as a fluorescent probe [14] to visualize biological factors such as cells and to play a role in cell biology and molecular diagnosis. Nowruzi et al[15] found that phaeoglobin has an anti-inflammatory effect on human fibroblasts. Finally, because phaeoglobin possesses biological activities such as antioxidant and immunomodulation[16], it has important research implications in clinical medicine for anti-cancer and antitumor studies.

1.2 Overview of phycoerythrin-activated peptides

Bioactive peptides are usually peptide compounds with specific biological activities, such as dipeptides or polypeptides, which are composed of 20 amino acids connected by peptide bonds and formed in different combinations and arrangements[17]. Because of the ocean's large footprint in the earth, its diverse ecological environment, and the abundance of species present, marine-derived active peptides have become a research hotspot. Marine-derived bioactive peptides are small molecule specific functional peptides obtained from marine organisms or enzymatically cleaved from proteins present in marine organisms. Most of the marine-derived bioactive peptides have antioxidant, antimicrobial, antifreeze, immunomodulatory, and metal elements required for the survival and growth of organisms[18], and have great potential for development[19]. Phycoerythrin peptide is one kind of marine bioactive peptide produced by marine algae. Phycoerythrin active peptides are peptides extracted from phycoerythrin as raw material by enzymatic and hydrolysis processes, which are commonly used in the fields of food, pharmaceuticals, cosmetics, and clinical medicine[20], and they can play a role in antioxidant, genetic engineering, and fluorescence immunoassay[21].

There are some special amino acid sequences in phycoerythrin that are not biologically active by themselves, but after hydrolysis by specific enzymes, they can release biologically active peptides with different mechanisms of action as well as functions. For example, in anti-tumour[22] function, in photodynamic therapy,

phaeophytin white based peptide can generate single-line oxygen with strong oxidative capacity, which can significantly enhance the killing effect of laser on tumor cells, destroy the tumor cell structure and induce tumor cell apoptosis; in antioxidant[23] function, the amino acid sequences or functional groups on the phaeophytin active peptide can play an antioxidant role by providing hydrogen ions to scavenge the free radicals (ABTS, DPPH, hydroxyl radicals) to exert antioxidant effects; in disease prevention[24] functions, phaeoglobin active peptides can prevent mental illnesses by activating TrkB receptor-ERK1/2 signaling and decreasing PFOS-mediated susceptibility to calcium dysregulation, and by enhancing the vitality of frontal cortex neurons; in blood pressure lowering functions, phaeoglobin active peptides can specifically bind to the active site of ACE, reduce the generation of angiotensin II by inhibiting the activity of angiotensin-converting enzyme (ACE), attenuate the contractile effect of small arteries and reduce peripheral vascular resistance, and reduce blood pressure. According to the function and mechanism of action of phycoerythrin above, phycoerythrin active peptide has important research and application value in practical application[25,26].

The main methods for the preparation of bioactive peptides include enzymatic hydrolysis, microbial fermentation and chemical synthesis[27]. Enzymatic method is the most commonly used method for the preparation of bioactive peptides[28] , which is the hydrolysis of proteins by different enzymes, and due to the different enzymes produce different specificity for proteins, which leads to the release of peptides with a variety of bioactivities, which has the advantages of low cost and high efficiency, etc.; microbial fermentation is the inoculation of microorganisms onto proteins, and the use of enzyme systems produced during the fermentation process to decompose proteins to produce peptides It has the advantages of non-toxicity and low cost [29], but it is difficult to control because it requires the enzyme system in the fermentation process; chemical synthesis is the method of chemically synthesizing peptides of known amino acid sequences in the laboratory using specific experimental equipment, and it has the advantage of high specificity because the synthesis of peptides requires known amino

acid sequences, which makes it more demanding for the peptides. In the preparation of active peptides of algal red protein, the academic research mainly focuses on the preparation of peptides by enzymatic method. Wu et al[30] used Trichoderma-erythro-rhizumabrachiatum as raw material, adopted 35-50% ammonium sulfate salting out and DEAE-Sepharose anion-exchange column purification to obtain high-purity phycoerythrin, and then used porcine pepsin and trypsin to carry out step-by-step enzymatic hydrolysis of phycoerythrin to prepare phycobiliprotein ACE inhibitory peptide; Xie et al.[31] prepared prolyl endopeptidase inhibitory peptides by enzymatic hydrolysis of phycoerythrin using gastric and intestinal digestive enzymes, which can play a significant role in clinical treatment; Wu et al[32] generated angiotensin I-converting enzyme (ACE) inhibitory peptide by hydrolysis of extracted phycoerythrin R-type by pepsin and trypsin, and purified the hydrolysate by gel permeation and reversed-phase chromatography in turn, and obtained two peptide chains, which were resistant to digestion of common proteases in gastrointestinal tract, and could be used as potential inhibitors for the digestion of common proteases. Both peptide chains can resist the digestion of common proteases in the gastrointestinal tract, which can be used as potential nutraceuticals for the development of functional foods and promote the development of China's food industry.

1.3 Bioinformatics Online Prediction Tool

At present, the main methods used in academia to prepare bioactive peptides are enzymatic hydrolysis, microbial fermentation, and chemical synthesis. These methods all require experimentation and are greatly influenced by external environments, resulting in high experimental costs and poor controllability of the reaction process, which may not achieve the expected results. Take enzymatic hydrolysis[33] as an example. Enzymatic hydrolysis is a traditional method for preparing bioactive peptides. Due to the different time, temperature, pH, etc. required for protein degradation by different proteases[34] finding suitable enzymatic hydrolysis

conditions require precise and extensive experiments on key sites, which is time-consuming and labor-intensive. In order to reduce experimental costs and improve experimental efficiency and success rate, it is necessary to combine traditional experimental methods with rapidly developing modern technology and use bioinformatics technology to obtain target active peptides. Bioinformatics prediction tools can predict the amino acid sequence of active peptides based on the enzyme cleavage sites of target proteins, analyze and judge the toxicity, water solubility, and other biological activities of active peptides, determine their affinity with ligands, and screen peptides with strong binding ability and high activity to the target. The main steps in searching for new bioactive peptides are conducting simulated enzyme digestion and molecular docking experiments.

1.4 Simulated digestion tools

Simulated zymotomy is a method in which a computer uses bioinformatics software to perform zymotomy using known protein sequences and recognisable sites of proteases to obtain the target amino acid sequence. Currently, the software that can perform simulated digestion include BIOPEP, Peptide Cutter and MaxQuant. The commonly used tools for simulated digestion are BIOPEP (https://biochemia.uwm.edu.pl/en/biopep-uwm-2) and Peptide Cutter (https:// web.expasy.org /peptide cutter/), which have their own advantages and disadvantages. BIOPEP in the field of bioactive peptides is biased towards the study of food peptides, and the database consists of six parts, including proteins, allergenic proteins, bioactive peptides and sensory peptides[35], which covers a wide range of protein activities, and can completely reflect the information contained in the protein molecule; the tool is also able to take into account the variability of the enzyme digestion of proteins and provide personalised enzyme digestion results; since the software can Because the software can support quantitative descriptive calculations and the use of multiple enzymes to jointly simulate the cutting of proteins, so it can be flexible and mobile

experiments, but due to the previous software mainly used as a teaching software for students, the database is not large enough, for example, it contains a protein database is much smaller than the main database of Uniprot[36]. The advantages of Peptide Cutter are that the webpage page is concise, and can be quickly and easily operated.

The advantages of Peptide Cutter are the simplicity of the web page, which can be operated quickly and easily; the large number of enzyme data in the database can provide a variety of choices for proteolytic digestion of proteins by proteases, which can be applied to a wide range of conditions; lastly, the tool can fully demonstrate the probability of enzyme digestion, which can provide the feasibility of the enzyme digestion and provide a basis for the later physical validation experiments.

However, the disadvantage of this procedure is that it is unable to perform parallel or automatic sequential digestion of different protein sequences to reduce the experimental efficiency, and the procedure cannot use different enzymes to perform digestion of the same protein sequence at the same time[37], which needs to be carried out several times to waste the experimental time. Since simulated digestion demonstrates the economy of cost and time in experiments, scholars have great interest in using simulated digestion software to study bioactive peptides. For example, Chen et al [38] used Peptide Cutter to simulate enzymatic cleavage of soybean 7s globulin; Zhou et al[39] used bioinformatics software (BIOPEP and Peptide Cutter) to find new active peptides in Macrobrachium nematodes, which can play a role in anti-hypertension and anti-diabetes.

