Synthesis, Characterization and Biospecific Degradation Behavior of Sulfated Chitin

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Summary: Chitin is a polysaccharide found in the outer skeleton of insects, crabs, shrimps, and lobsters and in the internal structures of other invertebrates. Sulfated chitin was prepared by reacting carboxymethyl chitin (CM-chitin) with 2-aminoethane sulfonic acid by using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) catalyst. The prepared sulfated chitin was characterized by FTIR, elemental analysis, thermogravimetric analysis (TGA) and X-ray diffraction (XRD). The degree of substitution was found to be 0.98 by elemental analysis. The TGA studies showed that sulfated chitin was less thermal stability than carboxymethyl chitin. This is due to the grafting reaction. The sulfated chitin membranes were prepared from sulfated chitin and then crosslink with glutaradehyde. The biodegradation process was performed in PBS (pH 7.4) containing lysozyme (10 μg/ml) at 37 °C in an incubator. Experimental results from weight loss throughout the study showed that the biospecific degradation occur on the membrane by lysozyme.

Keywords: biomaterials; biospecific degradation; sulfated chitin; thermal properties

Introduction

Chitin, a naturally abundant polysaccharide, and the supporting material of invertebrates such as crustaceans, insects, consist of a homopolymer of (1→4) linked 2-acetamido-2-deoxy-β-D-glucopyranose residues. Chitin and chitosan are recommended as suitable functional materials, because these natural polymers have excellent properties such as biocompatibility, biodegradability, non-toxicity and adsorption properties. Chitin and chitosan derivatives have been used in various fields such as treating water, biomedical, cosmetic and agricultural or food industrial. It shows some biological activities such as immunological, antibacterial, wound healing activity, drug delivery and has been proposed for tissue engineering applications.

Chemical modification of chitin and chitosan derivatives to generate new biofunctional materials is of primary interest because such procedure would not change the fundamental skeleton of chitin and chitosan would keep the original physicochemical and biochemical properties depending on the nature of the group introduced.[1–4] The site specifies chemical modification of the amino and hydroxyl groups in chitin and chitosan with sulfate can generate products for pharmaceutical applications, because the structure of sulfated chitin served as nearest structural analogues of the natural blood anticoagulant heparin, demonstrate biomolecular mechanism of anticoagulant activity, antisclerotic and antiviral activities.[5–8] Making biodegradable constructs including sulfated chitin for drug delivery and tissue engineering material, proper control of its degradation in hard tissue is of critical importance

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for osteogenesis. In growth of fibrous and connective tissue into bone defects is induced by the rapid degradation of implant, while residual remains in tissue for a long period of time and subsequent fibrous capsule formation around material causes delay of bone remodeling. Therefore, the method of control of degradation properties of medical implants made of biodegradable polymer is a key to therapeutic success. Crosslinking of molecules using chemical agents has been widely studied to increase or enhance stability against biological degradation and mechanical properties. However, residual activity of reagents frequently results in toxic damage to tissues such as excessive inflammation. In this study, in order to use sulfated chitin as drug delivery or tissue engineering material; we systematically investigated the biodegradability behavior of sulfated chitin membranes. In this paper we described about the synthesis, characterization and *in vitro* biospecific degradation studies of sulfated chitin.

Experimental Part

Materials

CM-chitin (M_W-199,250) was received from Koyo Chemicals Ltd. EDC, glutaraldehyde, lysozyme and 2-aminoethyl sulfonic acid were received from Wako Pure Chemicals. All other materials used were of analytical grade.

Synthesis of Sulfated Chitin

Sulafated chitin was prepared by the following method. In briefly, CM-chitin (1 mole) was grafted with 2-aminoethyl sulfonic acid (1 mole) by using EDC catalyst (1 mole) in the aqueous medium at room temperature for 24 h. After 24 h the reaction mixture was precipitated in excess of acetone and washed several times with methanol. The sulfated chitin was insoluble in water. So, that sulfated chitin was treated with 1M NaOH solution. The synthesis method of sulfated chitin was shown in Figure 1.

Synthesis of Sulfated Chitin Membranes

The sulfated chitin membranes were prepared by using 2 g of Na salt of sulfated chitin was dissolved in 100 ml of water and then poured into Petri dish and dried at 37 °C for 3 days. After 3 days the membranes were crosslinked with glutaraldehyde. The crosslinked membranes were treated with $NaBH_4$ solution to remove the excess of glutaraldehyde.

In Vitro Degradation Studies

The sulfated chitin membranes of known weight were immersed in sterilized phosphate-buffered saline (PBS) or 10 μg/ml of egg-white lysozyme (Merck, Darmstadt, Germany) solution in 25 ml of PBS solution and kept in incubator at 37 °C for 14, 21, 28 & 35 days. After determined intervals of time, specimens were taken from each solution. The membranes were washed with water and then dried and weighed. The extent of degradation was expressed as the percentage of the dry-weight of the remainder after immersion against initial weight, and data are expressed as the mean ± standard deviation ($n = 3$).

