Carbohydrate Polymers 145 (2016) 78–85

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

Synthesis and characterization of chitosan alkyl urea

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ARTICLE INFO

Article history:
Received 12 November 2015
Received in revised form 31 January 2016
Accepted 11 March 2016
Available online 15 March 2016

Keywords:
Chitosan
N-methoxyformylation
Amine-ester exchange
Chitosan alkyl urea
Theoretical calculation

ABSTRACT

Chitosan is a versatile material employed for various purposes in many fields including the development of chiral stationary phases for enantioseparation. Chitosan alkyl urea is a kind of intermediate used to prepare enantioseparation materials. In order to synthesize the intermediates, in the present work, a new way to prepare chitosan alkyl urea has been established: chitosan was first reacted with methyl chloroformate yielding *N*-methoxyformylated chitosan, which was then converted to chitosan alkyl urea through amine-ester exchange reaction. With a large excess of methyl chloroformate and primary amine of low stereohindrance, the amino group in chitosan could be almost completely converted to ureido group. The as-prepared chitosan alkyl urea derivatives were characterized by IR, ^{1}H NMR, ^{13}C NMR,^{1}H-^{1}H COSY and ^{1}H-^{13}C HSQC NMR spectra. The chemical shifts of hydrogen and carbon atoms of glucose unit were assigned. It was found that the degree of substitution was obviously lower if cyclopropyl amine, aniline, *tert*-butyl amine and diethyl amine were used as reactants for the amine-ester exchange reaction. The reason was explained with the aid of theoretical calculations.

1. Introduction

Chitin is a polysaccharide widely existing in nature. After deacetylation, chitin converts to chitosan, which is biocompatible to living body (Asthana et al., 2015; Huang et al., 2015; Monsalve, Sierra, & Lopez, 2015), easily biodegradable in environment (Dananjaya, Godahewa, Jayasooriya, Lee, & De Zoysa, 2016; Duttagupta, Jadhav, & Kadam, 2015; Li et al., 2015), and has antibacterial property (Abu-Elala, Mohamed, Zaki, & Eissa, 2015; Mirhashemi et al., 2013; Jiří, Věra, Ingrid, & Jiří, 2015). As a result, chitosan is usually used in industry (Suyanto, Koesoemo, Ruriyanti, & Anggara, 2015), agriculture (Roshanravan, Soltani, Abdul Rashid, Mahdavi, & Yusop, 2015; Santos, Bacalhau, Pereira, Souza, & Faez, 2015), environmental protection (Suyanto, 2015; Luo, Zeng, Liu, & Zhang, 2015), medicine (Chen, Pan, Zhang, Zhong, & Zhang, 2015; Yhee et al., 2014) and food manufacture (Genskowsky et al., 2015; Gudjonsdottir et al., 2015). In order to broaden its application, it is necessary to modify the hydroxyl and amino groups of glucose unit, and selectively modify the amino group in particular. After modification, new functionalized compounds will be developed, which may meet various needs such as improving the solubility of chitosan, etc.

It is well known that supra-structure sometimes is critical for a polymer to exert its function. For instance, cellulose or amylose tris(phenylcarbamate) derivatives are very effective selectors for enantioseparation of chiral compounds (Chen, Duan, Fan, Bai, & Huang, 2014; Shen, Ikai, & Okamoto, 2014). Their powerful separation ability depends on their highly ordered structures, which result from full derivatization of three hydroxyl groups in cellulose or amylose skeleton (Ikai, Yamamoto, Kamigaito, & Okamoto, 2008). Chitosan is similar to cellulose in primary structure. If chitosan is modified with high substitution, the resulted derivatives may also possess highly ordered structure that probably bring about satisfactory enantioseparation ability. The purpose of this work is to develop a way to synthesize a series of intermediates for chitosan-based enantioseparation materials. Specifically, these intermediates are chitosan alkyl urea. Hydrogen bonding is a universal interaction for adsorption, molecular recognition (Michael, 2010), etc. There are amido groups contained in the urea, which can form hydrogen bond with the enantiomers to be separated. Meanwhile, the remaining hydroxyl groups in the intermediates can still be modified with various phenyl isocyanates providing structurally diversified enantioseparation materials (Zhang, Shen, Zuo, & Okamoto, 2014). In order to prepare chitosan derivatives with a highly ordered structure, chitosan of nearly complete deacetylation was chosen as the starting material. Chitosan was first reacted with

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http://dx.doi.org/10.1016/j.carbpol.2016.03.022
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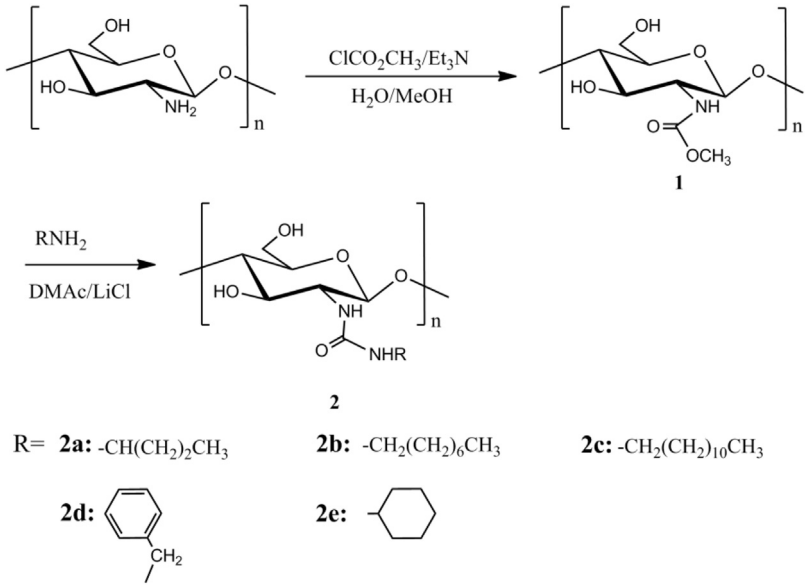

