Dissolution of Chitosan in Dimethyl Sulfoxide by Salt Formation

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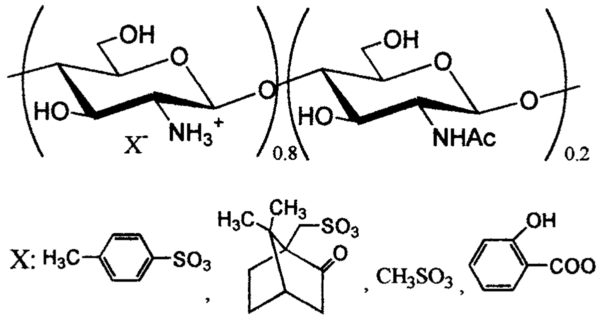
Chitosan dissolved in dimethyl sulfoxide by salt formation with some acids such as *p*-toluenesulfonic acid (*p*TsOH), (*1R*)-(–)-10-camphorsulfonic acid (CSA), methanesulfonic acid, and salicylic acid.

Chitin is a mucopolysaccharide composed of repeating β-(1-4)-2-acetamide-2-deoxy-D-glucopyranose unit (*N*-acetyl-D-glucosamine) and chitosan is a polysaccharide composed of repeating β-(1-4)-2-amino-2-deoxy-D-glucopyranose unit (D-glucosamine) which may contain small amount of residual *N*-acetyl-D-glucosamine unit. Generally, chitosan dissolves in dilute acidic water such as HCl, acetic acid, and some organic acids owing to a salt formation of amino group. It has been reported that chitin dissolved in organic solvent such as LiCl / *N*,*N*-dimethylacetamide,[1] sat. $CaCl_2 \cdot 2H_2O$/MeOH,[2] etc. On the other hand, there have been no reports on the dissolution of chitosan in organic solvent until now. Dissolution of chitosan in organic solvent, needless to say, is one of the important factor for the chemical modification of chitosan. Most recently, we found that chitosan-acid salts such as *p*-toluenesulfonic acid (*p*TsOH), (*1R*)-(–)-10-camphorsulfonic acid (CSA), methanesulfonic acid, and salicylic acid dissolved in dimethyl sulfoxide (DMSO). Herein we report the dissolution of chitosan-acid salts in DMSO and some organic solvents.



Chitosan (Flonac C, NHAc = 0.2, Mn = 24000) was purchased from Kyowa Tecnos Co., Japan. High MW of chitosan (Chitosan 10 B, NHAc = 0.04, Mn = 200000) was also purchased from Funakoshi Co., Japan. The degree of *N*-acetylation was determined by ^1H NMR spectrum and the Mn was also determined by GPC with pullulan as standard. Typical procedure for the preparation of chitosan salt is as follows. Chitosan (400 mg: 1.9 mmol of NH_2) was suspended in water (30 cm^3) and then equimolar of acid was added. After stirring for 1 h, the suspension was gradually turned to clear solution. The solution was dialyzed with dialysis membrane (MW cut off 12000) against distilled water for 2 days, and lyophilized to give chitosan-acid salt in a quantitative recovery. Selected ^1H NMR data is as follows. Chitosan-*p*TsOH salt (NHAc = 0.2, $NH_3^+TsO^-$ = 0.8): δ 1.82 (s, 0.6 H, NHAc), 2.29 (s, 2.4 H, CH_3 of Ts), 2.79 (br, 0.8 H, H-2 of GlcN unit), 3.2–3.8 (brm, 5.2 H, H-2 of GlcNAc and H-3,4,5,6 of hexosamine unit), 4.47 (br, H-1 of GlcNAc unit), 4.7 (br, 0.8 H, H-1 of GlcN unit), 7.12 (d, *J* = 8.0 Hz, 1.6 H, H-3,5 of Ts), 7.50 (d, *J* = 8.0 Hz, 1.6 H, H-2,6 of Ts).

Table 1. Solubility of chitosan salts in organic solvent[a]

Acid	Solvent		
	H_2O	DMSO	Others[b]
*p*TsOH	O	O	X
CSA	O	O	X
CSA[c]	O	O	X
$MeSO_3H$	O	S	X
H_2SO_4	X	X	X
2-HOC_6H_4COOH	O	O	X
PhCOOH	O	X	X
PhC_2H_4COOH	O	X	X
2-HOOCpyridine	O	X	X
AcOH	O	X	X
HCl	O	X	X

[a]Chitosan (NHAc=0.2, Mn=24000). Samples (10 mg) were dispersed in solvent (1 cm^3) and stood for 1 day; O, dissolved; S, partially dissolved, X, undissolved. [b]Other solvents are DMF, MeOH, pyridine, and $CHCl_3$. [c]Chitosan (NHAc=0.04, Mn=200000) was used.

