Immobilization of Bromelain onto Electrospun Functionalized Regenerated Cellulose Nanofibers

การตรึงเอนไซม์โบรมิเลนบนเส้นใยนาโนเซลลูโลซที่เกิดขึ้นใหม่ที่ได้จากกระบวนการปั่นเส้นใยด้วยไฟฟ้าสถิต

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ABSTRACT

Stem bromelain, a cysteine proteinase isolated from stem of pineapple plant *(Ananas comosus)*, was successfully immobilized onto regenerated cellulose (RC) fibers with diameters of 296±54 nm. To obtain RC fibers, the electrospun cellulose acetate (CA) fibers were firstly prepared from 17%w/w CA mixed with 5%w/w Tween 80 dissolved in a mixed solvent of water (25% w/w) and acetic acid (75% w/w) at an applied voltage of 25 kV and a fiber collection distance of 10 cm. Before being immobilized with bromelain, the surface of RC fibers was further functionalized by coupling with 12% w/v glutaraldehyde using aluminium sulfate as a catalyst. Scanning electron microscope (SEM) and Fourier transform infrared spectroscopy (FTIR) results indicated that the aldehyde groups on the fiber surface reacted with amino groups of bromelain and the fibrous structure of bromelain-RC was still intact.

บทคัดย่อ

เส้นใยนาโนเซลลูโลซถูกเตรียมขึ้นจากกระบวนการปั่นเส้นใยด้วยไฟฟ้าสถิตที่มีการตรึงเอนไซม์โบรมิเลน ซึ่งเป็นเอนไซม์ตามธรรมชาติที่พบในสับปะรด สามารถย่อยโปรตีนให้โมเลกุลเล็กลง เส้นใยขนาด 296 นาโนเมตร ได้ถูกเตรียมสำเร็จจากสารละลายเซลลูโลซอะซีเตต ความเข้มข้นร้อยละ 17 โดยมวลต่อมวล ในตัวทำละลายน้ำและกรดอะซิติกและมีการเติม Tween 80 เข้มข้นร้อยละ 5 โดยมวลต่อมวล สภาวะที่เหมาะสมที่จะทำให้เส้นใยมีขนาดเล็กและเรียบไม่เกิดเป็นปม คือ ศักย์ไฟฟ้า 25 กิโลโวต์ ระยะห่างปลายเข็มกับฉากรับ 10 เซนติเมตร และมีการปรับปรุงพื้นผิวเส้นใยนาโนให้เกิดหมู่ฟังก์ชันแอลดีไฮด์ ก่อนนำไปตรึงรูปเอนไซม์ ผลการศึกษาโดยใช้กล้องจุลทรรศน์อิเล็กตรอนแบบส่องกราด (SEM) และฟูเรียร์ทรานฟอร์ม อินฟราเรดสเปคโตรสโกปี (FTIR) พบว่าสามารถตรึงเอนไซม์โบรมิเลนลงบนเส้นใยนาโนเซลลูโลซและเส้นใยยังคงรูปอยู่ได้

Key Words: Bromelain, Enzyme immobilization, Cellulose, Electrospinning

คำสำคัญ: โบรมิเลน การตรึงเอนไซม์ เซลลูโลซ การปั่นเส้นใยด้วยไฟฟ้าสถิต

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Introduction

Stem bromelain (EC. 3.4.22.32) is a cysteine proteinase extracted from stem of pineapple plant *(Ananas comosus)* with molecular weight 23.8 kD (Maurer et al., 2001; Gupta et al., 2006). Free stem bromelain has been used in food processing, including meat tenderization and applications in the baking industry (Carlos et al., 2012). This protease is also widely used for haze prevention in beer, being active at the normal pH of this beverage (Benucci et al., 2012). Even though bromelain is a highly specific, selective, and efficient catalyst, it has some weak points (Huang et al., 2011). The enzyme are normally homogeneous catalysts which is difficult to be reused. To solve this problem, enzymes are normally immobilized onto the solid support which make it easy to be handled, separating easily from the reaction mixture and providing the opportunity to be reused (Eliane et al., 2014). Therefore, the enzyme immobilization could reduce cost and time in the production line and provide the ability to stop the reaction rapidly (Tischer et al., 1999). However, the activity of enzymes immobilized on the surface of solid support is normally reduced. Therefore, nanomaterials with high surface area to volume ratio are suitable for being the solid support because they provide the highly amount of the bound enzyme (Shinya et al., 2003). Several types of nanostructure materials have been used as enzyme support such as nanoparticle, mesoporous material and nanofibers. However, these nanomaterials also possessed their owned drawbacks. The nanoparticles are hard to be manage and difficult to be reused (Madan et al., 2013). The porous materials usually confine enzyme molecules and limit the diffusion which results in the reduction of enzyme activity (Hiroshi et al., 1998). Thus, nanofibers have a great potential as a supporter because they could overcome disadvantages of other nanomaterials. In addition, we can process the nanofibers to be a membrane sheet that can be touched and removed from reaction mixture easily. Generally, nanofibers could be prepared from an electrospinning technique, which is simple and inexpensive. Moreover, the fiber dimension, porosity and pore size could be tuned (Wang et al., 2009; Nandana et al., 2010).

