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Supporting Information

Rapid dissolution of chitin and chitosan with degree of deacetylation less than 80% in KOH/urea aqueous solution

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1. Experimental Section

1.1 Chemicals and Materials.

Shrimp shells purchased from Golden-Shell Biochemical Co. Ltd. (Zhejiang, China) were used as the raw material for *α*-chitin. Before use, the shrimp shells were treated with NaOH (1 mol/L) and HCl (1 mol/L) at an ambient temperature overnight, a 0.3 wt% $NaClO_2$ aqueous solution (buffered to pH 4.0) at 80 °C for 3 h, and thoroughly rinsed with deionized water. After freeze-drying, purified *α*-chitin was stored in a desiccator until use; its viscosity-average molecular weight (M_η) was determined by viscometry at 25 °C to be 2.03×10^5 g/mol in LiCl/DMAc (5 wt%). According to two abrupt-change conductometric titrations, the degree of deacetylation (*DD*) of the purified *α*-chitin was 5%. D-glucosamine hydrochloride (GlcN·HCl), D_2O (99.9%), and 3-(trimethylsilyl)-propionic-2,2,3,3-d4 acid sodium salts were obtained from Sigma-Aldrich. KOH, NaOH, ethanol, $NaClO_2$, and urea were purchased from Shanghai Chemical Reagent (China).

1.2 Preparation of deacetylated α-chitin and chitosan.

Purified *α*-chitin was dispersed in KOH aqueous solutions (30–60 wt%) at 80 °C for 30–120 min. After deacetylation, the deacetylated *α*-chitin and chitosan were rinsed with deionized water to remove alkaline residues, freeze-dried, and stored in a desiccator until use. The physical properties of deacetylated *α*-chitin and chitosan are listed in Table S1. Depending on their *DD* values, deacetylated *α*-chitin was labeled CT-15 and CT-32, while chitosan was labeled CS-69 and CS-79.

1.3 Dissolution of deacetylated α-chitin and chitosan.

Deacetylated α-chitin was dispersed in a 20 wt% KOH/4 wt% urea aqueous solution, followed by stirring in a cryogenic bath at −30 °C for 30 min for complete dissolution[1]. In contrast, chitosan was dispersed in a 16 wt% KOH/8 wt% urea aqueous solution, followed by stirring at 15 °C for 30 min for complete dissolution[2]. The resultant chitin and chitosan solutions were centrifuged for 5 min at 5000 rpm to eliminate air bubbles, forming clear and viscous solutions. No precipitation of insoluble or gelatinous particles of chitin or chitosan was observed. For comparison, aqueous solutions of LiOH (6.8 wt%), NaOH (11.4 wt%), and KOH (16 wt%) with same molar concentrations were used to study the effects of cations on dissolution.

1.4 Characterization.

Fourier transform infrared (FT-IR) spectra were recorded in the range of 4000–400 cm^{-1} using a Fourier transform infrared spectrometer (Nicolet 5700 FT-IR Spectrometer, Thermo Fisher Scientific); samples were prepared using the KBr-disk method.

The X-ray diffraction (XRD) profiles of deacetylated α-chitin and chitosan were recorded in the reflection mode with a diffractometer (Rigaku SmartLab, Japan) using Cu$K\alpha$ radiation ($\lambda = 1.542$ Å); a scanning rate of 2°/min and step size of 0.01° were used, with a 2θ range of 5–60°. Deacetylated α-chitin and chitosan were ground into fine particles to eliminate the effects of crystalline orientation, and the degree of crystallinity (χ_c) of deacetylated α-chitin and chitosan were determined by the multipeak fitting of their XRD profiles. The apparent crystal size (*ACS*) of deacetylated α-chitin and chitosan were calculated according to the Scherrer equation:

$$\mathrm{ACS} = \frac{\mathrm{K}\lambda}{\beta \cos\theta} \tag{1}$$

, where k is the Scherrer constant (0.89), λ is the wavelength of the CuKa radiation source, θ is the azimuth angle of the lattice plane, and β is the full width at half maximum of the related lattice plane.

