The Secondary Structure Analysis and Protein Identification of Esterase Were Performed by Circular Dichroism

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Keywords: Esterifying Enzyme, Protein Structure, Round Two Chromatographic.

Abstract: In order to improve the yield of esterase and achieve the purpose of improving liquor quality rate and aroma, the structure of esterase was studied by circular dichroism method and the protein was identified by liquid mass spectrometry (LC-MS/MS). The results showed that the content of esterification enzyme Helix (Helix) was 17.10%, anti-parallel β -folding structure was 29.70%, parallel β -folding structure was 5.40%, β -rotation structure was 18.60%, irregular coil structure was 37.80%. This indicates that esterase is a kind of protein with irregular curl. The β -folding structure of esterase accounted for 35.10%, indicating that the secondary structure of esterase had a certain rigidity, but the β -rotation and random curl structure accounted for 56.40%, indicating that the various residues in esterase peptide segment had a large degree of freedom, good flexibility, but poor stability. Protein identification results showed that RHICH Lipase peptide had the highest content, 906, coverage rate of 76.35%, total theoretical amino acids of 389 protein. The number of PSEFL DNA-Binding Response regulator peptide was 14, the coverage rate was 32.93%, and the total number of theoretical amino acids was 246. Then, the number of BURPL 50S ribosomal protein L5 peptide was 10, the coverage was 18.99%, and the total number of theoretical protein amino acids was 179; Then there was 9GAMM 30S ribosomal protein S19 peptide, the number was 7, the peptide coverage was 15.22% and the total number of theoretical amino acids was 92; Finally, the peptide of 9GAMM Succinate dehydrogenase iron-sulfur subunit was 7, the coverage rate was 16.03, and the total number of protein theoretical amino acids was 237.

1 INTRODUCTION

Esterase (esterase.C.3.1.1.1), also known as carboxyl esterase, is an enzyme that can hydrolyze carboxyl ester bonds and catalyze the synthesis of low grade fatty acid esters (Chen, 2017), which has the ability to catalyze ester synthesis and decomposition. Therefore, liquor industry is used to call it esterase or ester decomposition enzyme. Protein refers to the polymer formed by the connection of 20 different amino acids (Yao, 2006; Xu, 2011; S Sirén, 2020) The structure of protein includes the chemical structure and spatial structure of protein (Xu, 2020; Gao, 2010). The methods (Zhou, 2021; Wei, 2021) to study the secondary structure and advanced structure of protein include X-ray crystal diffraction technology, nuclear magnetic resonance technology and circular dichroism technology (Xiao, 2020). But the first two methods are limited by many factors, it is difficult to analyze. The analytical scanning of circular dichroism spectrometer plays an important role in studying the secondary structure and advanced structure of proteins, which is a special absorption spectrum (Huang, 2019). The circular dichroism spectrum of biological macromolecules such as proteins detected by circular dichroism chromatography is used to obtain the secondary structure of biological macromolecules (Tong, 2018). Therefore, CIRCULAR dichroism is widely used in protein folding and conformation research (Liu, 2012).

This paper uses circular dichroism to analyze the secondary structure of esterase and identify the esterase protein to provide a theoretical basis for the application of esterase in wine industry.

Cao, X., Zhong, L., Li, S., Zhou, A., Zhang, K. and Song, Z.
The Secondary Structure Analysis and Protein Identification of Esterase Were Performed by Circular Dichroism.
DOI: 10.5220/0012002200003625
In *Proceedings of the 1st International Conference on Food Science and Biotechnology (FSB 2022)*, pages 76-79
ISBN: 978-989-758-638-5

2 MATERIALS AND METHODS

2.1 Strains and Chemical Reagents

Staphylococcus aureus: staphylococcus aureus was isolated and screened from Wuliangye Daqu, and the strain producing esterification enzyme was identified as *STaphylococcus aureus* according to the morphological characteristics of the fungus colony and molecular biology.

Acetonitrile, formic acid and ammonium bicarbonate were all ms grade. Dithiothreitol and iodoacetamide were analytically pure. Trypsin sequencing grade.

2.2 Circular Dichroism Detection

The sample was dissolved in water at a concentration of 0.2 ug /uL. The initial wavelength was set at 180 nm, the end wavelength was set at 260 nm, the step size was 1 nm, the collection time was 1 s/ point, and the cuvette width was 0.1 cm. The blank control solution was sampled with 300 uL and deducted after measurement. Then test the sample with 300 uL, and save the data after test.

