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Preparation of highly regioselective amphiprotic chitosan derivative via "click chemistry"

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ABSTRACT

Synthesis of a highly regioselective amphiprotic chitosan derivative was achieved by click chemistry. The Huisgen cycloaddition between 6-azido-6-deoxy-*N*-pthaloyl-chitosan and methyl propiolate was successfully carried out in the presence of Cu(I) catalyst. After the reaction, both the phthaloyl protecting group and methyl group were completely removed by hydrazine. FT-IR and NMR spectroscopy as well as elemental analysis strongly support the structural uniformity of the desired amphiprotic chitosan derivative, which has both a carboxylic group with a 1,4-triazole linker at the C-6 position and an amino group at the C-2 position per repeating unit. The amphiprotic chitosan derivative was soluble under both acidic and basic aqueous conditions. In contrast, it formed nanoparticle under neutral condition due to the interaction between the positive ($-NH_3^+$) and negative ($-COO^-$) ions on the chitosan derivative.

1. Introduction

Chitosan is produced by deacetylation of chitin, which is the main component of exoskeletons of crustaceans such as crab and shrimp. Chitosan has a linear polysaccharide structure composed of a β-(1-4)-linked D-glucosamine. Its unique structure with reactive amino and hydroxyl groups per anhydroglucosamine repeating unit has potential for designing advanced materials through chemical modification. In particular, chemical modification with a well-controlled macromolecular structure is a promising way to provide a novel chitosan derivative with reliable properties [1].

Among the chitosan derivatives, amphiprotic chitosan, which can either donate or accept protons, make it possible to offer several applications. Carboxymethylchitosan is the most typical amphiprotic chitosan derivative, having amine and carboxylic acid groups in the molecule. The unique macromolecular structure provides characteristic properties such as antibacterial activity [2], biocompatibility [3], and solubility [4,5], as well as chelating [6], aggregation [7], and membrane properties [8]. However, it is difficult to prepare regioselective amphiprotic chitosan by *N*- and *O*-carboxymethyl etherification using monochloroacetic acid.

We have recently developed the first successful preparation method for highly regioselective chitosan derivatives by Cu(I)-catalyzed azide alkyne Huisgen cycloaddition [9]. The reaction is known to be the most ideal "click chemistry" reaction from the perspective of its high regioselectivity, quantitative yield, and mild reaction conditions without generation of by-products [10]. Application of click chemistry with chitosan allows us to design various chitosan-based materials from azido-chitosan derivative, which leads to the creation of finely designed novel chitosan derivatives.

Accordingly, we present herein the preparation of a highly regioselective amphiprotic chitosan derivative via click chemistry using 6-azido-6-deoxy chitosan derivative.

2. Experimental

2.1. Materials

All chemicals were purchased from Kanto Chemical or Wako Pure Chemical and used without further purification. Chitosan with a 99% degree of deacetylation (DDA) and a number average molecular weight (M_n) of 40,000 was obtained from the complete deacetylation of chitosan (DDA = 90%, M_n = 55,300, Koyo Chemical) using sodium hydroxide, and was thoroughly freeze-dried prior to use.

2.2. Characterization

^{1}H and ^{13}C NMR spectra were recorded using a JEOL JNM-LA400 and Bruker AVANCEII600 spectrometer, respectively. Chemical shifts were referenced to tetramethyl silane (TMS; 0.0 ppm).

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Fourier Transform Infrared (FT-IR) spectra were recorded with a FT-IR spectrometer (Spectrum 65, PerkinElmer Japan Co., Ltd.) equipped with an ATR attachment (Universal ATR, PerkinElmer Japan Co., Ltd.). All the spectra were obtained by accumulation of 16 scans with a resolution of 4 cm^{-1} at 400–4000 cm^{-1}. Degree of substitution (DS) values of the series of chitosan derivatives were calculated from the C and N content in the elemental analysis (EA) data obtained using an elemental analyzer (Elementar Vario EL III, Elementar) or peak areas of the ^{1}H NMR spectrum. Water solubility of the amphiprotic chitosan (**6**) was evaluated from the transmittance of the aqueous solution at 600 nm, using a UV-Vis spectrophotometer (JASCO-V550). The sample concentration was 0.1 wt.%. The pH value of the water solvent was adjusted by acetic acid and sodium hydroxide. For field emission scanning electron microscope (FE-SEM) observation, the prepared chitin aggregates were collected by filtration and dried in an oven. The sample was coated with an approximately 2 nm layer of Pt by an ion sputter coater and observed by FE-SEM (JSM-6700F; JEOL, Ltd.) operating at 2.0 kV. The average particle size of the nanoparticles was estimated by image analysis.

