Colloids and Surfaces B: Biointerfaces 121 (2014) 417–424

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

Fabrication of glycopolymer/MWCNTs composite nanofibers and its enzyme immobilization applications

Jing Quan [a], Zhongqing Liu [a], Christopher Branford-White [b], Huali Nie [a], Limin Zhu [a,*]

[a] College of Chemistry, Chemical Engineering and Biotechnology, Donghua University, Shanghai 201620, PR China
[b] Institute for Health Research and Policy, London Metropolitan University, London N7 8DB, UK

ARTICLE INFO

Article history:
Received 25 March 2014
Received in revised form 16 May 2014
Accepted 12 June 2014
Available online 19 June 2014

Keywords:
Glycopolymer
Composite Nanofibers
MWCNTs
Electrospun
CAT immobilization

ABSTRACT

Glycopolymer (poly(AN-*co*-OVSEG))/MWCNTs (multiwalled carbon nanotubes) composite nanofibers are fabricated using a facile approach combining enzymatic synthesis, radical polymerization and electrospinning. The structure of the glycopolymer was confirmed by FT-IR and ^{1}H NMR. Poly(AN-*co*-OVSEG)/MWCNTs composite nanofibers were prepared using electrospinning and characterized by scanning electron microscopy (SEM) and transmission electron microscopy (TEM). The hydrophilic properties of the composite nanofibers surfaces were increased since the contact angle of poly(AN-*co*-OVSEG)/MWCNTs composite was reduced from 65.5° to 37° compared to (PAN). As an enzymatic model catalase (CAT) was loaded (ca. 55.0 mg/g) to the poly(AN-*co*-OVSEG)/MWCNTs nanofibers. The optimum temperature for poly(AN-*co*-OVSEG)/MWCNTs nanofibers increased from 25 °C to 45 °C compared to free CAT. The covalently immobilized enzymes conjugate exhibited 60% activity at 60 °C, while the free enzyme was entirely inactivity after 5 min heat treatment. The immobilized CAT retained 70% of its initial activity after 5 cycles of decomposition of hydrogen peroxide.

1. Introduction

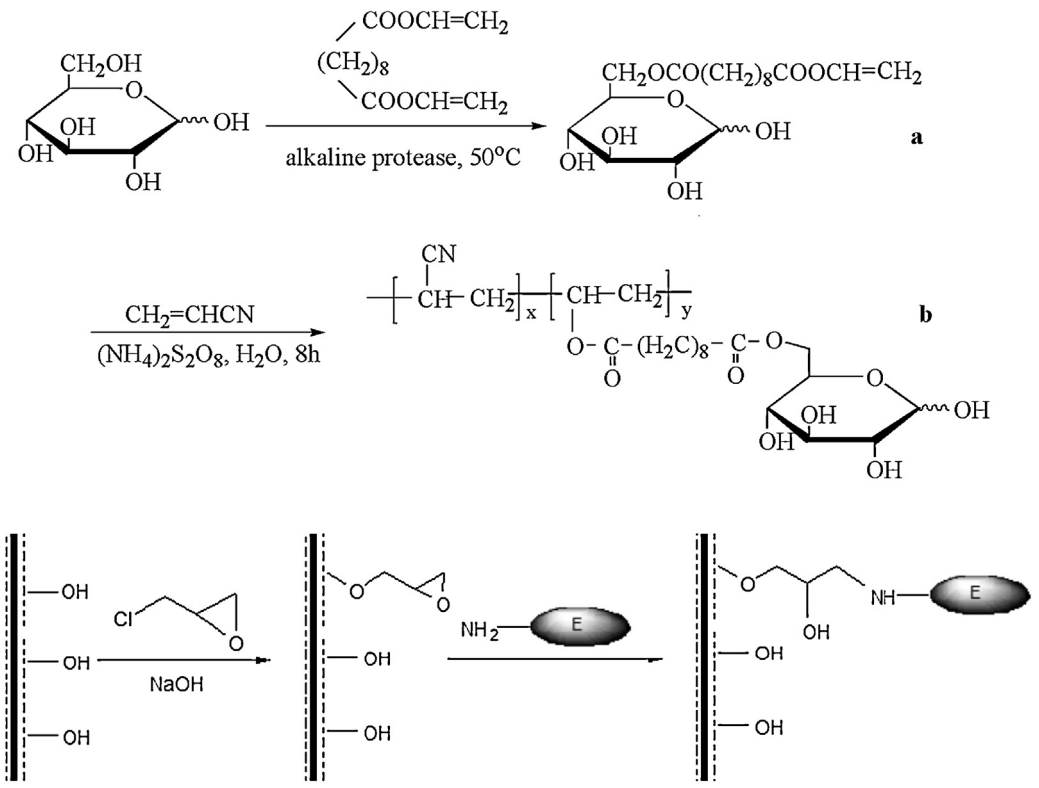
Glycopolymers, are polymers with pendant saccharides, which can be prepared from natural and synthetic polymers. They usually exhibit strong interactions with proteins due to their high multivalency [1–4]. Saccharides moieties contained in glycopolymers act as specific biological functional groups similar to those of naturally occurring glycoconjugates. They have the advantages of being biocompatible and being biodegradable [5,6]. Therefore, special attention has been focused on the biomimetic strategies of glycopolymers to reconstruct the environments for evaluating bioactive molecular interactions within a given matrix structure. Glycopolymer nanocarriers have been prepared in different forms including vesicles, micelles, nanoparticles and nanofibers, for the delivery of bioactive molecules, such as folic acid, biotin–avidin, antibodies and DNA sequences [7–11].

