

MOLECULAR ASPECTS OF HYDRATED CHITIN

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Abstract

As chitin is known to be poorly reactive due to rigid crystalline structure arranged by binary hydrogen bonds, the destruction of the crystalline structure has been attempted through the dissolution or granulation of chitin molecule. α -Chitin, the tightly assembled chitin molecules with rigid crystalline structure, and β -chitin, loosely packed chitin molecules were dissolved in calcium chloride dehydrate saturated methanol. (Ca solvent). The crystalline structure of non-woven fabrics were analyzed to contain α -type crystalline structure. On the other hand, β -chitin was found to be converted to hydrated chitin granule by vigorous mechanical stirring in water followed by the preparation of β -type chitin non-woven fabrics. But α -chitin doesn't give any smooth granule with mechanical agitation probably due to tight crystalline structure. The hydration mechanism was suggested to be quite different, because the chitin hydrogel prepared by dissolution procedure aggregated by freezing process and the β -chitin granule remained unchanged. Both chitin non-woven fabrics displayed a typical cationic behavior almost similar level of chitosan films on the adsorption of ionic dyes, although the non-woven fabrics of β -chitin were slightly poorer than those of α -chitin fabri A remarkable susceptibility of chitin hydrogel was shown by chitinase, but not by lysozyme and chitosanase among chitinolytic enzymes probably due to different hydration behavior. The reactivity enhancement was also shown by chitin hydrogel on the several chemical modifications such as preparations of alkali-chitin and phosphoryl chitin.

Keywords: Chitin, Calcium chloride, Solubility, Hydrogel, Fabrication

Introduction

Although chitin, a natural mucopolysaccharide from *Crustaceans*, is known to show various functions especially in the biomedical field (Tokura & Azuma, 1992; Brine *et al.*, 1993), it has a drawback which is the extreme difficulty to regenerate the chitin particle because of its rigid crystalline structure consisted of hydrogen bonds establishing between amide bond and hydroxyl groups in contrast to cellulose having a close chemical structure (Muzzarelli, 1977). Several solvent systems such as dinitrogen tetroxide-*N,N*-dimethylformamide (DMF) (Allan *et al.*, 1971), lithium chloride-*N,N*-dimethylacetamide (DMAc) (Austin, 1988), formic acid (Foster & Webber, 1960), hexafluoroisopropanol-hexafluoroacetone (Capozza, 1975), etc., have been demonstrated to regenerate chitin. However, they require fairly drastic condition to dissolve chitin and it is hard to maintain chitin molecule without depolymerization during dissolution process. If a milder solvent system is available to dissolve chitin, we can expect that the physicochemical measurements will be easier even at high temperatures. The calcium chloride-saturated methanol has been reported to be a good solvent system to dissolve nylons (Schupp, 1944). The dissolution mechanism of nylon is considered to imply the destruction of hydrogen bonds by the chelation of calcium ion to amide bonds.

In this paper, the preparation of chitin solution has been achieved successfully through the destruction of rigid crystalline structure of chitin molecule without any drastic conditions by the application of calcium chloride-methanol (Ca-methanol) system. Using the solvent, intrinsic viscosity of reacetylated chitosans of various molecular weights were measured to establish the viscosity equation for the estimation of molecular weight of chitin directly together with a brief analysis for the dissolution mechanism.

Materials and methods

Chitin powder

α -Chitin was prepared from Queen Crab shells according to the method of Hackman (Hackman, 1954) and the resulting chitin flake was powdered to 60 mesh pass before use. A lyophilized β -chitin from the pens of *Loligo* squid was kindly supplied by Nihon Suisan Co. Ltd. and a 80% deacetylated chitosan was obtained from Kyowa Technos Co. Ltd. The reagents of reagent class were purchased from Wako Pure Chemical Industries Co. and used without further purification.

Solubility Assay

Chitinous materials were suspended in various saturated salt solutions at the concentration of 1.0%(w/v) and refluxed for several hours depending on the molecular weight. The insoluble part was removed by centrifugation (10,000 rpm, 20 min at room temperature) and the supernatant was poured into distilled water to precipitate chitin. The precipitate was treated by 20% of aqueous sodium citrate to remove calcium followed by air drying. The weight of precipitate was counted as the solubility.

Preparation of \square -chitin sludge (Heterogeneous process)

\square -chitin powder from squid pen was converted to sludge following to step by step mechanical agitation in water applying Wearing Blender at room temperature (Takai, 1992).

Preparation of chitin hydrogel (Homogeneous process)

A 50ml of chitin solution was added dropwisely into 500ml of distilled water under vigorous stirring at room temperature. The precipitate was collected by centrifugation followed by several rinses with distilled water and then homogenized by Wearing Blender. The homogenized gel was dialyzed against distilled water until no calcium ion was detected in outer solution applying dialyzing bag (Tamura & Hamaguchi).

