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An efficient synthesis of ampicillin on magnetically separable immobilized penicillin G acylase

Ping Xue *, Xiao Dan Song, Xue Rong Cao

Key Laboratory of Energy Resources & Chemical Engineering, Ningxia University, Yinchuan 750021, China

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Abstract

Penicillin G acylase (PGA) was immobilized on the magnetic hydrophilic polymer microspheres with average pore size of 17.1 nm, specific surface area of 128.2 m^2/g and saturate magnetization of 6.4 emu/g. The 96.7% ampicillin yield with 1.60 of the synthesis/hydrolysis (S/H) ratio from 6-aminopenicillanic acid (6-APA) and D-(−)-alpha-phenylglycine methyl ester (D-PGME) can be achieved using the resultant magnetic biocatalyst in ethylene glycol, where only 82.1% yield with 1.40 of the S/H ratio was obtained using the free PGA under the identical reaction conditions. The immobilized PGA can be separated magnetically and recycled for five times without obvious loss of its catalytic activity.

Keywords: Immobilized penicillin G acylase; Magnetic polymer microspheres; Ampicillin synthesis; Reusability

As an industrial enzyme, the penicillin G acylase plays an important role in the manufacturing of semi-synthetic antibiotics due to its capability of catalyzing the hydrolysis of penicillin G to 6-APA and acylation of 6-APA to synthesize semi-synthetic penicillins [1]. The free enzyme is known to have a limited reusability, a very low tolerance toward organic solvents. Now much effort to develop newer type of supports is taken to improve the enzymatic catalytic efficiency.

Ampicillin is one of the most widely used β-lactam antibiotics and suitable for a broad spectrum of bacterial infections. In the enzymatic synthesis of ampicillin, there is an inherent drawback of a large excess of the acyl donor required to the reaction in aqueous media owing to the competing hydrolysis of the side chain donor and the secondary hydrolysis of the products. For this reason, an important parameter—the so-called synthesis/hydrolysis ratio (S/H, the molar ratio of product and hydrolyzed side chain donor formed) is often used to evaluate the economic viability of the process. Recently, it was found that the S/H ratio depends not only on the nature of the side chain and the reaction conditions (solvents, pH, temperature) but also on the support of immobilizing enzyme and method [2]. Therefore, the ampicillin synthesis catalyzed by PGA is usually carried out in organic solvents or cosolvents to improve the yield of ampicillin and S/H ratio by reducing water activity.

In this work, the PGA immobilized on the magnetic hydrophilic polymer microspheres containing active oxirane groups, prepared by inverse suspension polymerization of glycidyl methacrylate (GMA) and N,N′-methylene

* Corresponding author.
E-mail address: ping@nxu.edu.cn (P. Xue).

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bisacrylamide (MBAA) in the presence of Fe^{3+} and Fe^{2+} dispersed in formamide, was used for ampicillin synthesis for the first time, and a high yield and the S/H ratio were successfully achieved in ethylene glycol at 20 °C. The immobilized PGA can be separated magnetically and recycled effectively.

1. Experimental

Preparation of magnetic hydrophilic polymer microspheres: 0.67 g of $FeCl_2 \cdot 4H_2O$ and 1.8 g of $FeCl_3 \cdot 6H_2O$ were dissolved in 25 mL formamide, and then concentrated aqueous ammonia was slowly added to adjust the pH to 9. After heating to 90 °C and stirring for 2 h, followed by another 4 h at 60 °C, 2.6 g of GMA and 1.9 g of MBAA were added to form a polymerizing mixture. The mixture was dispersed in the suspension which consisted of 109 mL of *n*-heptane, 35 mL of tetrachloroethylene and 0.1 g of span 60 as well as 5.0 g of calcium stearate. The polymerization was initiated with 2,2-azobis(2-methylpropionitrile) under nitrogen at 60 °C for 5 h. The resultant microspheres were separated magnetically, washed with acetone, dried under vacuum for 24 h. The sample was named MHPM.

Immobilization of PGA on MHPM: 0.06 g of MHPM support was immersed in 2 mL of solution prepared by diluting ten times original enzyme solution with pH 7.0 phosphate buffer (PB). The mixture above was stirred gently for 48 h at 30 °C. The immobilized PGA on MHPM was separated by a magnet and washed with phosphate buffer eight times. The sample was named PGA/MHPM.

Typical synthesis of ampicillin: 50 mmol/L of 6-APA, 100 mmol/L of D-PGME and 2 mL of solvent were mixed in a reactor. The wet PGA/MHPM was added and shaken at certain temperature. The PGA/MHPM was magnetically separated at the end of reaction and kept at 4 °C for further use. The mixed solution was analyzed by HPLC (FL2200) equipped with a UV detector and Venusil XBP reversed-phase C_{18} column (4.6 mm × 200 mm).

