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Solubilization of a Rigid Polysaccharide: Controlled Partial *N*-Acetylation of Chitosan to Develop Solubility

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ABSTRACT

The solubility–structure relationship was studied in the acetylation of chitosan to elucidate key factors for solubilization of rigid polysaccharides. N*-Acetylation of chitosan proceeded smoothly and reproducibly in a highly swollen state, and the degree of substitution was easily controlled. Evaluation of the properties of the resulting acetylated chitosans indicated the importance of the distribution mode of acetyl groups as well as the extent of substitution for solubilization. The chitosan derivatives with about 50% substitution prepared under appropriate conditions exhibited high solubility in water, and the presence of half a molar amount of randomly distributed substituents on the amino groups was confirmed to be an essential requirement in developing solubility. The present procedure is much simpler to prepare the water-soluble chitin than the conventional method involving partial deacetylation of chitin.*

INTRODUCTION

Chitin is annually produced in a huge amount in nature and is still an almost unutilized biomass resource in spite of the structural closeness to cellulose. It is, however, beginning to attract much attention in various fields, including medicine, pharmacology, biochemistry and materials science as a natural polymer with interesting properties associated with the peculiar structure. Chitin chemistry is hence being explored extensively by an increasing number of groups in both basic and applied aspects. Though many possibilities as a novel material for advanced uses have been pointed out (Muzzarelli, 1977; Kurita, 1986), the intractabil-

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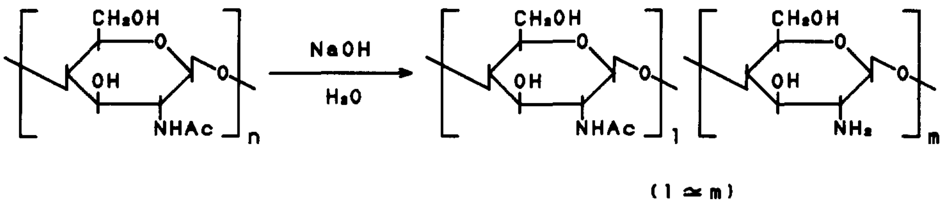
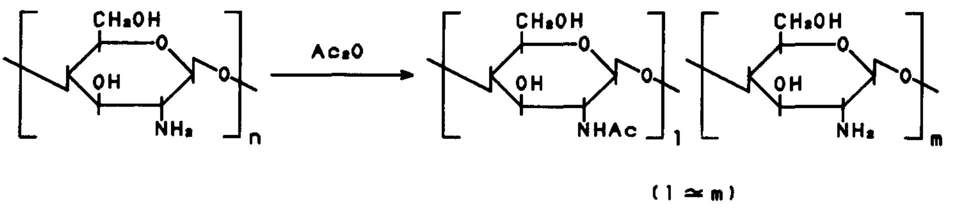
ity has undoubtedly delayed its utilization. It is infusible and insoluble in solvents except some special ones (Capozza, 1976; Rutherford & Austin, 1978; Ando & Kataoka, 1980; Uragami *et al.*, 1981). Chitosan, a deacetylated form of chitin, is soluble in some dilute aqueous acids, but it precipitates out on neutralization.

A partially deacetylated chitin with a specific narrow range of degree of deacetylation, 0·45 to 0·55, which is prepared by alkaline hydrolysis in homogeneous solution (Scheme 1), is readily soluble in water, and the solution is stable in all pH regions (Sannan *et al.*, 1976). This modified chitin is of interest as a water-soluble polycation and moreover, has many advantages in chemical modifications, since reactions can be performed in homogeneous aqueous solution or in a highly swollen state in organic solvents (Kurita *et al.*, 1977*a*; 1988*a*). Samples with the same degree of deacetylation prepared under conventional heterogeneous conditions are naturally insoluble. These results and some other data suggest that the solubility or high affinity to solvents is attributable to random distribution of specific amounts of substituents along the backbone (Kurita *et al.*, 1977*b*; 1988*b*; 1990).