2.3 Protein Identification

2.3.1 LC - MS/MS Detection

Packed with Acclaim PepMap RPLC C18, 5 μm, 100A; 150 μm I.D. × 150 mm, Packed with Acclaim PepMap RPLC C18, 1.9 μm, 100A; Mobile phase A: 0.1% formic acid; Mobile phase B: 0.1% formic acid, 80% ACN; Flow rate: 600 nL/min; Analysis time of each component: 60 min.

2.3.2 Mass Spectrometry Conditions

Primary mass spectrometry parameters: Resolution: 70,000; AGCtarget: 3 e6; MaximumIT: 100 ms; Scanrange: 300 to 1400 m/z.

Secondary mass spectrometry parameters: Resolution: 17,500; AGCtarget: 1 e5; MaximumIT: 50 ms; TopN: 20; An NCE/steppedNCE: 28.

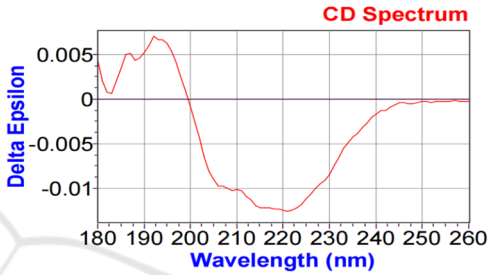
2.3.3 Database Search

The mass spectrometry raw file retrieves the target protein database using Byonic.

3 RESULTS AND DISCUSSION

3.1 CD Spectrum of Esterase in Far ULTRAVIOLET Region

The UV CD spectrum of esterification enzyme was shown in Fig.1. According to Fig.1, there was a positive polarization peak at 195 nm and a small shoulder at 187 nm, while the control group had a wide negative shoulder at 210 nm and a wide negative peak at 220 nm.



The abscissa represents the scanning wavelength and the ordinate represents the ellipticity

Figure 1: Far-ultraviolet (180-260 nm) scanning of esterification enzyme.

3.2 Secondary Structure of Esterification Enzyme

The secondary structure of the sample was fitted with CDNN software, including Helix, Antiparallel, Parallel, beta-turn and RNDM.coil.

The analysis results were as follows: esterification enzyme helix structure 17.10%, antiparallel structure 29.70%, parallel β -folding structure 5.40%, β -rotation structure 18.60%, random coil structure 37.80%. This indicates that esterase is a kind of protein with irregular curl.

3.3 Esterase Protein Identification

Raw files generated by esterase through LC-MS/MS data collection were opened with Xcalibur, and total ion flow chromatography fig. 2 could be seen as follows:

Table 1: Calculation of secondary structure ratio in esterification enzyme.

The sample	Helix	Antiparallel	Parallel	Beta-Turn	Rndm. Coil
Esterifying enzyme	17.10%	29.70%	5.40%	18.60%	37.80%

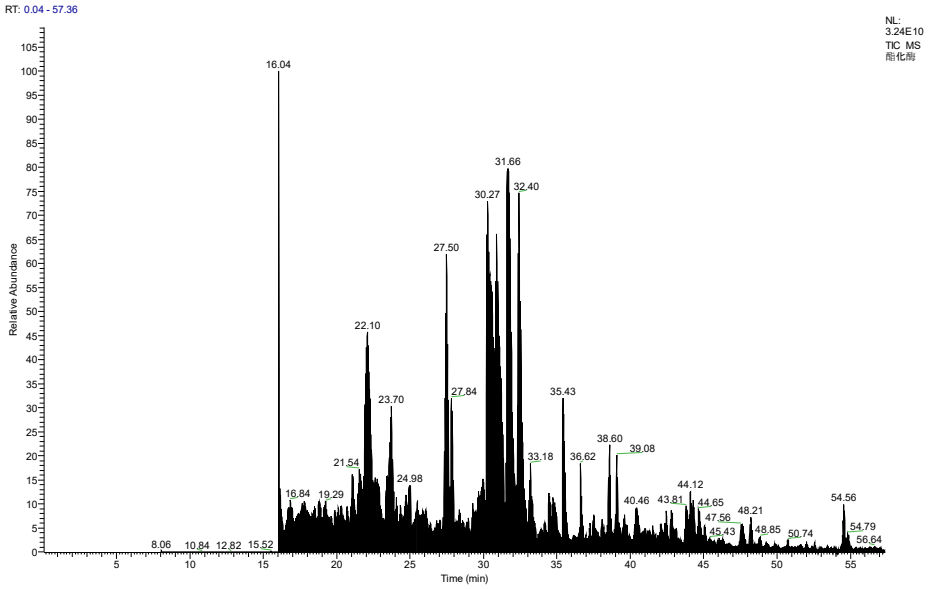


Figure 2: Total ion flow chromatogram of esterification enzyme.