2. Results and discussion

The designed and prepared MHPM support for immobilizing PGA possesses the multiple functional actions. Fig. 1a shows that the MHPM has perfect spherical shape and the average size of 100–200 μm. A typical magnetization curve for the MHPM is observed in Fig. 1b in which there is no hysteresis in the magnetization with both remanence and coercivity being zero as well as 6.4 emu/g of saturation magnetization. Therefore, the MHPM displays superparamagnetic behavior, and their separation in liquid media can be controlled by magnetic fields. Furthermore, there is an abundance of the active oxirane groups on the surface of MHPM that can couple covalently the enzyme under mild conditions. The MHPM has high specific surface area and large average pore diameter (128.2 m^2/g and 17.1 nm, respectively), which is the advantage for the immobilization of PGA molecular with bulk volume.

The apparent activity and enzyme coupled to the PGA/MHPM was 569 IU/g and 34.6 mg/g according to literature [3,4], respectively. The results of the PGA/MHPM catalyzed 6-APA and D-PGME to synthesize ampicillin in some conventional solvents are listed in Table 1. The ampicillin yield and S/H ratio reach 96.7% and 1.60 using PGA/MHPM in pure ethylene glycol. While using free PGA, the values were 82.1% and 1.40, respectively. This result shows that the synthesis activity of PGA can be effectively improved by the immobilization on the MHPM support, especially in the S/H ratio. Furthermore, the reaction medium of the ampicillin synthesis by PGA/MHPM is a significant factor.

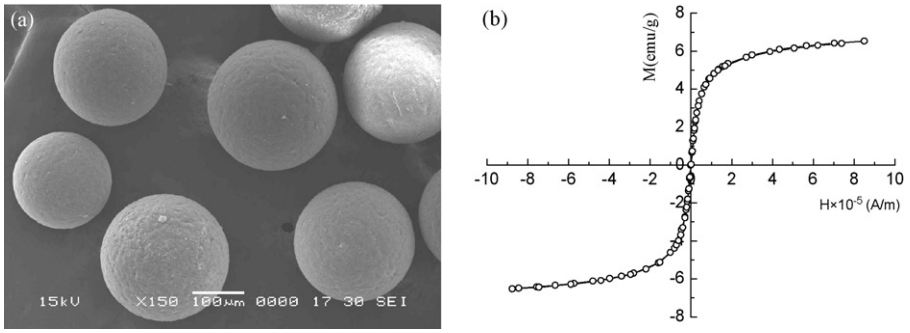

Fig. 1. (a) SEM photograph and (b) magnetization curves of MHPM.

Table 1
The results of ampicillin synthesis by PGA/MHPM.[a].

PGA	Solvent	Ampicillin yield (%)	S/H
Free PGA	Ethylene glycol	82.1	1.40
PGA/MHPM	Ethylene glycol	96.7	1.60
PGA/MHPM	Ethyl acetate	2.0	0.16
PGA/MHPM	40% (v/v) ethylene glycol–PB	2.9	0.24

[a] *Reaction conditions*: temperature 20 °C, time 48 h, and mole ratio of D-PGME to 6-APA = 2:1.

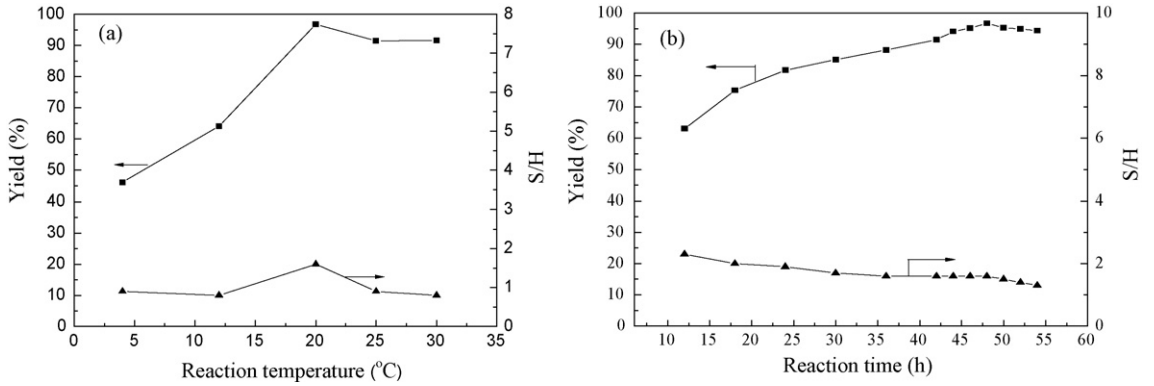

Fig. 2. Effect of (a) temperature and (b) time on ampicillin yield and S/H ratio.

