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 **Study on the catalytic mechanism of polypeptide-based polyethylene terephthalate mimics**

First (Bachelor's) level of higher education

Specialty 162 "Biotechnology and Bioengineering"

Educational and professional program "Biotechnology"

Completed: student of group BEBT-20
Boda JIANG

Scientific supervisor
Olena OKHMAT, Ph.D., Assoc. Prof.

Reviewer
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Educational and professional program Biotechnology

APPROVE
Head of Department of Biotechnology, Leather and Fur, Professor,
Doctor of Technical Science
Olena MOKROUSOVA
_____
«___»_____2024

ASSIGNMENTS
FOR THE QUALIFICATION THESIS
Jiang Boda

1. Thesis topic **Study on the catalytic mechanism of polypeptide-based polyethylene terephthalate mimics**
Scientific supervisor Olena Okhmat, Ph.D., Assoc. Prof.

approved by the order of KNUTD "____"_____2024, №____

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_____

EXECUTION SCHEDULE

№	The name of the stages of the qualification thesis	Terms of performance of stage	Note on performance
1	Introduction	From 01 April 2024 to 11 April 2024	
2	Chapter 1. Literature review	From 06 April 2024 to 20 April 2024	
3	Chapter 2. Object, purpose, and methods of the study	From 21 April 2024 to 30 April 2024	
4	Chapter 3. Experimental part	From 01 May 2024 to 10 May 2024	
5	Conclusions	From 07 May 2024 to 12 May 2024	
6	Draw up a bachelor's thesis (final version)	From 20 May 2024 to 25 May 2024	
7	Submission of qualification work to the supervisor for feedback	From 25 May 2024 to 27 May 2024	
8	Submission of bachelor's thesis to the department for review (14 days before the defense)	27 May 2024	
9	Checking the bachelor's thesis for signs of plagiarism (10 days before the defense)	31 May 2024	
10	Submission of bachelor's thesis for approval by the head of the department (from 7 days before the defense)	03 June 2024	

I am familiar with the task:

Student _____ Boda JIANG

Scientific supervisor _____ Olena OKHMAT

SUMMARY

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Polyethylene terephthalate (PET) is a polyethylene plastic with a large amount. Due to its good heat resistance, plasticity, toughness and other advantages, PET is widely used in various fields. However, due to the stability of its polymer structure, PET plastic waste is difficult to degrade naturally, resulting in a large amount of plastic waste accumulation, which poses a serious threat to human health and the ecological environment. Enzymatic degradation means that the enzyme molecule binds to the binding site of the PET molecule to form the enzyme-host complex to achieve the purpose of decomposing PET. Enzymatic degradation, as a green and sustainable method, has brought a new idea to solve the environmental pollution caused by waste plastics. However, the preparation process of natural enzymes is complicated, the production cost is high, the stability is poor, the reaction medium is strict and other conditions limit their wide application. In this experiment, PETase, a natural enzyme with high PET degradation activity, was used as the simulated object. Based on the structural and catalytic characteristics of the natural enzyme, PET degradation enzyme was constructed with polypeptides. The structure of the simulated enzyme was characterized by CD, TEM and THT methods. The catalytic activity of PET degradation by simulated enzyme was studied by HPLC. Molecular docking was used to simulate the interaction between enzymes and substrates. The experimental results showed that the simulated enzyme could self-assemble to form β-folded secondary structure, and had good stability

under high temperature conditions. The simulated enzyme can effectively catalyze PET degradation at a temperature of 65 ℃ and PH of 9, and the degradation products are TPA and MEHT. Further through molecular docking, it was found that the simulated enzyme interacts with the substrate mainly through hydrogen bonding and hydrophobic interaction. The simulated enzyme constructed in this experiment can provide a theoretical reference for the degradation of industrial and daily waste plastics.

Keywords: self-assembling peptide; Enzymatic degradation; Polyethylene terephthalate (PET); mimicase

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INTRODUCTION

Enzymatic degradation of PET has little pollution to the environment and high efficiency, which can be used as the main idea to solve the degradation problem. But it is not easy to obtain, high production cost and poor environmental adaptability. Therefore, it is necessary to further research and development of efficient and stable artificial enzymes to improve degradation efficiency, reduce production costs, and expand its applicability in different media, so as to promote the practical application and promotion of enzymatic PET degradation technology. In this study, polypeptides were used as the main substrate material to construct a simulated enzyme for PET degradation, and the structure, degradation activity and interaction mode of the simulated enzyme with the substrate were further studied, providing more in-depth theoretical guidance and practical basis for the construction and study of simulated enzyme for PET degradation and its catalytic mechanism.

The purpose of the study is the production of highly efficient PET degradation simulation enzyme, reduce cost and improve efficiency.

The objectives of the study is the simulated enzyme degradation of PET

The object of the study is the mimicase

The subject of the study is the properties and applicable conditions of PET catalyzed by enzyme were simulated

Research methods is the preparation of enzyme mimics →CD method. The effect of temperature on the structure was observed by TEM, FTIR.→ The difference of catalytic activity of PET particles as substrate was studied by HPLC. → The catalytic mechanism was studied by molecular docking.

The scientific novelty a self-assembled simulated enzyme was used.

The practical significance of the results obtained is the reduce the degradation cost of PET and increase the degradation efficiency

CHAPTER 1
LITERATURE REVIEW

1.1 Overview of PET (Polyethylene terephthalate)

Polyethylene terephthalate (PET) is composed of terephthalic acid and ethylene glycol. The two monomers are linked together by ester bond, forming a stable polymer structure, and because of its heat resistance and high strength hardness, it plays a key role in many fields such as plastic bottle manufacturing, textile fibers, and films. However, despite its wide application, PET has a very high tolerance to many solvents and a relatively low biodegradation performance, which leads to a large number of waste PET products that are difficult to decompose naturally, posing a persistent and serious pollution hazard to the earth's environment.

1.2 PET degradation method

1.2.1 Physical degradation of PET

Physical method is a common PET waste recycling and treatment method, and its process includes sorting and crushing PET waste, and then secondary manufacturing or modified granulation to produce fiber [11]. In the recycling process of PET products, PET products need to be crushed and cleaned first, then sorted, dewatered and granulated, then injected, stretched and blown, and finally formed into packaging containers and other products [20]. For example, Jiang et al. prepared R-PET fibers using melt spinning technology and compared their properties with native PET (O-PET) fibers. The research results show that R-PET fiber has lower crystallinity and higher orientation. Due to cross-linking of molecular chains caused by impurities, its fracture strength is increased, but its elongation at break is decreased [21].

In addition, Majumdar[22] and his team used the melt extrusion process to convert PET bottles into textile grade polyester fibers, and compared the performance differences between recycled fibers and non-recycled fibers. It was found that the crystallinity and tensile strength of recycled polyester fiber were lower than that of non-recycled polyester fiber. Although the air permeability and moisture permeability of the fabric did not change significantly with the increase of recycled polyester proportion, the shear stiffness and flexural stiffness of the fabric showed an increasing trend. These processes are common strategies in the field of physical recycling due to their ease of use and cost effectiveness. However, the disadvantages of this method include the residue of harmful substances and the destruction of the PET molecular chain, which in turn affects the quality and performance of PET products.