2.3. Preparation of 6-azido-6-deoxy-N-phthaloyl-chitosan (*4*)

N-Phthaloyl-chitosan (**2**) was prepared in aqueous acid referring to the previously reported procedure [11]. Fully deacetylated chitosan (**1**) (1.0 g, 6.2 mmol of anhydro glucosamine unit) was refluxed with phthalic anhydride (4.2 g, 18.6 mmol) in 50 mL of AcOH/H_2O (1.0%, v/v) for 24 h. After the reaction, the precipitate was collected by centrifugation. The product was dispersed in methanol, filtered, washed thoroughly with methanol, and dried at 80 °C under vacuum. Yield: 1.7 g. DS_{phth} 1.00. FTIR (ATR): ν (cm^{-1}) = 3439 (O—H), 2935 (C—H), 1774, 1702 (C=O_{imide}), 718 (arom).

6-Bromo-6-deoxy-*N*-phthaloyl chitosan (**3**) and subsequently 6-azido-6-deoxy-*N*-phthaloyl chitosan (**4**) were prepared according to a previously reported method [9]. *N*-Phthaloyl chitosan (**2**) (3.9 g, 13.4 mmol) was dispersed in 390 mL of *N*-methyl-2-pyrrolidone (NMP) and *N*-bromosuccinimide (23.5 g, 132 mmol), and then with triphenylphosphine (35.0 g, 134 mmol) were added to an ice bath; the mixture was then heated to 80 °C and stirred for 2 h under an argon atmosphere. The reaction mixture was poured into ethanol, and the precipitate was collected by centrifugation. The polymer was precipitated into ethanol again, filtered, and washed with ethanol, distilled water, and acetone, and then dried at 80 °C under vacuum. Yield: 4.3 g. DS_{phth} 1.00. ^{1}H NMR (DMSO-d_6): δ (ppm) = 7.79 (arom), 5.26–3.41 (H-1–H-6). FTIR (ATR): ν (cm^{-1}) = 3476 (O—H), 2942 (C—H), 1775, 1706 (C=O_{imide}), 717 (arom).

For 6-azido-6-deoxy-*N*-phthaloyl-chitosan (**4**), 6-bromo-6-deoxy-*N*-phthaloyl chitosan (**3**) (4.2 g, 11.9 mmol) was dissolved in 420 mL of NMP, and sodium azide (7.7 g, 118 mmol) was added to the solution. The mixture was stirred at 80 °C for 4 h under an argon atmosphere in order to remove the salts, and the filtrate was precipitated into ethanol. The precipitate was collected by centrifugation, and the product was dispersed into ethanol, filtered, and washed again with ethanol, distilled water, and acetone. After drying at 100 °C under vacuum, 6-azido-6-deoxy-*N*-phthaloyl-chitosan (**4**) was obtained. Yield: 3.3 g. DS_{azide} 0.93; elemental analysis: Calcd.: C 53.17, H 3.82, N 17.72%; found: C 52.08, H 4.32, N 16.45%. ^{1}H NMR (DMSO-d_6): δ (ppm) = 7.83 (arom), 5.20–2.96 (H-1–H-6). ^{13}C NMR (DMSO-d_6): δ (ppm) = 169.60 (C=O_{phth}), 136.33, 133.15, 124.87 (arom), 98.59 (C-1), 80.05, 74.53, 70.10 (C-3–C-5), 59.18 (C-2), 52.14 (C-6). FTIR (ATR): ν (cm^{-1}) = 3470 (O—H), 2925 (C—H), 2102 (N_3), 1775, 1706 (C=O_{imide}), 718 (arom).

