Synthesis and Characterization of Chitosan Derivatives Carrying Galactose Residues

Can Zhang, Qineng Ping, Ya Ding

College of Pharmacy, China Pharmaceutical University, Nanjing 210009, People’s Republic of China

Received 1 May 2004; accepted 24 January 2005
DOI 10.1002/app.21975
Published online in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: A series of water-soluble chitosan derivatives, carrying galactose residues, were synthesized by using an alternative method in which the galactose groups were introduced into amino groups of the derivatives. First, hydroxyethyl chitosan (HECS) and hydroxypropyl chitosan (HPCS) were synthesized under alkaline conditions by using chitosan and propylene or chitosan and CICH₂CH₂OH as the starting materials, respectively. Then lactobionic acid was added into the systems so as to form galactosylated HECS (Gal-HECS) and galactosylated HPCS (Gal-HPCS) with substitution degrees of 53 and 47%, respectively. Lactosaminated HPCS (Lac-HPCS) and Lac-tosaminated HECS (Lac-HECS) were obtained with substitution degrees of 42 and 38%, respectively, by the reductive amination of the mixtures of lactose and HECS or lactose and HPCS with potassium borohydride present in the reaction. The chemical structures of new chitosan derivatives were characterized by FTIR, ¹H NMR, ¹³C NMR, and elemental analysis. Some physical properties were also analyzed by wide angle X-ray diffraction (WAXD) and differential scanning calorimetry (DSC). The novel chitosan derivatives carrying galactose residues may be used as additives for hepatic targeting delivery. © 2005 Wiley Periodicals, Inc. J Appl Polym Sci 97: 2161–2167, 2005

Key words: chitosan; synthesis; FT-IR; NMR; drug delivery systems

INTRODUCTION

It is well known that hepatocytes can only recognize the asialoglycoprotein receptor (ASGP-R) among the liver-associated cell surface receptors and that ASGP-R is present in several human hepatoma cell lines.¹² If a ligand binds to a galactose receptor, the ligand-receptor complex may be internalized rapidly and then the receptor would recycle back to the cell surface.³ The receptor shows a high binding capacity and efficient cellular uptake of galactosylated ligands. Several studies indicated that the delivery system of galactose receptor-mediated endocytosis would be useful for drug targeting to hepatocyte and hepatoma cells.⁴⁻⁵

Chitosan is a naturally abundant biodegradable polysaccharide produced by deacetylation of chitin. A number of cationic chitosans, due to their good properties such as nontoxicity of cell, biocompatibility, and low immunogenicity, were taken into consideration as potential additives in the pharmaceutical field for drug delivery systems.⁶⁻¹⁷ Taking advantage of chitosan, a few lactosaminated or galactosylated derivatives, which were chemically conjugated with drugs, have been reported. For example, the conjunct of lactosaminated N-succinyl-chitosan was utilized as a liver-specific drug carrier in mice.¹⁸ DNA complexation, of galactosylated quaternary chitosan, galactosylated chitosan-graft-dextran, galactosylated chitosan-graft-PEG, and galactosylated chitosan-graft-PVP, was investigated as a nonviral vector for gene delivery.¹⁹⁻²³ Zhang and coworkers also reported on the synthesis of galactosylated chitosan and the preparation of its microspheres, which may be used as a potential drug delivery system with passive and active hepatic targeting properties.²⁴

However, chitosan can be dissolved only in diluted acid solution, so that galactosylated or lactosaminated reactions of chitosan have to proceed in a heterogeneous system. As a result, the substitution degree of the reactive products is low and the further N-substitution degree of the residue amino group of chitosan is not high. In addition, the derivative with hydrophobic properties is usually taken up by a reticuloendothelial-system and has short residence time in blood circulation. For this article, a series of novel water-soluble galactosylated and lactosaminated chitosan derivatives were synthesized by an alternative method. First, two water-soluble O-substituted chitosan derivatives (HECS and HPCS) were obtained, and then the galactose groups were more easily introduced into the amino groups of HECS and HPCS with higher substitution degree. The chemical structures of the modified chitosan were characterized by FTIR, ¹H NMR, ¹³C NMR, and elemental analysis. Some physical properties were analyzed by WAXD and DSC.
EXPERIMENTAL

Materials

Chitosan was provided by Nantong Suanglin Biochemical Co. Ltd (China), with a degree of deacetylation of 91.5% and viscosity average molecular weight of 25KD. N, N, N, N'-tetramethylethylenediamine (TEMED) and lactobionic acid (LA) were purchased from Acros (USA). N, N'-Dicyclohexylcarbodiimide (DCC) was obtained from Shanghai Chemical Regent Company (China). All other chemical solvents and reagents were used without further purification.

