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Immobilization of Plant Esterase Purified from Wheat Flour on Porous Poly (GMA-AM-EGDMA) Polymer Particles Newly Made With Liquid and Solid Pore-Forming Reagents

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Abstract: *The Poly (GMA-AM-EGDMA) macrospheres were synthesized with glycidlymethacrylate (GMA), acrylamide (AM) as monomer and glycol dimethacrylate (EGDMA) as cross-linker, firstly with carbinol and distilled water as liquid pore-forming reagents and nano-calcium carbonate as solid one via suspension polymerization. Its exterior form was shown by scanning electron microscopy. Under the most suitable terms, the plant esterase isolated and purified from wheat flour was bounded on the particles above, the activity yield was 65.78% and the activity of it could reach 33.09 U/g dry carriers. Kinetic studies showed the pH operating range of the immobilized enzyme was wider and its thermal stability was better than those of the free enzyme, meanwhile, a good operational stability was also observed after being emploied 6 times repeatedly.*

Keywords: *Wheat esterase, Immobilization, Suspension polymerization, GMA-AM-EGDMA*

1. INTRODUCTION

In the past few decades, immobilized enzyme has been used in many fields, such as food, nourishment, pesticides and so on，and it is widely recognized that the bounded enzymes are very potential as industrial biocatalysts [1-5]. A lot of articles [6-8] described a number of materials for the immobilization of enzymes, among them, epoxy-activated carrier, which could display good reactivity under gentle terms, has aroused people's great interest in the immobilization of enzyme [9,10].

In this paper, Poly (GMA-AM-EGDMA), which has a large pore structure and reactive epoxy groups, was obtained well using glycidyl methacrylate (GMA), acrylamide (AM) as monomer and glycol dimethacrylate (EGDMA) as cross-linker, carbinol and distilled water as liquid pore-forming agents and nano-calcium carbonate as solid by suspension method. The exterior form of the carrier was seen by scanning electron microscopy, and then it was used to bound plant esterase purified from wheat flour. Under the optimum conditions, the enzyme activity and the activity yield were tested. Finally, the basic properties of the immobilized enzyme including optimum temperature, optimum pH, pH stability, thermal stability and operational stability were also researched.

2. EXPERIMENTAL

2.1. Apparatus and Reagents

DZ-6020 Vacuum Desiccator, JJ-2 Organization compactors, LG10-2.4 Centrifugal machine, Ultraviolet visible spectrophotometer (T6 new century), TG328B analytical balance, Thermostatic oscillator (SHA-B), Circulating water vacuum pump SHZ-D (Ⅲ), and Digital pH Meter (PHS-3C), KQ5200B Ultrasonic cleaning machine were applied in our research.

Wheat flour purchased from baidahuang china, Acrylamide (AM), glycidyl methacrylate (GMA) and Dimethyl Acrylate (EGDMA) were purchased from Shanghai Alading Reagent co. Ltd., Fast blue B salt was obtained from Shanghai Golden Harvest Biotech Co. Ltd.. The remaining chemical reagents used were all analytical. All water solutions were prepared by twice distilled water.

2.2. Enzyme Purification

All the processes of the purification were used according to Wu et al. reported [11]. 30g of wheat flour was weighted and mixed with 140-160 volumes of cold sodium phosphate buffer solution (PBS, 0.01 M), After being grounded with a high speed tissue gravity mill, the mixture obtained was stored in the

fridge for 12 hours，then it was centrifuged for half an hour with a speed of 8000 r/min, The transparent supernatant was retained and the precipitate was gave up. Mixed with ammonium sulfate (30%) with continuously stirring for 1-2 h, the solution was centrifuged as the first step. The sediment was gave up, and the clear part was take to 70% saturation by adding solid ammonium sulfate and then centrifuged as above. The sediment obtained was dissolved in 25.00 ml 0.01 M PBS (pH 6.4) and dialyzed for two days in the refrigerator, the enzymes obtained (159.90 U/ml) was stored to be used in the next step.