1.5 Bioinformatics tools for analysing physicochemical properties and biological activities

After simulated enzymatic cleavage of phycoerythrin, in order to obtain the target phycoerythrin peptide with high activity, it is necessary to analyse and screen the physicochemical properties and biological activities of the obtained peptide sequences, to ensure the safety and practicality of the obtained peptide chains. To

predict the physicochemical properties of peptides, three software programs, PepDraw, ToxinPred and Innovagen, are commonly used.

PepDraw (https://www2.tulane.edu/~biochem/WW/PepDraw/) is a tool for analysing the physicochemical properties of peptide sequences, which can be inputted into the amino acid peptide sequence and then analysed in real time. After inputting the amino acid peptide sequence, it can generate the primary structure of amino acids in real time and predict the physicochemical properties of peptide sequences such as isoelectric point, molecular weight, and hydrophobicity[40], for example, et al[41] used the PepDraw tool to predict the physicochemical properties of active peptides such as isoelectric point in the screening of snakehead-derived anti-inflammatory peptides, which facilitates the researchers to understand the structure of the peptide sequences and other physicochemical information fully.

ToxinPred (https://webs.iiitd.edu.in/raghava/toxinpred /index.html) is a tool to predict the toxicity of peptide sequences, which can guarantee the safety of peptides by predicting the potential toxicity of the products of protein digestion; Innovagen (http://www.innovagen.com / proteomics-tools) is a tool used to predict the water solubility of bioactive peptides, and the prediction of water solubility facilitates the homogeneous distribution of active peptides in aqueous solution and improves the absorption rate and utilisation efficiency of food or drugs. For example, Zhou et al[42] used ToxinPred and other software to analyse the toxicity and other bioactivities of the products of enzymatic digestion of rice gluten. For predicting the biological activities of peptides, four software, CAMPR3, IL2pred, IL4pred and AnOxPP, are usually used to predict the antimicrobial, immunomodulatory and antioxidant properties of the studied peptides.CAMPR3 (http://www.camp3.bicnirrh.res.in/) is a tool for predicting the antimicrobial properties of peptides, and users can freely choose one or more algorithms of Random Forest Classifier, Artificial Neural Network Classifier for prediction according to the data characteristics of antimicrobial peptides to increase the probability of successful prediction of antimicrobial peptides.

Zhu et al[43]used the CAMPR3 tool in the prediction of antimicrobial peptides present in sesame seeds; IL2pred (https: //webs.iiitd.edu.in/ raghava/il2pred/index.html) and IL4pred (https:// webs. iiitd.edu.in/ Raghava /il4pred /index.php) are tools for predicting the immunomodulatory properties of peptides, which can play an It can help to understand the target and mechanism of action of peptides in immunomodulation and promote immunological drug research; AnOxPP (http:// www.cqudfbp.net/AI-Tools/AnOxPP/index.jsp) is a tool for predicting the antioxidant property of peptides, which can better predict the antioxidant property of peptides by using the bi-directional long and short-term memory prediction mechanism. antioxidant properties of peptides, which allows better screening of antioxidant peptides[44]. In summary, these bioinformatics tools can be used to find peptide fragments with good physicochemical properties and some biological activities, which can help food and pharmaceutical companies to follow the relevant laws and regulations and prevent the safety risks, to ensure that the resulting products meet the safety and quality standards, so as to ensure that the companies can carry out their production and business activities normally.

1.6 Molecular docking tools

Molecular docking technology is a computer method to analyse the spatial matching and interaction mode between biological macromolecular substances and ligands, and to predict the binding mode and affinity of ligands and receptors. Nowadays, software capable of molecular docking include AutoDock, AutoDock Vina and Discovery Studio, etc. The commonly used molecular docking software are mainly AutoDock (https://autodock.scripps.edu/), AutoDock Vina (https:// github.com/ ccsb-scripps/ Auto Dock-Vina) and Discovery Studio (https://accelrys.com/products/discovery-studio/) are the three software. AutoDock adopts an empirical free ability field and can predict the correct arrangement of multiple hydrogen bonding groups[45] , has several search algorithms such as

Lamarckian Genetic Algorithm and Monte Carlo Simulated Annealing Algorithm, which can efficiently find the binding space of ligand and receptor. In the docking method, it uses semi-flexible docking, the ligand needs to make changes according to the receptor's conformation, and the docking accuracy is high, which is suitable for docking small molecules to large molecule models[46] .

The disadvantages of AutoDock are that the file input and ligand docking are slow and inefficient, and the program's application is in the form of a single thread, which is not conducive to analysing large and complex molecular structures[47]. AutoDock Vina software belongs to the same AutoDock suite as AutoDock, but it is faster than AutoDock in terms of calculation speed and accuracy of exported results, and supports simultaneous docking of multiple ligands and provides Python docking, which is convenient for researchers to carry out their work, but there are also drawbacks of AutoDock Vina[48], it is not able to support macrocyclic and flexible molecules, and it is not possible to drastically Discovery Studio is easy to operate, less specialized and suitable for a wide range of users; the docking method is flexible docking, the conformation of the receptor and the ligand can be changed freely, and small molecules can be docked with large and small molecules, which is suitable for a wide range of applications; the software is equipped with visualization functions and accepts customized output, which makes it easy for users to work with the software. The software has a visualization function and accepts customized output, which makes it easy to use the user's personalised settings[49].

The shortcomings of the Discovery Studio software are that it is a commercial paid software, which requires the payment of a high license fee, and because it needs to deal with the screening of biological macromolecules and the analysis of data, it has high requirements for computer hardware, and the economic cost requirements are high. Molecular docking technology can conveniently and accurately solve similar problems such as the interaction between biomolecules and ligands, so scholars often use molecular docking technology when studying related problems. For example, Yang et al[50] used bioinformatics software to screen a novel glucosidase inhibitory peptide

from walnuts, which played a role in alleviating diabetes.

Summary of the chapter I

This chapter elaborates on phycoerythrin and phycoerythrin active peptides, which are suitable for researching and developing potential bioactive peptides due to their various advantages.

This chapter elaborates on various bioinformatics tools, which are suitable for studying bioactive peptides due to their various advantages.

Chapter II

OBJECT, PURPOSE, AND METHODS OF THE STUDY

2.1 Experimental materials

UniProt database (https://www.uniprot.org/), BIOPEP tool (https:// biochemia. uwm.edu.pl/en/biopep-uwm-2/), Peptide Ranker tool (http:// distilldeep. ucd.ie/ PeptideR-anker /).

Table 2.1 **Phycocyanin raw material**

Uniprot serial number	Protein	Amino acid sequence	Amino acid length
1	*2*	*3*	*4*
		MKSVITTTISAADAAGRFPSSSDLESVQG	
P513 68	R-phycoerythrin alpha chain	NIQRAAARLEAAEKLASNHEAVVKEAG DACFAKYSYLKNPGEAGDSQEKVNKCY RDVDHYMRLVNYCLVVGGTGPVDEWG IAGAREVYRTLNLPTSAYVASFAFARDR	164
		LCVPRDMSAQAGVEYAGNLDYIINSLC	
		MKSVITTTISAADAAGRYPSTSDLQSVQ	
P848 61	R-phycoerythrin alpha chain	GNIQRAAARLEAAEKLGSNHEAVVKEA GDACFSKYGYNKNPGEAGENQEKINKC YRDIDHYMRLINYTLVVGGTGPLDEWGI AGAREVYRTLNLPSAAYIAAFVFTRDRL	164
		CIPRDMSAQAGVEFCTALDYLINSLS	
		MKSVITTVVSAADAAGRFPSNSDLESIQG	
P113 92	B-phycoerythrin alpha chain	NIQRSAARLEAAEKLAGNHEAVVKEAG DACFAKYAYLKNPGEAGENQEKINKCY RDVDHYMRLVNYDLVVGGTGPLDEWGI AGAREVYRTLNLPTSAYVASIAYTRDRL	164
		CVPRDMSAQAGVEFSAYLDYLINALS	
		MLDAFSRVVVNSDAKAAYVGGSDLQAL	
		KKFIADGNKRLDSVNAIVSNASCIVSDA	
P689 39	R-phycoerythrin beta chain	VSGMICENPGLIAPGGNCYTNRRMAACL RDGEIILRYVSYALLAGDPSVLEDRCLNG LKETYIALGVPTNSSVRAVSIMKAAAVA	177