In-situ two-dimensional wide-angle X-ray diffraction (2D WAXD) patterns of deacetylated α-chitin and chitosan were recorded using a single-crystal four-circle diffractometer (Bruker Smart APEX II) using CuKα radiation with $\lambda = 1.542$ Å (40 kV, 0.65 mA). The sample was inserted into a quartz capillary with a diameter of 0.7 mm for the *in-situ* analysis, and a solvent was added to the capillary before measurement. The temperature was controlled in the range of −60 to 10 °C for deacetylated α-chitin and 0 to 25 °C for chitosan using cryogenic equipment (Oxford cryostream-700). The distance between the detector and sample was 50 mm, with a 60 s exposure time for each image.

Cross-polarization/magnetic angle spinning (CP/MAS) solid-state ^{13}C NMR spectra of deacetylated α-chitin and chitosan were recorded at an ambient temperature using a Bruker Advance III 600 MHz spectrometer with a spin rate of 5.0 kHz. The repetition and contact times were 2.0 s and 1.0 μs, respectively. Adamantine was used to calibrate the ^{13}C chemical shifts, and a total of 2048 scans were collected for each sample. Prior to measurement, the samples were crushed to fine powders and completely dried at 80 °C in vacuum. The degree of acetylation was determined from the ratio of the integral value of the methyl carbon to that of the anomeric carbon signal.

The ^{1}H and ^{13}C NMR spectra of deacetylated α-chitin and chitosan in KOH/urea/D_2O

were recorded at 10 °C on a Bruker Advance III (500 MHz) instrument. The following NMR operating parameters and conditions were used: 15000 scans, a pulse width of 11.8 μs, and a 2-s relaxation-delay time. Sodium 3-(trimethylsilyl)-propionate-d4 (1%) was used as an internal standard to calibrate the chemical shifts. The deacetylated α-chitin and chitosan concentrations were 6 wt%, whereas the GlcNAc concentration was 10 wt%.

A Hitachi S-4000 microscope was used for the SEM analysis of the chitin and chitosan matrix microstructures. The α-chitin and chitosan matrices were solvent-exchanged to 100% ethanol, frozen in liquid nitrogen, and snapped immediately to create cross-sections, which were subsequently dried to avoid shrinkage by supercritical CO_2 (Samdri-PVT-3D, Tousimis). Thin lamellae of the chitin and chitosan matrices were repeatedly peeled off (parallel to the surface) to expose the inner surface of the samples.

Atomic force microscopy (AFM) (Cypher ES, Asylum Research) was used to analyze the morphology of deacetylated α-chitin and chitosan aqueous solutions; images were recorded in the AC mode. Silicon probes with a spring constant of 40 N/m, a resonance frequency of 300 kHz (RTESP-300, Bruker), and an average tip radius of 8 nm were used.

Transmission electron microscopy (TEM) images were recorded using a JEM-2100 transmission electron microscope (JEOL, Japan). For TEM analysis, a droplet of the deacetylated α-chitin or chitosan solution was deposited on a super-thin carbon-coated copper grid at an ambient temperature for several minutes before being blotted with filter paper, and subsequently rinsed with ethanol. The sample was then dried under a high-purity nitrogen gas flow.

A ZEISS Axioscope A1 microscope (Germany) equipped with a heating/cooling stage (Linkam LTS420, UK) was used for *in-situ* optical microscopy. The dissolution of deacetylated α-chitin and chitosan in the KOH/urea aqueous solution at different temperatures was analyzed using the optical-microscopy accessory software.

The viscosity-average molecular weight (M_η) of deacetylated α-chitin was determined using viscometry at 25 °C in an LiCl/DMAc solution (5 wt%). The M_η was calculated using the Mark-Houwink equation[3]:

$$[\eta] = 0.24 \times M_\eta^{0.69} \tag{2}$$

The weight-average molecular weight (M_w) of chitosan was determined by size exclusion chromatography (SEC) using multi-angle light scattering (MALS) (DAWN HELLEOS-II equipped with a He-Ne laser (λ = 663.4 nm)) and differential refractometry (RI) (Opitilab T-rEX, λ = 658.0 nm) using a 0.1 M NH_4Ac/0.15 M HAc aqueous solution with an eluent flow rate of 0.5 mL/min. The differential refractive-index increment (dn/dc) was 0.198 cm^3/g. [4]

The degree of deacetylation (*DD*) of deacetylated α-chitin and chitosan were measured using two abrupt-change conductometric titrations. Before titration, the sample was mixed in an HCl aqueous solution (0.1 mol/L) for 6 h. The *DD* of deacetylated α-chitin and chitosan were calculated according to the following equation[5]:

$$\mathrm{DD} = \frac{\Delta \mathrm{V} \times \mathrm{c} \times 203}{\mathrm{m} + \Delta \mathrm{V} \times \mathrm{c} \times 42} \times 100\% \tag{3}$$

where, ΔV, c, and m represent the volumes of NaOH aqueous solution consumed between the first and second abrupt-change points, the concentration of NaOH aqueous solution, and the mass of the sample, respectively.

