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Comparative Study on Purification Methods of Alfalfa Esterase

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Abstract **Original Research Article**

The alfalfa esterase was purified by ammonium sulfate precipitation method, cellulose method and sephadex method respectively. The result showed that the esterase activity purified by sephadex was higher than that of the other two purification methods. Infrared spectroscopy technology was used to study alfalfa esterase purified by different methods, it was found that absorption peaks of the infrared spectrum of crude extracts before purification was plentiful and disordered, and the absorption peaks of purified samples showed obvious and clear, indicating that all three methods could be used to purify esterase, it's just the peak of esterase purity of sephadex was the highest.

Keywords: Infrared spectrum; alfalfa esterase; purification.

INTRODUCTION

Alfalfa esterase belongs to carboxylic acid hydrolase, which can hydrolyze 2,6-dichloroacetylindophenol and turn the solution blue. Its activity can be inhibited by organophophorus pesticides and carbamate pesticides, so that the chromogenic reaction is weakened or even no reaction occurs at all [1]. In order to obtain purified alfalfa esterase, assisted by infrared spectroscopy technology [2-5], this paper explored better purification method of alfalfa esterase by comparing the spectra of alfalfa esterase with different purification methods, so as to lay a foundation for the application of alfalfa esterase in the detection of pesticide residues.

1. MATERIALS AND METHODS

1.1 MATERIALS

Alfalfa was collected from the scientific experiment field of Heilongjiang Bayi Agricultural University.

1.2 Reagents and equipment

Sephadex G-100 was purchased from Pharmacia Co.,Ltd; Cellulose DE-32 was purchased from Biosharp Life Science Co.,Ltd; Bovine serum protein was purchased from Shanghai Aladdin Biochemical Technology Co.,Ltd; 2,6-dichloroindophenol was purchased from BOC Science Co.,Ltd. All the reagents were of AR grade.

Nicolet is 5 Fourier Transform Infrared Spectrometer was from Thermo Fisher Co.,Ltd; JY92-IIDN Ultrasonic Cell Disrupter was from NINGBO SCIENTZ BIOTECHONOLOGY Co.,Ltd; AR224N Analytical Balance was from Ohaus US Co.,Ltd; Ultraviolet Visible Spectrophotometer TU-1901was from Beijing Purkinje General Instrument Co.,Ltd; FD-1D-50 Freeze Dryer was from Shanghai Bilon Co.,Ltd; H2050R-1 High-speed Refri Gerated Centrifuge was from Hunan meter centrifuge instrument limited Co.,Ltd.

1.3 METHODS

1.3.1 Alfalfa esterase extraction

At the temperature of 4 ℃, 5g alfalfa, together with 0.1mol·L^{-1} phosphate buffer solution with pH 7.0, was added according to the ratio of material to liquid of (m:v) 1:10. The mixture was extracted with ultrasonic disrupter for 2 minutes, and the obtained crude enzyme solution was centrifuged at 4000r·min-1 for 20 minutes, the supernatant was taken for standby.

1.3.2 Purification of alfalfa esterase

10.0 mL of the above mentioned crude enzyme solution was purified by ammonium sulfate precipitation method, DEAE-32 Cellulose and Sephadex G-100 column chromatography method, and collect the activity peaks.

1.3.3 Determination of esterase activity and specific activity

3.50 mL of purified enzyme solution and 4.00 mL distilled water were added into a conical flask, the conical flask was kept in a water bath for at the temperature of 40℃ for 10 minutes. Subsequently, add 0.50 mL chromogenic agent, the solution was kept at the temperature of 40℃ for 20 minutes, and the absorbance value was determined at 610 nm. Solution without esterase was taken as the reference solution.

Take 1.00 mL of the above mentioned purified enzyme solution, determined the total protein content by biuret method. The specific activity of esterase was calculated as shown below:

Specific activity=Esterase Activity (ΔA)/Total Protein Content (m)

1.3.4 Infrared determination of purified esterase

1.00mL of the above mentioned esterase purified solution with the highest specific activity respectively was freeze-dried to remove the extractant. The obtained powder was mixed with KBr of the same quality, and was compressed into tablets for infrared testing. The above KBr tablets were dried at a suitable temperature before testing in case of the impact of CO_2 and H_2O.