1.2.2 Chemical degradation of PET

Chemical method is the use of chemical methods to depolymerize PET, and the monomer or oligomer formed by depolymerization can be used as a raw material for reproduction. At present, chemical methods are the main methods for industrial PET degradation, including three methods: hydrolysis, alcoholysis and ammonolysis [12]. Chemical methods realize the recycling of PET, but the degradation cost is high and the degradation process will cause secondary pollution to the environment [10]. Shu Xiaotong's team used ethylene glycol as the alcoholysis agent to study the process of chemical recovery of PET, and screened the catalyst for alcoholysis of PET, and found that tetrabutyl titanate as the catalyst not only had a high alcoholysis rate but also did not affect the synthesis of polyester elastomers [3]. The methanol depolymerization method of PET has the advantages of mild reaction conditions, short process flow and easy use of existing industrial equipment for production, which is favored by the industry. The alcoholysis products of PET methanol are dimethyl terephthalate

(DMT) and ethylene glycol (EG), which can be used as materials for recycled PET and as raw materials for other processes [4]. QiXinhua et al. constructed an artificial microbial community composed of Rhodococcusjostii, Pseudomonas putiodus and two strains of Bacillus subtilis modified by metabolic engineering to degrade PET[6]. Mishra et al. [23] used methanol to depolymerize PET and optimized the particle size and reaction time of PET.

1.2.3 Biodegradation of PET

Enzyme catalysis has the characteristics of high efficiency, specificity and mild reaction conditions, and is widely used in chemical engineering, medical medicine, food hygiene and agricultural production. Natural enzymes generally have high catalytic efficiency, are able to react under mild conditions, and are usually highly selective to substrates. At the same time, specific substrate molecules can be recognized, which makes them highly specific in the organism and avoids non-target reactions. Its stability can be improved under appropriate conditions, such as low temperature, stable pH value or binding to the support, compared to chemical catalysts, natural enzymes react under mild conditions and can usually be degraded by enzymes in the organism without lasting environmental effects.

At present, there are many researches on enzymatic degradation of PET. Tokiwa et al. [13] found several enzymes that can hydrolyze various polyesters. With the continuous development of bioengineering technology, people have turned their attention to biodegradation. Microorganisms first secrete extracellular enzymes, which bind to macromolecule polymer PET outside the cell, and convert macromolecule PET into water-soluble small molecule compounds, which are absorbed into the body. These water-soluble small molecules are further decomposed in the microorganism and finally decomposed into carbon dioxide, water and other substances [14]. Chunchi et al.

used the industrial yeast strain Pichiapastoris to produce a highly efficient PET hydrolase named FAST-PETase, which further improved the performance of the enzyme by removing the glycosylation of two N-links through molecular modification[5]. The XueRui team utilized thermophilic polyester hydrolases, such as the cleavase (LCC), isolated from the leafy compost metagenome and its variants. The chitin binding domain (ChBD) was fused from the plum chitin-degrading bacterium SYBC-H1 to the C-terminal of the reported LCCICCG variant, showing higher adsorption capacity for PET substrates[7]. Moog et al. [24] successfully integrated PET-ASE enzyme (derived from Ideonella sakaiensis) into photosynthetic microalgae Phaeodactyla triangulata, and the results showed that the PET-degrading activity of this enzyme in brine environment was still effective against industrial PET fragments at a suitable temperature of 21°C, and the products were TPA and MHET. This opens up a new way for large-scale industrial application of bioenzymatic PET. Then, Then[25] et al. introduced disulfide bonds into the calcium-binding site of PET hydrolase, and innovated a kind of high-temperature polyester hydrolase, raising the optimal operating temperature of the enzyme from 69.8°C to 94.7°C, thus greatly increasing the decomposition rate of PET. Ronkvist et al. [26] used HiC, a keratinase with excellent thermal stability, to hydrolyze PET with a crystallinity of 7%, and found that the hydrolysis rate was optimal at 70°C, and the products were also TPA and EG. Impressively, HI-C catalyzed a low-crystallinity PET film at 70°C for only 96 hours, achieving a 97% weight loss. These reviews show that the possibility and efficiency of biodegrading PET has been significantly improved by improving the properties of the enzyme.

1.3 Simulated enzymes

Simulated enzymes are a class of non-protein molecules synthesized by organic chemical methods, which are simpler than natural enzymes. They are

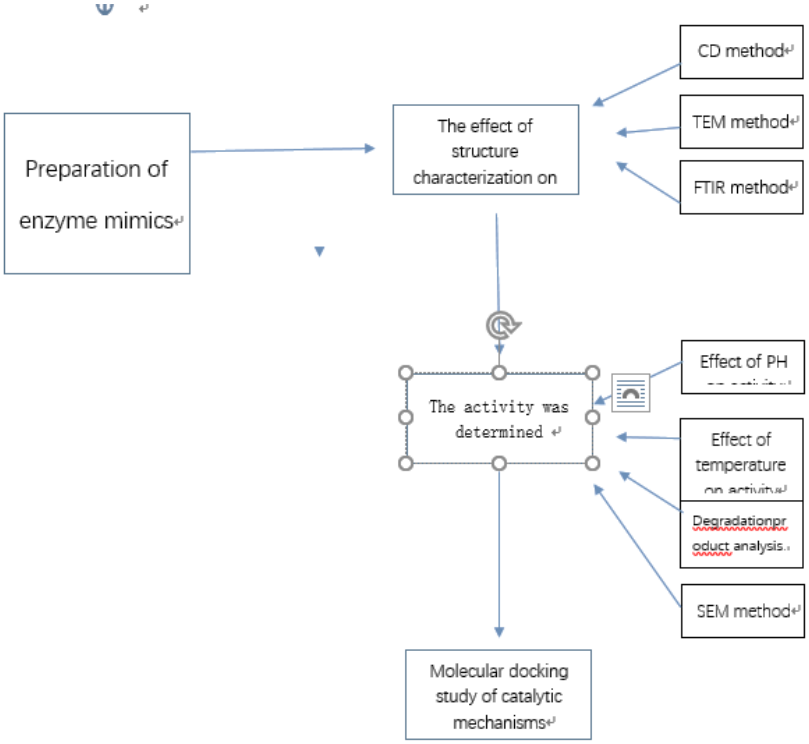
synthesized according to the working characteristics and structural characteristics of the required enzymes, and are a class of catalysts with enzyme properties made by artificial methods. Compared with natural enzymes, the advantage of simulated enzymes is that the structure is simple and more stable, with purpose and higher production efficiency, and the cost is lower and more conducive to production applications. Up to now, there have been many applications of simulated enzymes: metal-organic framework [19], graphitic acetylene [27], nanomaterial biosensors [28] and peptides [29]. In these materials, peptides, as an ideal biomolecular, are mainly composed of amino acid residues, which can be produced according to the active site of natural enzymes, and the driving force of their self-assembly is non-covalent interactions (hydrogen bonding, van der Waals forces, hydrophobic or electrostatic interactions, etc.). These interactions are also present in natural enzymes and the peptides have simpler structures that facilitate modification, thus often making the reaction more complete and producing a favorable microenvironment. In addition, compared with natural enzymes which are more difficult to extract, the production cost of simulated enzymes is lower and the biocompatibility is better.

Conclusions to chapter 1

In this study, natural enzymes with high PET degradation activity were used as the simulated objects. Based on the rational design of amino acid sequence and combined with non-amino acids, polypeptide mimicase was constructed for PET degradation, and the mechanism of PET catalyzed by the enzyme mimicase was analyzed by means of physicochemical characterization and molecular dynamics calculation techniques.

CHAPTER 2
OBJECT, PURPOSE AND METHODS OF THE STUDY

Research the technical route:



2.1 Experimental materials and instruments

Experimental materials

Polypeptide Ac - SHDSGVKVKVKVKVDPTKVKVKVKV - NH2

Reagents: dimethyl sulfoxide (DMSO), buffer, distilled water, acetonitrile, p-nitrophenylacetate, p-nitrophenol, trimethylol aminomethane, hydrochloric acid, 4-hydroxyethyl piperazine ethanesulfonic acid (hepse), 2-morpholine ethanesulfonic acid (MES). Thioflavin T

Experimental instruments

Instruments: Circular dichroic spectrum, transmission electron microscope, spectrometer, infrared lamp, photometer, tablet press, agate mortar, analytical balance, constant temperature incubator, pipette gun.