References

(1) Cai, J.; Huang, J.; Zhang, L. Solvent Composition for Dissolving Chitin. CN103059320B, 2013.

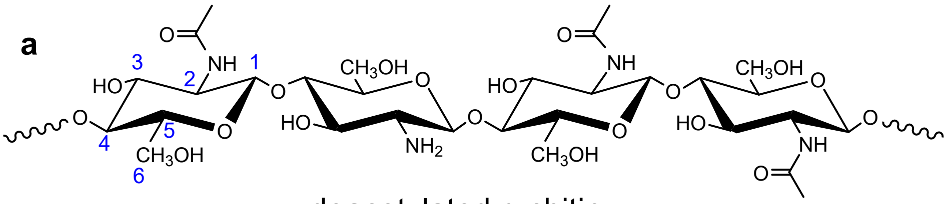
(2) Cai, J.; Zhong, Y.; Huang, J.; Zhang, L. Method for Continuously Preparing Chitin/Chitosan Solutions with Different Deacetylation Degrees. ZL 201711023349.7, **2017**.

(3) Terbojevich, M.; Carraro, C.; Cosani, A.; Marsano, E. Solution Studies of the Chitin-lithium Chloride-N,N-di-methylacetamide System. *Carbohyd. Res.* **1988,** *180* (1), 73-86.

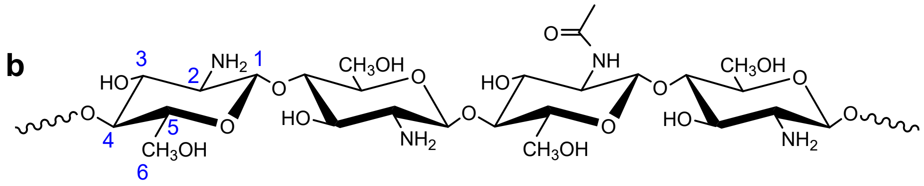
(4) Ghazy, A. R.; Shaheen, M.; Kenawy, E.-R.; El-Mekawy, F. Determination of Chitosan Scattering Parameters by Using Multi Angle Static Laser Light Scattering (MASLLS). *Delta J. Sci.* **2016,** *37* (2), 112-118.

(5) Bai, L.; Kämäräinen, T.; Xiang, W.; Majoinen, J.; Seitsonen, J.; Grande, R.; Huan, S.; Liu, L.; Fan, Y.; Rojas, O. J. Chirality from Cryo-electron Tomograms of Nanocrystals Obtained by Lateral Disassembly and Surface Etching of Never-dried Chitin. *ACS Nano* **2020,** *14* (6), 6921-6930.

2. Additional Figures and Tables



deacetylated *α*-chitin



chitosan

Figure S1. The molecular structure of (a) deacetylated *α*-chitin and (b) chitosan.