Continued Table 2.1

1	*2*	*3*	*4*
		FITNTASQRKMATADGDCSALASEVASY CDRVAAAIS	
Q020 37	B-phycoerythrin beta chain	MLDAFSRVVVNSDTKAAYVGGSDLQAL KKFIADGNKRLDSVNAIVSNASCVVSDA VSGMICENPGLITPGGNCYTNRRMAACL RDGEIIIRYVSYALLSGDPSVLEDRCLNG LKETYIALGVPTNSNARAVDIMKASVVA LINNTATLRKMPTPSGDCSALAAEAGSY FDRVNSALS	177
P848 62	R-phycoerythrin beta chain	MLDAFSRVVVNSDSKAAYVSGSDLQAL KTFINDGNKRLDAVNYIVSNSSCIVSDAI SGMICENPGLITPGGNCYTNRRMAACLR DGEIILRYVSYALLAGDASVLEDRCLNG LKETYIALGVPTNSTVRAVSIMKAAAVC FISNTASQRKVEVIEGDCSALASEVASYC DRVVAAVS	177
P347 84	R-phycoerythrin gamma chain, chloroplastic	MASPAFAVNGMFTPVKLSGSFTASMPV DSKPAASATGVRMVVDPLQRKYQSIGKI GVDYSRPKKLATYVRSGYSVGMEFPNTP SMAGHYSLTDCDKAGGAAKILMKYDEY CAKGMLQVGKRAACRTGVYTTKCTEGT QPQMAFDVRVFNRTQAFRQAQKPVAAR LREQYEARKACFVLAHNCSREEAQFKE MPMSCATFLASKMEATGACYRTVRPTS VAEDYMAGSVRAQLYTKLNPKGVYGV GACEDGHAKGDADQRRVIALASEYRAA AQSPSTVTGQQYKSAQLATQLFAHDCH HEQEQIYEYPAVAAAMCRY	317

2.2 Experimental Methods

2.2.1 Searching for Phycoerythrin sequences

Using a computer to search for the keyword ‘Phycoerythrin’ in the UniProt (https://www.uniprot.org/) database to find the verified amino acid sequences of phycoerythrin, seven different phycoerythrin chains were identified in Table 2-1. Among them, the amino acid sequences used in selecting the raw materials of

phycoerythrin included the red algae Phycoerythrin (P51368 and P68939), Polytubularia (P84861 and P84862), Chlorella vulgaris (P11392 and Q02037) and Red algae (P34784); and the sources of phycoerythrin included the α (P51368, P84861 and P11392), β (P51368 and P11392), β (P51368 and P11392), and α (P51368, P84861, and P11392) of phycoerythrin. and P11392), β (P68939, Q02037 and P84862) and γ chains (P34784) of phycoerythrin.

2.2.2 Simulated enzymatic digestion

Using the Enzyme action panel of the BIOPEP (https :// biochemia. uwm.edu.pl/en/biopep-uwm-2/) tool, pepsin PH 1.3 (EC3.4.23.1), trypsin (EC3.4.21.4), papain (EC3.4.22.2) and proteinase K (EC3.4.21.64), four common proteases, were used to simulate the enzymatic cleavage of the found phycoerythrin chains, and the enzymatic cleavage of phycoerythrin by various proteases was analysed to find two suitable proteases to simulate the enzymatic cleavage of phycoerythrin. The digested peptides of the two proteases were used to predict the potential biological activities using the Peptide Ranker (http://distilldeep.ucd.ie/PeptideRanker/) program, and the peptide sequences with scores >0.5 were used for the next step of physicochemical properties and biological activities.

2.3 Results and analyses

2.3.1 Analysis of phaeoglobin mimicry digestion results

The seven phycoerythrin chains found were subjected to simulated enzymatic digestion using pepsin, trypsin, papain and proteinase K, respectively, and a total of 910 peptides and 193 active peptides were obtained, and the resulting peptides are shown in Figure 2.1. Due to the different cleavage sites of each enzyme, the composition and number of peptides obtained were different, of which the most

peptides obtained by enzymatic digestion with papain were 360, and the least peptides obtained by enzymatic digestion with pepsin were 123; after screening with the activity prediction tool Peptide-Ranker, the most active peptides were obtained by enzymatic digestion with papain, which were 80, and the least active peptides were obtained by enzymatic digestion with trypsin, which were 25.

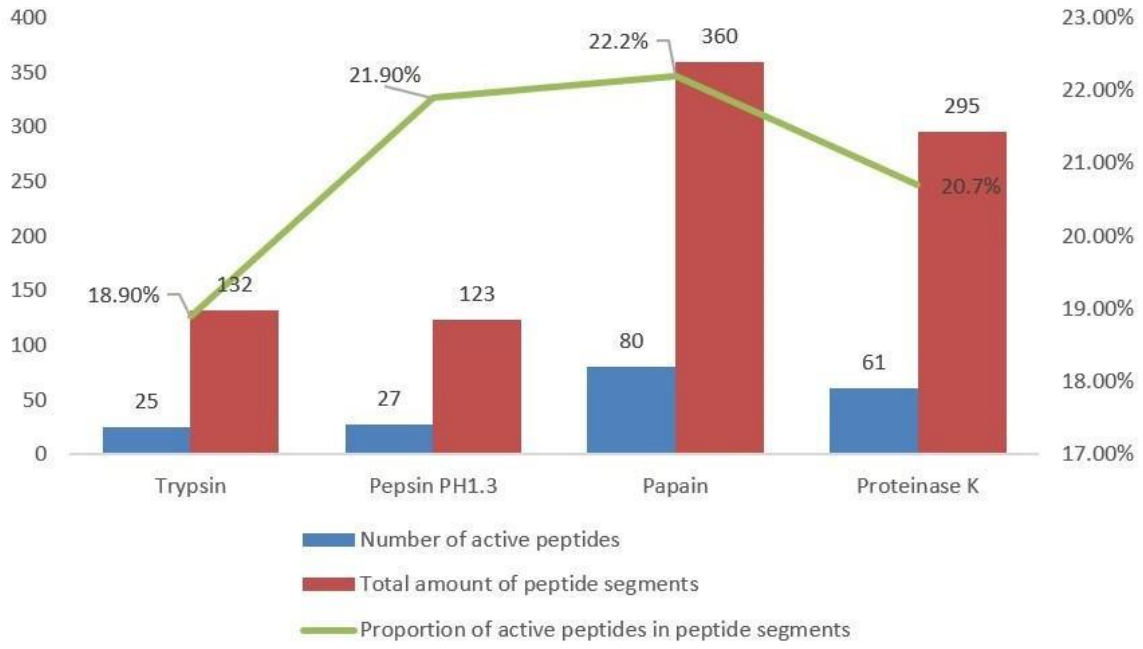

Figure 2.1 The results of simulated enzyme digestion of phycoerythrin by different enzymes

The least number of active peptides was 25. According to the number of obtained peptide fragments and active peptides, the percentage of active peptides in peptide fragments was obtained, and the papain cleavage product accounted for the highest percentage of 22.2%, and the trypsin cleavage product accounted for the lowest percentage of 18.9%. Due to the papain cleaved peptides are too fragmented (the majority of biologically active dipeptides), and there are a large number of repetitive fragments (49 repetitive fragments), the actual usable fragments are less, so in this paper to simulate the work of enzyme cleavage selection of the protease used in the protease, comprehensively consider the protease on the alginate red protein cleavage activity as well as after the enzyme peptides of the length of the peptide, how many

and other factors, the use of pepsin and proteinase K, the joint enzymatic degradation of the alginate red protein. Pepsin and proteinase K were used for the enzymatic digestion of alginate.

A total of 302 peptides were obtained after simulated enzymatic cleavage of the alginate chains using pepsin and proteinase K together, and the resulting peptides (with two or more amino acids) for each part are shown in Table 2.2.

Table 2.2 **Simulated enzymatic digestion results of phycoerythrin**

Uniprot serial number	Name of phycoerythrin chain	Peptide results (two or more)	Number (s)
1	*2*	*3*	*4*
P5136 8	R-phycoerythrin alpha chain	KSV、TTTI、SAADAAGRF、SSSDL、ESV、QGNI、QRAAARL、EAAEKL、ASNHEAV、KEAGDACF、AKY、SY、KNP、GEAGDSQEKV、NKCY、RDV、DHY、RL、NY、CL、GGTGP、DEW、GI、AGAREV、RTL、NL、TSAY、ASF、AF、ARDRL、CV、RDM、SAQAGV、EY、AGNL、DY、NSL	37
P8486 1	R-phycoerythrin alpha chain	KSV、TTTI、SAADAAGRY、STSDL、QSV、QGNI、QRAAARL、EAAEKL、GSNHEAV、KEAGDACF、SKY、GY、NKNP、GEAGENQEKI、NKCY、RDI、DHY、RL、NY、TL、GGTGP、DEW、GI、AGAREV、RTL、NL、SAAY、AAF、TRDRL、CI、RDM、SAQAGV、EF、CTAL、DY、NSL	36
P1139 2	B-phycoerythrin alpha chain	KSV、TTV、SAADAAGRF、SNSDL、ESI、QGNI、QRSAARL、EAAEKL、AGNHEAV、KEAGDACF、AKY、AY、KNP、GEAGENQEKI、NKCY、RDV、DHY、RL、NY、DL、GGTGP、DEW、GI、AGAREV、RTL、NL、TSAY、ASI、AY、TRDRL、CV、RDM、SAQAGV、EF、SAY、DY、NAL DAF、SRV、NSDAKAAY、GGSDL、QAL、KKF、ADGNKRL、DSV、NAI、	37
		SNASCI、SDAV、SGM、CENP、GL、	