Measurements

The IR spectra of the polymers were recorded in a Perkin Elmer FT-IR 2000 series spectrophotometer at room tempera-

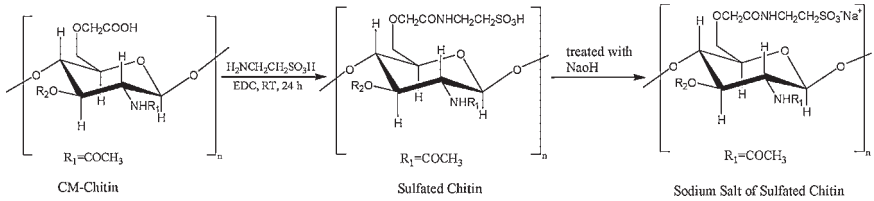

Figure 1.
Synthesis of sulfated chitin.

ture with the KBr pellet method. TGA was performed with a SII TG-DTA 6200 thermal analyzer using 2 mg of the sample at a heating rate of 10 °C/min in nitrogen. X-ray diffractograms were recorded according to a powder method with a Mac Science M_3X (model no. 1030) diffractometer using CuKα radiation. A Perkin-Elmer 2400 carbon-hydrogen analyzer was used for elemental analysis.

Results and Discussion

Synthesis of Sulfated Chitin

The sulfated chitin was prepared by graft copolymerization method under mild conditions. From this method the polymer degradation is less comparing to the other methods. From the elemental analysis the degree of substitution was found to be 0.99. Anal. Cal. For $[C_{12}H_{19}O_6N_2\ (SO_3H)\ H_2O]_n$: Calculated (%). C-37.02; H-5.14; N-7.20; S-9.00. Found (%): C-36.85; H-6.53; N-6.98; S-9.05. The prepared sulfated chitin was insoluble in water. It may be due the high degree substitution of sulfonic acid group. But the sodium salt of sulfated chitin was soluble in water.

FT-IR Spectra

The FT-IR spectra of CM-chitin and sulfated chitin were shown in Figure 2. The peak at 3439 cm^{-1} is due to –NH stretching. The formation of amide stretching was shown at 1667 & 1591 cm^{-1}. The peak at 1202 cm^{-1} is due to $–SO_2$ stretching. The FTIR studies confirmed the formation of sulfated chitin.

TGA Studies

The TGA studies of CM-chitin and sulfated chitin was shown in Figure 3. In the thermogram of CM-chitin two decomposition steps could be observed, the first occurs in the range of 50–100 °C, and is attributed to water evaporation. The second occurs in the range of 240–300 °C and could be attributed to the degradation of the saccharide structure of the molecule, including the dehydration of saccharide rings and the polymerization and decomposition of the acetylated and deacetylated units of chitin. The sulfated chitin showed the second decomposition step occurred at a lower temperature than CM-chitin decomposition. So, the sulfated chitin was less thermal stability than CM-chitin. This is due to the grafting reaction.

XRD Studies

CM-chitin exhibits a sharp diffraction peaks at 20 2θ values. It indicates that CM-chitin have a crystalline structure. In the case of sulfated chitin, we cannot found any sharp diffraction peaks. This is due to the grafting reaction. Introduction of substituents into polysaccharide structure

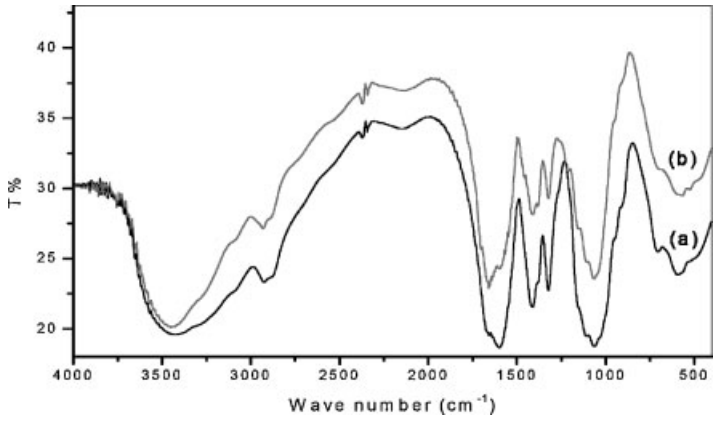

Figure 2.
FT-IR Spectra of (a) CM-Chitin and (b) Sulfated chitin.