2.2 Experimental methods

2.2.1 Peptide self-assembly simulation enzyme construction

A 2m short peptide powder was dissolved in 20μLDMSO, after full shock, a certain volume of 5mM pH9 Tris-HCL buffer was added, mixed with ultrasonic 90s, and left for 24 hours at 25℃, away from light, to slowly fibrosis and form a gel solution, and a peptide solution with a final concentration of 5 mm was obtained. Before use, the gel solution was violently swirled and shaken for 1 min to obtain peptide nanofiber solution and set aside.

2.2.2 Structural characterization

2.2.2.1 Circular Dichroism Spectroscopy (CD)

CD spectroscopy can provide information about the structure of a protein, including its secondary structure and chiral properties. Turn on the circular dichroism spectrometer at room temperature and adjust the Settings after preheating: wavelength 190nm to 260nm, standard sensitivity, 0.1 data spacing, 100nm/min scan speed, 1 nm bandwidth and 1nm step size 8. The color plate specification is a quartz color plate polarized at 1mm optical path. Three blank control groups were set up, and the polypeptide reaction solution at different culture temperatures was put into the chromatograph to observe the data.

2.2.2.2 Transmission Electron Microscopy (TEM) technology

TEM technology can provide high-resolution images to help observe and analyze the microstructure of materials. Drop 7μL of the peptide self-assembly solution prepared under different conditions on the TEM copper net at room temperature for 30min, absorb excess sample solution with filter paper, then immediately add 7μL of freshly prepared 2% phosphotungstic acid, and stain for 1.5min. Then the excess 2% phosphotungstic acid is absorbed with filter paper, and finally washed three times with ultra-pure water and placed in a dry

petri dish to air dry. The prepared TEM copper mesh was placed in the sample chamber, vacuumed, and the self-assembled peptides were observed in a suitable field of view at 200kV.

2.2.2.3 Fourier Transform Infrared Spectroscopy (FTIR) technology

Fourier infrared spectroscopy is a commonly used analytical technique to determine the structure and chemical composition of a substance. It can convert the infrared signal absorbed by the sample into interference signal, and then convert the interference signal into spectrogram by Fourier transform, so as to obtain the infrared spectrum information of the sample. By analyzing the absorption peaks of samples under different wave numbers, information such as functional groups and chemical bond types in the simulated enzymes can be determined, so as to analyze the structure and composition of the simulated enzymes.

2.2.3 High Performance Liquid Chromatography Technology for detection of activity of polypeptide mimics

Under different pH (5.5 ~ 10.0) and temperature (25 ~ 70℃), 0.5mM enzyme mimics and 2mg/L-1PET microplastics were cultured in glass bottles. The control group without enzyme mimics was subjected to the same reaction conditions as the experimental group. After the specified time for hydrolysis, the reaction mixture was heat treated at 100 ° C for 10min to stop the hydrolysis reaction, and then centrifuged (18,000×g, 5min).

The supernatant was analyzed by HPLC system equipped with WelchUltimateXB-C18 column (4.6×250mm, 5μm), and the mobile phase was set as methanol-phosphate buffer (60:40) at a flow rate of 0.5mL/min. The separation temperature is 35℃ and the detection wavelength is 240nm for testing [9].

2.2.4 Study on catalytic mechanism of polypeptide mimics

Molecular docking technology is a computational method used to predict binding patterns and binding energies between small molecules (such as drug molecules) and large molecules (such as proteins, peptides). When studying the mechanism of PET degradation catalyzed by polypeptide mimics, molecular docking technology can be used to simulate the binding mode between polypeptide and PET mimics, and predict the binding energy between them, so as to reveal the interaction mechanism between polypeptide and PET. A PET substrate model 2-hydroxyethyl-(monohydroxyethyl terephthalate)3 (2-HE(MHET)3) was established using Gauss 09 software. The AutoDockTools1.5.6rc3 software generates PDBQT format files for all polypeptides and 2-HE(MHET)3. Then, use the AutoDockVina program

The interaction between them was investigated. Finally, Chimera1.12 and LigPlus2.1 software packages were used to visualize the molecular docking results [9].

2.2.5 The degradation of PET by simulated enzyme was observed by SEM

The 0.5mM enzyme simulant and PET film (10 mm × 10 mm) were placed in glass bottles for hydrolysis at pH 9.0 and temperature 65℃. The control group without enzyme mimics was subjected to the same reaction conditions as the experimental group. The surface morphology of the hydrolyzed PET film was analyzed by SEM.

CHAPTER 3
EXPERIMENTAL PART

3.1 Design of polypeptide self-assembly bionic enzyme

At the active site of PETase, serine, histidine, and aspartic acid form a catalytic triad to decompose PET through an acid-alkali-nucleophilic mechanism. Therefore, S-H-D active site residues were first integrated into the peptide design to simulate the PET-degrading enzyme. The MAX peptide consists of a valine-lysine (VK) repeat sequence with a discontinuous tetrapeptide (-VDPPT-) on each side, which can self-assemble into stable β-hairpin structures such as fibers and hydrogels under suitable conditions. In the designed polypeptide, S-H-D was chemically connected to the N terminal of MAX peptide, and GS was used as the connecting unit. The polypeptide was designed to form a supramolecular β sheet structure rich in electrostatic, van der Waals forces, hydrogen bonds and hydrophobic interactions, in which valine, due to its hydrophobicity, contributes to the formation of dihedral β sheets. The ends of the polypeptide, the N-terminal and the C-terminal, are sealed by acetyl and carboxyamide groups, respectively, to enhance their stability in solution. Under certain conditions, polypeptides self-assemble into β-transfiber through non-covalent interactions, with hydrophobic valine and threonine amino acids as substrate binding sites, exposed around the catalytic core of the fiber S-H-D. The NHs on the skeleton of glycine and serine residues forms a charge transfer system with the oxygen anions in the aqueous environment, which acts as a synergistic catalyst to shorten the distance between the substrate and the active site. Overall, our design strategy aims to replicate the active characteristics of PETase, enabling efficient and stable PET degradation through carefully arranged amino acid sequences.

3.2 Characterization of enzyme mimics

Temperature plays a crucial role in the biochemistry of the assembled peptides. First, temperature has a significant effect on the conformational stability of the assembled peptides, since the three-dimensional structure of the protein is maintained by non-covalent and hydrogen bonds between its amino acid residues. At lower temperatures, the assembled peptide may be in a more stable α-helix or β-folded state, and as the temperature increases, the thermal motion increases, which may cause the local or global conformation of the peptide chain to change, or even change from the folded state to a disordered extended state, a phenomenon called denaturation, which may reduce its biological activity.

In the experiment, the ideal temperature conditions should be able to maintain the structural stability and functional activity of the simulated enzyme. Too high a temperature may lead to permanent deactivation of the peptide chain, while too low a temperature may limit its function. Therefore, when designing and studying simulated enzymes, precisely controlling the experimental temperature is crucial,

Studying the effect of temperature on the thermodynamic properties of simulated enzymes, such as specific heat capacity and thermal stability, can deepen the understanding of their thermodynamics. The specific heat capacity reflects the ability of the system to absorb heat, while thermal stability measures the stability of the peptide at different temperatures. Using techniques such as transmission electron microscopy (TEM), Circular Dichroism (CD) spectroscopy and fluorescence spectroscopy, scientists can monitor the conformational changes of assembled peptides at different temperatures in real time, thereby optimizing their application in biological systems.