2.3. Synthesis of Macroporous Poly (GMA-AM-EGDMA) Bead Carriers

The porous Poly (GMA-AM-EGDMA) was prepared by suspension copolymerization. In a typical procedure, 0.8 ml of porogenic agent was prepared by mixing 0.6 ml carbinol and 0.20 ml distilled water. The porogenic agent was added to a mixture of monomers (1.90 ml GMA, 1.5737 g AM and 2.80 ml EGDMA) in which the free radical initiator AIBN (0.0848 g) was dissolved, then 0.1200 g of nanosize calcium carbonate was added for another 20 min. After the solution described above was placed in a 40 °C water bath temperature oscillator to prepolymerize for 12 h, it was put into a four_necked flask having 75.00 ml n-heptane, tetrachloroethylene (40.00ml) in which 0.0120 g span-60 and 0.30 ml Twain-80 were dissolved, and mechanically stirred for 0.5 h under a nitrogen atmosphere at 60 °C, then the reaction mixture was allowed to proceed at 65 °C for 4 h and at 75 °C for 2.5 h. The polymer particles obtained were stored in ethanol for 24 h to remove the porogen and in 0.10 M hydrochloric acid solution for another 24 h to get rid of nanosize calcium carbonate, and then the carrier obtained was washed completely with distilled water and dried under vacuum at room temperature.

2.4. Immobilization Process

0.0500 g polymer particles were mixed with 1.00 ml enzyme solution (pH 10, 5.03 U/ml) obtained above. With gently stirring, the reaction was allowed to proceed at 35 °C in water constant temperature oscillator. After 8 h, the bounded enzyme was separated and cleaned completely with double distilled water until no protein was detected.

2.5. Enzyme Activity Assay

The activities of the free and the immobilized enzyme were determined according to the reference (G.R. Craven, 1965) using α-naphthyl acetate as substrate. For the activity of free enzyme, aliquots of it (0.05 ml) were added to the mixture of 2.25 ml 0.10 M phosphate butter solution (pH 7.5), 0.10 ml fast blue B solution (2.337 mmol/l) and 0.10 ml α-naphthyl acetate (1.500 mmol/l). After precisely 5 min at 35 °C, the reaction was stopped by adding 1.00 ml SDS solution (3%), and then being incubated for another 10 min to ensure the product α-naphthol to react completely with fast blue B salt, the amount of t α-naphthol was tested at 500 nm.

For the immobilized enzyme, 0.0500 g was weighed and soaked in the mixture of 2.30 ml 0.1 M phosphate butter solution (pH 7.5) and 0.1 ml fast blue B salt (2.337 mmol/l) and the reaction began by putting in 0.10 ml α-naphthyl acetate (1.500 mmol/l), After 5 min at 35 °C, the reaction was ended and tested as that of the free enzyme.

The activity (1 U) was defined as the amount of enzyme that released 1 μmol α-naphthol in 1 min at 35 °C.

2.6. Operational Stability

1.0000 g of the bounded enzyme was weighted and immersed in the mixture including 46.00 ml of 0.1 M phosphate butter solution (pH 7.5) and 2.00 ml fast blue B solution (2.337 mmol/l), the reaction began when 10mL α-naphthyl acetate (1.500 mmol/L) was added at 35 °C, and the product was tested after 5 min, then the solid was separated and cleaned completely. After being dried completely, the immobilized enzyme was used for the next determination. The upper experiment was replicated 6 times according to the same terms.

3. RESULT AND DISCUSSION

3.1. Discussion about the Porous Poly (GMA-AM-EGDMA) Beads

According to experiments listed above, the beads obtained were characterized by the scanning electron micrographs （Fig.1）, it could be seen from Fig.1 that the beads have a much porous surface structure. Meanwhile, the carrier was used to immobilize the plant esterase purified from wheat flour as described in the experiment, under the best terms, the activity yield of the bounded enzyme was 65.78% and its activity could reach 33.09 U/g dry carriers.