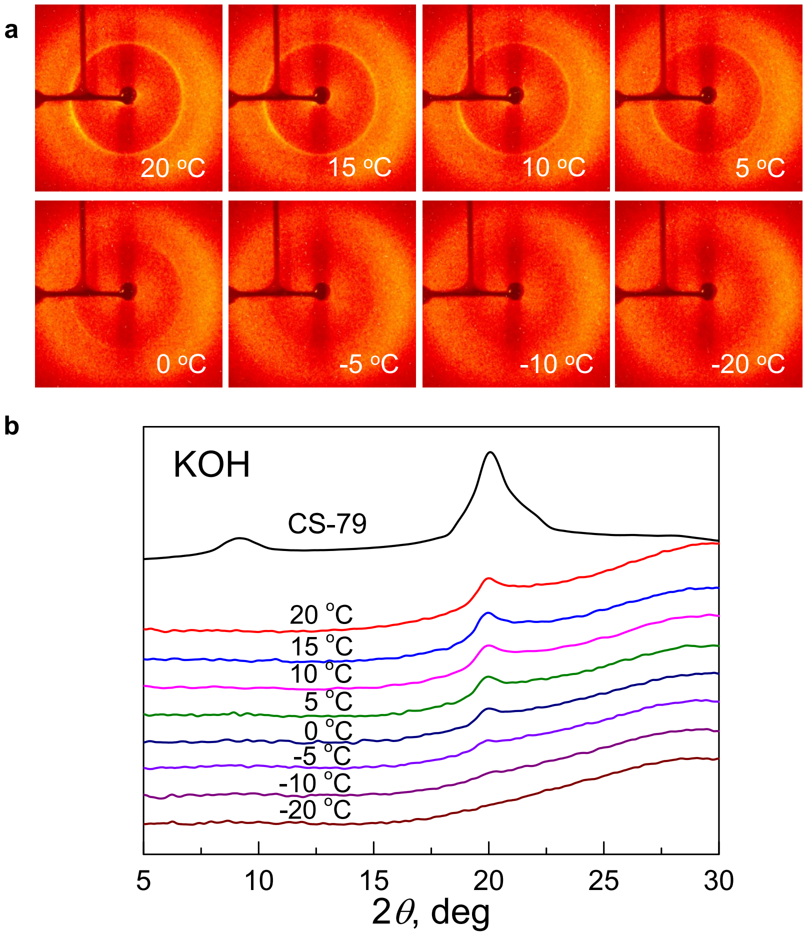


Figure S2. (a) 2D WAXD patterns and (b) Azimuthal-averaging XRD profiles of the chitosan powder CS-79 in a 16 wt% KOH aqueous solution (2.85 M) at different temperatures.

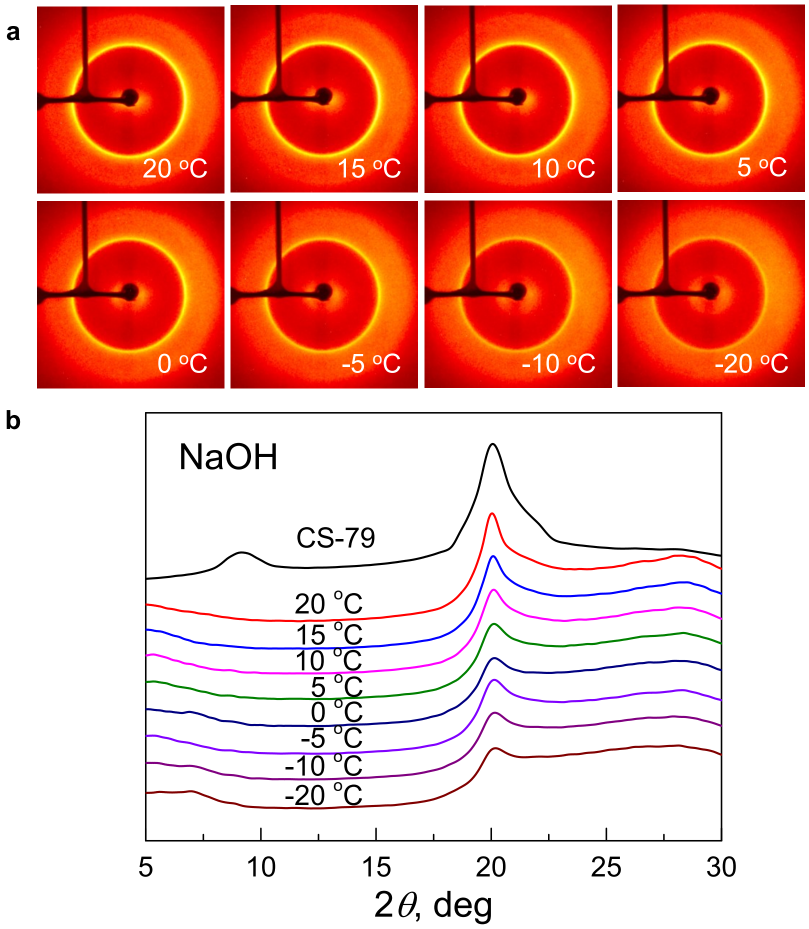


Figure S3. (a) 2D WAXD patterns and (b) Azimuthal-averaging XRD profiles of the chitosan powder CS-79 in an 11.4 wt% NaOH aqueous solution (2.85 M) at different temperatures.