Scheme I

The authors' interest in the solubility–structure relationship has prompted the examination of the influence of *N*-acetylation of chitosan on the solubility to investigate the possibility of preparing water-soluble chitin by an alternative procedure (Scheme 2), and some preliminary results were reported (Kurita *et al.*, 1989). The resulting partially



Scheme II

acetylated chitosan is expected to show high solubility, if the degree of substitution (*ds*) is controlled at a value of 0·5 and the acetyl groups are distributed randomly. The data obtained here would be helpful in disussing the solubility behaviour and in elucidating the key factors for solubilization of intractable rigid polysaccharides. Furthermore, partial acetylation of chitosan is much simpler than partial deacetylation of chitin, suggesting the possibilities of facile and efficient preparation of this water-soluble polycation.

EXPERIMENTAL

General

IR spectra were recorded with a JASCO IRA-1 spectrophotometer (Japan Spectroscopic Co. Ltd, Tokyo). The absorbance at 600 nm was determined with a JASCO UVIDEC-4. X-Ray diffraction diagrams were obtained by the powder method using nickel-filtered CuKα radiation with a Rigaku RAD-IA diffractometer (Rigaku Corporation, Tokyo). The degree of deacetylation was determined by IR spectroscopy (Sannan *et al.*, 1978).

Chitosan

Chitin was isolated from the shells of *Penaeus japonicus* and purified by treating with 1 mol/litre sodium hydroxide at 100°C for 6 h in nitrogen. The alkaline treatment was repeated several times with fresh 1 N sodium hydroxide until complete decoloration was attained, and the resulting purified chitin was pulverized with an automatic mortar to 100 mesh pass (Sannan *et al.*, 1976). It was then deacetylated with 50% sodium hydroxide at 135°C for 3·5 h in nitrogen to give white powdery chitosan with a degree of deacetylation of 90%.

Acetylation of chitosan: Method A

To a solution of 0·1 g of chitosan in 6 ml 10% aqueous acetic acid was added 24 ml methanol. A given amount of acetic anhydride was added at room temperature with stirring. The resulting gel was left standing overnight, broken into small pieces, and stirred with 180 ml methanol overnight. It was washed with acetone thoroughly and dried. The yield was quantitative.

Acetylation of chitosan: Method B

A solution of 0·3 g of chitosan in 8 ml 10% aqueous acetic acid was diluted with 8 ml methanol and poured into 100 ml pyridine to give a highly swollen precipitate. A given amount of acetic anhydride was added at room temperature. After stirring for 5 h, the mixture was poured into 300 ml acetone. The precipitate was collected by filtration, washed with acetone, and dried. It was treated with 100 ml 0·1 mol/litre methanolic potassium hydroxide solution at room temperature for 6 h, filtered, washed with methanol thoroughly, and then dried. The yield was 0·22 to 0·24 g.

Acetylation of chitosan: Methods C and D

A chitosan solution in aqueous acetic acid/methanol prepared as described in method B was added to a mixture of 10 ml pyridine and a given amount of acetic anhydride (method C) or to a mixture of 100 ml pyridine and a given amount of acetic anhydride (method D) with stirring. The reaction and work up of the product were carried out in the same manner. The yield was 0·24–0·29 g (method C) or 0·27–0·29 g (method D).

The molecular weight of the resulting water-soluble chitin was determined by gel filtration with Sepharose CL4B-16 in 1·2% aqueous sodium hydroxide using dextran standards. A typical sample with a degree of acetylation of 0·46 prepared by method D was shown to have Mn = 48200 and Mw = 158000.

Solubility test based on turbidity

A partially acetylated chitosan (100 mg) was added to 50 ml of 5% aqueous acetic acid, and the insoluble part was filtered. To the filtrate was added 1 mol/litre aqueous sodium hydroxide to pH 12, and the solution was poured into 300 ml of acetone/water (7:1) to precipitate the product. It was washed with the same mixed solvent thoroughly and dried.

Part of the product obtained above (15 mg) was dissolved in 30 ml of 5% aqueous acetic acid, and the pH of the solution was adjusted to 8·9. Deionized water was added to make the total volume 60 ml (0·025 wt%). The absorbance of the solution at 600 nm was determined as a measure of turbidity.

