Available online at www.sciencedirect.com

SCIENCE DIRECT®

Carbohydrate Research 339 (2004) 2321–2324

Carbohydrate
RESEARCH

Note

Influence of alkali-freezing treatment on the solid state structure of chitin

Fang Feng,* Yu Liu and Keao Hu

State Key Laboratory of Metal Matrix Composite, Shanghai Jiaotong University, Shanghai 200030, PR China

Received 1 March 2004; received in revised form 29 June 2004; accepted 29 June 2004

Abstract—Chitin was soaked in frozen sodium hydroxide for further modification. The solid state structural changes of the resulting chitin powders were studied by X-ray diffraction (XRD) and infrared spectroscopy measurements. During the alkali-freezing process, the crystal space parameters of chitin changed, and the order of the hydrogen bonds of chitin was modified. The study explains how the treatment is beneficial for further modification of chitin. The crystallinity of chitin was assessed according to XRD results. It decreased by half after the alkali-freezing treatment for the first 3 days, and then a few changes happened on the following days while the crystallinity of chitin was kept in the scale of 13.5–18%. That means that the alkali-freezing treatment of chitin for several days is suitable and sufficient.

Keywords: Chitin; Crystallinity; Alkali-freezing treatment; Hydrogen bond

Chitin [(1→4)-linked 2-acetamido-2-deoxy-β-D-glucan] is one of the most abundant biopolymers. It occurs in nature as the fibrous component of the exoskeleton of insects, crustaceans, and invertebrates and also in the cell walls of fungi.[1,2] Chitin and its derivatives are particularly attractive linear aminopolysaccharides, not only as abundant biomass resources, but also as speciality biopolymers for preparing advanced functional materials.[3–6] Chitin is, however, not soluble in common solvents, and this has caused serious difficulties for chemical modification reactions. To fully develop the high potential of chitin, such methodologies are needed: Alkali-freezing treatment of chitin is one of the methods. Frozen alkali chitin has already been chosen as a convenient precursor for efficient modifications.[7–10]

The aim of this study was to understand how the alkali-freezing treatment influences the solid state structure of chitin. The changes in the crystal plane of chitin during the soaking process were monitored using XRD. Infrared spectroscopy measurements showed that hydrogen bonding was modified by the treatment.

There are two polymorphs in chitin: the more abundant is α-chitin, whereas β-chitin is very rare.[11–13] X-ray and infrared spectroscopy studies have established that the two polymorphs have a different packing and arrangement of their molecular chains and have different hydrogen bonding systems. α-Chitin isolated from crab and shrimp shells has been studied extensively owing to its easy accessibility. It has a two-chain unit cell with $P2_12_12_1$ symmetry, indicating an antiparallel arrangement of the chitin chain. β-Chitin has a one-chain unit cell with $P2_1$ symmetry and consists of a parallel chain arrangement. γ-Chitin was identified by Rudall[14] and has not been subjected to the detailed analysis given to the α- and β-forms, and it has not yet been established that this is a true third structure.[13] There are N, H, and O atoms in the molecules, which allows chitin to form a large number of intermolecular and intramolecular hydrogen bonds. These hydrogen bonds cause chitin to have no melting point and not to dissolve in common solvents at normal temperatures, which makes modification difficult. A convenient way to overcome this shortcoming is to soak chitin in an alkaline solution and then freeze it in order to promote its reactivity.

---

* Corresponding author. Tel.: +86-21-62933751; fax: +86-21-62822012; e-mail: fengfanghaha@yahoo.com

0008-6215/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carres.2004.06.017

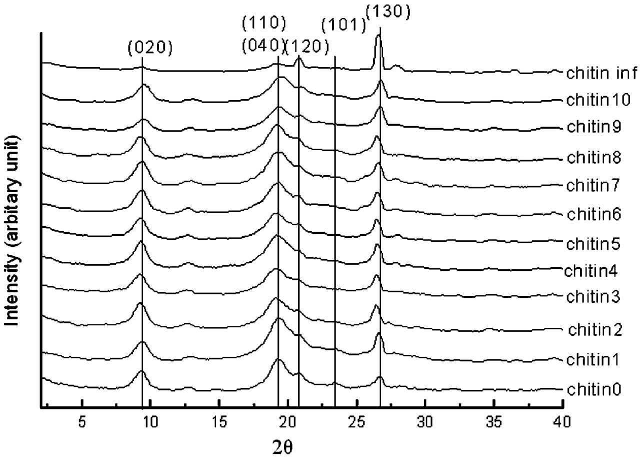

Figure 1. X-ray diffraction profiles of chitin at different alkali-freezing treatment time. Chitin0 is native chitin; chitin1∼chitin10 are samples with 1–10 days treatment; chitininf is a sample with three months treatment.