Measurements

$^1$H NMR and $^{13}$C NMR spectra were performed on a Bruker (AVACE) AV-500, AV-300 spectrometer; the sample was dissolved in the mixed solvent D$_2$O and F$_3$CCOOH. Zgpr pulse for suppressing water was used in $^1$H NMR.

IR spectra were recorded on a Fourier-transform infrared spectrometer (Nicolet 2000) in KBr discs. X-ray diffraction spectrometry was obtained by using an XD-3A powder diffraction meter with CuKα radiation in the range 5–40° at 40 kV and 30 mA. The results of differential scanning calorimetry (DSC) were obtained with NETZSCH DSC 204 equipment in a nitrogen atmosphere at a heating rate of 10°C/min.

HECS and HPCS synthesis

Preparation of HECS was according to the method reported by Huang and coworkers. Briefly, 1 g of chitosan, mixed with 20 mL 50 wt % NaOH solution, was lyophilized for 48 h. Then, alkali chitosan and isopropyl alcohol 30 mL were mixed and stirred for 1 h at 40°C. 30 mL of propylene epoxide was added and refluxed 2 h at 50°C with continuous stirring. Afterwards, the reaction mixture was treated by the same procedures described as above to give 0.5 g HPCS.

Gal-HECS and Gal-HPCS synthesis

1 g of HECS or HPCS was dissolved in 50 mL distilled water with stirring, and then the mixed solution of LA (2.6 g, 7.4 mmol) in 5 mL water and DCC (1.5 g, 7.3 mmol) in 1 mL of TEMED, was dropped into the HECS solution and stirred for 72 h at room temperature. The reaction solution was filtered, dialyzed (MWCO 10,000) against distilled water for 5 days, and then lyophilized to give 0.3 g of Gal-HECS or Gal-HPCS.

Lac-HECS and Lac-HPCS synthesis

HECS or HPCS (0.5 g, 2.0 mmol) was dissolved in 20 mL methanol, and then α-lactose (1.3 g, 3.6 mmol) in 5 mL of distilled water was added. After stirring for 24 h, potassium borohydride (0.2 g, 3.6 mmol), dissolved in 5 mL of distilled water, was mixed with the solution, which was stirred at room temperature for 144 h. At the end of the reaction, a part of the methanol was removed on a rotary evaporator under vacuum. The residual solution was dialyzed (MWCO 10,000) against distilled water for 24 h and then lyophilized to give 0.1 g of Lac-HECS or Lac-HPCS.

RESULTS AND DISCUSSION

Preparation and characterization of chitosan derivatives

Figure 1 displays the synthetic route of chitosan derivatives. The substitution degree of chitosan derivatives was calculated by comparing C and N molar ratios obtained from results of elemental analysis (Table I). The substitution degrees of the hydroxyethyl and hydroxypropyl groups were 95 and 100%, respectively. The degrees of galactosylated substitution were 53% (Gal-HECS) and 47% (Gal-HPCS), respectively. The degrees of lactosaminated substitution were 42% (Lac-HECS) and 38% (Lac-HPCS), respectively.

In the spectra of FTIR spectroscopy (Figs. 2 and 3), for chitosan, distinctive absorption bands appear at 1622 cm$^{-1}$ (Amide I), 1590 cm$^{-1}$ (–NH$_2$ bending), and 1380 cm$^{-1}$ (Amide III). The absorption bands at 1556 cm$^{-1}$ (asymmetric stretching of the C-O-C bridge),
1075, and 1033 cm\(^{-1}\) (skeletal vibration involving C-O stretching) are characteristic of its saccharine structure.\(^{27}\)

Compared with chitosan, the IR spectrum of HECS shows that the intensity of the peak at 3400 cm\(^{-1}\) was increased while the absorption peak at the N-H bond was not changed. This suggests that the hydroxyethyl moiety was introduced into 6-OH of chitosan.