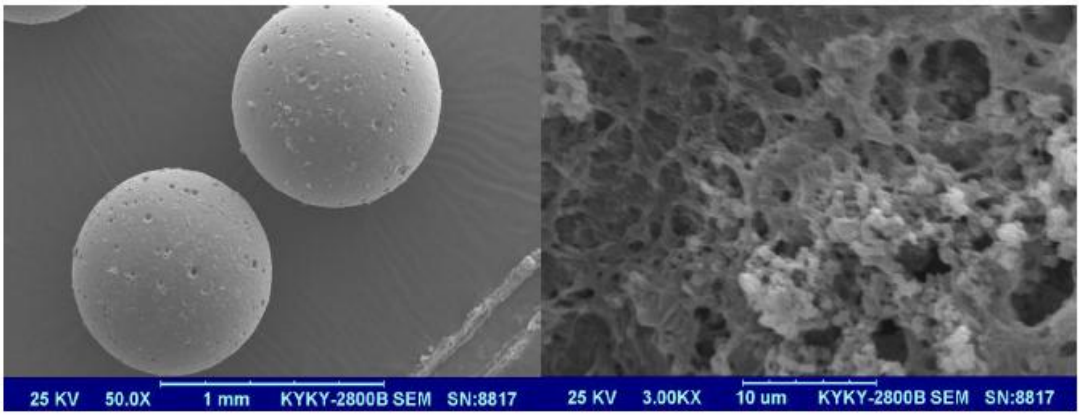

Fig1. *SEM photographs of the GMA-AM-EGDMA polymer particles*

3.2. Basic qualities of wheat esterases

3.2.1. Temperature Optima and Thermostability

At the temperatures ranging from 30 °C to 60 °C, the enzyme activity was determined at pH 7.5. The data obtained indicated that the suitable temperature of both enzymes including the free and the immobilized was 35 °C.

Respectively at 40 °C, 50 °C and 60 °C, the thermostability of both enzymes was also tested. After being placed in the water bath for 8 h at above temperature, the activities of them were determined, and the data revealed that the remaining activities were 85.77%, 77.68% and 16.03% for the bounded enzyme and 85.2%, 74.67% and 10.05% for the free separately at 40 °C, 50 °C and 60 °C. The experimental data above indicated that the immobilized enzyme had a better thermal stability.

3.2.2. pH optima and stability

In different pH range of 3.0-10.0, the enzyme activities were analyzed at 35 °C, the highest activity was found at pH 7.5 for both kinds of enzymes. Meanwhile, the influence of pH on the enzyme activities was also studied in our experiments. Experimental data investigated that the free enzyme was stable in the pH range of 6.0-7.5, while the immobilized enzyme was good in the range of 5.0-8.0, which showed that a much wider pH stability was found for the immobilized enzyme.

3.2.3. Operational stability of wheat esterase

The experiments were carried out according to the experimental and the results were plotted in Fig.2, From Fig.2, it could be seen that no obvious activity loss was found for the immobilized enzyme after being employed repeatedly 6 times, which means that Plant Esterase was combined closely with the carrier and a good operational stability was obtained.

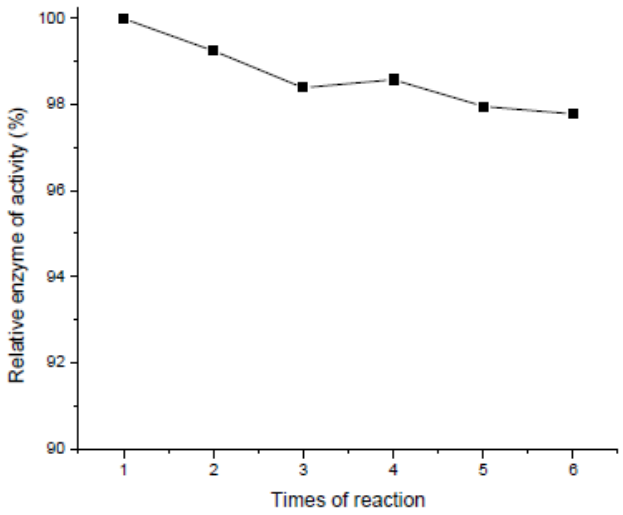

Fig2. *The operational stability of the immobilized enzyme*

4. CONCLUSION

In this paper, the poly (GMA-AM-EGDMA), firstly with carbinol and distilled water as liquid and nano-calcium carbonate as solid pore-forming reagents, was obtained from glycidyl methacrylate (GMA) and acrylamide (AM) as monomer, Glycol Dimethyl Acrylate (EGDMA) as cross-linking agent via suspension polymerization. The result obtained from the SEM investigated that carrier has a much more porous exterior form. Under the optimum conditions, the plant esterase purified from wheat flour was bounded on the over carrier, the activity yield was 65.78% and the activity of it could reach 33.09 U/g dry carriers, kinetic data showed that the enzyme immobilized on the beads demonstrated a good thermal and pH stability, meanwhile a nice operational stability was also observed. The result above showed the poly (GMA-AM-EGDMA) prepared here was very valuable to be studied further in enzyme immobilization fields.

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Huan Ge, born in 1990, is a graduate student in physical chemistry at Hebei University, Baoding, P. R. China. The main research work in our laboratory was the synthesis of polymer carriers, which will be used to immobilize enzyme, and the chromatographic detection of the drug. My work is the immobilization of the enzyme.