3. RESULTS AND DISCUSSION

3.1 Specific activity of purified esterase purified by different methods

Specific activity of purified esterase purified by ammonium sulfate precipitation method, cellulose method and sephadex method was 0.434, 0.249, 0.619 respectively.

3.2 IR spectra of purified esterase purified by different methods

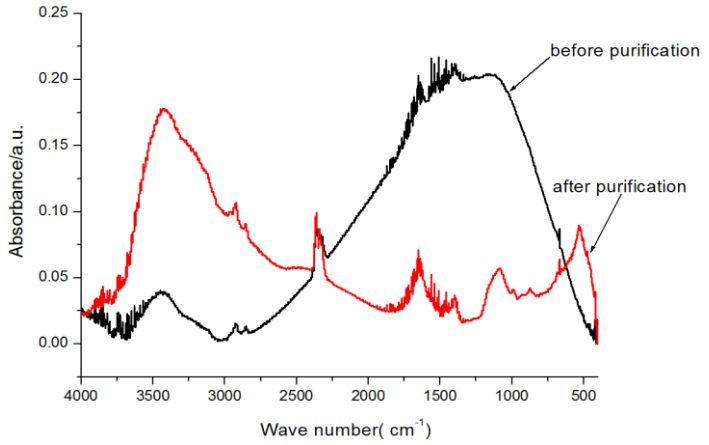

Fig 1: Comparison of IR spectra of esterase before and after purification

It was shown in Fig 1 that the IR absorption peaks before purification were numerous and disordered, and the IR absorption peaks of purified samples were obvious and clear, which suggested that the purification methods were effective.

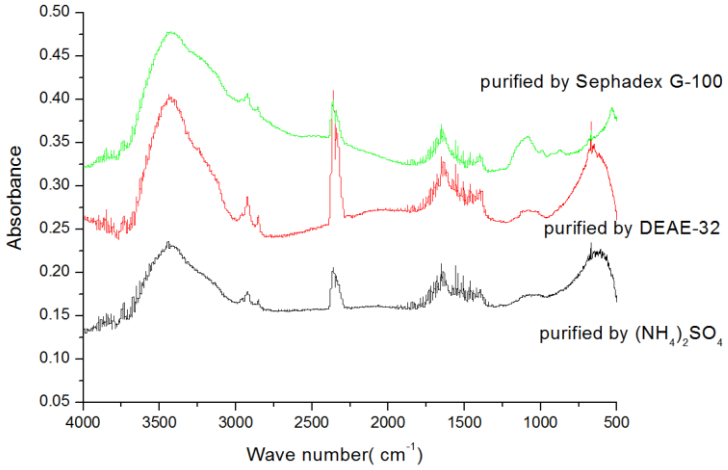

Fig 2: IR spectra of purified esterase purified by $(NH_4)_2SO_4$, DEAE-32 and Sephadex G-100

The infrared spectrum peaks of purified alfalfa esterase mainly distributed in the following four areas:

1. In the range of 3000 cm^{-1}-3700 cm^{-1}, the peak was high and wide with high intensity, which was attributed to the stretching vibration of -OH, the stretching vibration of -OH of intermolecular hydrogen bond, or the stretching vibration of -NH or the alone carboxylic acid also had a sharp edge at about 3350cm^{-1}.
2. In the range of 2250 cm^{-1}-2300 cm^{-1}, the peak was small and narrow. Regardless the impact of CO_2, triple bond and cumulative double bond were absorbed in this range, such as -CN,-N=C=O and so on.
3. In the range of 1400 cm^{-1}-1800 cm^{-1}, the peak was low and wide with medium intensity. The C=O stretching vibration of carboxylic acid and ester, C=O stretching vibration in amide (band I), -NH in-plane bending vibration (band II) and C-N stretching vibration in amide (band III) all appeared absorption peaks in this range.
4. In the fingerprint region of 1000 cm^{-1}-1250 cm^{-1},the peak was small and wide with weak intensity. The C-O stretching vibration peak of carboxylic acid was medium here, so as the C-O-C stretching vibration peak of ester.

4. CONCLUSION

Alfalfa esterase is a kind of carboxylic acid hydrolase. As an active enzyme, its activity is greatly affected by the environment. Therefore, the purification methods of biologically active enzymes need to be further studied for sure.

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