3.2.1 Circular dichroic analysis of simulated enzymes

The secondary structures of self-assembled peptides at different temperatures were analyzed by CD spectroscopy. In Figure 2-2, the assembled peptides form irregular curls at roughly the same wavelength between 25 ° C and 80 ° C, with a positive peak near 195 nm and a negative peak near 216 nm. In this experiment, the pH of the solution was kept around 8.5, and the range of changes shown by the peptide in response to changes in temperature was most easily observed. (Fig. 3.1) The structure of the peptide did not change significantly with the increase of temperature, but initially formed a β structure at room temperature, and this relatively stable structure could be maintained even when the temperature was up to 80 ℃.

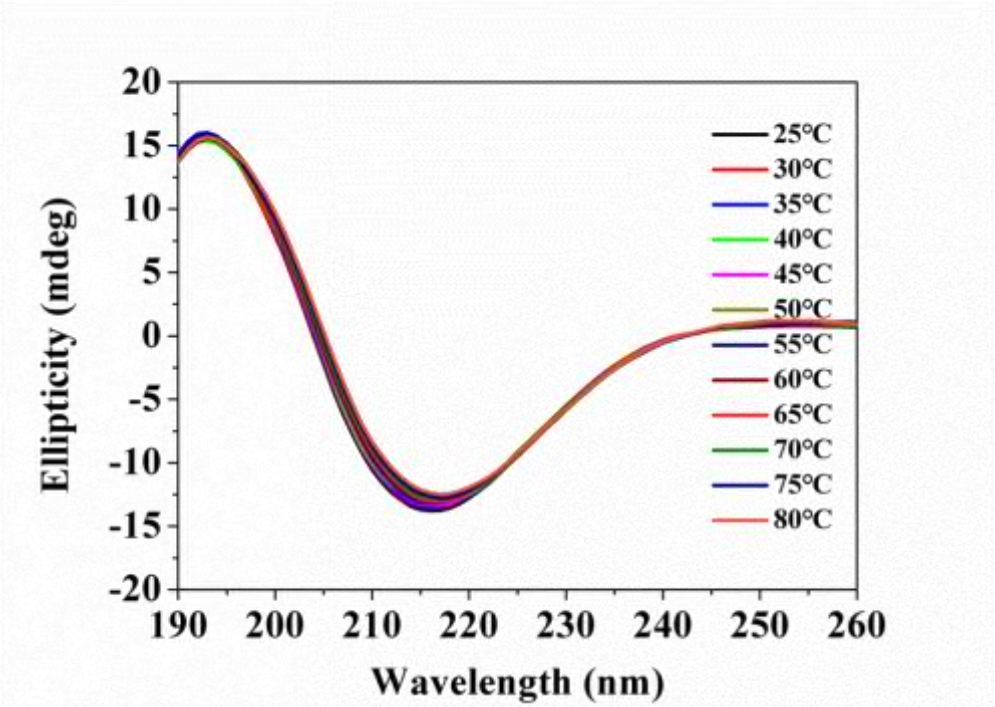


Figure 3.1 – CD diagram of the temperature

3.2.2 Morphology analysis of simulated enzymes

Transmission Electron Microscopy (TEM) is an advanced microscopic technique that uses high-energy electron beams to image a sample, revealing the fine structure of a material at the atomic scale. (Fig. 3.2) Therefore, through TEM observation, we can not only intuitively see the effect of temperature on the molecular microstructure of this peptide, but also infer the importance of the stability of the substance and possible structural optimization for its physical properties and functionality at specific temperatures.

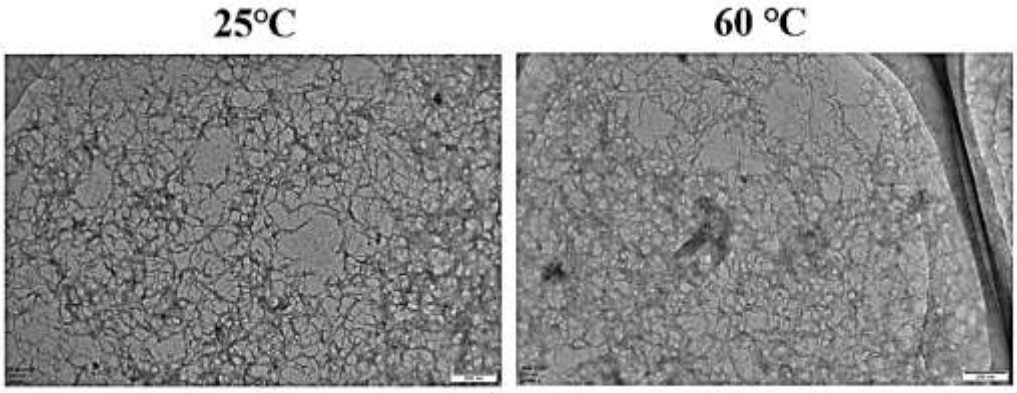


Figure 3.2 –TEM temperature view

At the beginning of the experiment, when the sample was observed in an environment of 25℃, the results showed that its microstructure showed a regular and stable shape. When the temperature was raised to 60℃, the secondary structure and morphology did not change much. This indicated that the peptide had a certain tolerance and stability to temperature changes.

3.2.3 Infrared analysis of simulated enzymes

Fourier Transform infrared spectroscopy (FTIR) was applied to further study the structure of the peptides incubated at 60°C (Fig. 3.3, left). There is a peak in the amide II band near 1533cm^{-1}, two peaks in the amide I band, a strong signal near 1625cm^{-1} and a weak signal at 1674 cm^{-1}, all of which indicate the existence of an anti-parallel β-sheet structure [18]. As can be seen

(Fig. 3.3, right), there is a β-fold structure peak at 1620 cm^{-1}, followed by a random, irregular structure without a specific secondary structure, revealing the character of its amino acid sequence.

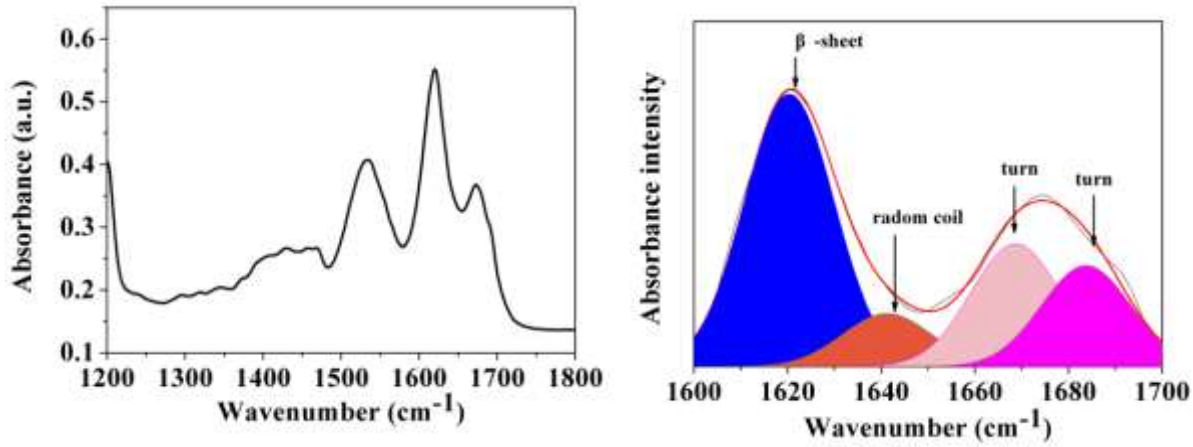


Figure 3.3 – FTIR diagram

3.3 Hydrolysis activity of simulated enzyme

Under different pH (5.5 ~ 10.0) and temperature (25 ~ 70℃), 0.5mM enzyme mimics and 2mg/L-1PET microplastics were cultured in glass bottles. The control group without enzyme mimics was subjected to the same reaction conditions as the experimental group. After the specified time for hydrolysis, the reaction mixture was heat treated at 100 ° C for 10min to stop the hydrolysis reaction, and then centrifuged (18,000×g, 5min). The supernatant was analyzed by HPLC system equipped with WelchUltimateXB-C18 column (4.6×250mm, 5μm), and the mobile phase was set as methanol-phosphate buffer (60:40) at a flow rate of 0.5mL/min. The separation temperature is 35℃ and the detection wavelength is 240nm for testing[9].