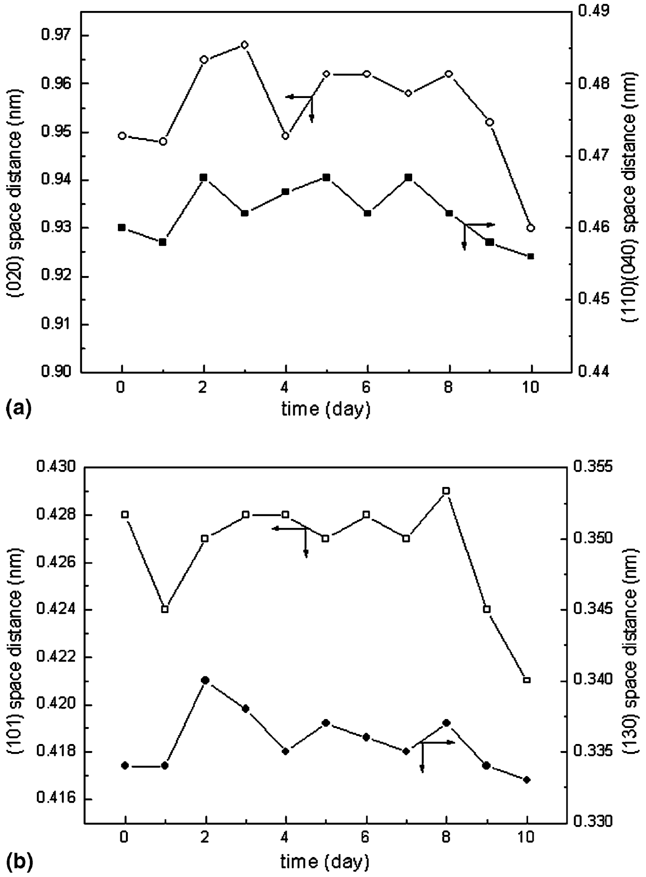

Figure 2. Changes in the *d*-spacing of chitin during the alkali-freezing process: (a) (020) and (110)(040) crystal plane; (b) (101) and (130) crystal plane.

Figure 1 shows the X-ray diffraction profiles of α-chitin submitted to soaking conditions at different treatment times, including the native chitin, samples from 1 to 10 days, and after three months (assumed as ∞). Five crystalline reflections were observed in the 2θ range of 5°–27°. They were indexed as 020, 110, 120, 101 and 130 from the lower angle.[15,16] The 040 reflection was considered being mixed with the 110 reflection because it was close to the 110 reflection, and its intensity was much weaker than those of the 110 and 020 reflections. Each crystalline reflection shifted with increasing treatment time. The intensity and the full width at half-maximum (FWHM) of the reflections were altered during the measurement. By peak fitting of the diffraction profiles in Figure 1, we calculated the accurate *d*-spacing and relative intensity at each specific treatment time, as shown in Table 1 and Figure 2. Each *d*-spacing increased and then diminished while increasing the alkali freezing time. The *d*-spacing change of the (020) plane is the most obvious. Almost on the third or the fourth day, most of the *d*-spacings became the largest. This change in *d*-spacing indicated that there were expansions of the crystal lattices in alkali chitin. Because sodium ions combined with the chitin molecules while NaOH penetrated into the crystal structure, especially the (020) plane, chitin kept the original crystal structure

Table 1. Variation of *d*-spacings of main peaks in X-ray diffraction of chitin during the alkali-freezing process

Chitin sample name	(020)		(110) (040)		(120)		(101)		(130)	
	d (Å)	Relative intensity	*d* (Å)	Relative intensity	*d* (Å)	Relative intensity	*d* (Å)	Relative intensity	*d* (Å)	Relative intensity
0	9.49	68.1	4.60	100	4.28	69.3	3.80	39.9	3.34	63.9
1	9.48	53.2	4.58	100	4.20	65.5			3.34	90.6
2	9.65	83.3	4.67*	100	4.27	69.4			3.40	
3	9.68*	68.2	4.62	90.8	4.28	63.0			3.38*	100
4	9.49	80.7	4.65	100	4.28	57.0			3.35	95.9
5	9.62	74.3	4.67	97	4.27	69.4			3.37	100
6	9.62	75.5	4.62	100	4.28	69.1			3.36	84.0
7	9.58	74.1	4.67		4.27	64.8			3.35	87.0
8	9.62	69.6	4.62	100	4.29*	70.4			3.37	87.8
9	9.52	52.0	4.58	88.7	4.24	60.0			3.34	100
10	9.30	74	4.56	94.6	4.21	62.5	3.78	35	3.33	100
∞	9.58	15.5	4.62	19.2	4.27	41.2			3.35	100

*Stands for the maximum space distance in the crystal plane.