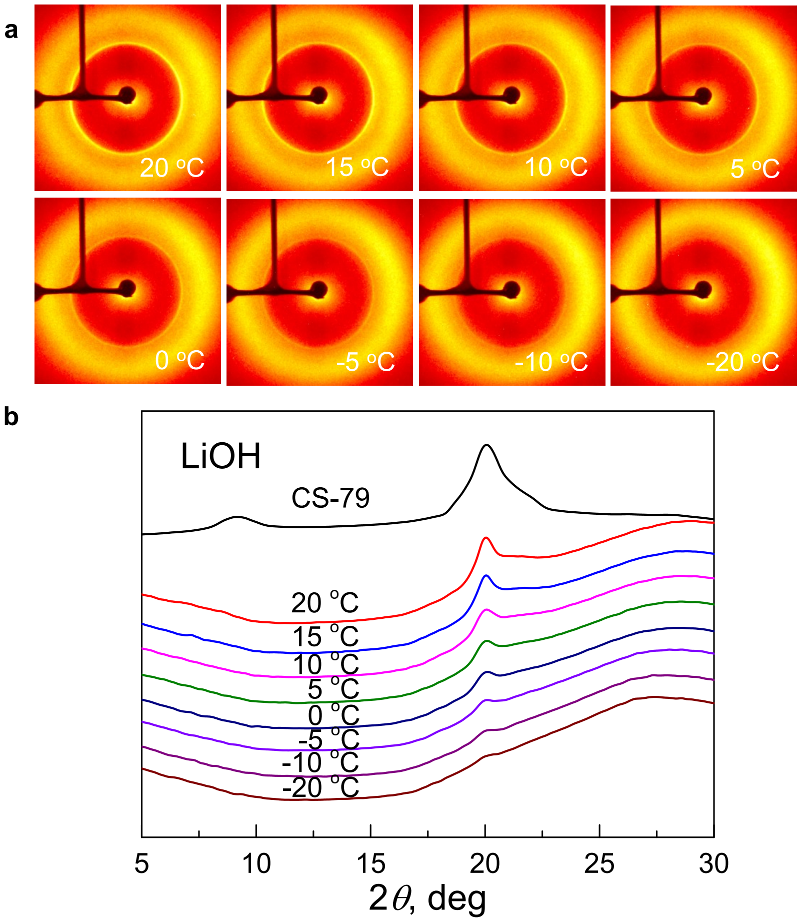


Figure S4. (a) 2D WAXD patterns and (b) Azimuthal-averaging XRD profiles of the chitosan powder CS-79 in a 6.8 wt% LiOH aqueous solution at different temperatures.

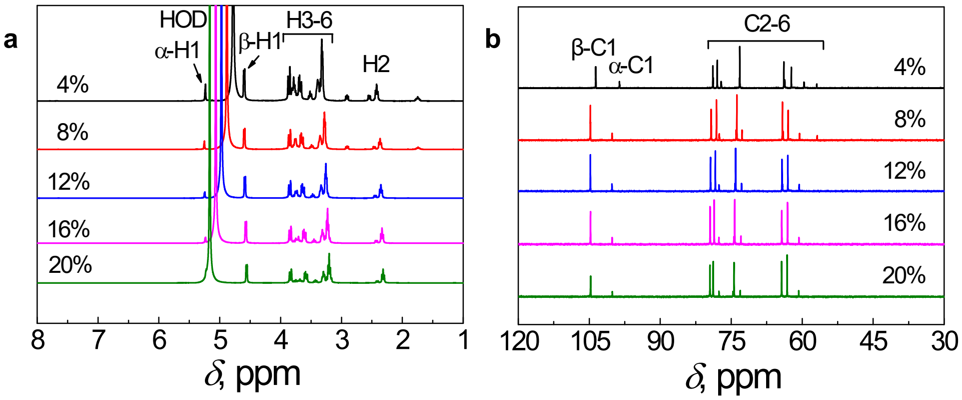


Figure S5. (a) ^{1}H NMR and (b) ^{13}C NMR spectra of GlcN in KOH/D_2O solutions with different KOH concentrations.

