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The depolymerization mechanism of chitosan by hydrogen peroxide

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Water-soluble chitosan with low molecular weight was prepared by the depolymerization of chitosan with aqueous H_2O_2 solution. The IR and ^{1}H-NMR studies verify that depolymerization leads to the breakage of 1,4-β-D-glucoside bonds of chitosan. X-ray analysis shows the depolymerization takes place at the surface of the chitosan in crystal region by so called peeling-off process while the amorphous portion is depolymerized by penetrating pattern.

1. Introduction

Chitosan, the N-deacetylated chitin, is made up of D-glucosamine units linked by β $(1 \rightarrow 4)$ bonds. It has been shown to be useful as drug carriers, water treatment additives, wound-healing agents and for a number of other important applications [1, 2]. However, the applications of chitosan are limited by its poor solubility and long period degradation. In recent years studies have revealed that the properties of chitosan depended on its molecular weight [3–5]. Chitosan with low molecular weight has some special physiological functions, such as binding lipid [3], affecting the mitogenic response [4] and restraining the growth of tumors [5]. So it is essential to obtain a water-soluble low molecular weight chitosan (LWCS).

LWCS is commonly obtained by enzymatic depolymerization of chitosan with hydrolytic enzymes or by acidic depolymerization with different acids such as hydrochloric acid and sulfuric acid [6–8]. Recently LWCS was also formed by oxidative depolymerization with some oxidative systems [9–12]. The depolymerization patterns of chitosan using O_3 and $NaNO_2$ have been reported [9, 10]. However to date there have been very few reports about the depolymerization mechanism of chitosan by H_2O_2. Tanioka *et al.* [11] and Chang *et al.* [12] respectively reported that Cu(II)-UV-H_2O_2 and H_2O_2-Fe^{2+} systems could decrease the molecular weights of chitosan. They postulated that metal ions induced the decomposition of H_2O_2, which caused the depolymerization, however they did not explain the feasibility of producing LWCS by using hydrogen peroxide. In this paper a peeling-off depolymerization pattern is advanced to explain how the depolymerization of chitosan by hydrogen peroxide occurs.

2. Experimental

2.1. Materials

Chitin, 100 meshes, was purchased from Yuhuan Biochemical Co. (Zhejiang, PRC). The following chemicals were used and were all of reagent grade: sodium hydroxide, hydrogen peroxide, hydrochloric acid and ethanol.

Chitosan, deacetylated from chitin, whose degree of deacetylation is 91% was analyzed by Element Analysis [13] (Perkin-Elmer 2400-II). Its M_v is 1.96×10^7, analyzed by Viscometry Measurement [14].

2.2. Depolymerization of chitosan

Chitosan was completely dissolved in hydrochloric acid solution, then H_2O_2 aqueous solution was added. The solution was stirred and reacted at the desired temperature for different durations. After the reaction, the solution was filtrated. The collected solid was washed with distilled water until reaching pH = 7, and then dried in vaccum. The obtained product was the water-insoluble chitosan. The filtrate was adjusted to pH 7.0 with NaOH solution and a precipitate was obtained by adding ethanol. The water-soluble low molecular weight chitosan (LWCS) was collected after drying the precipitate in vaccum.

2.3. Infrared spectrometry

Samples were ground with i.r.-grade potassium bromide in an agate mortar. Spectra were recorded with a Perkin-Elmer infrared spectrometer Model Equinox 55.

2.4. ^{1}H-NMR

Samples were dissolved in D_2O, and transferred to 5 mm NMR tubes. The measurements were performed

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on a Dpx400 Bruker Avance spectrometer. All chemical shifts were determined relative to internal standard tetramethyl silane (TMS). Typical conditions for the acquisition of ^{1}H-NMR spectra were 400 MHz at 25°C.

2.5. X-ray diffraction

X-ray diffraction measurements were carried out on a *D*/max-3A diffractometer with Cu K_α radiation (λ = 1.54 Å) over the range 5–40°2θ at a scan rate of 2°2θ per minute. The spectra were recorded at 40 kV, 25 mA.