The IR spectrum of HPCS exhibited a strong absorption at 1460 cm\(^{-1}\), which was assigned to the CH\(_3\) symmetrical deformation vibration. In addition, the peak at 1100 cm\(^{-1}\), assigned to the C-O adsorption peak of the secondary hydroxyl group, and the new peak at 2970 cm\(^{-1}\) indicated the incorporation of the hydroxypropyl moiety. The absorption peak at the N-H bond, however, was not changed. The results confirmed that substitution occurs at the C6 position.

The IR spectra of Gal-HECS and Gal-HPCS all showed new signals, at 1657 cm\(^{-1}\), assigned to the acylamino group, and a new peak at 1590 cm\(^{-1}\), that...
were attributed to the unreacted amino groups. The peak intensity at 3400 cm$^{-1}$ was increased largely due to the increase of the hydroxyl groups. This indicated that the amino groups of HECS and HPCS were partly substituted by the galactose groups.

The IR spectra of Lac-HECS and Lac-HPCS showed that the peak intensity at 3400 cm$^{-1}$ was increased largely due to the increase of hydroxyl groups. In contrast, the absorption peak for the N-H bond was greatly weakened. This confirmed that lactosaminated groups were introduced into the amino groups of HECS and HPCS.

The $^1$H NMR spectra of chitosan and modified chitosan are given in Figures 4 and 5. The $^1$H NMR assignments of chitosan are as follows: $^1$H NMR (D$_2$O) spectra of Gal-HECS and Gal-HPCS all showed new signals at $\delta = 3.5$ (H3, H4, H5, H6), $\delta = 2.0$ (NHCOCH$_3$). The other protons of Gal-HPCS were assigned to $\delta = 2.9$ (H$_2$), $\delta = 0.9$ (NHCOCH$_3$), $\delta = 1.1$ (H9).

The $^1$H NMR (D$_2$O) spectra of Gal-HECS and Gal-HPCS all showed new signals at $\delta = 4.6$, which was assigned to the protons of H1, H1', and Hc, and that the others of Gal-HECS were assigned to $\delta = 3.2 - 3.6$ (H3, H4, H5, H6, H7, H8, H2', H3', H4', H5', H6', Ha, Hb, Hc, Hc', Hf). The other protons of Gal-HPCS were assigned to $\delta = 2.9$ (H$_2$), $\delta = 2.0$ (NHCOCH$_3$), $\delta = 1.96$ (NHCOCH$_3$). Furthermore, the others of Lac-HPCS were assigned to $\delta = 5.03$ (H1), $\delta = 4.7 - 4.6$ (H1'), $\delta = 4.1 - 3.5$ (H3, H4, H5, H6, H7, H8, H2', H3', H4', H5', H6', Hb, Hc, Hc', Hf), $\delta = 3.0$ (H2), $\delta = 2.0$ (NHCOCH$_3$), $\delta = 127$ (H9). This suggested that the lactosaminated reaction had occurred.

Figures 6 and 7 show spectra of $^{13}$C NMR of chitosan, modified HECS, and modified HPCS. Signals at $\delta = 127$ (C1), $\delta = 76.5$ (C4), $\delta = 75$ (C5), $\delta = 70$ (C3), $\delta = 60$ (C6), $\delta = 55.6$ (C2), of the $^{13}$C NMR (D$_2$O/F$_3$CCOOD) spectrum of chitosan, were detected. The $^1$H NMR (D$_2$O) spectra of Lac-HECS and Lac-HPCS showed new peaks at $\delta = 68$, which...
was attributed to C8, and the peak intensity at $\delta = 60$ was increased. The chemical shifts of the other carbons of Gal-HECS were attributed as follows: $\delta = 98.5$ (C1), $\delta = 77.5$ (C4), $\delta = 75$ (C5), $\delta = 70$ (C3), $\delta = 60$ (C6, C7), $\delta = 55.6$ (C2).

The 13C NMR (D2O) spectrum of HPCS showed new peaks at $\delta = 18.2$ and 78.1, which were assigned to the carbons of the methyl groups (C9) and (C8), respectively. The chemical shifts of the other carbons of HPCS were attributed as follows: $\delta = 102.2$ (C1), $\delta = 76.3$ (C4), $\delta = 74.8 \sim 73.8$ (C5, C8), $\delta = 69.4$ (C3), $\delta = 60.7$ (C6, C7), $\delta = 57.4$ (C2).