Continued Table 2.2

1	*2*	*3*	*4*
P6893 9	R-phycoerythrin beta chain	AP、GGNCY、TNRRM、AACL、RDGEI、RY、SY、AL、AGDP、SV、EDRCL、NGL、KETY、AL、GV、TNSSV、RAV、SI、KAAAV、AF、TNTASQRKM、ATADGDCSAL、ASEV、ASY、CDRV、AAAI	40
Q0203 7	B-phycoerythrin beta chain	DAF、SRV、NSDTKAAY、GGSDL、QAL、KKF、ADGNKRL、DSV、NAI、SNASCV、SDAV、SGM、CENP、GL、TP、GGNCY、TNRRM、AACL、RDGEI、RY、SY、AL、SGDP、SV、EDRCL、NGL、KETY、AL、GV、TNSNARAV、DI、KASV、AL、NNTATL、RKM、TP、SGDCSAL、AAEAGSY、DRV、NSAL	40
P8486 2	R-phycoerythrin beta chain	DAF、SRV、NSDSKAAY、SGSDL、QAL、KTF、NDGNKRL、DAV、NY、SNSSCI、SDAI、SGM、CENP、GL、TP、GGNCY、TNRRM、AACL、RDGEI、RY、SY、AL、AGDASV、EDRCL、NGL、KETY、AL、GV、TNSTV、RAV、SI、KAAAV	40
		、CF、SNTASQRKV、EV、EGDCSAL	
P3478 4	R-phycoerythrin gamma chain, chloroplastic	、ASEV、ASY、CDRV、AAV ASP、AF、AV、NGM、TP、KL、SGSF、TASM、DSKP、AASATGV、RM、DP、QRKY、QSI、GKI、GV、DY、SRP、KKL、ATY、RSGY、SV、GM、EF、NTP、SM、AGHY、SL、TDCDKAGGAAKI、KY、DEY、CAKGM、QV、GKRAACRTGV、TTKCTEGTQP、QM、AF、DV、RV、NRTQAF、RQAQKP、AARL、REQY、EARKACF、AHNCSREEAQF、KEM、SCATF、ASKM、EATGACY、RTV、RP、TSV、AEDY、AGSV、RAQL、TKL、NP、KGV、GV、GACEDGHAKGDADQRRV、AL、ASEY、RAAAQSP、STV、TGQQY、KSAQL、ATQL、AHDCHHEQEQI、EY、AV、AAAM、CRY	72

In order to find biologically active peptides among the enzymatically digested peptides, the peptides obtained in Table 2.2 need to be scored for activity using the

Peptide Ranker database, and peptides with a final test result score of >0.5 are considered to be biologically active, as shown in Table 2.3.

Table 2.3 **Biological activity score of peptide segments**

Uniprot serial number	Name of phycoerythrin chain	Peptide sequence	The numerical value of Peptide Banker score (>0.5)	Number (s)
1	*2*	*3*	*4*	*5*
P51268	R-phycoerythrin alpha chain	AF	0.973259	8
		CL	0.879917	
		ASF	0.772311	
		SAADAAGRF	0.757443	
		RL	0.626352	
		RDM	0.571844	
		GGTGP	0.558284	
		GI	0.521628	
P84861	R-phycoerythrin alpha chain	AAF	0.833322	8
		GY	0.741592	
		CI	0.660168	
		RL	0.626352	
		EF	0.598976	
		RDM	0.571844	
		GGTGP	0.558284	
		GI	0.521628	
P11392	B-phycoerythrin alpha chain	SAADAAGRF	0.757443	6
		RL	0.626352	
		EF	0.598976	
		RDM	0.571844	
		GGTGP	0.558284	
		GI	0.521628	
P68939	R-phycoerythrin beta chain	AF	0.973259	11
		GL	0.808777	
		DAF	0.804969	
		SGM	0.778471	
		GGNCY	0.718971	
		AACL	0.673804	
		AP	0.626856	
		AGDP	0.559689	
		RY	0.543741	
		KKF	0.532541	
		NGL	0.507526	

Continued Table 2.3

1	*2*	*3*	*4*	*5*
Q02037	B-phycoerythrin beta chain	GL	0.808777	10
		DAF	0.804969	
		SGM	0.778471	
		GGNCY	0.718971	
		AACL	0.673804	
		SGDCSAL	0.571482	
		RY	0.543741	
		SGDP	0.540729	
		KKF	0.532541	
		NGL	0.507526	
P84862	R-phycoerythrin beta chain	CF	0.99641	8
		GL	0.808777	
		DAF	0.804969	
		SGM	0.778471	
		GGNCY	0.718971	
		AACL	0.673804	
		RY	0.543741	
		NGL	0.507526	
P34784	R-phycoerythrin gamma chain, chloroplastic	AF	0.973259	14
		AF	0.973259	
		GM	0.953169	
		RM	0.847822	
		RP	0.822267	
		SGSF	0.810608	
		CRY	0.793881	
		SCATF	0.782501	
		NGM	0.755652	
		SM	0.628268	
		SRP	0.616922	
		QM	0.607122	
		CAKGM	0.600706	
		EF	0.598976	

By using this tool, it was found that out of a total of 302 peptides, the peptides were scored in the range of 0.03 to 0.99, and the biological activity of the obtained peptides varied greatly. Of these, 65 scored above 0.5 and were considered to be potentially biologically active and were target peptides that could be used for further manipulation. Among these available peptides, according to the length of the amino acid sequence of the peptides, there are 29 dipeptides and 36 tripeptides and above, and the peptides have a high degree of complexity, which can carry more biological information, which is conducive to the stabilisation of the peptide structure and rich

biological activity. According to the different sources of peptides, it can be concluded that there are 22 peptides of α-source, 29 peptides of β-source and 14 peptides of γ-source. From the joint Table 2.2 and Table 2.3, β-source active peptides accounted for the largest proportion of the overall peptides of the source, 24.2%; γ-source active peptides accounted for the smallest proportion of the overall peptides of the γ-source, 19.4%, which indicates that in general, compared with the α and γ chains of phycoerythrin chain, β-chain active peptides can play a better role due to the high proportion of the β-chain active peptides, and the large number of peptides that can be involved in the expression of the function of a specific physiological activity, thereby. The overall biological activity of the β-chain of phycoerythrin is better, which is more valuable in the extraction and study of bioactive peptides.

Summary of the chapter II

1. The BIOPEP tool can simulate enzyme digestion of proteins to obtain a large number of peptide segments, while the Peptide Ranker tool can screen for biologically active peptide segments from a large number of peptide segments

2. Using the BIOPEP tool, 7 phycoerythrin chains were simulated for enzymatic cleavage using pepsin (pH=1.3) and proteinase K, resulting in a total of 302 phycoerythrin peptide segments.

3. The biological activity of the obtained peptide segments was predicted using the Peptide Anker tool, and 65 peptide segments with predicted scores>0.5 were considered to have biological activity. Among them, the proportion of beta source active peptide segments was the highest.

Chapter III
EXPERIMENTAL PART

3.1 Analysis of biological activity and physicochemical properties of phycoerythrin peptides

3.1.1 Experimental materials

ToxinPred tool (https://webs.iiitd.edu.in /raghava /toxinpred /index.html), Innovagen tool (http://www.innovagen. com /proteomics-tools), CAMPR3 tools (http:// www.camp3. bicnirrh.res.in/), IL2pred tools (https://webs.iiitd.edu.in /raghava/ il2pred/index.html), IL4pred tools (https :// webs. ii i td. edu .in/ Raghava /il4pred /index.php), AnOxPP tool (http://www.cqudfbp. net/AI-Tools /AnOxPP /index.php), PepDraw tool (https:// www 2.tulane.edu / biochem/ WW/ Pep Draw/).