Objective of the study

The aim of this study was to modify the regenerated cellulose (RC) nanofibers membrane making them suitable for immobilizing bromelain enzyme.

Methodology

Materials

Acetic acid (99.7%) (RCI Labscan; Thailand), aluminum sulfate hexadecahydrate (AS) (≥95.0%) (Fluka; USA), CA (acetyl content = 39.8 %w/w) with MW of 3 x 10^4 g/mol (Sigma-Aldrich; USA), glutaraldehyde (GA) (50% w/v) (Sigma-Aldrich; USA), and potassium hydroxide (KOH) (95%) (UNID; South Korea) were used as received.

Preparation of cellulose acetate (CA) fibers

CA powders and Tween 80 were dissolved in acetic acid:water (3:1 by weight) mixed solvent to obtain 15, 16, 17, 18 and 19% w/w CA solutions with 5% Tween 80. The mixtures were stirred for 2 h. The applied electrical potential, the fiber collection distance the fiber collection time, the flow rate and the inner diameter of needle were +25 kV, 10 cm, 4 h, 0.6 ml/h and 0.9 mm, respectively. The morphology of CA ultrafine fibers was observed by scanning electron microscope (SEM). An

average diameter of the electrospun fiber mats was measured directly from SEM images using a Photoshop CS3 ($n \geq 50$).

Fabrication of RC fibers

The CA nanofiber membrane was immersed in 0.5M potassium hydroxide (KOH) in ethanol (20 ml) at 25 °C for 1 h to obtain the RC fibers, followed by washing with deionized water for several times to remove the residual KOH, and dried in air overnight. Then, they were subjected to attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-TIR) to confirm the success of deacetylation of CA fibers.

Immobilization of bromelain onto RC fibers

The amino groups of bromelain were activated by immersing the fiber mats into the mixture of 12% w/v glutaraldehyde (GA) solution and aluminium sulphate (0.02 g) for 20 min. The fiber mats were dried at 80 °C for 20 min, cured at 120 °C for 3 min, washed with deionized water thrice, and dried in air. The obtained membrane was submerged in free bromelain enzyme (10 mg in 1 ml PBS) at 4 °C for 5 days. When the reaction completed, the mats were taken out. The excess enzyme was washed with water thrice. The bromelain-immobilized RC fibers were analyzed by ATR-FTIR.

Results and Discussion

Fabrication of CA nanofibers

The electrospun CA fibers prepared from 15, 16, 17, 18 and 19% w/w CA were observed under SEM and the images are shown in Figures 1(a)-(e), respectively. At 15 and 16% CA the beaded structure were found. These bead structures led to the decrease in surface area per volume. The uniform fibers with smooth surface prepared from 17, 18, and 19% w/w CA with diameters of 296±54, 385±78, and 473±75 nm, respectively, were obtained. At 17% w/w, the thinnest uniform fibers were generated.

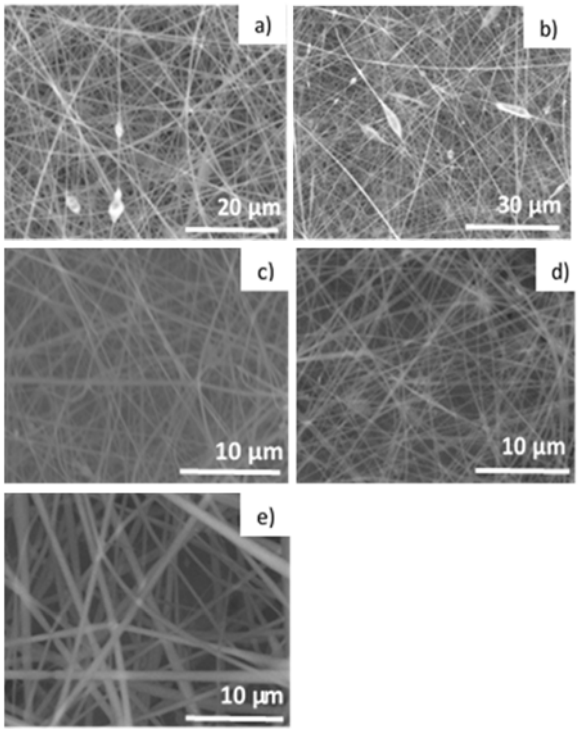

Figure 1 SEM images of electrospun CA fibers prepared from (a) 15, (b) 16, (c) 17, (d) 18, and (e) 19% w/w CA

Immobilization of bromelain onto RC fibers

The electrospun CA fibers prepared from 17% w/w CA at 25 kV were then deacetylyzed to generate the RC. After being reacted with GA, the aldehyde groups generated on the fiber surface allowed the immobilization with bromelain or bromelain-RC.