3. Results and discussion

3.1. FT-IR analysis of water-soluble and water-insoluble chitosans

The FT-IR spectra of chitosan, LWCS and water-insoluble chitosan are shown in Fig. 1 (a, b and c), respectively. In Fig. 1a, the characteristic absorptions of the original chitosan at 1597 cm^{-1} (–NH_2), 1648 cm^{-1} (amide I band) and 1555 cm^{-1} (amide II band) but with no absorption at 1480 cm^{-1} (C=O of $COCH_3$), are similar to those of high deacetylation degree chitosan that has been reported in literature [15]. The peaks at 3200–3500 cm^{-1} (O–H, N–H), 1423 cm^{-1} (symmetrical deformation of –CH_3 and –CH_2), 1254 cm^{-1} (twisting vibration of O–H), 1089 cm^{-1} (streching vibration of hydroxyl), 1028 cm^{-1} (streching vibration of the C–O–C in glucose circle), 1153 cm^{-1} and 895 cm^{-1} (the special absorb peaks of β (1 → 4) glucoside bond in chitosan) are all shown in Fig. 1a and b. These data shows the structures of the main chain of chitosan and LWCS are the same.

In comparison with the FT-IR spectrum of chitosan, that of the water-soluble product shows a new peak at 1623 cm^{-1}, which is assigned to the absorbance of C=O. It might be the new side group of LWCS.

In the FT-IR spectrum of water-soluble chitosan (Fig. 1c), the peak at 777.5 cm^{-1} is assigned to the out-of-plane deformation of the N=CHCOO$^-$ group, which is a consequence of the N=C double bond [16].

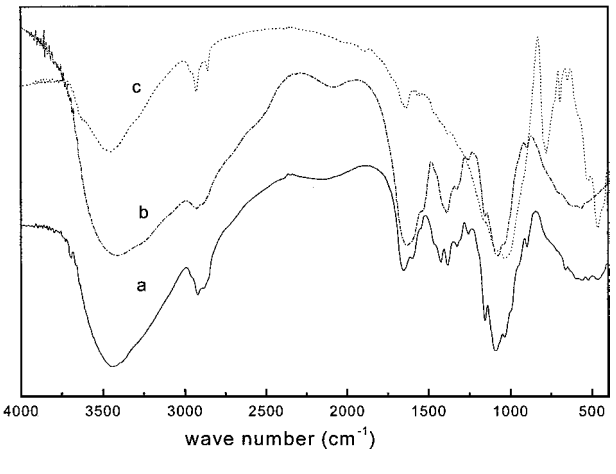


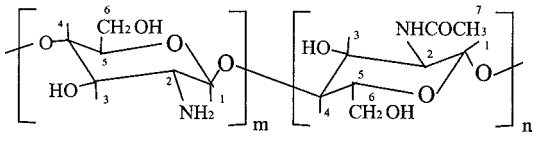
Figure 1 IR spectra of (a) chitosan, (b) LWCS, and (c) water-insoluble chitosan.

TABLE I ^{1}H-NMR chemical shifts (ppm) for chitosan and water-soluble chitosan in D_2O solution

	H-1	H-2	H-3	H-4	H-5	H-6	H-7
Chitosan	4.87	3.18	3.78	3.83	3.74	3.87	2.07
Water-soluble chitosan	4.71	3.00	3.46	3.65	3.39	3.76	1.94

3.2. ^{1}H-NMR spectra analysis

The ^{1}H-NMR chemical shifts for chitosan and LWCS are listed in Table I. While chitosan shows a singlet at 3.8 ppm (H2) and multiplets at 3.7–3.9 ppm (H3, H4, H5, H6) corresponding to the ring methane protons together with a singlet at 2.0 ppm (H7), which is due to the N-acetyl glucosamine units having survived the saponification chitin, and a small signal at 4.8 ppm (H1). In the ^{1}H-NMR spectrum of the LWCS, the shift of each ^{1}H of LWCS corresponds to the shift of chitosan, so no significant changes take place in characteristic shifts of LWCS compared with the original chitosan. In another word the structure of LWCS remains the same as that of the chitosan.



3.3. Discussion of the depolymerization mechanism

The studies of Tanioka [11] and Chang [12] suggest that the depolymerization was predominantly caused by radical reactions. The metal ions induced the depolymerization of H_2O_2, which caused chitosan degradation. Thus in the study of Chang [12], the content of Fe in the chitosan was a trace (14 ppm), however even when free radical scavengers and metal chelator were added, the depolymerization still took place. Tanioka [11] suggested chitosan was depolymerized by hydroxy radical generated through Cu (II)- ascorbate- H_2O_2 in ultraviolet at room temperature. Nevertheless they did not explain how HO$^\bullet$ generates. Most probably the principle reason is not the presence of extraneous matter, but rather in the nature of H_2O_2. In fact there are many ways of the formation of HO$^\bullet$ decomposed by H_2O_2. Fang [17] suggested that in the degradation of hemicellulose by H_2O_2, the highly reactive HO$^\bullet$ was formed in the base. But they did not explain the feasibility of producing LWCS by using H_2O_2 and how the depolymerization occurs.