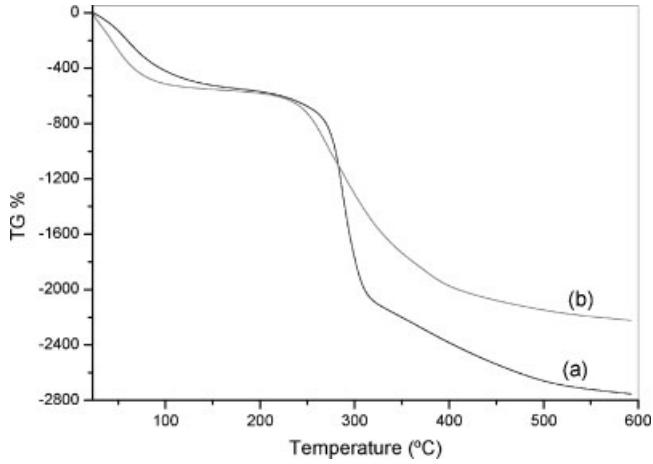

Figure 3.
TGA of (a) CM-chitin & (b) Sulfated chitin.

should disrupt the crystalline structure of the polymer, especially by the loss of hydrogen bonding.[3]

In vitro Degradation Studies

The *in vitro* degradation behavior of sulfated chitin membrane was studied by degradation with lysozyme, which is naturally present in body fluid. The weight loss of sulfated chitin after lysozyme degradation is shown in Figure 4. It was observed that about 13% weight loss of membrane was lost after 14 days incubation in PBS. At the same time, 20% weight loss was observed in the lysozyme degraded membranes. After 14 days incubation, the membranes in PBS were stable up to 35 days incubation. Therefore it can be proved that about 13% of starting materials was out of the cross linked groups. The rate of degradation of membrane by lysozyme was faster up to 35 days. The degradation was increased from 20% to 30%. Weight loss about 30% was detected on the membrane at 35 days.

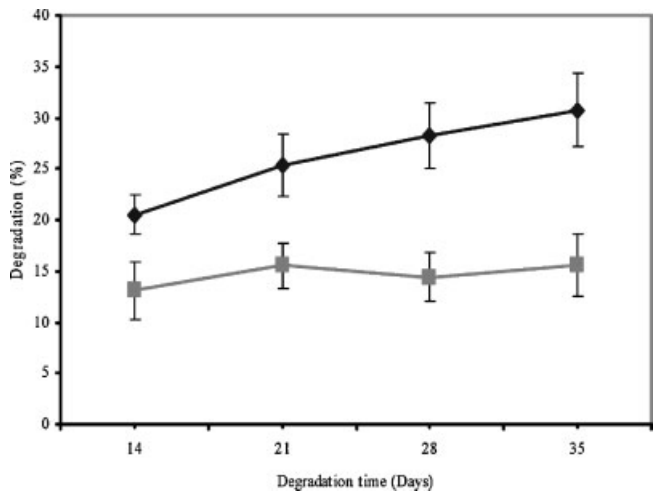

Figure 4.
Percent degradation of sulfated chitin membrane by lysozyme (◆) and without lysozyme (■). Each point represents the mean value of three replicates.

After 35 days, the membranes were broken into small species and they are not able to continuous the study. But the membranes in PBS were not broken. According to these results, it can be concluded that sulfated chitin membranes can be easily degraded by lysozyme.

Conclusions

The sulfated chitin was prepared by grafting method under mild conditions. The sulfated chitin was characterized by FT-IR, elemental analysis, TGA and XRD studies. And also, the biospecific degradation behaviour of sulfated chitin membranes by using lysozyme was studied. The degradation studies showed that the sulfated chitin was degraded by lysozyme. The sulfated chitin showed some biospecific degradation, it is interesting implications in tissue engineering and drug delivery.

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[1] R. A. A. Muzzarelli, *Natural Chelating Polymers*, Pergaman, New York **1973**, pp. 83.
[2] R. Jayakumar, M. Prabaharan, R. L. Reis, J. F. Mano, *Carbohydr. Polym.* **2005**, *62*, 142.
[3] R. Jayakumar, R. L. Reis, J. F. Mano, *E-Polymers* **2006**, 35.
[4] J. Berger, M. Reist, J. M. Mayer, O. Felt, N. A. Peppas, R. Gurney, *Eur. J. Pharam. Biopharam.* **2004**, *57*, 19.
[5] D. K. Kweon, S. B. Song, Y. Y. Park, *Biomaterials* **2003**, *24*, 1595.
[6] R. Jayakumar, N. Nwe, S. Tokura, H. Tamura, *Int. J. Biol. Macromol.* **2007**, *40*, 175.
[7] S. Nishimura, H. Kai, K. Shinada, T. Yoshida, S. Tokura, K. Kurita, *Carbohydr. Res.* **1998**, *306*, 427.
[8] P. Vongchan, W. Sajomsang, D. Subyen, P. Kongtawelert, *Carbohydr. Res.* **2002**, *337*, 1233.