3.1.2 Searching for Phycoerythrin sequences

ToxinPred (https://webs.iiitd.edu.in/raghava/toxinpred /index.html) was used to predict the potential toxicity of phycoerythrin released after enzymatic digestion to ensure the safety of the peptide fragments; Innovagen (http:// www. innovagen. com / proteomics-tools) to predict the water solubility of active peptides and find peptide fragments that are easily soluble in water to ensure that the peptides are easily absorbed. The antimicrobial properties of the peptides were predicted using CAMPR3 (http://www.camp3.bicnirrh.res.in/); the antimicrobial properties of the peptides were predicted using IL2pred (https: //webs. iiitd.edu. in/raghava/ il2pred /index.html), IL4pred (https: // webs. iiitd.edu .in/ raghava/il4pred /index.php) to predict the immunoinducing activity of the peptide segment, thus demonstrating immunomodulatory properties: the peptide was predicted using AnOxPP (http://www.cqudfbp .net/AI-Tools /AnOxPP/index. jsp) to predict the antioxidant properties of the peptides; by using several of the above tools, the peptide specificity

was sufficiently predicted to find peptides with certain biological activities. The screened peptides were analysed by PepDraw (https:// www2.tulane.edu/~biochem/WW/PepDraw/) for molecular weight, hydrophobicity and other physicochemical properties of the peptide sequences to gain a deeper understanding of the peptides found.

3.1.3 Results and analyses

After predicting the biological activity of the peptides, the potential toxicity and water solubility of the peptides with biological activity need to be predicted to find a safe and water-soluble peptide, and the specifics of each peptide are shown in Table 3.1. Among them, potential toxicity and allergenicity can predict the danger in advance to ensure the safe use of the active peptide; good water solubility and easy to dissolve in water can ensure that the peptide is better digested and absorbed, and the peptide's bioactivity can be better utilised.

After screening through the above table, a total of 16 non-toxic and water-soluble peptide segments (no duplicated peptide segments) were found. Due to its safety and solubility in water, the 16 peptide segments have the basic conditions for research as bioactive peptides. Compare 16 peptide segments with validated peptide segments in the BIOPEP database and identify 9 potential peptide segments. To identify the biological efficacy of the 9 peptide segments, CAMPR3 tool was used to predict antibacterial activity (SVM classifier and artificial neural network classifier both predicted), AnOxPP tool was used to predict antioxidant activity, and IL2Pred and IL4red tools were used to predict immune regulation. The details are shown in Table 3.2.

After predicting the efficacy of the peptide segments, it was found that all 9 peptide segments have one or more biological characteristics. According to the characteristics of the peptide segments, there are a total of 4 peptide segments with antibacterial properties. There are a total of 9 peptide segments with immunomodulatory properties, including 9 peptide segments with IL2 immune

induction activity, 7 peptide segments with IL4 immune induction activity, and 7 peptide segments with both IL2 and IL4 immune induction activities.

Table 3.1 **Toxicity and water solubility testing of peptide segments**

Name of phycoerythrin chain	Peptide sequence	ToxinPred toxicity	Innovagen water-soluble
1	*2*	*3*	*4*
R-phycoerythrin alpha chain	AF	Nontoxic	Poor water solubility
	CL	Nontoxic	
	ASF	Nontoxic	
R-phycoerythrin alpha chain	SAADAAGR F	Nontoxic	Poor water solubility
	RL	Nontoxic	
	RDM	Nontoxic	
	GGTGP	Nontoxic	
	GI	Nontoxic	
	AAF	Nontoxic	
	GY	Nontoxic	
	CI	Nontoxic	
	RL	Nontoxic	
	EF	Nontoxic	
	RDM	Nontoxic	
R-phycoerythrin alpha chain	GGTGP	Nontoxic	Poor water solubility
	GI	Nontoxic	
R-phycoerythrin alpha chain	SAADAAGRF	Nontoxic	Poor water solubility
	RL	Nontoxic	
	EF	Nontoxic	
	RDM	Nontoxic	
	GGTGP	Nontoxic	
	GI	Nontoxic	
R-phycoerythrin beta chain	AF	Nontoxic	Poor water solubility
	GL	Nontoxic	
	DAF	Nontoxic	
R-phycoerythrin beta chain	SGM	Nontoxic	Poor water solubility
	GGNCY	Nontoxic	
	AACL	Nontoxic	
	AP	Nontoxic	
	AGDP	Nontoxic	Good water solubility
	RY	Nontoxic	
	KKF	Nontoxic	
	NGL	Nontoxic	Poor water solubility

Continued Table 3.1

1	*2*	*3*	*4*
R-phycoerythrin beta chain	GL	Nontoxic	Poor water solubility
	DAF	Nontoxic	Good water solubility
	SGM	Nontoxic	Poor water solubility
R-phycoerythrin beta chain	GGNCY	Nontoxic	Poor water solubility
	AACL	Nontoxic	
	SGDCSAL	Nontoxic	Good water solubility
	RY	Nontoxic	Poor water solubility
R-phycoerythrin beta chain	SDRP	Nontoxic	Good water solubility
	KKF	Nontoxic	
	NGL	Nontoxic	Poor water solubility
R-phycoerythrin beta chain	CF	Nontoxic	Poor water solubility
	GL	Nontoxic	
	DAF	Nontoxic	Good water solubility
	SGM	Nontoxic	Poor water solubility
R-phycoerythrin beta chain	GGNCY	Nontoxic	Poor water solubility
	AACL	Nontoxic	
	RY	Nontoxic	Good water solubility
	NGL	Nontoxic	Poor water solubility
R-phycoerythrin gamma chain, chloroplastic	AF	Nontoxic	Poor water solubility
	AF	Nontoxic	
	GM	Nontoxic	
	RM	Nontoxic	Good water solubility
	RP	Nontoxic	
	SGSF	Nontoxic	Poor water solubility
	CRY	Nontoxic	Good water solubility
	SCATF	Nontoxic	Poor water solubility
	NGM	Nontoxic	
	SM	Nontoxic	Good water solubility
	SRP	Nontoxic	
	QM	Nontoxic	Poor water solubility
	CAKGM	Nontoxic	Good water solubility
	EF	Nontoxic	

Table 3.2 **Prediction of antibacterial, antioxidant, and immunomodulatory properties of peptide segments**

Peptide sequence	Predicting antibacterial activity	Predicting antioxidant activity	IL2 immune induction activity	IL4 immune induction activity
SAADAAGRF	not have	have	have	not have
RDM	have	not have	have	have
DAF	have	have	have	have
AGDP	not have	have	have	have
KKF	have	have	have	have
SGDCSAL	not have	have	have	not have
SGDP	not have	have	have	have
CRY	not have	not have	have	have
CAKGM	have	not have	have	have

There are a total of 6 peptide segments with antioxidant properties. To better

understand these 9 peptide segments, the physicochemical properties and structural images of each peptide segment were obtained using the PepDraw database, as shown in Table 3.3 and Figure 3.1- Figure 3.9.

Table 3.3 **Physical and chemical properties of peptide segments**

Peptide sequence	Length	Dumpling	Isoelectric point	Net charge	Hydrophobicity	Extinction coefficient [1]	Extinction coefficient [2]
SAADAAGRF	9	864.4077	6.56	0	+15.25 Kcal * mol -1	0 M-1 * cm-1	0 M-1 * cm-1
RDM	3	420.1785	6.54	0	+12.68 Kcal * mol -1	0 M-1 * cm-1	0 M-1 * cm-1
DAF	3	351.1425	2.95	-1	+10.33 Kcal * mol -1	0 M-1 * cm-1	0 M-1 * cm-1
AGDP	4	358.1483	3.13	-1	+13.33 Kcal * mol -1	0 M-1 * cm-1	0 M-1 * cm-1
KKF	3	421.2681	10.59	+2	+11.79 Kcal * mol -1	0 M-1 * cm-1	0 M-1 * cm-1
SGDCSAL	7	651.2524	3.05	-1	+12.84 Kcal * mol -1	0 M-1 * cm-1	0 M-1 * cm-1
SGDP	4	374.1432	3.05	-1	+13.29 Kcal * mol -1	0 M-1 * cm-1	0 M-1 * cm-1
CRY	3	440.1836	8.71	+1	+8.98 Kcal * mol -1	1490 M-1 * cm-1	1490 M-1 * cm-1
CAKGM	5	508.2130	8.77	+1	+11.66 Kcal * mol -1	0 M-1 * cm-1	0 M-1 * cm-1