The yield and the S/H ratio are both much higher in ethylene glycol than in a cosolvent of 40% (v/v) ethylene glycol–phosphate buffer (0.1 mol/L, pH 7.0) due to the suppression of D-PGME and ampicillin hydrolysis in favor of synthesis in ethylene glycol. In addition, it was observed that the yield and S/H ratio were very low in ethyl acetate (2.0% and 0.16, respectively). The reason for the low yield in ethyl acetate may be the strong hydrophilicity of the MHPM support from large numbers of hydrophilic imide groups on it and the very poor solubility of substrate 6-APA in ethyl acetate. Unlike hydrophobic ethyl acetate, the hydrophilic ethylene glycol can not only swell the MHPM but also dissolve substrates leading to the good compatibility between the enzyme and the MHPM as well as the high diffusion of substrates close to the immobilized PGA. As a result, PGA/MHPM displays high catalytic activity for ampicillin synthesis in ethylene glycol.

The effects of reaction temperature and time on ampicillin synthesis in ethylene glycol by PGA/MHPM are shown in Fig. 2a and b. With the increase of the reaction temperature under the same reaction time of 48 h, the ampicillin yield and S/H ratio increased rapidly and reached the maximal values at 20 °C. As the temperature was above 20 °C, the yield and S/H ratio descended gradually. This indicates that the immobilized PGA for ampicillin synthesis exhibits a high catalytic activity at near room temperature. As shown in Fig. 2b, the S/H ratio reduced slowly all along with the increase of time at 20 °C. However, the maximal yield of 96.7% was observed in 48 h and followed by a slight decrease. Therefore, the optimal temperature and time for ampicillin synthesis over PGA/MHPM are 20 °C and 48 h, respectively.

When 6-APA concentration was fixed at 50 mM, synthesis of ampicillin depended on the D-PGME concentration (Table 2). The low yield and S/H ratio were obtained at low D-PGME concentration, which indicated that much faster

Table 2
The effect of mole ratio of D-PGME to 6-APA on ampicillin synthesis by PGA/MHPM.[a].

Mole ratio of D-PGME to 6-APA	Ampicillin yield (%)	S/H
1:1	33.3	1.21
1.5:1	61.0	2.00
2:1	96.7	1.60
2.5:1	98.5	1.43

[a] *Reaction conditions*: temperature 20 °C, time 48 h, and ethylene glycol as solvent.

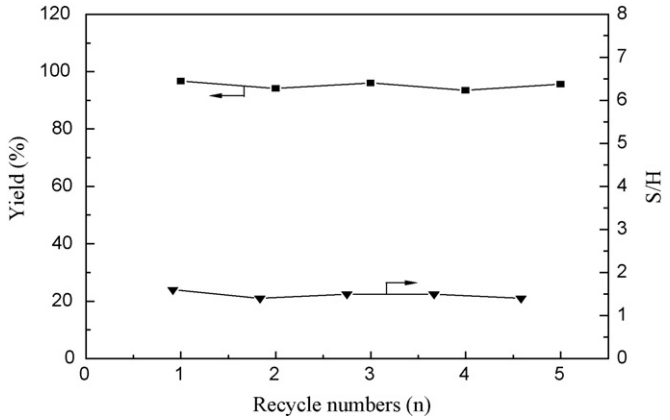

Fig. 3. Reusability of PGA/MHPM.

D-PGME hydrolysis than ampicillin synthesis occurred in this situation. When the ratio was 2:1, ampicillin synthesis dominated the process so that higher yield and the maximum S/H ratio were obtained. However, no significant improvement was obtained but the S/H value decreased with the further increase of the mole ratio of D-PGME to 6-APA. Therefore, the appropriate molar ratio was 2:1.

In general, the major causes for poor reusability of immobilized enzyme are the leaching enzyme from support and mechanical loss of immobilized enzyme in repeating operations. The MHPM has strong magnetic response and its magnetic properties were not affected by the immobilization of PGA. Consequently, the PGA/MHPM can be separated quickly without any loss under an external magnetic field.

The reusability of the PGA/MHPM for ampicillin synthesis in ethylene glycol is shown in Fig. 3. The yield and S/H ratio changed without significant decreases during 5 consecutive operations at 20 °C, which indicates that PGA immobilized on MHPM performed an excellent reusability. This demonstrates that PGA has been coupled covalently to the MHPM support and the PGA/MHPM is a promising biocatalyst in industrial applications.

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References

[1] M.I. Youshko, V.K. Svedas, Adv. Synth. Catal. 344 (8) (2003) 894.
[2] L. Cao, F.V. Rantwijk, R.A. Shelon, Org. Lett. 2 (10) (2000) 1361.
[3] P. Xue, G.Z. Lu, W.Y. Liu, Chin. Chem. Lett. 17 (1) (2006) 129.
[4] M.M. Bradford, Anal. Biochem. 72 (1) (1976) 248.