RESULTS AND DISCUSSION

Controlled acetylation was carried out on thoroughly purified chitosan to establish high reproducibility in acetylation and the solubility test, and the structure–solubility relationship is explored. Two of the most important factors for developing solubility would most likely be (1) introduction of acetyl groups into a half the number of the amino groups and (2) random distribution of the acetyl groups along the backbone.

Acetylation in acetic acid/methanol

Acetylation was first attempted in solution in aqueous acetic acid/methanol (method A), but the mixture generally became a gel 1–2 min after the addition of acetic anhydride. The *ds* values of the products prepared with two-fold and four-fold excess acetic anhydride were high, but almost in the same level, 0·85 and 0·86, respectively. This acetylation procedure was, however, not suitable for the present purpose of solubilization, since the reaction is often accompanied by gel formation, which would make the control of reaction difficult both in the extent of substitution and in the random distribution of substituents.

Acetylation in a highly swollen state

Chitosan was then subjected to acetylation in a highly swollen state that allows almost homogeneous reactions and usually brings about no gelation. A highly swollen chitosan precipitate was prepared by pouring a chitosan solution in aqueous acetic acid/methanol into pyridine. The precipitate obtained from the above mixed solvent was more highly swollen than that from aqueous acetic acid alone, due to the higher affinity of the mixed solvent for pyridine. A large amount of methanol, however, somewhat interfered with the reaction, resulting in a slight decrease in the *ds* value, as shown in Table 1, and moreover, caused the whole reaction mixture to set to a gel instantaneously on addition of acetic anhydride. This indicates that methanol should be used in such an amount that is large enough to ensure high swelling of chitosan and is small enough to prevent gelation. The reaction was thus conducted in 8 ml each of aqueous acetic acid and methanol for 0·3 g of chitosan (method B).

The acetylation reaction proceeded smoothly and reproducibly under these conditions without gel formation. The products, however, showed a weak shoulder band of ester at 1730 cm^{-1} in the IR spectra. They were

TABLE 1
Effect of the Amount of Methanol on the Degree of Acetylation[a]

MeOH (ml)	*Pyridine (ml)*	*Degree of substitution*
8	100	0·45
10	50	0·30
30	50	0·27
50	50	0·24

[a] Chitosan: 0·3 g, 10% AcOH: 8 ml, Ac_2O (mol)/ $-NH_2$ (mol) = 20, temperature: room temperature; time: 5 h.

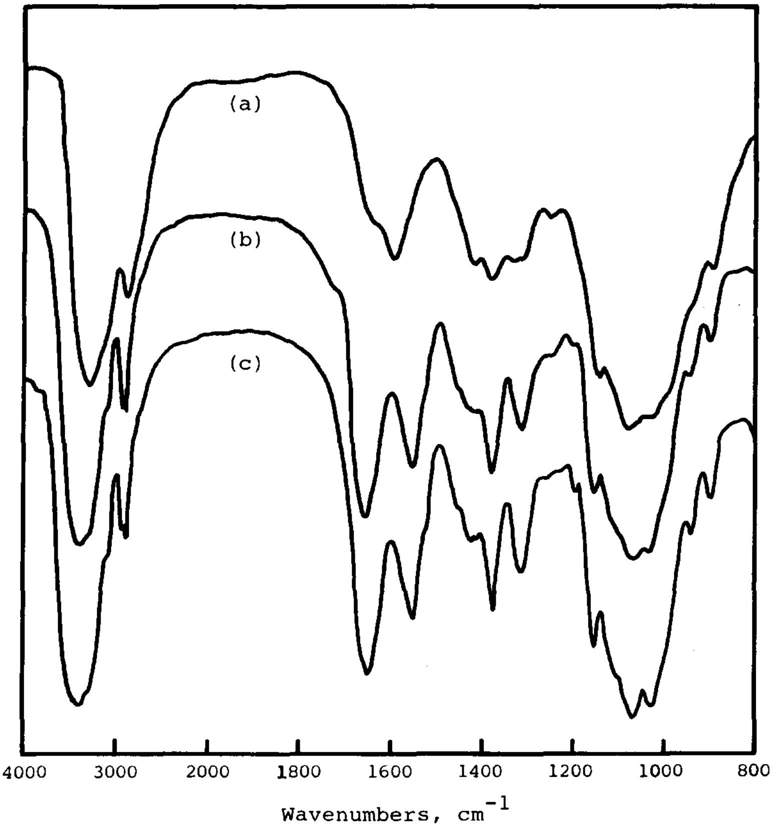


Fig. 1. IR spectra of (a) chitosan, (b) acetylated chitosan before alkaline treatment and (c) acetylated chitosan after alkaline treatment with a *ds* value of 0·55 (KBr method).