Chitosan (NHAc = 0.2, Mn = 24000) dissolved in water in the presence of equimolar of acid listed in Table 1. After freeze drying of aq chitosan salt solution, a part of chitosan-acid salts such as *p*TsOH, CSA, $MeSO_3H$, and salicylic acid (2-HOC_6H_4COOH) dissolve in DMSO. High MW of chitosan (NH = 0.04, Mn = 200000)-CSA salt was also dissolved in DMSO. Since these chitosan-acid salts were recovered quantitatively after dialysis with dialysis membrane (MW cut off 12000), the degradation of chitosan with acid did not occur. Non-freeze dried chitosan powder, however, did not perfectly dissolve in DMSO by addition of equimolar or excess amount of acids. Only a part of chitosan powder was confirmed to dissolve directly in DMSO after collection of DMSO soluble fraction.[3] Furthermore, chitosan-salt film, which was prepared by drying procedure from aqueous chitosan-acid solution, was not shown perfect dissolution. Therefore, freeze drying process is an important factor for the perfect dissolution of chitosan salt in DMSO. These freeze dried chitosan-acid salts, however, did not dissolve other organic solvents such as DMF, methanol, pyridine, and chloroform. From the ^1H NMR spectra in DMSO-d_6, typical signals assigned as chitosan and corresponding acids were observed. These results suggest that alkyl or allyl sulfonic acid was effective for the dissolution of chitosan in DMSO. The SO_3H group in chitosan-acid salts would be advantageous for the solvation in DMSO. Chitosan-acid salt with divalent sulfonic acid like H_2SO_4 was insoluble even in water. On the other hand, only chitosan-salicilic acid salt was dissolved in DMSO, although other carboxylic acids listed in Table 1 were not. Although the reason why only salicilic acid was effctive for the dissolution of chitosan in DMSO are not clear, the phenolic hydroxyl group at *ortho* position should be important role for the solvation of chitosan-acid salt in DMSO.

These chitosan-acid salts were diluted from the DMSO solution against various organic solvents and these results were shown in Table 2. Almost all of chitosan-acid salts in DMSO solution were diluted in DMF, pyridine, and MeOH. In particular, chitosan-CSA salt solution was diluted even in chloroform and acetone (data not shown). Chitosan-salicilic acid, however, was formed precipitate in pyridine which would be caused by the neutralization of chitosan-acid salt by pyridine. In the case of triethylamine, all of samples were formed precipitate owing to the change of NH_3^+ to NH_2 of chitosan (pKa = 6.5). These chitosan-acid salts were not diluted in diethyl ether or ethyl acetate. From these results, the chemical modification of chitosan could be carried out under the acidic homogeneous conditions with these diluted solvent systems.

Table 2. Dilution of DMSO solution of chitosan-acid salts with organic solvent[a]

Acid	Solvent[b]			
	MeOH	Pyridine	$CHCl_3$	Et_3N
*p*TsOH	O	O	X	X
CSA	O	O	O	X
$MeSO_3H$	O	O	X	X
Salicilic acid	O	X	X	X

[a] Samples (10 mg·cm^{-3}; 0.5 cm^3 in DMSO) were diluted with solvent (5 cm^3) and stood for 1 day; O, homogeneous, X, precipitation was formed. [b] All samples were diluted with DMF, but precipitate was formed with diethyl ether and ethyl acetate.

In this study, we found a novel method for the dissolution of chitosan in organic solvent without any chemical modification. At the present stage, one weak point is that these solvent systems are kept homogeneous only under the acidic conditions. Now we are studying the chemical modification of chitosan using these solvent systems and will report in near future.

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References and Notes

1 P. A. Austin, U. S. Patent 4059457 (1977); *Chem. Abstr.*, **88**, 63496d (1978).

2 S. Tokura, S. Nishimura, N. Sakairi, and N. Nishi, *Macromol. Symp.*, **101**, 389 (1996).

3 Undissolved chitosan was removed by filtration and the supernatant was diluted in water, dialyzed, and lyohpilized.