The results of protein identification were retrieved by software Byonic database as shown in the attached page:

From the attached pages: The results showed that the top ten proteins with high scores were RHICH Lipase, PSEFL DNA-Binding Response regulator, BURPL 50S ribosomal protein L5 and 9GAMM 30S Ribosomal protein S19, 9GAMM Succinate dehydrogenase iron-sulfur subunit, 9GAMM Succinate--CoA ligase [ADP-forming] Subunit beta, ARHIZD Lipase, BURPL 50S Ribosomal protein L14, 9GAMM 2-methylisocitrate lyase, 9GAMM Sulfate Adenylyl transferase subunit 2. The number of RHICH Lipase peptide was 906, the coverage rate was 76.35%, and the total number of theoretical amino acids was 389. The number of peptide in PSEFL DNA-Binding Response regulator was 14, the coverage rate was 32.93%, and the total number of theoretical amino acids was 246. Then the number of peptides in the BURPL 50S Ribosomal protein L5 was 10, the coverage was 18.99%, and the total number of theoretical amino acids in the protein was 179; Then 9GAMM 30S ribosomal protein S19 had a peptide number of 7 and a peptide coverage of 15.22% with a theoretical total of 92 amino acids; Fifth, the number of peptide of 9GAMM Succinate dehydrogenase iron-sulfur subunit was 7, the coverage rate was 16.03, and the total number of protein theoretical amino acids was 237; The sixth score was 9GAMM Succinate-- the maximum number of peptides in CoA ligase [ADP-forming] subunit beta was 2, the coverage rate was 7.22%, and the total number of theoretical amino acids in protein was 388. In the seventh place, ARHIZD Lipase had a maximum of 16 peptides, a coverage rate of 9.77%, and a total of 389 theoretical amino acids; For the eighth BURPL 50S ribosomal protein L14, the maximum number of peptides was 9, the coverage rate was 22.95%, and the total number of theoretical amino acids was 122; The number of 9GAMM 2-methylisocitrate lyase is 3, the coverage rate is 5.19%, and the total number of protein theoretical amino acids is 289; The number of peptides in 9GAMM Sulfate adenylyl transferase subunit 2 is 3 at most, the coverage rate is 8.50%, and the total number of theoretical amino acids in protein is 306.

4 CONCLUSIONS

Using circular dichroism to analyze esterase, the β -fold structure of esterase accounted for 35.10%, β -rotation and random curl structure accounted for 56.40%, indicating that esterase is a protein mainly with random curl, and each residue in esterase peptide segment has a large degree of freedom, good flexibility, poor stability. Protein identification results showed that RHICH Lipase had the highest protein content, with 906 peptides, 76.35% coverage rate and 389 theoretical amino acids. PSEFL DNA-Binding Response Regulator had 14 peptides, the coverage rate was 32.93%, and the total number of theoretical amino acids was 246. Then, the number of peptide fragments in the BURPL 50S

Ribosomal protein L5 was 10, the coverage was 18.99%, and the total number of theoretical amino acids in the protein was 179. Then the number of peptides in 9GAMM 30S ribosomal protein S19 was 7, the coverage rate was 15.22%, and the total number of theoretical amino acids in protein was 92. Finally, the peptide number of 9GAMM Succinate dehydrogenase iron-sulfur subunit was 7, the coverage rate was 16.03, and the total number of protein theoretical amino acids was 237. Different methods and molecular mechanisms are used to change the properties of esterification enzyme. Therefore, protein identification combined with esterification enzyme can provide ideas for the experimental design of improving the properties of esterification enzyme, so as to promote the high-quality development of wine industry.

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ACKNOWLEDGMENTS

Fund Project: Cooperation project of Wuliangye Yibin Co., Ltd (CXY2019ZR012

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