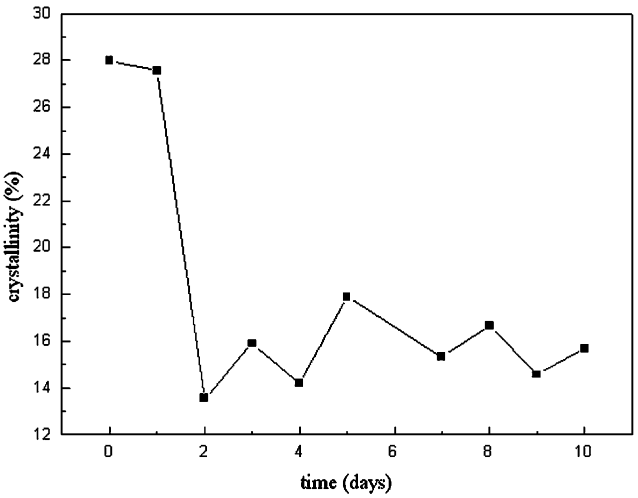

Figure 3. Crystallinity changes of chitin during the alkali-freezing process, as deduced from XRD measurements.

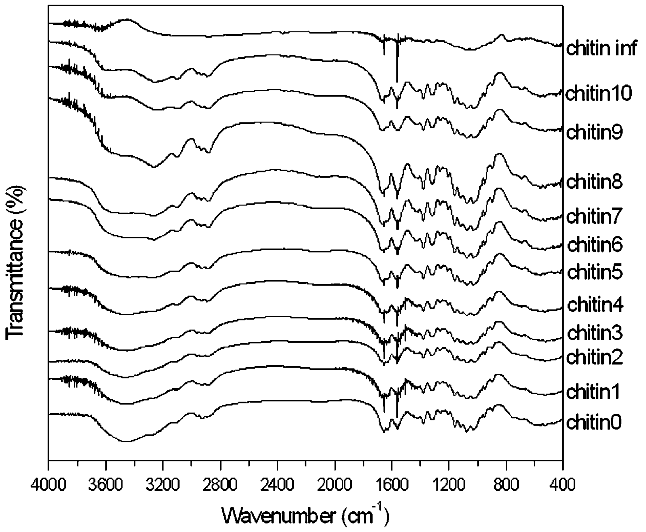

Figure 4. IR spectra of chitin during the alkali-freezing process (see Fig. 1 for legend).

even after being neutralized.[16] The expansion of the crystal plane is useful for small molecules to impregnate and permeate and allows further reaction to take place more thoroughly, which was shown in the hydroxypropyl modification of chitin.[7,17]

The crystallinity change of the chitin samples with alkali-freezing time is shown in Figure 3. During the freezing treatment period, the crystallinity decreased by half in the initial 3 days and then underwent a few changes in the range of 13.5–18%. The crystallinity of alkali chitin for 3 months was 17.92% and then in the previous range. The results show that the alkali-freezing process reduces the crystallinity of chitin but the effect is limited and not further modified by a prolonged treatment.

Infrared spectroscopy is useful to study hydrogen bonding and other interactions, as well as the miscibility. IR spectra of the alkali-frozen chitin samples is shown in Figure 4. The frequencies of the IR absorption bands in the region from 3480 to 800 cm^{-1} were consistent with the assignments for α-chitin.[13] The bands at around 3440 cm^{-1} for the OH and NH stretching vibrations became wider and moved towards lower wavenumbers because of the change in intermolecular and intramolecular hydrogen bonds during the alkali freezing process.[18,19] This implies that the association through hydrogen bonding and the crystal structure of chitin was modified after alkali-freezing treatment.

In summary, the alkali-freezing process of chitin was studied in detail. It was discovered that the distance of each crystal plane of chitin increased and subsequently decreased during the alkali-freezing treatment period. That is the crystal plane of chitin expanded for the first several days, which is useful for small molecules to permeate and make further reaction to take place more thoroughly. Meanwhile, it was discovered that the crystallinity of chitin decreased dramatically in the initial 3 days of the process, but it was kept in the range of 13.5–18% on the following days.

1. Experimental

1.1. Materials

Commercially available chitin from shrimp shells was supplied by Zhejiang Yuhuan Chitin Co. (PRC). The degree of deacetylation was 10%, as determined by IR spectroscopy.[20] All other chemicals were of reagent grade and were used as received.

1.2. Alkali-freezing treatment of chitin

Chitin (20 g) was soaked by stirring in aq 50% NaOH (200 mL) at room temperature. The samples in the sealed container were kept frozen at −18 °C for various times, from 1 to 10 days and 3 months. The samples were melted and were dispersed and neutralized using aqueous HCl for 24 h at room temperature. The precipitated samples were filtered and washed with distilled water and then dried under diminished pressure.