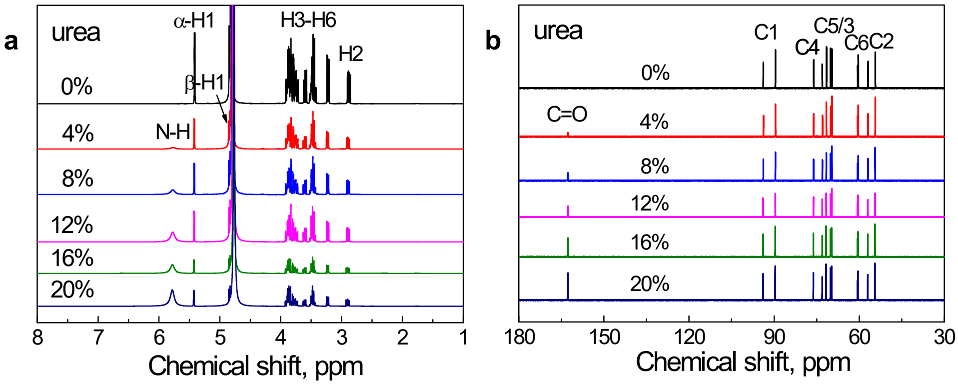


Figure S6. (a) ^{1}H NMR and (b) ^{13}C NMR spectra of GlcN in urea/D_2O solutions with different urea concentrations.

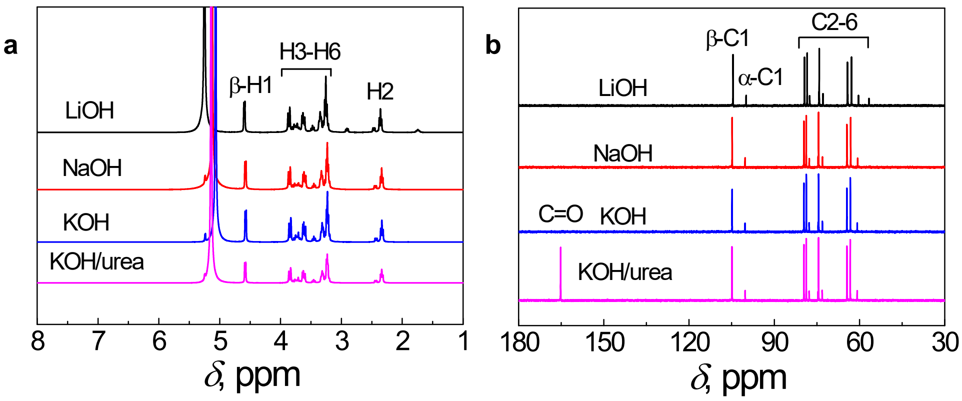


Figure S7. (a) ^{1}H NMR and (b) ^{13}C NMR spectra of GlcN in LiOH/D_2O, NaOH/D_2O, KOH/D_2O, and KOH/urea/D_2O solutions.

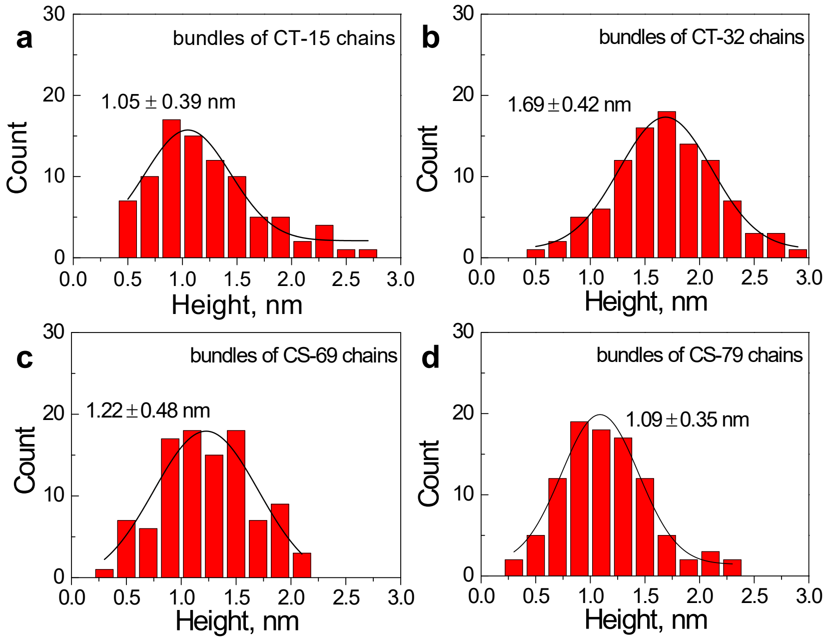


Figure S8. Statistical height histograms of bundles of deacetylated *α*-chitin chains for (a) CT-15 and (b) CT-32, and bundles of chitosan chains for (c) CS-69 and (d) CS-79.