Scheme 1. Synthesis of chitosan alkyl urea derivatives.

methyl chloroformate to form a carbamate that further reacted with a primary amine yielding chitosan alkyl urea. The influence of electronic cloud density of nitrogen atom in the amines used as reactants for the amine-ester exchange reaction was studied by density functional theory (DFT) calculation. In addition, the influence of stereohindrance of the amines on the reaction was discussed in detail.

2. Experimental

2.1. Chemicals

All amines were purchased from Energy Chemicals (China). Methyl chloroformate, *N*,*N*-dimethyl acetamide (DMAc), lithium chloride, triethylamine, sodium hydroxide, acetic acid, pentanol and methanol were of analytical grade and purchased from Sinopharm Chemical Reagents Co., Ltd. (China). Trifluoroacetic acid-*d* (TFA-D) (D, 99.5%) was purchased from Cambridge Isotope Laboratories Inc. Chitin (from shrimp shell) was commercially available from Hai Zhi Yuan Biological Products Co., Ltd. (China). Chitosan was prepared by deacetylation of the chitin in 45% NaOH aqueous solution and in 30% NaOH alcoholic solution in sequence (Mima, Miya, Iwamoto & Yoshikawa, 1983; Tolaimate, Desbrieres, Rhazi & Alagui, 2003). The viscosity-average molecular weight ($\overline{M}$v) of chitosan was 1.5×10^5, and the deacetylation degree (*DD*) was 99.3%.

2.2. Preparation of chitosan alkyl urea

The synthetic scheme is shown in Scheme 1.

Chitosan (1.0 g, 6.2 mmol) was dissolved in a diluted HCl solution (30 g, 0.8%), and the resulting solution was cooled in an ice bath. When the temperature of the solution was below 10 °C, methanol (30 g) was added with stirring. The temperature was adjusted below 5 °C, and methyl chloroformate (4.66 g, 49.6 mmol) was added. The mixture was stirred at 2–10 °C for 7 h. During this period, the acidity of reaction mixture was maintained within a range of pH 2–7 by adding triethylamine. After the completion of the reaction, the product was filtered and washed thoroughly with ethanol. After drying, *N*-methoxyformylated chitosan (**1**) (1.3 g) was obtained in a yield of 95%.

Above prepared *N*-methoxyformylated chitosan (1.0 g, 4.57 mmol) was dissolved in a 10% solution of LiCl in DMAc at 80 °C, then *n*-butyl amine (2.67 g, 36.6 mmol) was added. The reaction solution was stirred at 110 °C for 12 h. The formed gel was treated with ethanol and grinded. The solid was filtered and washed with ethanol to afford **2a** i.e. chitosan *n*-butyl urea (1.1 g) in a yield of 92%. Chitosan *n*-octyl urea (**2b**), chitosan *n*-dodecyl urea (**2c**), chitosan benzyl urea (**2d**) and chitosan cyclohexyl urea (**2e**) were prepared by the same method.

2.3. Characterization of chitosan and chitosan derivatives

IR spectra of chitosan and chitosan derivatives were recorded on a Nicolet FT-IR instrument (USA) with KBr pellet. ^{1}H NMR spectra of chitosan and the chitosan derivatives prepared with cyclopropyl amine, aniline, *tert*-butyl amine and diethyl amine were measured with a NMR 400 MHz spectrometer of Varian (USA). ^{1}H-^{1}H COSY NMR, ^{1}H-^{13}C HSQC NMR, ^{1}H and ^{13}C NMR spectra of **2a**–**e** were measured on a high-resolution liquid NMR 600 MHz spectrometer of Bruker Avance III (Sweden) with a 5 mm TCI CryoProbe equipped with Z-gradients up to 53 G/cm. Sample solutions (20 mg/ml) were prepared with TFA-D as solvent. TFA-D was also employed as reference: proton (δ 11.50 ppm) and carbon (δ 164.1 ppm). Detection temperature was set at 25 °C. *DD* of chitosan (Fig. S1), degree of substitution (*DS*) of *N*-methoxyformylated chitosan and chitosan alkyl urea were determined according to integrals in corresponding ^{1}H NMR spectra (Figs. 2 and 3, Figs. S2–S9). Viscosity-average molecular weight of chitosan was measured with a Ubbelohde viscometer (i.d. 0.5 mm) with 0.1 M aqueous acetic acid solution as solvent (Maghami and Roberts, 1988).

2.4. Computational details

Theoretical calculations concerning the geometry optimization, energy differences and natural bond orbital (NBO) charges were performed with Gaussian 09 software package (Gaussian 09 et al., 2010). Optimization of the molecular structures was conducted by means of density functional theory (DFT) with the Becke's 3 parameters (B3) and the Lee-Yang-Parr's nonlocal correlation functional (LYP) (Jiang, Ou-yang, Zhu, Zou, & Tang, 2014). The basis sets for C, N and H were 6-311++G** with polarization functions for C, N and H, and with diffuse function only for C and N atoms (Jiang et al.,