1.3. Measurements

Infrared spectra of treated chitin powder samples were recorded with a Perkin–Elmer infrared spectrometer (Model EQUINOX 55). X-ray diffraction patterns were obtained from a Bruker AXS D8 Advance X-ray generator. Ni-filtered Cu Kα radiation ($\lambda = 0.154$ nm), generated at 40 kV and 400 mA, was collimated with a 1.0 mm ∅ pinhole.

1.4. Data analysis

The degree of crystallinity measured by X-rays is given by

$$W_{c,x} = \frac{I_c}{I_c + K_x I_a}, \tag{1}$$

where I_c and I_a are, respectively, the crystalline and amorphous intensities at certain angles, K_x is the collimator.

The X-ray diffraction profiles were deconvoluted with the Levenberg–Marquardt algorithm of the nonlinear least square fitting method. A fifth-degree polynomial function was used for the background function, and a pseudo-Voigt (pV) function was used to fit the crystalline reflections. The pV function $P(2\theta)_{pV}$ was

$$PV(2\theta) = \eta P(2\theta)_L + (1-\eta)P(2\theta)_G, \tag{2}$$

where η is a parameter varying from 0 to 1 and $P(2\theta)_G$, and $P(2\theta)_L$ are the Gaussian and Lorentzian functions, respectively, represented by the following equations:

$$PV(2\theta)_G = \frac{2}{H}\left(\frac{\ln 2}{\pi}\right)^{1/2} \exp\left[-4\ln 2\left(\frac{2\theta - 2T}{H}\right)^2\right], \tag{3}$$

$$P(2\theta)_L = \frac{2}{\pi H}\left(1 + 4\left(\frac{2\theta - 2T}{H}\right)^2\right)^{-1}, \tag{4}$$

where T is the Bragg angle and H is FWHM. Accurate d-spacings were calculated from the refined parameter B.[15]

References

1. *Chitin*; Muzzarelli, R. A., Ed.; Pergamon: Oxford, 1977; pp 45–51.
2. *Chitin Chemistry*; Roberts, G. A. F., Ed.; Macmillan: London, 1992; pp 21–35.
3. Khor, E.; Lim, L. Y. *Biomate* **2003**, *24*, 2339–2349.
4. Majeti, N. V.; Ravi, K. *React. Funct. Polym.* **2000**, *46*, 1–27.
5. Felse, P. A.; Panda, T. *Bioprocess. Eng.* **1999**, *20*, 505–512.
6. Mattioli, B. M.; Muzzarelli, B.; Muzzarelli, R. A. A. *Carbohydr. Eur.* **1997**, *19*, 30–36.
7. Dong, Y. M.; Wu, Y. S.; Wang, J. W. *Eur. Polym. J.* **2001**, *37*, 1713–1720.
8. Tokura, S.; Tamura, H. *Biomacromolecules* **2001**, *2*, 417–421.
9. Tokura, T.; Nishi, N.; Tsutsumi, A.; Somorin, O. *Polym. J.* **1983**, *15*, 485–489.
10. Hirano, S. Water-Soluble Glycol Chitin and Carboxymethylchitin, In *Methods in Enzymology*; Wood, W. A., Kellogy, S. T., Eds.; Academic: San Diego, 1988; Vol. 161, pp 408–410.
11. Salmon, S.; Hudson, S. M. *J. Mater. Sci.: Rev. Macromol. Chem. Phys.* **1997**, *C37*, 199–276.
12. Tolaimate, A.; Desbrieres, J.; Rhazi, M. *Polymer* **2000**, *41*, 2463–2469.
13. Blakwell, J. Physical Methods for the Determination of Chitin Structure and Conformation. In *Methods in Enzymology*; Wood, W. A., Kellogy, S. T., Eds.; Academic: San Diego, 1988; Vol. 161, pp 435–442.
14. Rudall, K. M. *Adv. Insect. Physiol.* **1963**, *1*, 257–313.
15. Wada, M.; Saito, Y. *J. Polym. Sci., Part B: Polym. Phys.* **2001**, *39*, 168–174.
16. Li, J.; Revol, J. F.; Marchessault, R. H. *J. Appl. Polym. Sci.* **1997**, *65*, 373–380.
17. Kim, S. S.; Kim, S. J.; Moon, Y. D. *Polymer* **1994**, *35*, 3212–3216.
18. Maréchal, Y.; Chanzy, H. *J. Mol. Struct.* **2000**, *523*, 183–196.
19. Ngono, Y.; Maréchal, Y.; Mermilliod, N. *J. Phys. Chem. B* **1999**, *103*, 4979–4985.
20. Sannan, T.; Kurita, K.; Ogura, K.; Iwakaru, T. *Polymer* **1978**, *19*, 458–459.