International Journal of Biological Macromolecules 65 (2014) 234–240

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

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

Synthesis, characterization and antibacterial activity of new fluorescent chitosan derivatives

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ARTICLE INFO

Article history:
Received 9 December 2013
Received in revised form 17 January 2014
Accepted 19 January 2014
Available online 25 January 2014

Keywords:
Chitosan derivatives
Fluorescence
Antibacterial activity

ABSTRACT

The present work aims to the development of innovative new derivatives of chitosan that can be used for medical applications. This innovation is based on the synthesis and characterization of chitosan-g-aminoanthracene derivatives. Thus, *N-(anthracen-9-yl)-4,6-dichloro-[1,3,5]-triazin-2-amine* (AT) reacted with chitosan by the following steps: at first, cyanuric chloride reacted with 9-aminoanthracene to obtain *N-(anthracen-9-yl)-4,6-dichloro-[1,3,5]-triazin-2-amine* (AT), then the AT reacted with chitosan to obtain (CH-g-AT). The final product of CH-g-AT was separated, purified and re-crystallized by dioxane. The structure of the prepared chitosan derivatives was confirmed by FTIR-ATR, solid-NMR, TGA, X-RD, and DSC. The new chitosan derivatives showed fluorescence spectra in liquid and in solid state as well. CH-g-AT showed also high antibacterial activity against gram −ve species (*Escherichia coli*).

1. Introduction

The term modification of polymers is understood as the changes in terms of physical and chemical transformations of polymers. The chemical modification is based on the chemical reactions of active substances with functional groups of that polymer. A great attention is paid to such modification as well as to the preparation of synthetic polymers in order to obtain new potential biocompatible materials [1,2]. Considerable attention in recent years is paid also to the study of the preparation and properties of photoactive polymers. The interest of such modified polymers is connected with their wide range of applications in electronic and optoelectronic devices. Such polymers have great potential application in holographic recording, data storage, light-emitting diodes and solar systems, etc. [3]. Although most previously studied, fluorescent polymers are of synthetic origin. Recently, this research is focused on natural polymers having similar applications [4–7]. The advantages of natural polymers, compared to synthetic ones, represent in safety, biocompatibility, biodegradability, easy availability and relatively low cost. Chitosan is one of the natural and attractive polymers that exhibits a variety of biological activities with a wide potential of different applications.

Chitosan (CH) (β-1-4)-D-glucosamine) is the deacetylated structure of chitin, which is the second famous polymer found in nature after cellulose, that isolated from crabs, shrimps and other crustaceans [21,22]. Chitosan have unique properties like nontoxic, biodegradability, biocompatibility that has long been used as a natural polymer or a crude material in medicine, textile and food industry [8–12,23]. Due to the polycationic nature, chitosan has antimicrobial activity against several bacteria and fungi [10,24]. From the previous work [10,24] chitosan, can inhibit the growing of bacteria and fungi by inhibiting the normal metabolism of through the ionic interactions at cell surfaces and consequently leading at the end to the cell death [11]. The presence of a large number of primary amine and hydroxyl groups on the chitosan molecule

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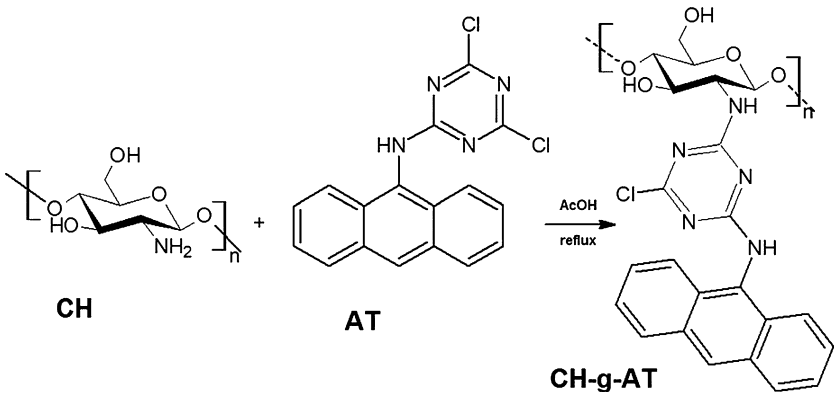
http://dx.doi.org/10.1016/j.ijbiomac.2014.01.050



Scheme 1. Preparation of CH-g-AT.

gives a possibility for many chemical modifications, by obtaining a new class of biomaterials. Recently, a large number of publications dealing with the labeling of chitosan by the introduction of a fluorophore in its molecule can be found, mostly via the primary amino group. Hence, fluorescent polymer could be obtained [13–15].

