Synthesis and characterization of new chitosan-based Schiff base compounds

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Chitin (Cn) was extracted from the armors of crustaceans Astacus leptodactylus (Lake Sevan, Armenia) and then converted to chitosan (Cs), its deacetylated derivative. Novel Schiff bases (CsSB) were synthesized by interaction of Cs with 4-(2-chloroethyl)benzaldehyde (aldehyde-1) and 4-(2-bromoethyl)benzaldehyde (aldehyde-2), and underwent dehydrohalogenation, under basic conditions (10 °C), to yield respective vinyl derivatives. All newly synthesized compounds were structurally characterized by solubility tests, elemental analysis, infrared spectroscopy (FTIR), thermogravimetry (TGA), proton nuclear magnetic resonance (1H NMR), and X-ray diffraction (XRD).

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1. Introduction

Chitosan (Cs) [β-(1 → 4)-2-amino-2-deoxy-d-glucopyranosyl] is the product of deacetylation of chitin (Cn) [β-(1 → 4)-2-acetamido-2-deoxy-d-glucopyranosyl]. Some products of Cs chemical transformations are biocompatible, biodegradable and non-toxic; they exhibit antioxidant properties and have been widely used in biomedicine (Kumirska, Weinhold, Thöming, & Stepnowski, 2011), environmental protection (Kocak, Sahin, Arslan, & Ucan, 2012), agriculture (Hadrami, Adam, Hadrami, & Daayf, 2010), and textile industry (Klaykruayat, Siralertmukul, & Srikulkit, 2010). Numerous derivatives of Cs were synthesized due to the pending amino and hydroxyl groups peripherally attached to the macromolecule backbone (Mourya, Inamdar, & Tiwari, 2010; Santos, Dockal, & Cavalheiro, 2005; Sajomsang, Tantayanon, Tangpasuthadol, Thatte, & Daly, 2008). The Schiff bases that can be obtained by interaction of the Cs amino groups with aldehydes and ketones are of immediate interest as biomaterials endowed with a set of unique properties (Jiao et al., 2011; Sashiwa & Aiba, 2004). In particular, the newly formed imino groups increase the physiological activity of chitosan Schiff bases (CsSB), so that they can act as antibacterial (Soliman, El-Kousy, Abd-Elgary, & Abou-zeid, 2013), antitumor (Park, Chung, Choi, & Park, 2011), antimicrobial and anticrotrophic (Mohamed & Fekry, 2011) agents. CsSB were also synthesized with aromatic aldehydes as carbonyl components (Jiao et al., 2011; Santos et al., 2005; Sajomsang et al., 2008). The existence of electron-donating and electron-withdrawing substituents at the aromatic ring exerted a noticeable influence on the electrophilicity of the carbonyl group and on the degree of substitution (DS) of CsSB. As a carbonyl compound, to the best of our knowledge, 4-(2-chloroethyl) benzaldehyde (aldehyde-1) and 4-(2-bromoethyl) benzaldehyde (aldehyde-2) have not been used in the CsSB synthesis. Structurally, an attractive feature in aldehydes chosen is the presence of a CH₂CH₂-group that allows for elimination reaction and for introducing an unsaturation in the select positions of the molecular assembly. In particular, pending vinyl groups would allow for the synthesis of new polymeric systems with potentially valuable properties. Herein we report on the synthesis of new CsSB derivatives and their structural characterization by means of TGA, 1H NMR, FTIR, and XRD as well as elemental analysis.
2. Experimental