3.3.1 Influence of temperature on hydrolysis activity

The activity of the enzyme is affected by the change of reaction temperature, the temperature rises, the thermal motion of the substrate molecule accelerates, the collision frequency increases, the enzyme activity gradually

increases, and the reaction rate also gradually accelerates. However, too high temperature will lead to a decrease in the stability of the protein, which in turn will affect the spatial structure of the enzyme, and the possibility of denaturation and inactivation will gradually increase. As can be seen from Fig. 3.4, the enzyme activity tends to increase before 65°C and reaches a maximum at 65°C. This shows that the activity of the enzyme can be effectively improved at a temperature of about 65°C, which can accelerate the reaction rate and improve the product generation efficiency.

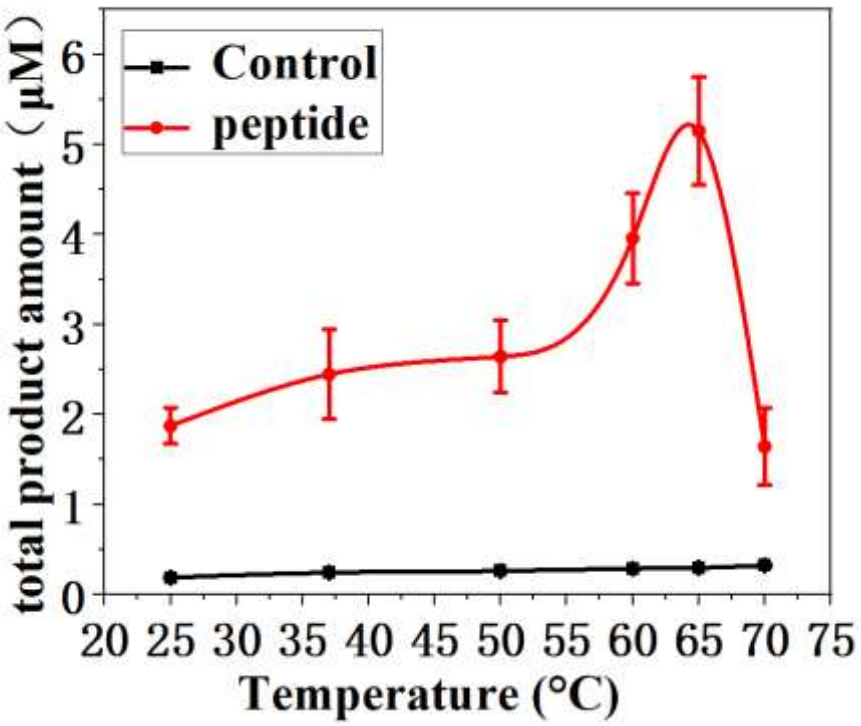


Figure 3.4 – Effect of temperature on enzyme activity

3.3.2 Influence of pH on hydrolysis activity

According to the results of the study, pH has a significant effect on the reaction rate and enzyme activity. Before the pH value was below 9, the reaction rate showed an upward trend, indicating that the enzyme was more active at lower pH, which facilitated the reaction. However, when the pH is close to 9, the reaction intensity reaches its maximum and the product

generation is most complete. This indicates that in this pH range, the enzyme is catalytic most efficient and is able to catalyze the reaction more efficiently, resulting in the largest amount of product. However, when the pH value exceeds 9, the simulated enzyme begins to denature, the hydrolytic activity begins to decrease, and the amount of product decreases, indicating that too high pH value will inhibit the activity of the enzyme, thereby reducing the catalytic efficiency and reducing the production of products. Therefore, proper pH conditions are crucial for the catalytic efficiency of enzymes, and too high or too low pH will affect the activity of enzymes, which in turn will affect the progress of the reaction (Fig. 3.5).

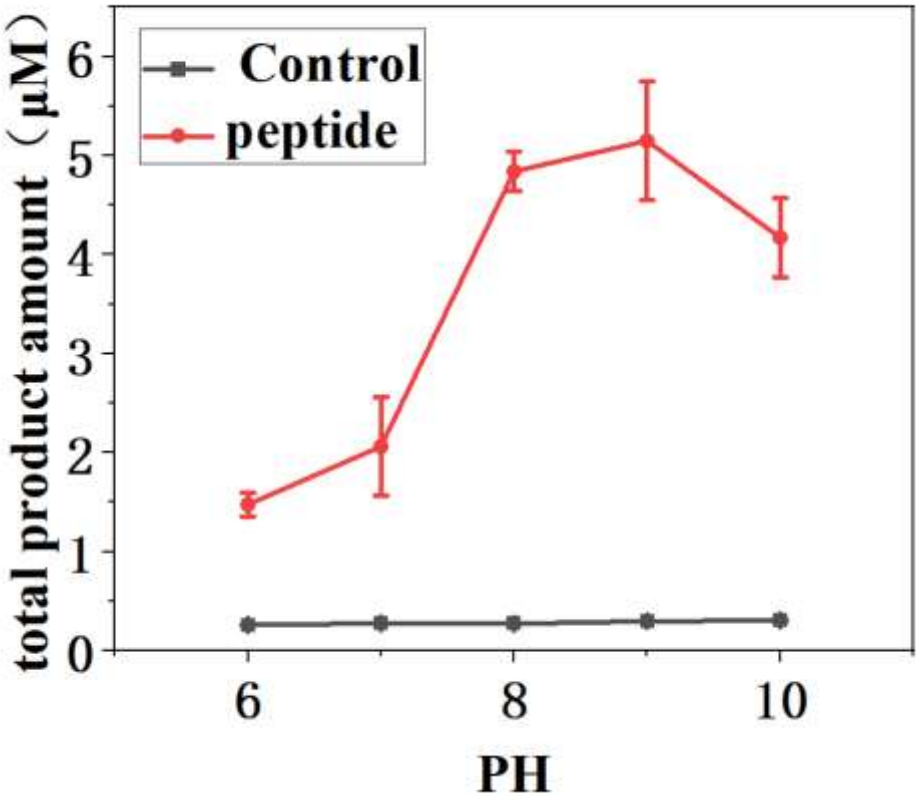


Figure 3.5 – Effect of pH on enzyme activity

In summary, the results show that under suitable pH and temperature conditions, the simulated enzyme exhibits high catalytic activity and can effectively degrade PET and generate target products, demonstrating its

important application in environmentally friendly chemical synthesis. In addition, the results highlight the value of simulated enzymes in optimizing process design and increasing production efficiency. By fine-tuning reaction conditions, we are able to continuously improve catalytic efficiency, which is important for scaling up industrial production and reducing costs. Therefore, the application of simulated enzymes in environmentally friendly chemical synthesis has broad prospects and is expected to play an important role in the development of environmental protection technology in the future.

