Preparation of Immobilized Recombinant Tubulin Beta(TuBb) on Chitosan Nanoparticles by Covalent Binding Method

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Abstract. Objective In order to screen TuBb inhibitors, this paper describes the preparation of immobilized TuBb on chitosan nanoparticles. Methods TuBb was immobilized onto chitosan nanoparticles by covalent binding method. Results The results of the univariate test indicated that the highest immobilized yield can be obtained when the optimal immobilization condition was 1 mg of TuBb, 0.5 mol/L of buffer solution with pH 6.5, immobilization 30 min and immobilization at 0-4 ℃. Conclusions The authors conclude that the immobilized TuBb maintain the catalysis properties and can be used as the screening of TuBb inhibitors.
Keywords: TuBb; Chitosan nanoparticles; Immobilization;

1 Introduction

Microtubule is an important component of the cytoskeleton, which has been demonstrated to be critical in many cellular processes.TuBb as the basic unit composed of microtubule, has become an important target for anticancer drug research[1]. TuBb inhibitors have been considered to be an effective therapeutic approach in the treatment of tumor, such as Taxol and Colchicine[2-3]. Traditional Chinese medicines(TCMs) are rich in resources and nowadays, many researchs have been focused on sceening enzyme inhibitors from TCMs[4-5]. However, the complex composition make it difficult to develop a simple, rapid and reliable method for screening TuBb inhibitors from TCMs.

The immobilization of enzyme on suitable carriers have been proved effective in improve TuBb stability and reusability, while the catalysis properties are maintained. The immobilized enzyme with good performance can be used as the screening of TuBb inhibitors[6-8].

There are many enzyme immobilization methods and immobilization methods can be roughly summarized as follows: vector adsorption method, including physical adsorption method, covalent

binding method and ionic binding method; crosslinking; embedding method, including microcapsules type and grid type [9-10].

In this dissertation, TuBb was immobilized on chitosan nanoparticles by covalent binding method. The advantage of covalent binding method is firm combination between

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TuBb and chitosan nanaparticles, allowing TuBb to be reused. And a series of studies were conducted,about the methods for TuBb immobilization and the investigation of immobilization conditions.

2 Materials and Instruments

Recombinant tubulin beta was purchased from Cloud-Clone Co., Ltd.. Bovine serum albumin was purchased from Jiangsu Anhui Biotechnology Co., Ltd.. Coomassie brilliant blue G250 was purchased from Wuhan More Biotechnology Co., Ltd.. Glutaraldehyde solution(25%) was purchased from Sinopharm Chemical Reagent Co.,Ltd.. Morpholineethanesulfonic acid, Magnesium chloride hexahydrate and Ethylenebis(oxyethylenenitrilo) tetraacetic acid was purchased from Aladdin Industrial Co.,Ltd.. Chitosan nanoparticles were prepared by the laboratory own. Distilled water was prepared with demimeralized water.

CHY-2 Temperature oscillator was purchased from Jintan Fu Instrument Co., Ltd.. Sartorius B125s electronic balance was purchased from Sartorius Co.,Ltd.. HJ-5 Multi-functional stirrer and HH-2 Digital thermostat water bath were purchased from Changzhou Guohua Electric Co.,Ltd.. KQ-500B Ultrasonic cleaning machine was purchased from Kunshan Ultrasonic Instrument Co.,Ltd.. GT-10-1 High-speed desktop centrifuge was purchased from Beijing Era Beili centrifuge Co.,Ltd.. PHS-29A PH Meter was purchased from Shanghai REX Instrument Factory. UV2102 UV-visible spectrophotometer was purchased from Unico,American.

3 Methods

3.1 Immobilization of Recombinant Tubulin Beta (TUBb)

Add 40μL gutaraldehyde solution(25%) into the chitosan nanoparticles suspension (0.5g/mL,1mL) and shake 90 min at 30℃. Centrifugate, remove the supernatant and wash the precipitate three times. Then, the precipitate was ultrasonic dispersed with 1mL distilled water. Add 1mL 1 mg/mLl TuBb solution, which was prepared by ph 6.5 ,0.5mol/L cold MES buffer, into the suspension above and shake 30 min at 0-4℃. Centrifugate, remove the supernatant and wash the precipitate three times. After immobilization, the tubulin solution and flushing were collected and measure the amount of TuBb which was not immobilized by Coomassie Brilliant Blue. At last, the immobilized TuBb was stored at 4 ℃.