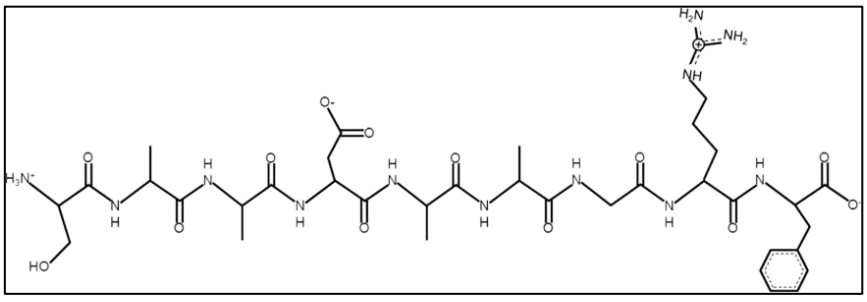

Figure 3.1 Chemical structure of peptide SAADAAGRF

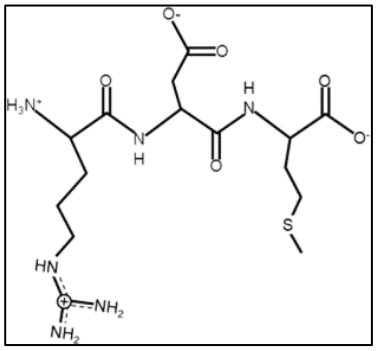

Figure 3.2 Chemical structure of peptide RDM

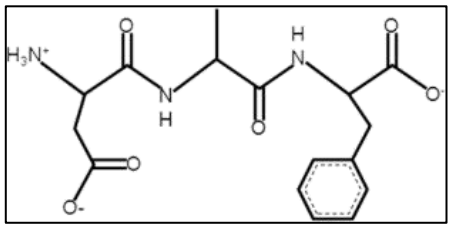

Figure 3.3 Chemical structure of peptide DAF

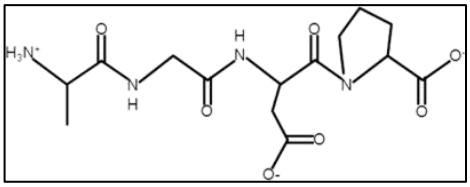

Figure 3.4 Chemical structure of peptide AGD

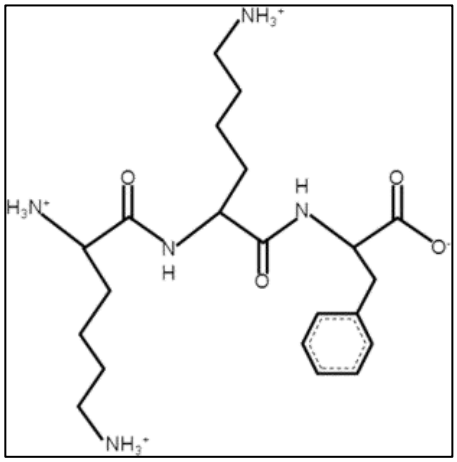

Figure 3.5 Chemical structure of peptide KKF

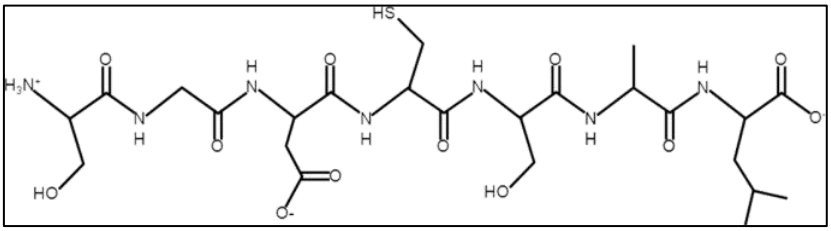

Figure 3.6 Chemical structure of peptide SGDCSAL

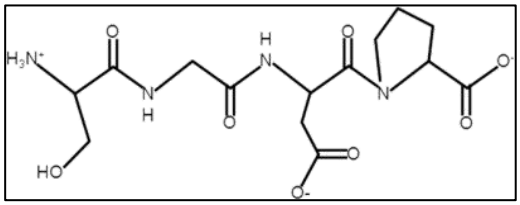

Figure 3.7 Chemical structure of peptide SGDP

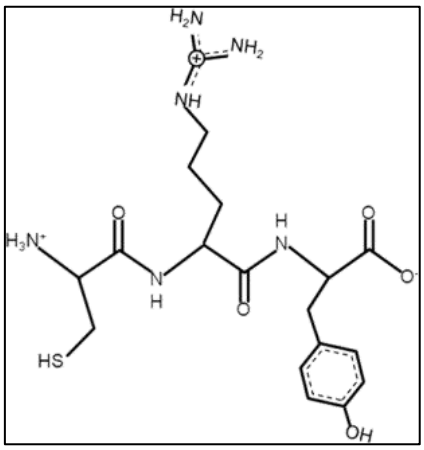

Figure 3.8 Chemical structure of peptide CRY

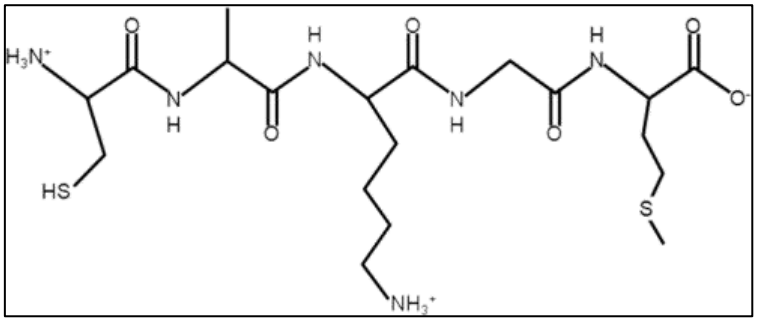

Figure 3.9 Chemical structure of peptide CAKGM

3.2 Analysis of molecular docking of phycoerythrin-active peptides

3.2.1 Experimental materials

RCSB PDB database (http : // www.rcsb.org/), AutoDockVina software and Pymol software.

3.2.2 Experimental method

The common receptor proteins TLR2 in Toll-like receptor and KEAP1 in Kelch-like ECH-associated proteins were selected for molecular docking with phycoerythrin-activated peptides. Firstly, the crystal structures of TLR2 (PDB ID:2z80) (accessed on 20 March 2025) and KEAP1 (PDB ID:1u6d) (accessed on 9 May 2025) receptor proteins were found through the RCSB PDB database (http://www.rcsb.org/), and the found crystal structures of the receptor proteins were de-duplicated using the AntoDock Vina software. The AntoDock Vina software was used to pre-process the crystal structure of the found receptor protein by dehydrogenation, hydrogenation, and modification of amino acids to promote the binding of the receptor protein to the peptide. Next, the processed receptor protein was molecularly docked with the active peptide of phycoerythrin using AutoDock Vina software to find a peptide with strong interaction with the receptor protein. Finally, Pymol software was used to export the docking system visualization graph and analyse the results.

3.2.3 Results and analyses

Nine peptides were molecularly docked to the receptors TLR2 (PDB ID:2z80) and KEAP1 (PDB ID:1u6d) using the AntoDock Vina software, and the magnitude of the interaction force between the resulting peptides and the receptor proteins can be derived from the values of the binding energy. At binding energies below -5.0 kcal/mol, the interaction force between the peptide and the receptor protein is usually regarded

as spontaneous, and binding energies in the range of -7.0 to -5.0 kcal/mol indicate that the peptide and the receptor protein bind to each other with good stability, and those less than -7.0 kcal/mol indicate that the receptor and the ligand can bind strongly to each other, and the entire system is in a The whole system is in a very stable state. The lower the binding energy, the more stable the receptor and ligand are, and the molecules can be more tightly bound to each other.

Combining the binding energy values in Table 3.4 and Table 3.5, the KEAP1 receptor interacts more strongly with the phycoerythrin-active peptide, and it is easier for the ligand to react with the receptor and carry out the relevant reactions to form a stable molecular conformation. Separately, when the peptides were molecularly docked to the TLR2 receptor, the binding energy scores of the individual peptides are shown in Table 3.4.

Table 3.4 **The binding energy score generated by molecular docking between each peptide segment and TLR2 receptor**

Peptide sequence	Binding energy
SAADAAGRF	-5.2kcal/mol
RDM	-4.7kcal/mol
DAF	-5.5kcal/mol
AGDP	-5.2kcal/mol
KKF	-4.8kcal/mol
SGDCSAL	-4.7kcal/mol
SGDP	-4.8kcal/mol
CRY	-4.9kcal/mol
CAKGM	-4.5kcal/mol

The binding energies of peptides SAADAAGRF, AGDP and DAF molecularly docked with the TLR2 receptor were -5.2 kcal/mol, -5.2 kcal/mol and -5.5 kcal/mol, respectively, which can spontaneously generate interaction force with the receptor and

have affinity with the TLR2 receptor, among which the peptide DAF generates the highest binding force with TLR2 receptor and both bind more stably. The peptide DAF produces the highest binding force with TLR2 receptor, and the two binding is more stable and better able to exert biological properties.

When the peptides were molecularly docked with KEAP1 receptor, the binding energy scores of each peptide are shown in Table 3.5.