2.4. Preparation of amphiprotic chitosan derivative (*6*)

The procedure for azide-alkyne [3 + 2] dipolar cycloaddition to prepare the polymer **5** was as follows: 6-azido-6-deoxy-*N*-phthaloyl-chitosan (**4**) (0.20 g, 0.63 mmol) was dissolved in dimethyl sulfoxide (DMSO, 40 mL), and copper(II) sulfate pentahydrate (8.0 mg, 0.032 mmol, in 0.2 mL of distilled water), sodium ascorbate (6.4 mg, 0.032 mmol in 0.1 mL of distilled water), and methyl propiolate (172 μL, 1.9 mmol) were added, and the mixture was stirred at 70 °C for 96 h. The mixture was precipitated in diethylether/2-propanol (1/1, v/v), collected by filtration, washed with diethylether/2-propanol (1/1, v/v), and dried in vacuum. Yield: 0.23 g. DS_{ester} 1.07; elemental analysis: Calcd.: C 54.00, H 4.53, N 14.00%; found: C 49.65, H 4.45, N 11.69%. ^{1}H NMR (DMSO-d_6): δ (ppm) = 8.37 (triazole), 7.78 (arom), 5.22–4.17 (H-1–H-6), 3.73 (methyl). ^{13}C NMR (DMSO-d_6): δ (ppm) = 167.47 (C=O_{phth}), 160.26 (C=O_{ester},), 138.40, 129.38 (C=$C_{triazole}$), 134.20–122.90 (arom), 95.74 (C-1), 77.23–56.89 (C-3–C-5), 56.89 (C-2), 51.25 (methyl), 49.68 (C-6). FTIR (ATR): ν (cm^{-1}) = 3475 (O—H), 2950 (C—H), 1776, 1709 (C=O_{imide}), 1667 (C=O_{ester}), 1213 (C—O_{ester}), 720 (arom).

To remove the phthaloyl protecting group and methyl group, polymer **5** (0.21 g, 0.53 mmol) was dissolved in NMP (20 mL), 20 mL of 4 M aqueous hydrazine monohydrate was added, and the mixture was stirred at 100 °C for 4 h under Ar. The mixture was precipitated into MeOH, and the precipitate was collected by filtration, washed with MeOH, and dried in vacuum. Yield: 0.12 g. DS_{acid} 1.06; elemental analysis: Calcd.: C 38.85, H 3.99, N 20.14%; found: C 38.21, H 5.08, N 19.44%. ^{1}H NMR (10 wt.% CD_3COOD in D_2O): δ (ppm) = 8.55 (triazole), 3.98–3.29 (H-1–H-6). ^{13}C NMR (10 wt.% CD_3COOD in D_2O): δ (ppm) = 163.56 (C=O_{acid}), 143.72, 131.58 (C=$C_{triazole}$), 99.29 (C-1), 78.23–72.56 (C-3–C-5), 58.63 (C-2), 53.15 (C-6). FTIR (ATR): ν (cm^{-1}) = 3292 (O—H), 2879 (C—H), 1662 (C=O), 1594 (N—H_{amine}).

3. Results and discussion

3.1. Preparation of 6-azido-6-deoxy-N-phthaloyl-chitosan (*4*)

6-Azido-6-deoxy-*N*-phthaloyl-chitosan (**4**) was prepared according to Scheme 1. We have reported highly chemoselective *N*-phthaloylations of chitosan in aqueous acetic acid media for protection of the amino group [11]. Thus, *N*-phthaloyl chitosan (**2**) was prepared using phthalic anhydride and fully deacetylated chitosan in aqueous AcOH with a concentration of 1.0%. The DS value of the phthaloyl group was 1.0.

Deoxyhalogenation of *N*-phthaloyl-chitosan (**2**) and subsequent azidation were carried out according to the previously described procedure [9]. That is, *N*-phthaloyl-chitosan (**2**) was brominated by reaction with *N*-bromosuccinimide and triphenylphosphine for 2 h at 80 °C. Due to steric hindrance by the bulky *N*-phthaloyl group at the C2 position of chitosan, selective deoxybromination at the C-6 primary hydroxyl group was achieved [12]. The DS value of the bromine group estimated by ^{1}H NMR spectrum was also 1.0.

Azidation of the 6-bromo-6-dexoy-*N*-phthaloyl chitosan (**3**) was achieved by a nucleophilic displacement reaction of the bromine group with sodium azide. The DS value of the azide group estimated by the C and N content of elemental analysis data was 0.93, supporting that almost all of the bromine groups of polymer **3** were substituted by an azide moiety. In the FTIR spectrum of polymer **4**, a significant absorption peak at 2102 cm^{-1} is evidence for the azide moiety (Fig. 1). In the ^{13}C NMR spectrum of polymer **4**, all peaks with well-resolved sharp signals were assignable to the respective carbon atoms (Fig. 2). The peak at 52.1 ppm is characteristic of the C-6 carbon of azide derivative **4**. Other pyranose ring carbons at C-1–C-5 were also clearly observed as five signals. Aromatic and carbonyl carbons of the phthaloyl moiety are observed