3.2 Determination of TuBb Immobilization Yield

Measure the amount of the free TuBb which was not immobilized by Coomassie Brilliant Blue. Immobilization yield was determined and calculated by Eq.1.

Immobilization Yield(%)=$(m-m_f-m_r)/m*100\%$ (Eq.1) where m is the initial amount of TuBb, m_f is the amount of TuBb in the flushing solution, m_r is the residual amount of TuBb in the TuBb solution after immobilization.

4 Investigation of immobilization conditions

4.1 Effects of time on immobilization yield

Add 1mL chitosan nanoparticle(0.5g) to the test tube and shake 30min at 30℃ . Add 1mL 1 mg/mL TuBb solution ,which was prepared by ph 6.5 ,0.5mol/L cold MES buffer , into the suspension above and shake 10,15,20,30,40,50 min at 0-4℃. Centrifugate, remove the supernatant and wash the precipitate three times. After immobilization, the tubulin solution and flushing were collected and measure the amount of TuBb which was not immobilized by Coomassie Brilliant Blue to calculate the immobilization yield. TuBb activity is represented by the relative percentages of the highest TuBb activity.

4.2 Effects of tempreture on immobilization yield

Add 1mL chitosan nanoparticle(0.5g) to the test tube and shake 30min at 25,30,35,40,45,50,60℃ . Add 1mL 1 mg/mL TuBb solution ,which was prepared by ph 6.5 ,0.5mol/L cold MES buffer , into the suspension above and shake 30 min at 0-4℃. Centrifugate, remove the supernatant and wash the precipitate three times. After immobilization, the tubulin solution and flushing were collected and measure the amount of TuBb which was not immobilized by Coomassie Brilliant Blue to calculate the immobilization yield. TuBb activity is represented by the relative percentages of the highest TuBb activity.

4.3 Effects of pH on immobilization yield

Add 1ml chitosan nanoparticle(0.5g) to the test tube ,adjust pH(4.0,4.5,5.0,5.5,6.0,6.5,7.0) and shake 30min at 30℃ . Add 1mL 1 mg/mL TuBb solution ,which was prepared by ph 6.5 ,0.5mol/L cold MES buffer , into the suspension above and shake 30 min at 0-4℃. Centrifugate, remove the supernatant and wash the precipitate three times. After immobilization, the tubulin solution and flushing were collected and measure the amount of TuBb which was not immobilized by Coomassie Brilliant Blue to calculate the immobilization yield. TuBb activity is represented by the relative percentages of the highest TuBb activity.

4.4 Effects of the amount of TuBb on immobilization yield

Add 1ml chitosan nanoparticle(0.5g) to the test tube and shake 30min at 30℃. Add 1Ml 0.5,0.8,1.0,1.3,1.5mg/mL TuBb solution ,which was prepared by ph 6.5 ,0.5mol/l cold MES buffer , into the suspension above and shake 30 min at 0-4℃. Centrifugate, remove the supernatant and wash the precipitate three times. After immobilization, the tubulin solution and flushing were collected and measure the amount of TuBb which was not immobilized by Coomassie Brilliant Blue to calculate the immobilization yield. TuBb activity is represented by the relative percentages of the highest TuBb activity.

5 Results and Discussions

5.1 Effects of time on immobilization yield

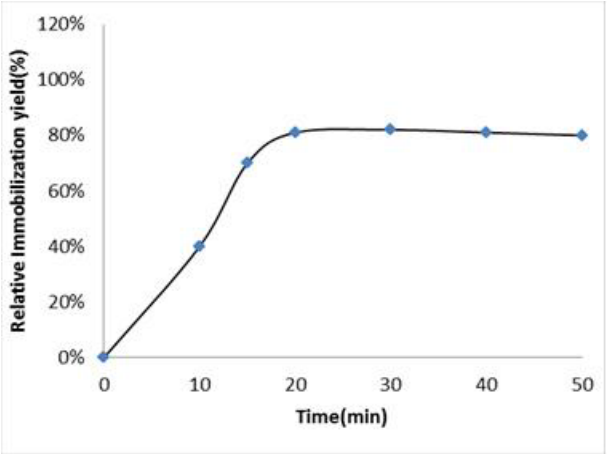

Fig.1 Effects of time on immobilization yield

The Fig.1 show that when the tempreture is during 0 to 30℃,relative immobilization yield is rising and when the tempreture is above 30℃,relative immobilization yield is unchanging. Therefore,we choose 30min as the immobilization time.