thus treated with methanolic potassium hydroxide, and the removal of the ester groups was confirmed by the IR spectra, as shown in Fig. 1. The substitution extent was controlled by the amount of acetic anhydride, and as illustrated in Fig. 2, *ds* values of 0·28 to 0·62 were attained with

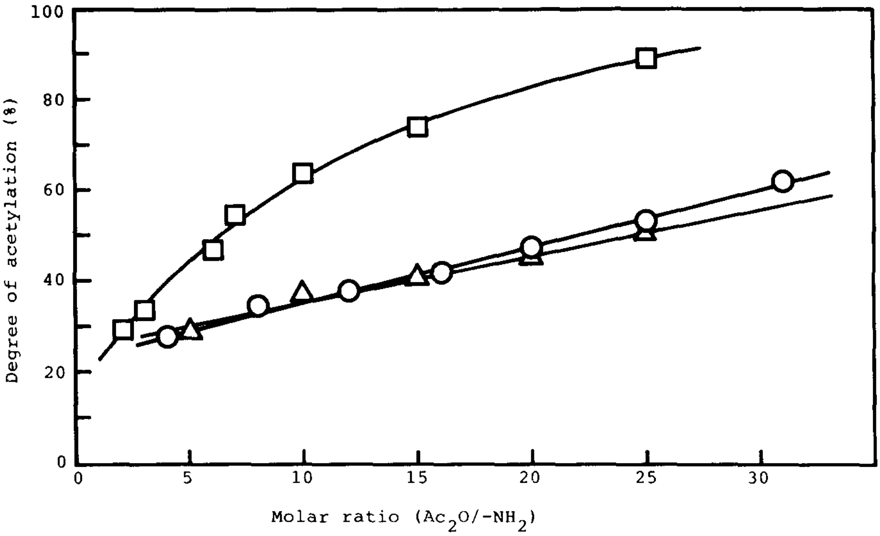


Fig. 2. *N*-Acetylation of chitosan with acetic anhydride in a highly swollen state: (○) method B, (△) method C and (□) method D.

four to 30-fold excess acetic anhydride. Solubility tests of the products, however, showed that even the samples with *ds* values of about 0·5 left small amounts of insoluble matter in water. This may be ascribable to the trace heterogeneity of the precipitate formed in pyridine, i.e. a very small portion of chitosan coagulates as a fibrous precipitate that may interfere with the subsequent acetylation to proceed randomly.

In order to perform the acetylation in a more improved swollen state to realize random substitution, the chitosan solution was poured into pyridine mixed previously with acetic anhydride. In this way, the appearance of the precipitate remained highly swollen, ensuring reaction took place under almost homogeneous conditions. When 10 ml of pyridine was used (method C), the products had almost the same *ds* values as those by method B, as shown in Fig. 2, although the mixture became rather viscous.

The reaction could be conducted under even more uniformly swollen conditions in 100 ml of pyridine (method D). Moreover, acetylation took place much more efficiently, as is evident from Fig. 2. This may be interpreted in terms of easy miscibility of chitosan solutions with pyridine/acetic anhydride mixtures as a result of the increased amount of pyridine, leading to instantaneous thorough mixing of the reactants. With 25-fold excess acetic anhydride, a product with a value of *ds* of 0·89 was obtained. In addition to the improved, almost homogeneous reaction conditions, methods C and D gave better yields (80–90%) than method B where they were 70–80%.