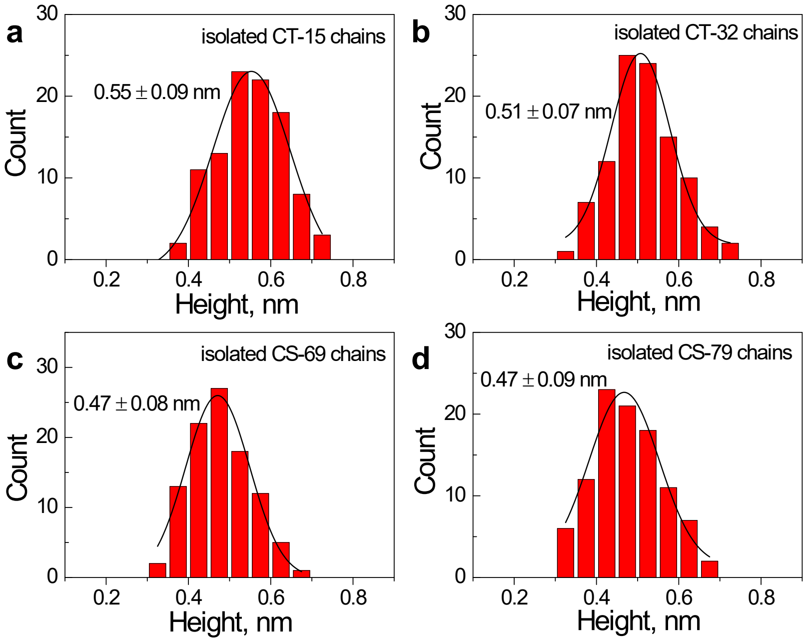


Figure S9. Statistical height histograms of isolated deacetylated *α*-chitin chains for (a) CT-15 and (b) CT-32, and isolated chitosan chains for (c) CS-69 and (d) CS-79.

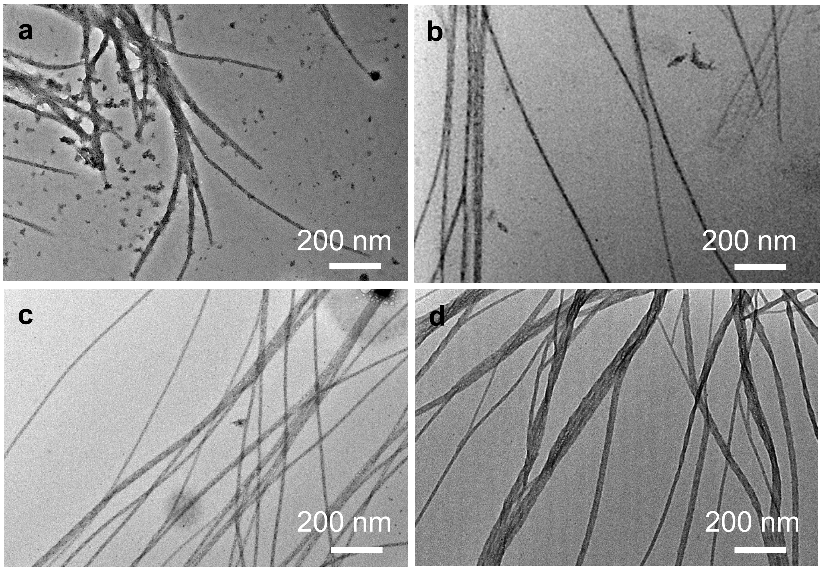


Figure S10. TEM images of self-assembled deacetylated *α*-chitin nanofibrils for (a) CT-15 and (b) CT-32, and chitosan nanofibrils for (c) CS-69 and (d) CS-79.

Table S1. Physical properties of deacetylated *α*-chitin and chitosan.

Sample	χ_c (%)	*DD* (%)		*M*
		NMR	potentiometric titration	($\times 10^5$ g/mol)
CT-15	77	15	17	1.98[a]
CT-32	72	32	29	1.92[a]
CS-69	71	69	67	1.70[b]
CS-79	61	79	78	1.59[b]
RCT-15	66	15	16	1.95[a]
RCT-32	60	32	30	1.90[a]
RCS-69	59	69	68	1.66[b]
RCS-79	52	79	78	1.56[b]

χ_c, degree of crystallinity; *DD*, degree of deacetylation. [a]The viscosity-average molecular weight (M_η) determined by viscometry. [b]The weight-average molecular weight (M_w) determined by SEC-MALS.