2.1. Materials and methods

Reagents and solvents were purchased from various vendors and used without further purification. Sodium hydroxide, potassium hydroxide, hydrochloric acid, sulfuric acid, glacial acetic acid, acetone, diethyl ether, methanol, ethanol, benzene, chloroform, dichloromethane, tetrahydrofuran, N,N-dimethylformamide, silver nitrate, and dimethyl sulfoxide were purchased from Reahim (chemically pure), tetrachloromethane and trifluoroacetic acid were delivered by Sigma-Aldrich, and sodium bicarbonate, hexamethylenetetramine, magnesium sulfate, aqueous ammonia, and sodium borohydride were obtained from Reahim (pure for analysis). Deuterium oxide, trifluoroacetic acid-d₄, and dimethyl sulfoxide-d₆ were acquired from Cambrige Isotope Laboratories (Andover, MA). In the solubility tests, the ratio of the product mass (mg) and a volume of solvent (mL) was equal to 1:2. Elemental analyses (C, H, N) were performed on Euro EA 3000 analyzer (Analytik Jena). Melting temperatures were measured using a Boehtius tipe microscope with a heating stage. Attenuated total reflection Fourier-transform infrared spectra (FTIR ATR) were registered by Nicolet 5700 spectrometer (ZnSe prism, Happ-Genzel apodization, correction of ATR distortion, number of scans 32, resolution 4 cm⁻¹). The thermogravimetry (TGA) was carried out in air atmosphere using Derivatograph Q-1500 (Hungary; temperature gradient: room temperature to 700 °C/10 °C/min). The initial weight of each sample was 100 mg. The proton nuclear magnetic resonance (¹H NMR) spectra were measured using Varian Mercury 300VX/300 MHz spectrometer at 303 K. X-ray diffraction (XRD) measurements were conducted using DRON-3 X-ray diffractometer with CuKα radiation (2θ, 10–80°).

2.2. Cn extraction

Armors of crayfish Astacus leptodactylus (Lake Sevan, Armenia) were cleaned of pectins, grinded to a fine powder, and extracted according to the literature protocol (Pertsutsunthiwong, How, Chandkrachang, & Stevens, 2002; “Process 2”). Demineralization was carried out with dilute HCl (3.5% HCl; powder mass to acid volume (w/v) was equal to 1:14) at RT for 2.5 h. Under continuous stirring, the acid solution was added portionwise until the release of carbon dioxide ceased off completely. The product was washed with distilled water until no chloride ions remained in the solution (AgNO₃ test), then dried at 60 °C for 1.5 h. Deproteinization was conducted with aq. NaOH (5%; w/v, 1:15) at 80 °C for 5 h. The product was then washed with distilled water until neutral reaction (pH 7), dried at 65 °C for 12 h, and decolorized in the Soxlet apparatus by refluxing a mixture of benzene and ethyl alcohol (v/v, 1:1) for 44 h. The isolated product was then dried at 65 °C for 22 h to yield pure Cn (28%). Elemental analysis, %: C, 46.33; N, 6.98; H, 6.36; ash, 0.08. The degree of acetylation (DA) of Cn (90.5%) was calculated by using elemental analysis data and an equation reported earlier (Kasaaai, Arul, & Charlet, 2000).

2.3. Cs synthesis

Under nitrogen atmosphere, deacylation of Cn (7 g) was carried out with aq. NaOH (50%; w/v, 1:14) and NaBH₄ (w/w, 10:1) at 117–120 °C for 2.5 h. The product was washed with distilled H₂O until neutral reaction (pH 7), then rinsed with ethyl alcohol (x3), acetone (x3), and dried in vacuum (10⁻¹ torr; RT, 30 h) to yield Cs (5.7 g). Elemental analysis, %: C, 45.39; N, 7.70; H, 6.58; ash, 0.2. DA of synthesized Cs (47.6%) was calculated by using elemental analysis data and an equation reported earlier (Kasaaai et al., 2000).

2.4. Cs purification

Cs (2.6 g) was dissolved in diluted acetic acid (0.19 M; 260 ml H₂O + 3 ml CH₃COOH) by continuously stirring the resulting suspension for 6.5 h. The mixture was then neutralized with NH₃OH (10%; 675 ml, 2.85 M; 1.5 h) and treated according to the literature protocol (Santos et al., 2005). The product was washed with distilled water until neutral reaction (pH 7) and dried in vacuum (10⁻¹ torr; RT, 5.5 h) to yield Cs-pure (2.2 g).

2.5. Synthesis of aromatic aldehydes

4-(2-Chloroethyl)benzaldehyde (aldehyde-1) and 4-(2-bromoethyl)benzaldehyde (aldehyde-2) were synthesized by method described in Pogosyan and Matsoyan (1983).