In the depolymerization system of chitosan with H_2O_2, the balances exist as shown in Equations 1 and 2, and the total reaction is shown in Equation 3.

$$\text{R-NH}_2 + \text{H}^+ = \text{R-NH}_3^+ \quad (1)$$

$$\text{H}_2\text{O}_2 = \text{H}^- + \text{HOO}^- \quad (2)$$

$$\text{H}_2\text{O}_2 + \text{R-NH}_2 + \text{H}^+ = \text{R-NH}_3^+ + \text{HOO}^- + \text{H}^+ \quad (3)$$

The hydroperoxide anion is very instable and easily decomposed to high reactive hydroxyl radical

($HO^{\bullet}$) [17],

$$HOO^- \rightarrow OH^- + O^{\bullet} \quad (4)$$

$$H_2O_2 + HOO^- \rightarrow HO^{\bullet} + O_{2^-}^{\bullet} + H_2O \quad (5)$$

The hydroxyl radical is a very powerful oxidant. The main chemical action of $HO^{\bullet}$ with polysaccharide has been demonstrated to be hydrogen abstraction [18]. It reacts with carbohydrates very quickly and the reaction is shown in Equations 6 and 7. $HO^{\bullet}$ pulls off a hydrogen atom and combines with it to form water.

$$(GlcN)_m - (GlcN)_n + HO^{\bullet} \rightarrow (GlcN^{\bullet})_m - (GlcN)_n + H_2O \quad (6)$$

$$(GlcN^{\bullet})_m - (GlcN)_n + H_2O \rightarrow (GlcN)_m + (GlcN)_n \quad (7)$$

During the treatment, the R-NH_2 preferentially reacts with H^+ to produce R-NH_3^+, which causes the decrease of $[H^+]$ and the increase of pH. In addition, HOO^- is rapidly decomposed to $HO^{\bullet}$, which means that H_2O_2 is continually decomposed as shown in Equation 3. These radicals undergo further reactions rapidly to form water-soluble oxidation products with low molecular weight.

In this work, when chitosan was depolymerized by 10% H_2O_2 at 60°C for 4 h the M_v of LWCS was 1.1×10^4, analysized by Viscometry Measurements. The reduction of M_v indicates the decrease of chitosan polysaccharide chain. However, the FT-IR spectra and ^{1}H-NMR spectra of the LWCS and the original chitosan shows no distinct difference, which indicates that no obvious change takes place in the structures of chitosan chain during the depolymerization. The results obtained verify the rupture of 1,4-β-D-glucoside bonds in the macromolecule to be the basic process during amino groups of chitosan protection by acids.

In dilute aqueous HCl solutions, chitosan is soluble. The depolymerization is a homogenous reaction. The hydrogen bonds of intermolecules and intramolecules in chitosan are broken and the molecules are extended, which cause the reactions between all the functional groups of chitosan and H_2O_2. Therefore, a random reaction with the glucosamine units is likely to have occurred when all amine groups are assumed to be equally active. The reaction was affected not only by the concentration of H^+ according to Equations 1–3, but also by the steric shield of the amino groups in the polyanion chitosan. In acid systems, most $-NH_2$ groups of the chitosan molecules can bond with H^+ to form R-NH_3^+ which is electron-absent. When $[H^+]$ is low, the degree of NH_2 protonation is also low. The repellency affected by protonated amine groups with low concentration makes the polyanion chitosan stretched, which causes the decrease of steric shield of NH_2, so the reaction readily occurred. With the increase of $[H^+]$, the degree of NH_2 protonation increases and the reverse electric field is formed around the polycation chitosan chains. The electric field weakens the repelling effect between the protonated amine groups in the macromolecules and decreases the stretch of the molecular chain. Therefore the curled molecular chains increase the shield of the NH_2, which makes the depolymerization be difficult. Thus, the pH value of the solution should be selected in a proper range.