Table 3.5 **The binding energy score generated by molecular docking between each peptide segment and KEAP1 receptor**

Peptide sequence	Binding energy
SAADAAGRF	-7.1kcal/mol
RDM	-7.0kcal/mol
DAF	-7.0kcal/mol
AGDP	-7.8kcal/mol
KKF	-6.9kcal/mol
SGDCSAL	-6.0kcal/mol
SGDP	-7.7kcal/mol
CRY	-7.8kcal/mol
CAKGM	-6.4kcal/mol

The binding energies of all peptides were lower than -5.0 kcal/mol, and all peptides could spontaneously interact with the receptor. The binding energies of peptides SAADAAGRF, RDM, DAF, AGDP, SGDP and CRY were lower than -7.0 kcal/mol, and the peptides were strongly bound to KEAP1 receptor. The binding energy of peptides AGDP and CRY is lower than -7.0kcal/mol, and they can strongly bind to the KEAP1 receptor, and the docking system is highly stable, and peptides AGDP and CRY have the highest binding force with KEAP1 receptor, with a binding energy of -7.8kcal/mol

After the above peptides that can spontaneously generate binding energy were visualized and analysed using Pymol software for molecular docking results, the interaction forces and bonding relationships between the peptides and the receptor proteins were obtained as shown in Figures 4.1, 4.2 and 4.3. When the

peptide binds to the TLR2 receptor, peptide SAADAAGRF forms the most hydrogen bonds with the receptor protein, with a total of 8 bonds, the longest bond length is 3.4 Å, and the shortest bond length is 1.9 Å.

Peptide DAF forms the least hydrogen bonds with the receptor protein, with a total of 1 hydrogen bond, and the shortest bond length is 2.1 Å.

Therefore, peptide SAADAAGRF can bind to the binding site of the TLR2 receptor protein in a better way, and the peptide can bind to the binding site of TLR2 receptor protein in a better way.

Therefore, SAADAAGRF can better bind to the binding site of TLR2 receptor protein and activate the body's immune response. When the peptide binds to KEAP1 receptor, peptides RDM and SAADAAGRF form the most hydrogen bonds with the receptor protein, with a total of 6 bonds, the longest bond length is 3.6 Å, and the shortest bond length is 1.8 Å.

Peptides CRY and AGDP form the fewest hydrogen bonds with the receptor protein, with a total of 2 bonds, and the bond lengths of both bonds are 2.0 Å. Peptides RDM and SAADAAGRF can more accurately bind to the KEAP1 receptor protein. The peptides RDM and SAADAAGRF can more precisely bind to the binding site of KEAP1 receptor protein and better activate the related reactions in the body.

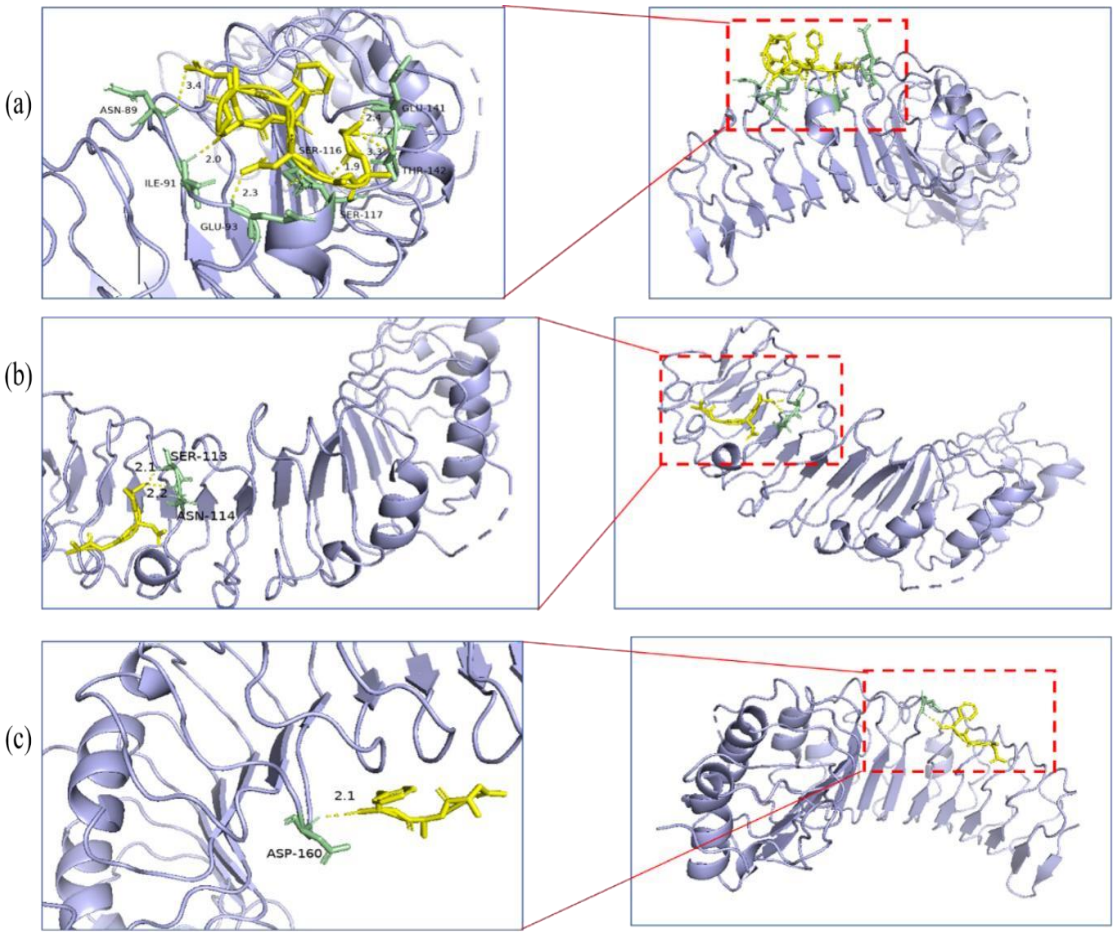

Figure 3.10 Visualization results of peptide docking with TLR2 receptor molecules

Note: (a)SAADAAGRF; (b)AGDP; (c)DAF;

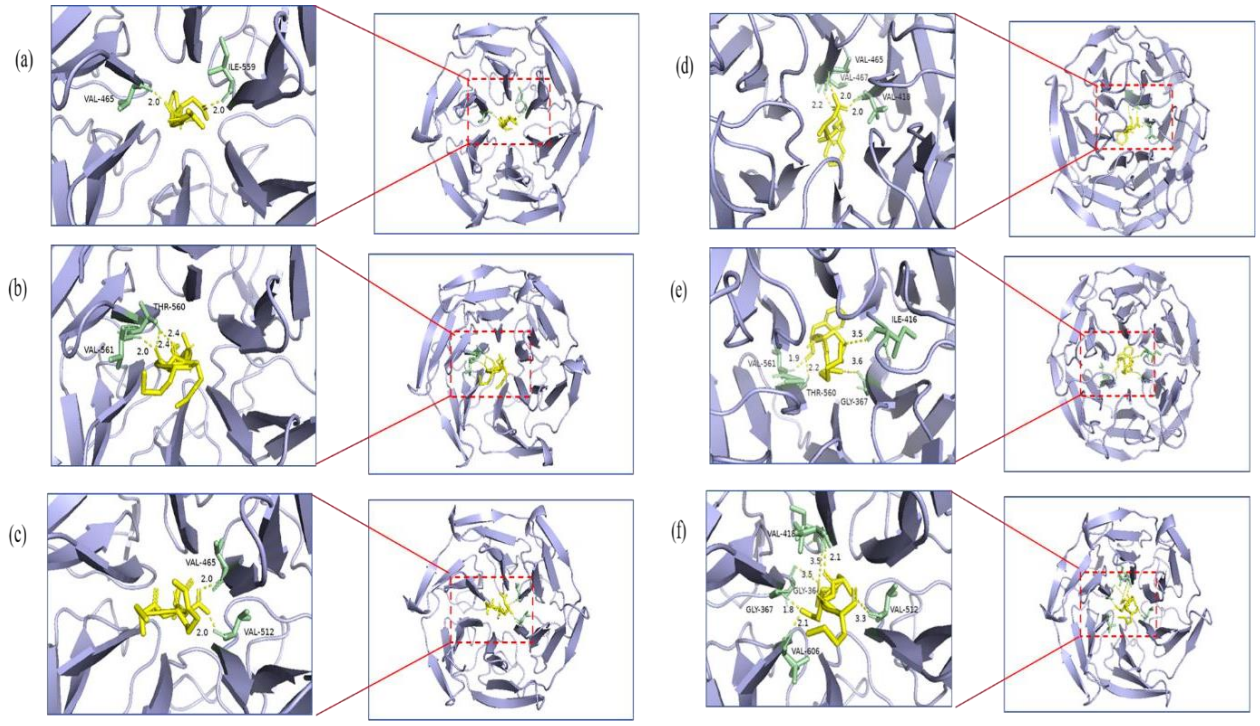

Figure 3.11 Visualization results of peptide docking with KEAP1 receptor molecule 1

