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Note

A chitosan oxalate gel: its conversion to an *N*-acetylchitosan gel *via* a chitosan gel*

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A number of neutral (*e.g.*, curdlan[2] and agarose[3]) and acidic (*e.g.*, carrageenans[4,5] and alginic acid[6]) polysaccharide gels are known, but little is known about basic (amino) polysaccharide gels. *N*-Acylchitosan gels[7], *N*-arylidene- and *N*-alkylidene-chitosan gels[8,9], and a chitin gel[10] are the only known basic polysaccharide gels, and all of these are thermally irreversible. These gels are generally known to be composed of a fibrous solid phase, which forms a framework of many pores by cross-linking with a liquid phase filling in the pores[11,12]. Our interest is to demonstrate, for purposes of analyzing the molecular organization of gels, an intramolecular conversion of one gel to another gel in the solid phase.

We now report a novel chitosan oxalate gel, which melts on heating and solidifies on cooling in aqueous oxalic acid solution, and undergoes an intramolecular conversion to an *N*-acetylchitosan gel *via* a chitosan gel. This is the first report dealing with a basic polysaccharide gel that is thermally reversible.

The thermally reversible gel was formed from a $>2\%$ solution of chitosan (<0.2 NAc/GlcN) in 3% aqueous oxalic acid (1.0–2.0 $\mathrm{mol \cdot GlcN^{-1}}$). The chitosan oxalate gel melted on heating at 80–90° and solidified again on cooling under these conditions. Furthermore, the heating and cooling cycle was repeated many times without affecting the gel properties. The elastic hardness of the gel increased almost linearly with chitosan concentration (3.0–4.5%) at a molar ratio of 1:1.5 for GlcN:oxalic acid (Fig. 1).

Maximum values for its elastic hardness and breaking point at 3% chitosan concentration were found in the range of 1.0–1.5 mol. equiv. of oxalic acid to GlcN (Fig. 2). The gel did not undergo syneresis, and it was stable in water, as well as in a 2% aqueous acetic acid solution at room temperature for several months. The excess of oxalic acid present in the gel was removed by dialysis in distilled water, and the chitosan oxalate gel was obtained. Its elemental analysis data proved that the chitosan oxalate xerogel is composed of one mol. equiv. of oxalic acid per D-glucosaminyl residue,

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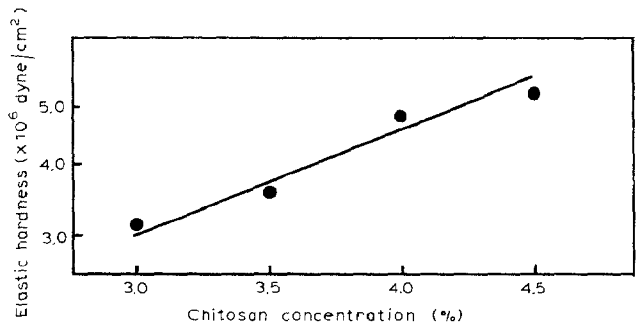

Fig. 1. Effect of chitosan concentration on the elastic hardness of the chitosan-oxalate gel at a molar ratio of GlcN:Oxalic acid = 1.0:1.5.

indicating the absence of the cross-linking of oxalic acid in chitosan chains. The structure was also confirmed by the i.r. data, which showed absorptions at 1700 (COOH), 2600, 1620, and 1400 cm^{-1} (ionic salt), and by ^{13}C-n.m.r. data, which showed resonances at δ 167 and 164 (C=O). Gel formation was inhibited by the presence of an *N*-acetyl group at d.s. >0.2/GlcN in chitosan. The corresponding gel did not form from chitosan when it was dissolved in either an aqueous solution of malonic acid (pK_1 2.86) or in an aqueous solution of succinic acid (pK_1 4.16), both of which are weaker acids than oxalic acid. The gelation is initiated by formation of the ionic salt with oxalic acid (pK_1 1.27) at d.s. >0.8/GlcN. The gelation mechanism of the chitosan oxalate gel seems to differ from the cross-linking of chitosan chains, because the minimum d.s. value required for gel formation by cross-linking was about 0.002, as previously reported in the reaction of chitosan with *m*- or *p*-phthalaldehyde[13]. The chitosan oxalate gel may be