3.3.3 Degradation products

As shown in Fig. 3.6, the regression equation for TPA is y=256938.247x-9238.4227, R^2=0,989. The regression equation for Fig. 3.7 is y=381542.548x+46916,183 and R^2 is 0.99377.

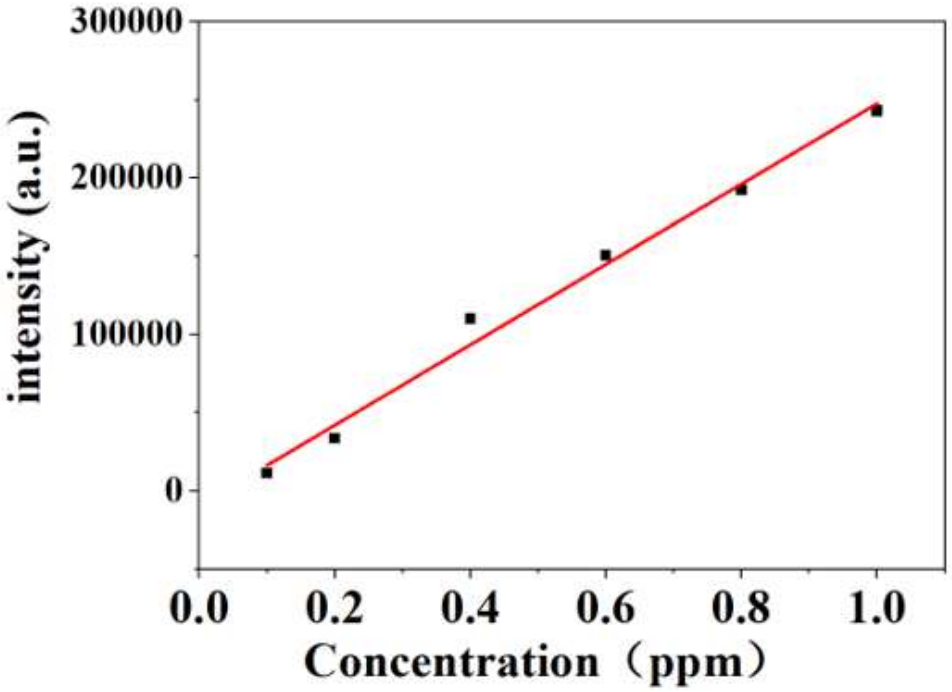


Figure 3.6 – TPA standard music chart

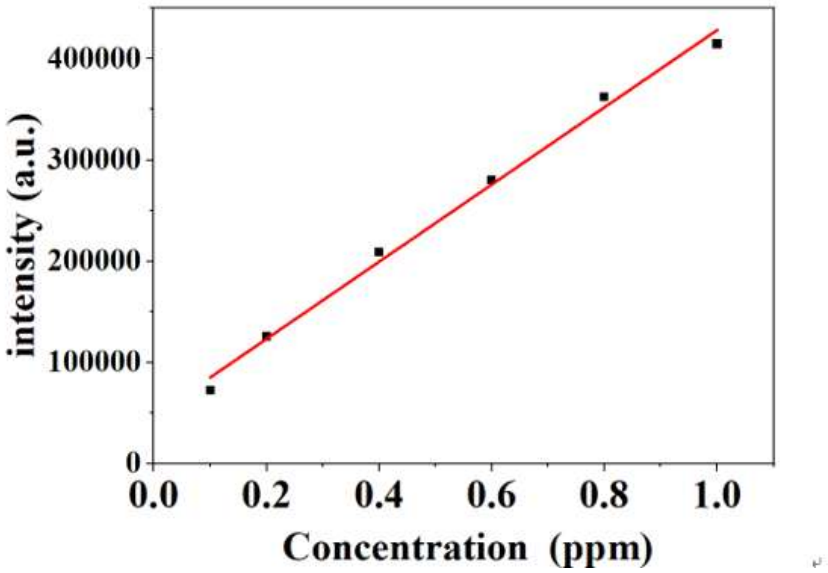


Figure 3.7 – MEHT curve chart

The products of PET degradation were further analyzed. By comparing the HPLC spectrum with the standard, it was found that there were double peaks in the liquid chromatogram, the corresponding peak was TPA at 5 min and MHET at 5.8 min, indicating that the hydrolysate products of peptide mimetic enzyme degrading PET were TPA and MHET (Fig. 3.8).

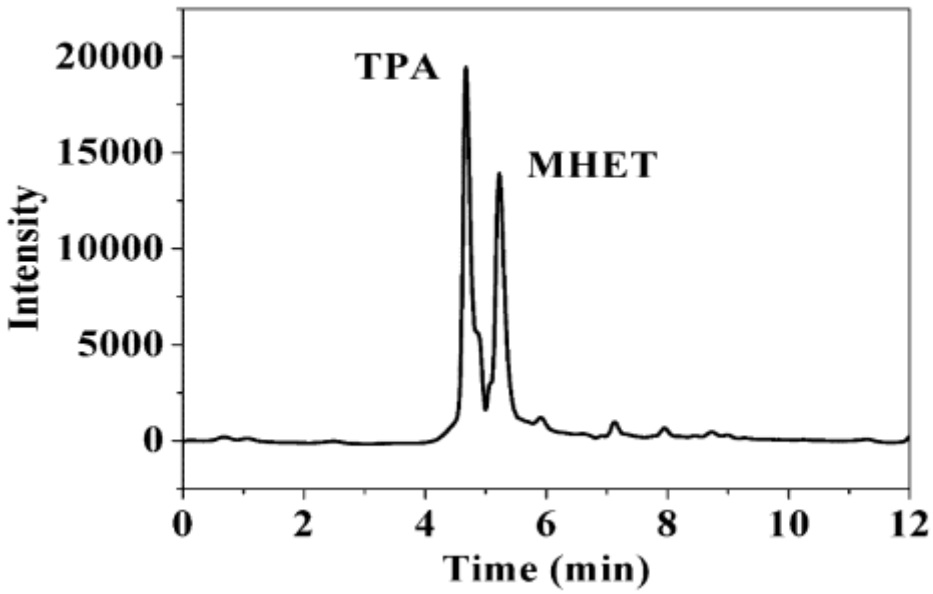


Figure 3.8 – Product diagram

3.3.4 Simulated enzyme degradation of PET film

According to the before-and-after control in Fig. 3.9, it can be bserved that compared with the smooth and uniform film of the control group, there are obvious traces of roughness and erosion on the surface of the PET film with a thickness of 100 μm after enzyme mimetic treatment, and a large number of holes appear. The results indicated that the mimetic enzyme had the ability to degrade PET.