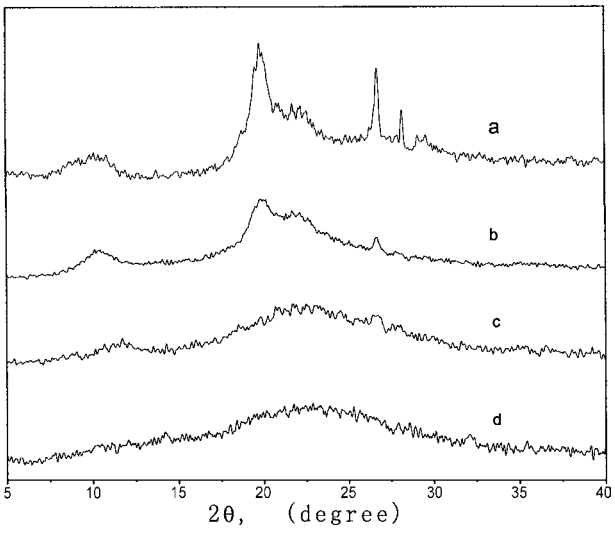


Figure 2 X-ray diffraction patterns of (a) initial chitosan, (b) LWCS-A (reacted at 60°C for 1 h), (c) LWCS-B (reacted at 60°C for 2 h), (c) LWCS-C (reacted at 60°C for 3 h).

Fig. 2 shows the X-ray scatting profiles of the LWCS-A, LWCS-B and LWCS-C samples as compared with that of chitosan.

Chitosan has two distinct crystal forms I and II [19]. The form I crystal is orthorhombic with a unit cell of $a = 7.76$ Å, $b = 10.91$ Å, and $c = 10.30$ Å. The strongest reflection appears at $2\theta = 11.4°$, which is assigned to (100) reflection. The form II crystal is also orthorhombic with a unit cell of $a = 4.4$ Å, $b = 10.0$ Å, and $c = 10.3$ Å (fiber axis). The strongest reflection appears at $2\theta = 20.1°$, which also corresponds to the (100) reflection. As shown in Fig. 2a, the original chitosan shows the strongest reflection at $2\theta = 19.84°$, which is consistent with the pattern of the form II crystal.

LWCS-A was the water soluble product of chitosan depolymerized with H_2O_2 solution at 60°C for 1 h. As shown in Fig. 2a and b, the peak intensities of LWCS-A were significantly lower than those of chitosan at $2\theta = 10.38°$, $2\theta = 19.84°$ and $2\theta = 26.72°$. When the reaction time reached 2 h, the water-soluble depolymerized product is LWCS-B, whose X-ray scattering profile is shown in Fig. 2c. The characteristic reflection of LWCS-B at $2\theta = 10.38°$, $2\theta = 19.84°$ and $2\theta = 26.72°$ decreased further and the peaks are broaden. Therefore, Fig. 2b and c show LWCS-B, LWCS-C still include a crystal portion. During the depolymerization, the change in peaks was caused by the amorphous part of chitosan being preferentially degraded while the crystal part was temporally maintained. The main chains of chitosan are not all coplanar and have difficulty in rotating relative to each other, so the main chains are constrained under hydrogen bonds in chitosan structures. The disordered packing of the side chains having rotational freedom prevents the occurrence of well-defined reflections for main chains. Consequently, in the beginning of the depolymerization some crystal part was temporally maintained.

With prolonged reaction time the chitosan was further depolymerized to amorphous LWCS; this is shown in Fig. 2d with only a decreased wide peak at $2\theta = 22.32^{\circ}$. Thus, it was assumed in the crystal portion, the reaction occurs at the surface of the chitosan powder. As soon as the outer layer is sufficiently depolymerized to become soluble in the reaction medium, it peels off. The depolymerization of the chitosan can proceed in the same manner. This so called peeling process probably applies mainly to the crystalline regions. The amorphous portion presumably is penetrated quite rapidly by the reaction medium, and becomes soluble without difficulty. Consequently, if the extent of the amorphous regions could be increased, the depolymerization rate of chitosan would become faster.

4. Conclusions

Water-soluble chitosan with low molecular weight (LWCS) has been prepared by the depolymerization of chitosan with H_2O_2. The depolymerization only leads to the changes of side groups while no distinct change takes place in the structures of main chain. This is supported by IR and ^{1}H-NMR studies. The results obtained verify the rupture of 1,4-β-D-glucoside bonds in chitosan to be the basic process during amino groups of chitosan protection by acids. In the X-ray work, we advance a peeling-off depolymerization pattern to explain how the depolymerization of chitosan by H_2O_2 occurs. It is proposed that in the crystal regions of chitosan, the depolymerization occurred by a peeling-off process while in the amorphous regions, it is occurred by penetrate. The depolymerization rate of chitosan would become faster if the extent of amorphous regions increased.

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