Preparation of chitin sheet

Chitin hydrogels were converted into sheet by filtration of chitin hydrogels on Nylon meshed cloth and the gel pad was air dried by pressing between Nylon cloths under pressure.

Adsorption behavior of chitin sheet with various kinds of dyes

Chitin sheet was immersed in various kinds of dye aqueous solutions at room temperature for 24 hr and then measured optical density of supernatant to estimate adsorbed dyes. A similar procedure was applied to chitin hydrogel to evaluate maximum amount of dye adsorption (Tamura & Okazaki).

Results and discussions

Non-harmful Solvent for Chitin

Solvent compositions for chitin have been investigated to prepare the clear chitin solution. Several metal salts of Group II of the periodic table were tested in combination with alcohols such as

methanol, ethanol, *n*-propanol, isopropanol, etc. The calcium chloride dihydrate-saturated methanol was found to be the most effective solvent system among those including calcium or magnesium salts. The solubility of chitin powder against Ca-methanol system was found to depend remarkably on the calcium content in methanol. The poor solubility observed for Ca-ethanol system might be due to the lower calcium content at the saturation in ethanol as compared to methanol. Faint solubility was observed for chitin powder in magnesium chloride, magnesium nitrate or calcium nitrate saturated in methanol solution. However, a slight solubility was observed for the calcium thiocyanate saturated methanol solution. Briefly, 4%(w/v) chitin solution was prepared 15 minutes reflux in the case of reacylated chitin (calculated molecular weight was ca. 5×10^4) and the solution showed hydrodynamic flow at 40°C, but almost gel form at lower than 15°C.

There is also clear difference between solubility of α - and β -chitins, as listed in Table 1. Lower solubility was found for β -chitin in spite of looser crystalline structure than that of α -chitin (Sundarajan & Marchessault, 1978; Gardoner & Blackwell, 1975). Since the gelation of β -chitin solution started at lower chitin concentration than that of α -chitin, the gelation mechanism might be related significantly with dissolution mechanism or the conformation of chitin molecules in solvent.

Though there was little solubility for chitosan, *N*-deacetylated derivative of chitin, and cellulose against the Ca-methanol solvent system, a bacterial cellulose and bacterial heteropolysaccharide containing a small amount of GlcNAc residue (Ogawa & Tokura, 1993), an ultra-pure cellulose of extra-cellular polysaccharide of *Actobacter xylinum*, adsorbed the solvent to form a gel like particles. The contribution of acetamide groups of chitin has been suggested to solubilize chitin molecule by the formation of complexes with calcium ion. Hydroxyl groups in GlcNAc residues are also assumed to associate with acetamide group to form calcium complexes, because 3,6-*O*-diacetyl-chitin (Nishi *et al.*, 1979) formed only a gel by the adsorption of solvent, whereas GlcNAc itself was clearly dissolved in Ca-methanol solvent. The solubility of chitin against the solvent was seriously depended on the molecular weight and the molecular conformation of chitin, because the better solubility was given by the smaller molecular weight fractions and also the better solubility was given by reacylated chitin from chitosan than that of natural chitin even if similar molecular weight. Since the reacylated chitin has less crystalline structure than that of natural chitin, calcium ions would be more easily form a complex with the chitin molecule. Furthermore, di-*O*-acetyl- or 6-*O*-monoacetyl-chitin was hardly present in the regenerated chitin powder which was recovered from chitin-calcium-methanol solution by the addition of large amount of water. But acetylation of recovered chitin powder was achieved smoothly following the EDTA treatment to remove calcium ion. These facts allowed us to assume that the hydroxyl groups of chitin molecule are blocked by calcium ion during the dissolution process. The chitin solution was stable for long periods at room temperature so that viscosity measurement could be done without any caution.

Preparation of chitin hydrogel

A swollen fibrous chitin (chitin hydrogel) was successfully prepared following by addition of chitin solution into large excess of water at room temperature to remove calcium completely. The gel contains 95-97% of water. The process was monitored by the transmittance measurement. The result is shown in Figure 1. Transmittance of chitin solution decreased drastically by the addition of water. About 0.2 fold water against chitin solution is sufficient for the completion of the precipitation. The result suggests that dissolution of chitin in calcium chloride dihydrate saturated methanol is persisted in the precise balance of the component.