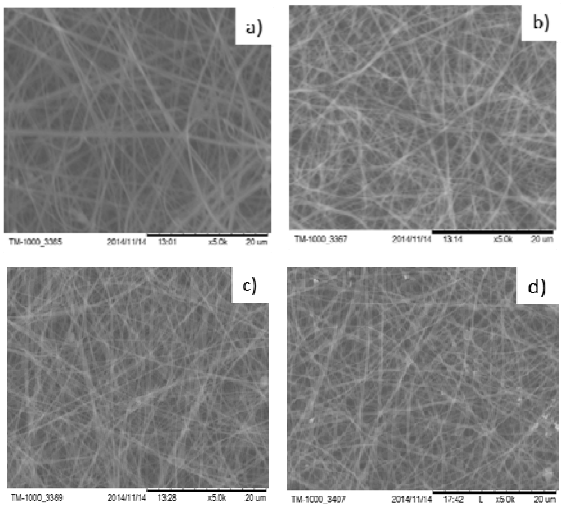

Figure 2 SEM images of electrospun (a) CA, (b) RC, (c) GA treated RC fibers and (d) RC-bromelain

Figure 2 reveals that the smooth and uniform ultrafine fibers with an average diameter of 296±54 nm (Figure 2 a) were observed. After the immobilization, the average diameter of bromelain-RC (274±56 nm) was lower than that of CA fibers possibly caused from shrank after deacytylation. However, the fibrous structure of bromelain-RC was still intact.

ATR-FTIR was used to confirm the success of each step of the material preparation. In the spectrum of CA (3a), three characteristic peaks at 1742 cm^{-1} (C=O stretching), 1369 cm^{-1} (C-CH_3 stretching), and 1234 cm^{-1}(C-O-C stretching) corresponding to the vibrations of the acetate groups were observed. The spectrum of RC (3b) shows that the characteristic absorption of acetate disappears and the absorption around at 3400 cm^{-1} attributing to OH stretching is presented instead. This result indicates that the CA fibers were successfully deacetylated. The characteristic peak of aldehyde groups at 1716 cm^{-1} in the spectrum of treated RC (3c) confirmed that the hydroxyl groups on RC fiber surface were reacted with GA. Last, the spectrum (3d) shows the peak at 1540 cm^{-1} corresponding to amide groups. This could be implied that the aldehyde groups on the fiber surface reacted with amino groups of bromelain.

Conclusions

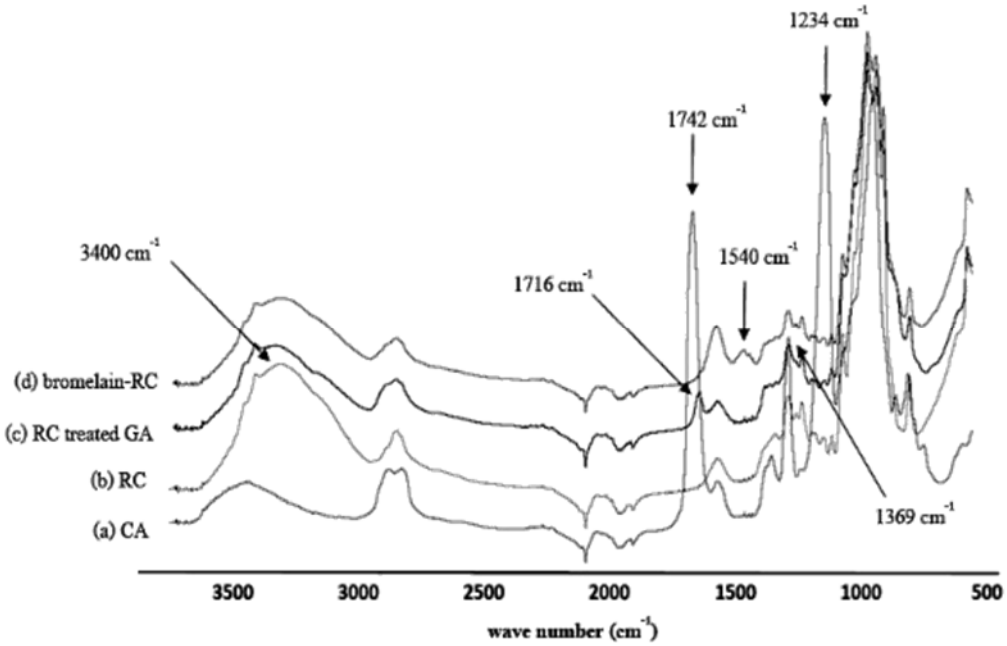

Figure 3 ATR-FTIR spectra of (a) CA, (b) RC, (c) RC treated GA and (d) bromelain-RC

Smooth CA fibers with diameter of 296±54 nm were successfully prepared. RC fiber was obtained by deacetylating the CA fibers and further functionalized by coupling with GA to generate the aldehyde groups on the fiber surface. The aldehyde group of the solid support reacted with amino groups of bromelain. Therefore, bromelain was successfully immobilized onto the treated RC fibers.

Acknowledgements

This study was granted by Department of Chemistry, Faculty of Science, Mahidol University, Thailand

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