The IR spectra of the partially *N*-acetylated chitosans with a *ds* value of about 0·5, a typical example being shown in Fig. 1, were identical with those of the water-soluble chitin prepared by the partial deacetylation of chitin. Crystallinity of the products obtained was examined by X-ray diffractometry, and they were completely amorphous, just like the conventional water-soluble chitin. This is in clear contrast to the high crystallinity of the starting chitosan, and the destruction of tight arrangement of the rigid polysaccharide molecules by introduction of appropriate substituents is quite desirable for solubilization. The amorphousness supports the random substitution as discussed previously (Kurita *et al.*, 1977*b*, 1988*b*, 1990).

Solubility behaviour

The solubility of the acetylated chitosans in water was examined to elucidate the effects of substitution. The samples with *ds* values above 0·6 or below 0·4 were only poorly soluble, as expected (Sannan *et al.*, 1976), and thus those with *ds* values around 0·5 were subjected to quantitative analysis of solubility. They were added to 5% aqueous acetic acid, and insoluble portions were filtered off. As summarized in Table 2, the soluble portions of the acetylated chitosans prepared by methods B and C were around 70%, whereas those prepared by method D were 90% or above. The filtrates were made alkaline, but the solutions were visibly clear. Turbidity was thus determined, and the results are included in Table 2. As expected, the samples prepared by method D showed very low turbidity even in alkaline regions, and the values indicate that the

TABLE 2
Solubility of *N*-Acetylated Chitosans with about 50% Substitution

Sample[a]	*Method*	*Ac_2O (mol)/ $-NH_2$ (mol)*	*Degree of substitution*	*Solubility (%)*[b]	*Turbidity*[c]
NAC-1	B	25	0·55	70·5	3·5
NAC-2	C	25	0·55	65·3	7·4
NAC-3	D	6	0·46	93·3	0·9
NAC-4	D	7	0·55	89·8	1·2
WSC	—	—	0·51	100	1·1

[a] NAC: *N*-Acetylated chitosan; WSC: water-soluble chitin by the conventional method.
[b] Portion soluble in 5% AcOH.
[c] Absorbance at 600 nm (*C* = 0·025%, pH 8·9).

same solubility as that of water-soluble chitin prepared by partial deacetylation of chitin was achieved. This confirms method D to be much superior to the others in solubility of the products and also to the conventional method in simplicity.

The *ds* values of the insoluble portions were high; the *ds* value was, for example, 0·71 for NAC-3, which was much higher than the average *ds* value of 0·46. Moreover, the acetyl groups originally present in chitosan are reasonably estimated to be distributed in a block fashion (Kurita *et al.*, 1977*b*). The formation of the insoluble portion was thus attributable primarily to the local heterogeneity of substitution. Although there remained small amounts of insoluble portions, filtration and reprecipitation gave completely soluble products in 80–90% yields, even starting from ordinary chitosan of 90% deacetylation. It is thus not necessary to use completely deacetylated chitosan, which may be prepared by repeated deacetylation (Domard & Rinaudo, 1983; Mima *et al.*, 1983).

CONCLUSIONS

Water solubility was developed by controlled partial *N*-acetylation of chitosan. It is noteworthy that the solubility of partially *N*-acetylated chitosans is drastically influenced by slight differences in the preparative conditions in spite of similar substitution degrees. The change in the conditions brings about some differences in the distribution mode of substituents, and the amount of insoluble portion could be reduced remarkably by improving the reaction conditions. The results obtained here indicate the importance of random substitution as well as the substitution extent in developing solubility of intractable rigid polysaccharides. Appropriate introduction of substituents plays a major role in efficiently destroying the crystalline structure, leading to enhanced hydrophilicity.

The conventional method to prepare water-soluble chitin by partial deacetylation of chitin involves some laborious processes including neutralization, which causes much trouble in the removal of the salt with large amounts of solvent. In contrast, method D described here is much simpler by far and quite suitable for production on both small and large scales. Moreover, this alternative procedure makes the preparation of water-soluble chitin of a desired molecular weight easy, starting from the appropriate chitosan, which is difficult by the conventional method because of the considerable degradation of the backbone during alkaline deacetylation (Sannan *et al.*, 1976).

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