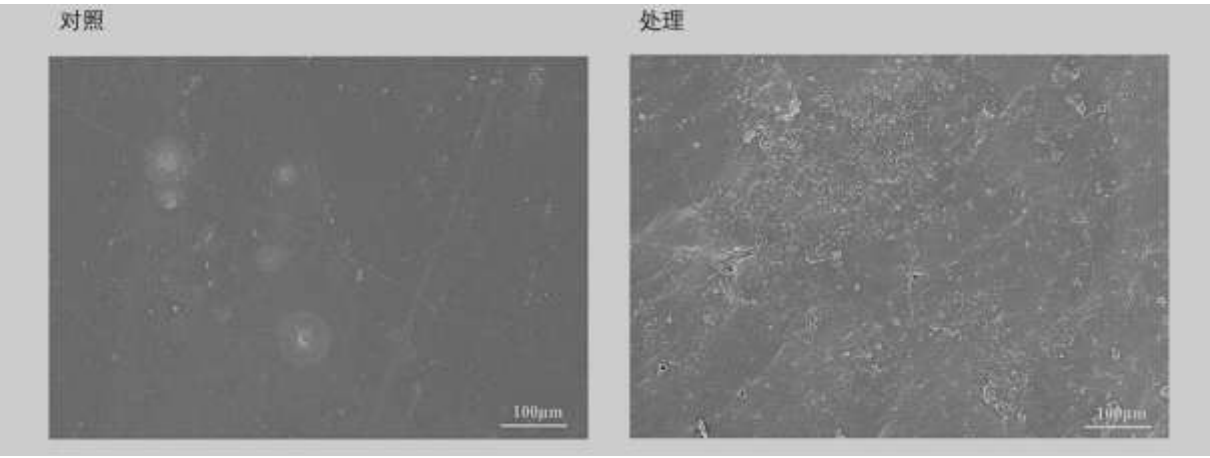


Figure 3.9 –SEM hydrolysis of 0.25mmPET membrane

3.4 Study on catalytic mechanism of polypeptide mimics

To gain a deeper understanding of the nature of the interaction between the enzyme mimics and PET, we analyzed the binding process of the PET model substrate 2-HE (MHET)3 using a molecular docking approach (Fig. 3.10). The substrate binds mainly to the long groove of the active site of the enzyme mimic, and the binding free energy between 2-HE(MHET)3 is -5.04 kcal/mol. The number of hydrogen bonds between the simulated enzyme and the substrate was 6, and the amino acids were His2, Thr17, Lys20, Lys24, and Val23. Hydrogen bonding is not only an important force for biological macromolecules to maintain structural stability, but also an important form of improving protein-ligand interaction, so that the binding has strong stability.

Hydrophobic interactions (Fig. 3.11, orange area) and Pi-sigma bonds with val19 as the acting amino acid are also present. The substrate binds to the active site of the enzyme mimic, indicating a specific spatial configuration and interaction pattern between them, and the resulting binding free energy and the number of hydrogen bonds further confirm the robustness and stability of this interaction. These results not only provide a deeper understanding of the binding mechanism between enzyme mimics and PET, but also provide important guidance for further design of mimics with higher affinity and stability.

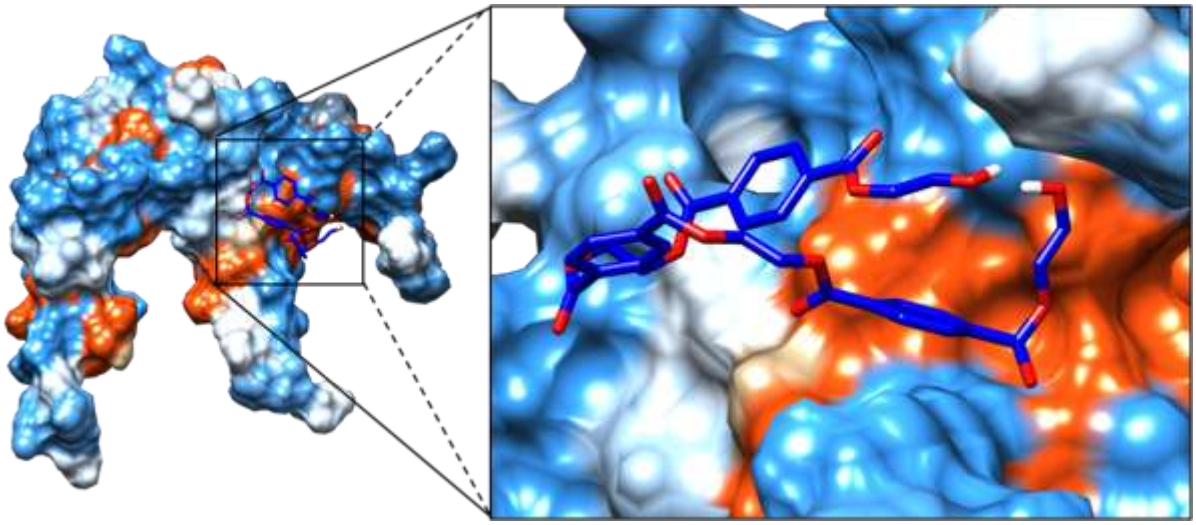


Figure 3.10 – Simulated 3D docking conformation of enzyme and substrate

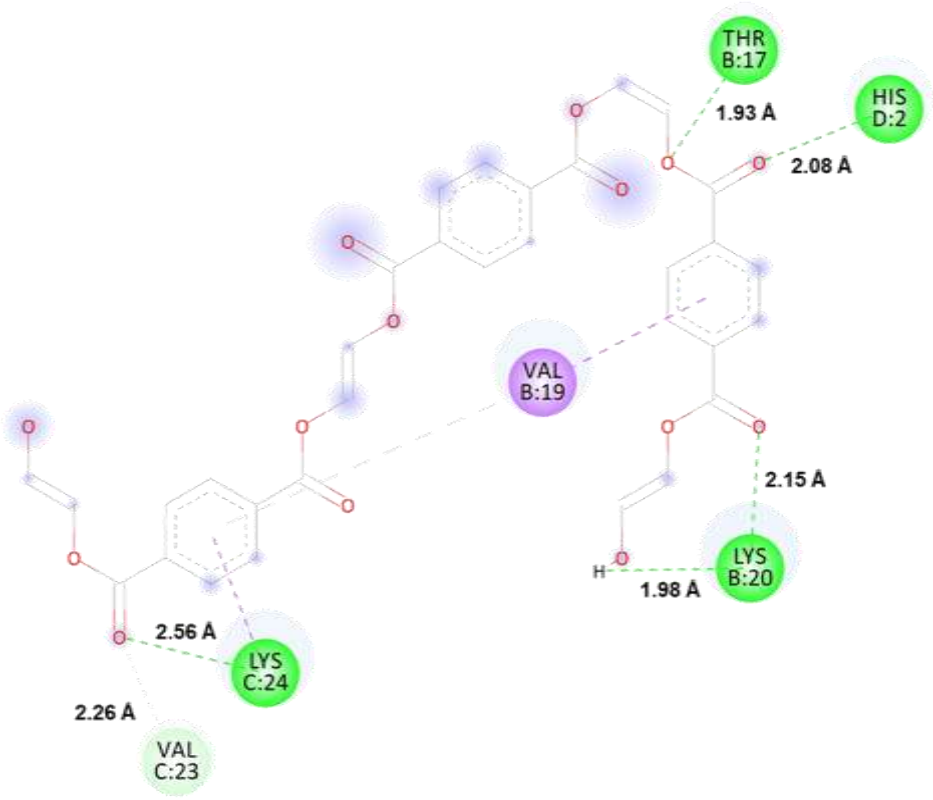


Figure 3.11 – 2D diagram of simulated enzyme-substrate interaction

Conclusions to chapter 3

These findings provide us with important insights into the interaction between enzyme mimics and PET. First, the substrate binds at the active site of the enzyme mimics, indicating that there is a specific spatial configuration and interaction pattern between them. Secondly, the obtained binding free energy and the number of hydrogen bonds further confirm the firmness and stability of the interaction. These results not only contribute to a deeper understanding of the binding mechanism between enzyme mimics and PET, but also provide important guidance for further design of mimics with higher affinity and stability. Based on these findings, we can further explore the interaction properties between enzyme mimics and PET, and provide more useful references and enlightenment for the development of related research fields.