The present work aims to the synthesis, characterization and fluorescence measurements of new chitosan derivatives (CH-g-AT). The final product was confirmed using FTIR-ATR, solid NMR, TGA, X-RD, DSC, and fluorescence spectra. The antibacterial activity was also evaluated by using gram −ve bacteria (*Escherichia coli*).

2. Experimental

2.1. Materials

Chitosan extracted from the Crawfish shell with the degree of deacetylation (95%, MW 100 kDa), 9-aminoanthracene, cyanuric chloride (Sigma–Aldrich), other solvents were used for the synthesis are received without further purification.

2.2. Methods

2.2.1. Preparation of *N-(anthracen-9-yl)-4,6-dichloro-[1,3,5]-triazin-2-amine (AT)*

The label *N-(anthracen-9-yl)-4,6-dichloro-[1,3,5]-triazin-2-amine* (AT) was prepared by the reaction of cyanuric chloride with 9-aminoanthracenes according to the procedure described in the previous work [16]. Briefly, cyanuric chloride (5 g, 27 mmol) was dissolved in 100 mL of acetone, sodium carbonate was added and the mixture was cooled to −5 °C. The solution of 9-aminoanthracene (5 g, 26 mmol) in 100 mL of acetone was added drop-wise under nitrogen atmosphere. The temperature of the reaction mixture was maintained between −5 to 10 °C and the reaction was continuously monitored by TLC. After 4 h, the reaction mixture was poured into 100 mL water and the precipitated crude product was filtered off and dried. The crude product was re-crystallized by using toluene.

2.2.2. Preparation of fluorescent *chitosan-g-(anthracen-9-yl)-4,6-dichloro-[1,3,5]-triazin-2-amine (CH-g-AT)*

Chitosan (0.2 g) was dissolved in 30–60 ml of 2% aqueous solution of acetic acid. Then, a solution of AT (10 mg, 0.0293 mmol, 20 mg, 0.0586 mmol, and 40 mg, 0.117 mmol) in 1,4-dioxane (10–25 ml) was added. The reaction mixture was heated under reflux for 3 h. Then, the heating was turned off and the reaction mixture was stirred at room temperature overnight, a mixture of 250 ml of acetone and 150 ml 1,4-dioxane was added. After addition of acetone the sample precipitate start to appear, filtered through pleated filter-papers, washed with acetone and dried at 50 °C, the crude product was purified and recrystallized by 1,4-dioxane.

2.3. Characterization of CH-g-AT

2.3.1. UV/vis spectroscopy

The absorption spectra were measured on a UV/vis spectra-Perkin-Elmer Lambda 35 spectrophotometer at room temperature. The instrument provides corrected excitation spectra directly.

2.3.2. Fluorescence spectra

Very weakly absorbing solutions (optical density ~0.05 at the excitation wavelength in 1 cm cell) were used. The fluorescence spectra were recorded by excitation at the wavelengths of the most intensive absorption vibronic band; the optical density at this wavelength was about 0.05 in a 1 cm cell. The fluorescence quantum yields in solution were measured using quinine sulphate (q_F = 0.54 in 0.5 mol/L H_2SO_4) [19] as the standard. The fluorescence spectra in solid phase were recorded from the surface of the pressed powder in special commercial cuvettes for spectrophotometer PE LS 55. Fluorescence spectra were corrected for the characteristics of the emission mono-chromator and for the photomultiplier response.

2.3.3. Solid-NMR spectroscopy

^{1}D solid-state NMR spectra were measured using a Bruker Avance III HD 500 NMR spectrometer. Magic angle spinning (MAS) frequency of the sample was 11 kHz. Amplitude modulated cross-polarization (CP) with a duration of 1 ms was used to obtain ^{13}C CP/MAS NMR spectra. The applied recycle delay was 5 s. The ^{13}C NMR scale was calibrated with glycine as an external standard (176.03 ppm – low-field carbonyl signal). To compensate for frictional heating of the spinning samples, all NMR experiments were measured under active cooling. The sample temperature was maintained at 298 K, and the temperature calibration was performed on Pb $(NO_3)_2$ using a calibration procedure described in the literature [20].

2.3.4. XRD diffraction

X-ray diffraction were collected on a device namely; D-8 Advance diffractometer (Bruker AXS, Germany) with Bragg–Brentano θ–θ goniometer (radius 217.5 mm) equipped with a secondary beam curved graphite mono-chromator and Na (Tl) I scintillation detector. The generator was operated at 40 kV and 30 mA. The scan was completed at room temperature from 5 to 60° (2θ) in 0.02° step with a counting time of 8 s per step.