β -chitin can be highly swelled in water by the vigorously stirring by blender, probably due to the loose packing of polymer chain. β -Chitin exists in a crystalline hydrate which accounts for its lower stability since water can penetrate between the chains of lattice⁵. Thus, due to the loose packing of polymer chain, β -chitin can be highly swelled by the vigorously stirring by blender. The chitin hydrogel composed of 92-95% of water was prepared following to dilution of chitin solution with large excess of water and then removal of calcium ion by dialysis. The chitin hydrogel is now under practical test on several skin reactions due to the formation of thinner protective barrier from air.

The direct preparation of chitin hydrogel from deproteinized Crab Shells is also now under investigation applying the dissolution property.

Preparation of sheet from various type of chitin gels

Chitin sheet were prepared only by press-air dry successfully without any binder. A α -chitin type crystalline structure was suggested for the chitin sheet from homogeneous procedure by XRD picture. But β -chitin type was for that from heterogeneous procedure of β -chitin powder.

Adsorption profiles of sheet against various dyes as model drug

Various dyes were applied to investigate the adsorption profiles of chitin sheet. The adsorbability of chitin sheet was almost similar level as much as those by chitosan sheet suggesting strong basicity of chitin molecule. Sulfate group, especially, was the most familiar group to adsorb on chitin among several functional groups. But the hydrophobic group was also one of adsorption factor though it was not so strong as to sulfate group. Although the higher adsorption of anionic dyes was observed for chitin hydrogels prepared with homogeneous procedure than that of the β -chitin sludge by heterogeneous procedure, there was hard to find out the difference on dye adsorption between sheet from α -Chitin and β -chitin. This might suggest that chitin sludge is composed of concrete β -chitin crystalline structure at inside and hydrogel at out side to suppress dye adsorption due to limited surface area comparing with α -chitin hydrogel. But suppression of surface area was achieved for α -chitin hydrogel due to the formation of hydrogen bonds to form sheet to make similar surface area to that of β -chitin.

Conclusion

α -Chitin, extracted from Crab or Shrimp shells, has been reported to dissolve in calcium chloride dihydrate saturated methanol probably due to destruction of intra and inter residual hydrogen bonds in chitin molecule. Resulted viscous chitin solution was converted to hydrogel containing 90 to 94% of water following to dilution of chitin solution with large excess of deionized water. β -Chitin, from Squid pen, has also reported to give a hydrogel from highly viscous chitin solution in calcium chloride dihydrate saturated methanol, although β -chitin could convert to hydrogel directly following to simple mechanical agitation in water because of poorer crystallinity than that of α -chitin. Thus dissolution of chitin is seriously influenced by the presence of calcium ion with a slight amount of water in alcohol.

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Table 1. Solubility of chitins and their derivatives in saturated $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ -MeOH

Solution		solubility ^a
a-chitin	1.0 g / 50 mL	+++
b-chitin	0.5 g / 40 mL	++
Chitosan	1.0 g / 20 mL	-
Bacterial cellulose	30 mg / 20 mL	±
Acetylated chitin	0.5 g / 40 mL	++
Nylon-6	1.0 g / 20 mL	+++

+++ : transparent solution, ++ : turbid solution or gelation,

± : gelation, - : insoluble

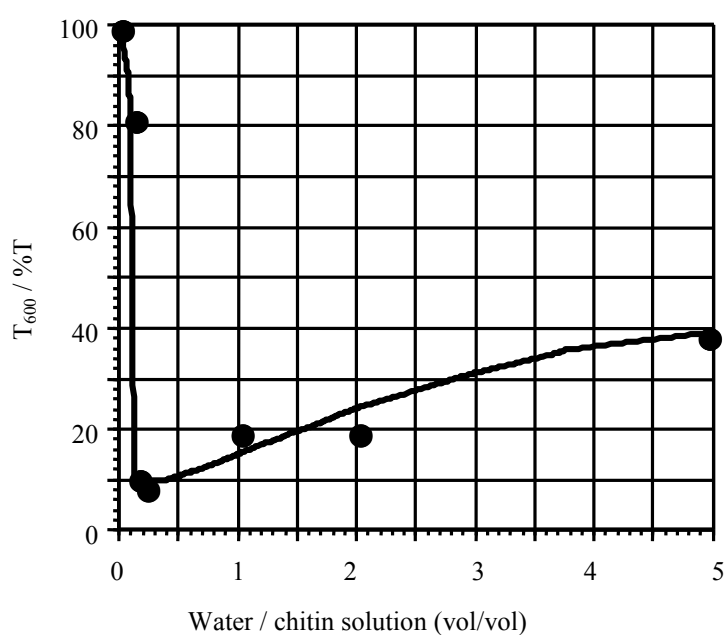


Figure 1. Transmittance measurement adding water into the chitin solution of calcium chloride dihydrate saturated methanol.