5.2 Effects of tempreture on immobilization yield

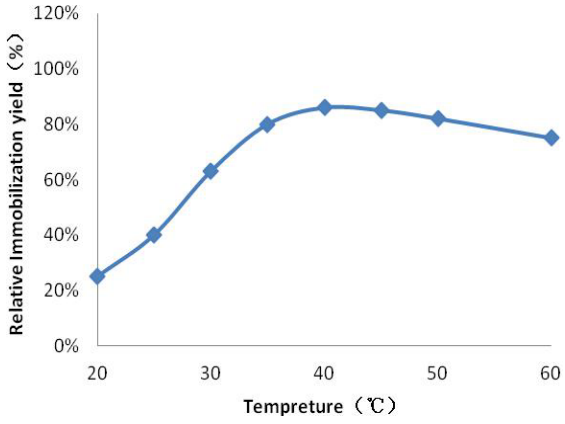

Fig. 2 Effects of tempreture on immobilization yield

The Fig.2 show that when the tempreture is during 0 to 40℃,relative immobilization yield is rising and when the tempreture is above 40℃, relative immobilization yield is unchanging.In order to retain the activity of TuBb, we choose 0-4℃ as the immobilization tempreture.

5.3 Effects of pH on immobilization yield

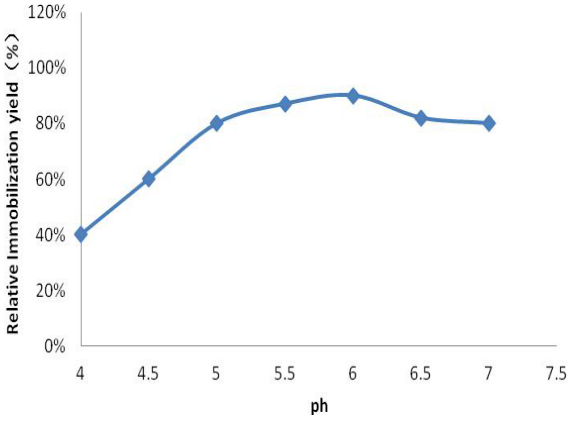

Fig. 3 Effects of pH on immobilization yield

The Fig.3 show that when pH is during 4.0 to 6.0, relative immobilization yield is rising and when the pH is above 6.0,relative immobilization yield is reducing.Therefore,we choose pH 6.0 as the pH of chitosan nanoparticle suspention.

5.4 Effects of the amount of TuBb on immobilization yield

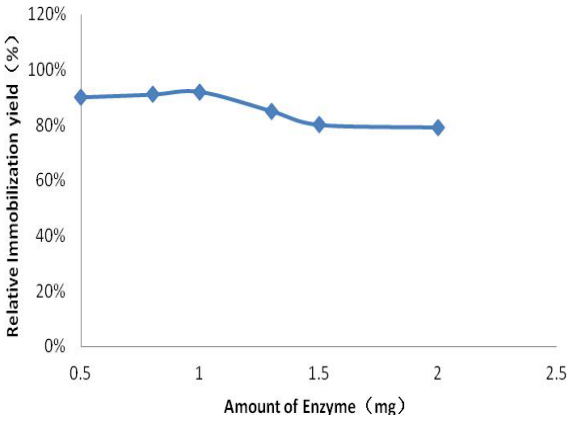

Fig. 4 Effects of the amount of TuBb on immobilization yield

The Fig.4 show that when the amount of TuBb is 1.0mg, relative immobilization yield is maxium.Therefore, we choose 1mg as the immobilization amount of TuBb.

The results of the univariate test indicated that the highest immobilized yield can be obtained when the optimal immobilization condition was 1mg of TuBb, 0.5mol/l of buffer solution with pH 6.5, immobilization 30 min and immobilization at 0-4℃.

6 Conclusions

In this study, TuBb was immobilized onto Chitosan nanoparticles and the screening model of TuBb inhibitors was developped. The screening model exhibited good stability which will prolong the validity of TuBb. Moreover, the model has the potential to be reused, allowing the protein cost to be saved.The method described in this study provides an application example of a rapid , simple and economic approach for the screening of TuBb inhibitors.

Acknowledge

This work was financially supported by the National Natural Science Foundation, PR China (Grant No.81260690),the Education Research Project of Science and Technology Foundation,Jiangxi Province,China (Grant No.GJJ13618,GJJ12518) and JiangXi Graduate Student Foundation(Grant No.YC2014-285).

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