A solution of 4-(2-chloroethyl) benzyl chloride (18.9 g, 0.1 mol) and hexamethylenetetramine (15.5 g, 0.11 mol) in dry chloroform (150 ml) was heated for 2 h at 70 °C. The reaction mixture was concentrated by 2/3 of the initial volume and diluted with ether (90 ml). The precipitate was filtered off, washed with ether, and refluxed in acetic acid (8 M; 90 ml) for 2 h. Hydrochloric acid was added (10 ml; 10 M) and the reaction mixture was refluxed for another 5 min. The crude mixture was extracted with ether, an organic layer was washed with water, then with aqueous sodium bicarbonate solution, then again with water and dried over MgSO₄. The reaction mixture was fractionated by vacuum distillation (103–105 °C/torr) to afford 4-(2-chloroethyl) benzaldehyde (aldehyde-1) (5.15 g, 31%), a colorless liquid. M.p. 32–33 °C. ¹H NMR (300 MHz, DMSO-δ₆/CCL₄; 1/3, δ, ppm, J, Hz): 3.15 (2H, t, J = 7.0, CH₂), 3.80 (2H, t, J = 7.0, CH₂Cl), 7.43–7.48 (2H, m) and 7.80–7.85 (2H, m, C₆H₄), 9.96 (1Hs, CHO).

4-(2-Bromoethyl)benzaldehyde (aldehyde-2) was synthesized analogously (7.23 g, 49%) by using 4-(2-bromoethyl) benzylchloride as a reagent. A colorless liquid, m.p. 36–37 °C. ¹H NMR (300 MHz, DMSO-δ₆/CCL₄; 1/3, δ, ppm, J, Hz): 3.15 (2H, t, J = 7.0, CH₂), 3.81 (2H, t, J = 7.4, CH₂Br), 7.43–7.48 (2H, m) and 7.80–7.85 (2H, m, C₆H₄), 9.96 (1Hs, CHO).

2.6. Synthesis of chitosan Schiff base (CsSB)

2.6.1. Cs-pure (Section 2.4) in the amount of 0.42 g (for the given DA of Cs of 47.6%, the amount of 2-amino–2-deoxy-2-glucopyranose units in the initial polysaccharide sample was 0.22 g, or 1.36 mmol) was stirred in a solution of H₂O (20 ml) and CH₃COOH (0.15 ml, 0.15 M) at RT for 7 h. A solution of aldehyde-1 (Section 2.5) (0.44 g; 2.61 mmol) in ethanol (5 ml) was added dropwise and the reaction mixture was stirred for additional 12 h. When adding aldehyde solution, the transparent and viscous (homogeneous) solution in the reactor gets cloudy, becomes heterogeneous, and light yellow residual matter gradually precipitates. The precipitate was filtered off, consecutively washed with acetone, ethanol and ether, and dried under reduced pressure (10⁻¹ torr, RT, 4 h) to yield CsSBRTC1 (0.46 g, 9.4%). The same synthesis was carried out at 80 °C by interaction of Cs-pure (Section 2.4) with aldehyde-1 (Section 2.5) to yield CsSBRTC1 (0.45 g, 7.1%).

2.6.2. Cs-pure (0.83 g; Section 2.4; for the given DA of Cs of 47.6%, the amount of 2-amino–2-deoxy-2-glucopyranose units in the initial polysaccharide sample was 0.43 g, or 2.7 mmol) was dissolved in a mixture of H₂O (40 ml) and CH₃COOH (0.3 ml, 0.15 M) and stirred at RT for 7 h. A solution of aldehyde-2 (Section 2.5) (1.06 g, 5.0 mmol) in ethanol (10 ml) was added dropwise, and the reaction mixture was stirred for additional 12 h. When adding aldehyde solution, the transparent and viscous (homogeneous) solution in the reactor gets cloudy, becomes heterogeneous, and tan residual matter gradually precipitates. The precipitate was filtered off, consecutively washed with acetone, ethanol and ether, then dried