From the 13C NMR (D2O) spectrum of the Gal-HECS and Gal-HPCS, the chemical shift at $\delta = 168.2$ showed the carbon signal of carbonyl groups. The chemical shifts of the other carbons of Gal-HECS were attributed as follows: $\delta = 97.6 \sim 97.4$ (C", C1", C1"), $\delta = 80.5 \sim 68.5$ (C"2, C3, C3", C4, C4", C5, C5", C8, Cb, Cc, Cd), $\delta = 60.0 \sim 59.6$ (C6, C6", C7, Ce), $\delta = 55.7$ (C2). Similarly, the other carbon signals of Gal-HPCS were assigned to $\delta = 99.9 \sim 98.4$ (C", C1, C1"), $\delta = 77.2 \sim 68.5$ (C"2, C3, C3", C4, C4", C5, C5", C8, Cb, Cc, Cd), $\delta = 61.9 \sim 60.1$ (C6, C6", C7, Ce), $\delta = 55.7$ (C2), $\delta = 18.2$ (C9). According to FTIR spectra of Gal-HECS and Gal-HPCS, acyl reaction occurred at the amino groups of HECE and HPCS.

Similarly, the other carbon signals of Lac-HECS and Lac-HPCS all showed a new signal, at about $\delta = 52$, attributed to Ca. The other carbon signals of Lac-HECS were attributed to $\delta = 102.1 \sim 100.2$ (C1, C1"), $\delta = 78.2 \sim 66.8$ (C3, C3", C4, C4", C5, C5", C8, Cb, Cc, Cd, Ce), $\delta = 60.4 \sim 59.9$ (C6, C6", C7, Cf), $\delta = 56.5$ (C2). The other carbon signals of Gal-HPCS were assigned to $\delta = 102.2 \sim 100.2$(C1, C1"), $\delta = 77.9 \sim 68.5$ (C"2, C3, C3", C4, C4", C5, C5", C8, Cb, Cc, Cd, Ce), $\delta = 61.9 \sim 60.1$ (C6, C6", C7, Cf), $\delta = 55.8$ (C2), $\delta = 18$(C9). Combined with FTIR results, this confirmed that lactosaminated groups were introduced into the amino groups of Lac-HECS and Lac-HPCS.

X-ray diffraction diagrams of chitosan and all the derivatives (Figs. 8 and 9) showed that chitosan exhibited three reflections falling at $2\theta = 11^\circ$, $2\theta = 20^\circ$, and $2\theta = 22^\circ$. Samuels and colleagues reported that the reflection falling at $2\theta = 11^\circ$ was assigned to crystal form I, and the strongest reflection appeared at $2\theta = 20^\circ$, corresponding to crystal form II.31 The WAXD patterns of the derivatives of galactosylated chitosan all showed only one broad peak at around $2\theta = 20^\circ$. This indicated that the ability to form hydrogen bonds within the chitosan ordered structure was decreased after chemical modification and the chitosan derivatives became amorphous.
DSC thermograms of chitosan derivatives are shown in Figures 10 and 11. The spectrum of chitosan showed a broad endothermic peak around 103.9°C and a sharp exothermic peak at 322.6°C. The former, endothermic peak may be due to the adsorbed water in chitosan and/or part of the polymer with low molecular weight. The latter may be attributed to the decomposition of chitosan. All modified chitosans showed endothermic peaks around 80°C and broad exothermic peaks around 280°C, due to moisture loss and thermal decomposition, respectively. The results also indicated that the chain structures of chitosan were changed, and the ability to crystallize was decreased by galactosylation and lactosamination.

CONCLUSIONS

Galactosylated or lactosaminated chitosan derivatives were synthesized in homogeneous reaction systems. In the processes, water-soluble chitosan derivatives, HECS and HPCS, were prepared for galactosylation and lactosamination. Homogeneous systems make synthesis easier, and high substitution degrees were obtained. The novel chitosan derivatives, carrying galactose residues, were characterized by FTIR, 1H NMR, 13C-NMR, and elemental analysis. Some physical properties were analyzed by WAXD and DSC. Taking advantage of water solubility and cellular uptake of galactosylated ligands, the novel chitosan derivatives may be used as effective additives for hepatic targeting drug delivery and prolonged residence in blood circulation. The potential uses of these derivatives, as the coating materials of nanoparticles, will be reported in future articles.

The authors gratefully acknowledge the National Natural Science Foundation of China (30271554), the National 863 project (2004AA2Z3232), and the Key Program of Science and Technology of the State Education Ministry of China (2003, 03090) for financial support.

References