CONCLUSIONS

1. In this research project, we designed a peptide-based polyethylene terephthalate (PET) mimic. Its secondary structure is stable, with high catalytic activity and strong hydrophobicity. As the temperature rises, it produces a β sheet structure and a fiber structure, which is highly resistant to temperature changes. The simulated enzyme temperature of 65°C and PH=9 could be used to efficiently treat PET, and the degradation products were TAP and MHET. The results showed that hydrogen bonding and hydrophobic interaction were the main forces of the interaction between the enzyme mimics and PET, and the amino acids were His2, Thr17, Lys20, Lys24, Val23, and Pi-sigma bonds with val19 as the acting amino acids.

2. Self-assembled simulated enzymes with similar functions of natural enzymes were used to conduct PET degradation studies. Combined with CD spectroscopy, TEM and FTIR technologies, the structural characteristics of polypeptide simulants were characterized from different perspectives, and activity detection and catalytic mechanism research were carried out. The results showed higher catalytic efficiency, lower cost and stronger environmental adaptability than natural catalytic enzymes. It is expected that it can be widely used in industry and life as a new material in the future.

3. This experiment only provides an idea of assembling enzymes, and lacks other forms of assembling enzymes for comparison. The accuracy of molecular docking may be limited by the simplification of substrate models, parameter selection and calculation methods, so its prediction effect in real environment needs to be further verified. Finally, this paper lacks in-depth discussion on the application prospects and potential problems of polypeptide mimics in real environment. Further corrections need to be made in the light of production practice.

LIST OF REFERENCES

1. Du, R. (2020). Synthesis of two novel simulated enzymes and their catalytic properties [Doctoral dissertation, Sichuan Agricultural University]
2. Zhou, Q., Huang, Q.-S., Li, J., et al. (2017). Isolation and characterization of a thermophilic polyglycol terephthalate degrading bacterium. Chinese Journal of Microbiology, 23(7), 2822-2834.
3. Shu, X. T. (2023). Preparation of polyester elastomer from waste PET and its properties [Doctoral dissertation, Beijing University of Chemical Technology, China].
4. Tang, S. (2022). Preparation and properties of PET alcoholysis catalyst [Doctoral dissertation, Hebei University of Technology]. (In Chinese)
5. Chen, C. C., Li, X., Min, J., Zeng, Z., et al. (2024). Complete decomposition of poly(ethylene terephthalate) by crude PET hydrolytic enzyme produced in Pichia pastoris. Chemical Engineering Journal, 481, 1385-8947. (In Chinese)
6. Qi, X., Ma, Y., Chang, H., et al. (2021). Evaluation of PET degradation using artificial microbial consortia. Frontiers in Microbiology, 12, 778-828.
7. Xue, R., Yinping, C., Huan, R., Ren, W., et al. (2021). Fusion of Chitin-Binding Domain from Chitinolyticbacter meiyuanensis SYBC-H1 to the Leaf-Branch Compost Cutinase for Enhanced PET Hydrolysis. Journal of Frontiers in Bioengineering and Biotechnology, 9, 762854-762854.Shan, L. X., Xiao-Ling, W., Min-Hua, Z., Wen-Yong, L.
8. Construction of Zn-heptapeptide bionanozymes with intrinsic hydrolase-like activity for degradation of di(2-ethylhexyl) phthalate. (2022). Journal of Journal of Colloid And Interface Science, 622, 860-870.

9. Li, X., Zhou, Y., Lu, Z., et al. (2023). Switchable enzyme mimics based on self-assembled peptides for polyethylene terephthalate degradation. Journal of Colloid and Interface Science, 646, 198-208.
10. Zhang, X., Bo, Y., Bai, X. (2023). Research progress of PET biodegrading enzymes. Journal of Inner Mongolia Forestry Science and Technology, 49(03), 59-64. (In Chinese)
11. Xing, K. (2020). Study on degradation of packaging waste PET plastics and properties of degradation products [Doctoral dissertation, Xi'an University of Technology].
12. Shi, K., Guo, L., Wang, Y., et al. (2019). Research progress of PET degradation methods and ionic liquid catalysts. Plastics, 48(3), 42-45. (In Chinese)
13. Tokiwa, Y., Suzuki, T. (1977). Hydrolysis of polyesters by lipases. Nature, 76-78.
14. Mueller, R.-J. (2006). Biological degradation of synthetic polyesters-Enzymes as potential catalysts for polyester recycling. Process Biochemistry, 9(10), 2124-2128.
15. Zhang, J. C. (2011). Preparation of carbon fiber supported Cobalt phthalocyanine catalyst and its catalytic performance by simulated enzyme [Doctoral dissertation, Zhejiang Sci-Tech University].
16. Jing, N., Wenhong, L., Qiang, L., et al. (2024). Research progress of PET plastic degradation and modification methods of degrading enzymes. Journal of Petrochemical Universities, 37(01), 16-24.
17. Micklitsch, C. M., Medina, S. H., Yucel, T., et al. (2015). Influence of hydrophobic face amino acids on the hydrogelation of β-hairpin peptide amphiphiles. Macromolecules, 48, 1281-1288.

18. Wang, Y., Yang, L., Wang, M., Zhang, J., Qi, W. (2021). Bioinspired phosphatase-like mimic built from the self-assembly of de novo designed helical short peptides. ACS Catal, 11, 5839-5849.
19. Ren, A. J. (2023). Investigation and analysis of simulated enzyme activity of metal-organic framework materials [Doctoral dissertation, Hubei University of Medicine].
20. Zhou, Y., Yunxuan, W., Caili, Z., et al. (2021). Recycling technology and standard status of polyethylene terephthalate. China Plastics, 35(08), 162-171. (In Chinese)
21. Jiang, Z., Guo, Z., Zhang, Z., et al. (2019). Preparation and Properties of Bottle-recycled Polyethylene Terephthalate (PET) Filaments. Textile Research Journal, 89(7), 1207-1214.
22. Majumdar, A., Shukla, S., Singh, A., et al. (2019). Circular Fashion: Properties of Fabrics Made from Mechanically Recycled Poly-ethylene terephthalate (PET) Bottles. Resources Conservation and Recycling, 161, 1-10.
23. Mishra, S., Goje, A. S. (2003). Kinetic and Thermodynamic Study of Methanolysis of Poly (ethylene terephthalate) Waste Powder. Polymer International, 52(3), 337-342.
24. Moog, D., Schmitt, J., Senger, J., et al. (2019). Using a Marine Microalga as A Chassis for Polyethylene Terephthalate (PET) Degradation. Microb Cell Fact, 18(171), 1-15.
25. Then, J., Wei, R., Oeser, T., et al. (2016). A Disulfide Bridge in the Calcium Binding Site of A Polyester Hydrolase Increases Its Thermal Stability and Activity Against Polyethylene Terephthalate. FEBs Open Bio, 6(5), 425-432. (In Chinese)

26. Ronkvist, A. M., Xie, W., Lu, W., et al. (2009). Cutinase-catalyzed Hydrolysis of Poly(ethylene terephthalate). Macromolecules, 42(14), 5128-5138.
27. Zhang, J. (2022). Preparation and bioenvironmental application of piezoelectric responsive graphite-alkynyl composites [Doctoral dissertation, Qingdao University of Science and Technology].
28. Wu, Y., Li, G., Zou, L., et al. (2017). Electrochemical Biosensors Based on Novel Nanocomposites and Polypeptide-analog Enzyme Signal Amplification. Chinese Chemical Society, National Natural Science Foundation of China, Chinese Society of Instrumentation. Abstracts of the 13th National Conference on Electroanalytical Chemistry, 1, 1.
29. Xia, L., Li, J., Hao, S., Han, A. (2021). Enzyme Mimics Based Membrane Reactor for Di(2-ethylhexyl) Phthalate Degradation. Journal of Hazardous Materials, 403, 124511.