Note：(a)AGDP; (b)CAKGM; (c)CRY; (d)DAF; (e)KKF; (f)RDM;

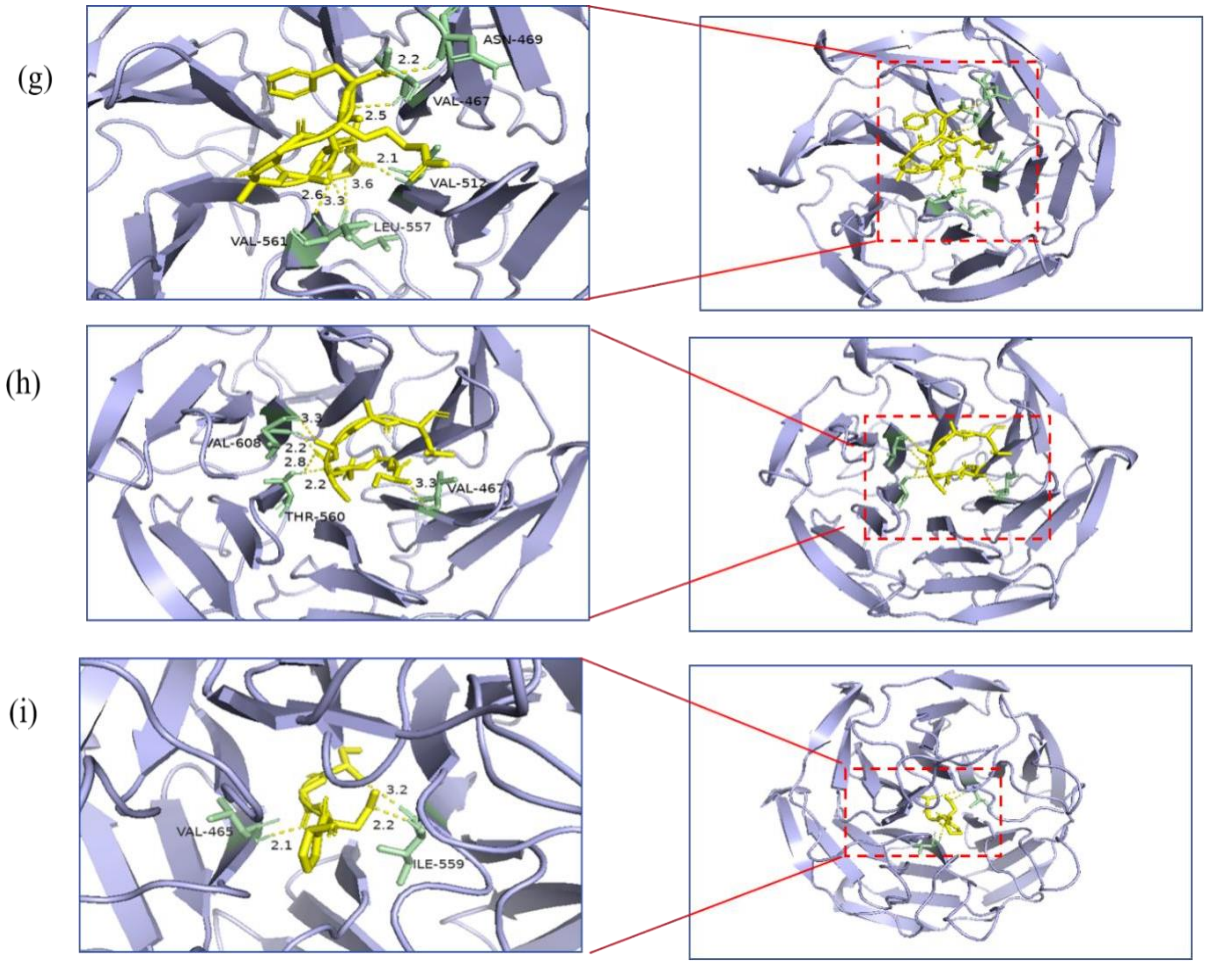

Figure 3.12 Visualization results of peptide docking with KEAP1 receptor molecule 2

Note: (g)SAADAAGRF; (h)SGDCSAL; (i)SGDP;

Summary of chapter III

1. Using ToxinPred and Innovation software to predict the toxicity and water solubility of biologically active peptide segments, 16 non-toxic and water-soluble potential peptide segments were identified, ensuring the safety and solubility of potential peptides.

2. Use CAMPR3 to predict the antibacterial activity of peptide segments, IL2pred and IL4red to predict the immunomodulatory activity of peptide segments, AnOxPP to predict the antioxidant activity of peptide segments, and identify the biological characteristics of each peptide segment.

3. Peptides with high affinity for TLR2 and KEAP1 receptors were screened by AntoDock Vina software, and the molecular docking results of the peptides were visualized and analysed by Pymol software. It was found that three peptides, SAADAAGRF, AGDP, and DAF, could spontaneously generate interaction force to TLR2 receptor, and nine peptides, SAADAAGRF, RDM, DAF, AGDP, KKF, SGDCSAL, SGDP, CRY, and CAKGM, could all spontaneously generate interaction force to KEAP1 receptor. Among them, peptide DAF produced the highest binding force with TLR2 receptor, and peptides AGDP and CRY produced the highest binding force with KEAP1 receptor, which were more capable of forming stable molecular conformation and could better exert the biological properties of the peptides.

CONCLUSIONS

This paper focuses on obtaining novel, potentially active peptides of phycocyanin from phycocyanin by using computer and bioinformatics software. The process mainly consists of three main steps: simulated enzymatic cleavage of phycoerythrin chains, analysis of the biological activity and physicochemical properties of the resulting peptides, and analysis of the peptide-receptor protein interaction force using molecular docking. Based on the study, the main conclusions are as follows;

1, 302 phycobiliprotein peptides were obtained by simulated enzymatic cleavage of seven phycobiliprotein chains using enzymatic functional pepsin (pH=1.3) and proteinase K together in the BIOPEP tool. The biological activity of the resulting peptides was predicted using the Peptide Ranker tool, of which 65 peptides had a prediction score of >0.5 and were considered to be biologically active. Among the biologically active peptides, β-source active peptides accounted for the highest proportion of β-source peptides, so β-source protein chains have high research value in studying biologically active peptides.

2. By using two bioinformatics tools, ToxinPred and Innovagen, to predict the toxicity and water solubility of the peptides, we found 16 potential peptides that were non-toxic and had good water solubility, which guaranteed the safety of the potential peptides as well as their solubility. By comparing with the validated peptides in the BIOPEP database, 9 potential active peptides of phycoerythrin were found. Then, CAMPR3 tool was used to predict the antimicrobial property of the peptides, IL2pred and IL4pred tools were used to predict the immunomodulatory property of the peptides, and AnOxPP tool was used to predict the antioxidant property of the peptides, so as to find out the biological properties belonging to each peptide. Finally, the chemical structure of each peptide was obtained by using

PepDraw software, which provided a deeper understanding of the peptides and facilitated the next step of molecular docking.

3. Peptides with high affinity for TLR2 and KEAP1 receptors were screened by AntoDock Vina software, and the molecular docking results of the peptides were visualized and analysed by Pymol software. It was found that three peptides, SAADAAGRF, AGDP, and DAF, could spontaneously generate interaction force to TLR2 receptor, and nine peptides, SAADAAGRF, RDM, DAF, AGDP, KKF, SGDCSAL, SGDP, CRY, and CAKGM, could all spontaneously generate interaction force to KEAP1 receptor. Among them, peptide DAF produced the highest binding force with TLR2 receptor, and peptides AGDP and CRY produced the highest binding force with KEAP1 receptor, which were more capable of forming stable molecular conformation and could better exert the biological properties of the peptides.

In summary, a total of 9 novel phaeoglobin active peptides were found to meet the expectations. Among these 9 peptides, 9 peptides such as SAADAAGRF, RDM, DAF, AGDP, KKF, SGDCSAL, SGDP, CRY, and CAKGM possessed immunomodulatory properties, 6 peptides such as SAADAAGRF, DAF, AGDP, KKF, SGDCSAL, and SGDP possessed antioxidant properties, and 4 peptides such as RDM, DAF, KKF, and CAKGM and 4 peptides such as RDM, DAF, KKF, SGDCSAL and SGDP have antimicrobial properties. The peptides SAADAAGRF, AGDP and DAF can spontaneously interact with TLR2 receptor, and all nine peptides can spontaneously interact with KEAP1 receptor. All nine peptides can be used as potential phycoerythrin-active peptides, which can contribute to the study of phycoerythrin and phycoerythrin-active peptides.

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