Enzymes, are highly specific, efficient, and “green” catalysts in chemical and biological fields [12]. In practical application, much attention has been placed on immobilizing enzymes onto or into an inert, insoluble material to overcome the limitations of instability and non-reusability of the free form [13–15]. Since immobilizing enzymes strongly depend on the properties and microstructures of the supporting system, the immobilization platform should be well organized to promote interaction with the biomolecule so providing optimum loading capacity that retains high stability [16]. It is widely accepted that nanoscale materials have large surface to volume ratios for enzyme loading [17]. Therefore, efforts to incorporate a biocompatible glycopolymer with nanoscale materials as a bioconjugation platform have been vigorously pursued [18,19].

Electrospun nanofibers are promising supports for enzyme immobilization due to their high specific surface area, fine porous structure allowing access to active sites and the low diffusion resistance. These systems also provide easy recoverability as well as potential applicability for continuous operations [20–25]. The electrospun nonwoven poly(acrylonitrile-*co*-acrylic acid) nanofiber mesh filled with carbon nanotubes has been successfully used for the immobilization of the oxidoreductase CAT where the immobilized enzyme displayed enhanced properties in terms of recycling and an increased stability [26]. CNTs can behave either as metals or as semiconductors, depending on their dimensions and electronic structure [27]. Their unique electronic properties suggest that CNTs have the ability to promote the electron transfer between the immobilized enzyme and material surface [28]. In addition, CNTs with specific thermal and mechanic properties, and high surface areas should provide a suitable microenvironment for immobilized enzyme especially in supporting redox specific enzymes [29,30].

* Corresponding author. Tel.: +86 21 67792655; fax: +86 21 67792655.
E-mail address: lzhu@dhu.edu.cn (L. Zhu).

http://dx.doi.org/10.1016/j.colsurfb.2014.06.030
0927-7765/



Scheme 1. Chemoenzymatic synthesis of glycopolymer poly(AN-*co*-OVSEG) and catalases immobilized onto the epoxidized modified composite nanofibrous membrane, (a) OVSEG; (b) poly(AN-*co*-OVSEG).

A promising strategy for designing novel composite nanofiber could include combining the advantages of glycopolymer and MWCNTs and attributing them into a single system. To achieve this objective, glycopolymer/MWCNTs nanofibers were designed, fabricated and investigated. We report the covalent immobilized of CAT onto composite nanofibers and evaluating the performance of the enzyme in terms of loading, optimum pH and temperature, storage stability and reusability. The outcomes from these studies firmly suggest the potential use of CNTs as successful supports systems for CAT.

2. Material and methods

6-*O*-vinylsebacyl-D-glucose (OVSEG) was synthesized in anhydrous pyridine at 50 °C, stirring at 250 rpm for 4 days [31]. Carboxyl graphitized multi-wall carbon nanotubes (MWCNTs) were purchased from Chengdu Organic Chemicals Co. Ltd. (Chinese Academy of Sciences). *N*,*N*′-dimethylformamide (DMF) and diethyl ether were provided by the Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Acrylonitrile (AN) and CAT from bovine liver (21,300 U/mg) were from Sigma.

2.1. Synthesis and characterization of poly(AN-co-OVSEG)

As shown (Scheme 1), poly(AN-*co*-OVSEG) was prepared by water phase precipitation polymerization of acrylonitrile (AN) and 6-*O*-vinylsebacyl-D-glucose (OVSEG). The molar ratio of acrylonitrile to OVSEG is 10:1, with H_2O and ammonium persulfate as solvent and initiator, respectively. The reaction was performed under N_2, at 60 °C, for 8 h. DMF and diethyl ether were used alternatively to purify the copolymer. The resulting product was dried under vacuum before being ground.

2.2. Preparation of the glycopolymer nanofibers

Poly(AN-*co*-OVSEG) and poly(AN-*co*-OVSEG)/MWCNTs nanofibers were prepared using a typical electrospinning process [32]. The spinning solution for poly(AN-*co*-OVSEG) was prepared by dissolving poly(AN-*co*-OVSEG) powder in DMF at ambient temperature gently stirring to form a homogeneous solution. The concentration of poly(AN-*co*-OVSEG) was 30 wt%. MWCNTs were added to poly(AN-*co*-OVSEG) using ultrasonication, for 30 min to obtain well-dispersed solution. The concentration of MWCNTs being 1 wt%.

After removing air bubbles, the homogeneous spinning solution was loaded into a 5 mL syringe and fed by a pump at 0.5 mL/h through a stainless steel spinneret with an internal diameter of 0.51 mm. A 14 kV voltage provided from a high-voltage power supply was applied to charge the solution which sprayed into finer jets and collected onto a grounded aluminum plate at a distance of 15 cm at 25 °C and 57% humidity. Finally, residual solvent was removed by drying the membranes under vacuum at 37 °C in a DZF-6050 Electric vacuum drying oven for 24 h.

2.3. Characterization of nanofibers

The surface morphology and diameters of all the prepared nanofibers were investigated using scanning electron microscopy (SEM; JSM-5600 LV microscope, JEOL, Tokyo, Japan) and transmission electron microscopy (TEM; JEM-2100F, JEOL, Tokyo, Japan). The hydrophobic properties of the nanofibers were evaluated on the basis of pure water contact angle measurements (DSA 10, Krüss, Hamburg, Germany).