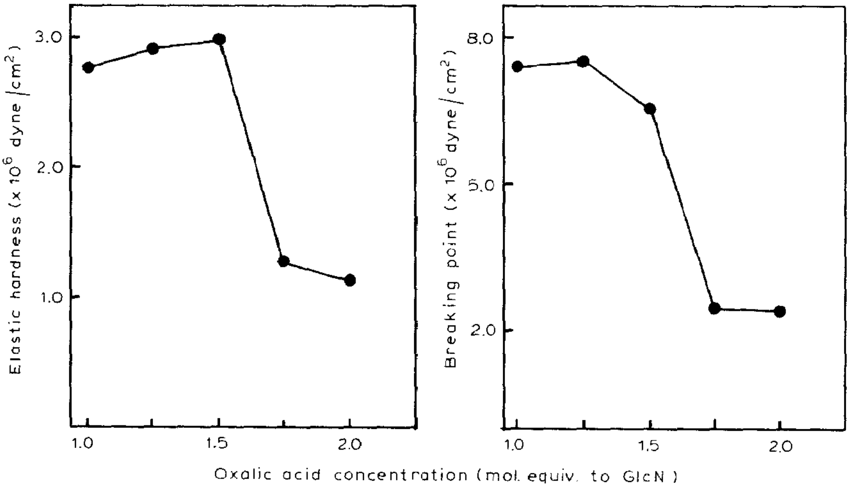

Fig. 2. Effects of oxalic acid concentration on the elastic hardness and the breaking point of the chitosan-oxalate gel at 3% chitosan concentration.

produced by salt formation at the amino groups of chitosan, and its gelation mechanism is similar to those of *N*-acylchitosan gels[14] and *N*-arylidene- and *N*-alkylidene-chitosan gels (monoaldehydes)[8,9] in which the d.s. for *N*-substitution should be higher than 0.8/GlcN.

The gel shape was little changed, even after the complete removal of oxalic acid by treating the gel with an aqueous NaOH solution; however, the gel turned opaque. The opaque xerogel was identified as chitosan on the basis of its i.r. and ^{13}C-c.p.-m.a.s.-n.m.r. spectra, and on basis of its elemental analysis data. The elastic hardness and the breaking point of the chitosan gel were about two times higher than that of the chitosan oxalate gel (Table I).

The opaque chitosan gel was treated with acetic anhydride in an aqueous methanol solution at room temperature overnight, and a transparent gel was obtained. The gel was indistinguishable from the *N*-acetylchitosan gel previously prepared[14] on the basis of its i.r. and ^{13}C-c.p.-m.a.s.-n.m.r. spectra, on the hydrolysis rate by lysozyme (Table II), and on a scanning electron microscopic observation of the xerogel[15]. The present study strongly indicates the presence of a common solid phase among the chitosan oxalate, chitosan, and *N*-acetylchitosan gels.

EXPERIMENTAL

General methods. — I.r. spectra (KBr) were recorded with a Hitachi 215 grating spectrometer, specific rotations with a JASCO Dip-181 digital polarimeter, and ^{13}C-c.p.-m.a.s. (cross polarization–magic angle spinning)-n.m.r. spectra with a JEOL FX-200 F.t.-n.m.r. spectrometer. The elastic hardness (dyne·cm^{-2}), and elastic break-

TABLE I

Some properties of a chitosan oxalate gel and its derived gels

Property	*Gels*[a]		
	Chitosan oxalate	*Chitosan*	N-*Acetylchitosan*
Color	Colorless	White	Colorless
N-Substituent	Oxalate	Free	Acetamido
Transparency	Transparent	Opaque	Transparent
Thermal property	Reversible	Irreversible	Irreversible
Stability in aqueous acids	Stable	Unstable	Stable
Stability in aqueous alkalis	Unstable	Stable	Stable
Elastic hardness (× 10^6 dyne·cm^{-2})	3.3	7.7	n.d.[b]
Breaking point (× 10^6 dyne·cm^{-2})	7.3	14.0	n.d.

[a] The chitosan oxalate gel was prepared from chitosan (0.9 g), oxalic acid (0.95 g, 1.25 mol. equiv. to GlcN), and water (30 mL) as described in the Experimental Section. The chitosan gel and the *N*-acetylchitosan gels were prepared from the chitosan oxalate gel. [b] Not determined.

TABLE II

Hydrolysis rate by lysozyme for an *N*-acetylchitosan xerogel derived from a chitosan gel

Product	*Reducing sugar value (mmol GlcN·mL^{-1})*
N-Acetylchitosan xerogel derived form the chitosan gel	0.31
N-Acetylchitosan xerogel prepared from chitosan[a]	0.32
Native chitin (crab shell)	0.06

[a] See Ref. 14.

ing point (dyne·cm^{-2}) of gels were measured with an Ilo M-302 curdmeter. A Hitachi S-500 scanning electron microscope was used, operating at an accelerating voltage of 20 kV.

Materials. — Partially *N*-acetylated derivatives (d.s. 0.6, 0.5, 0.4, 0.3, 0.2, and 0.1 for NAc) of chitosan were prepared from crab-shell chitosan $\{[\alpha]_D^{25}\ -9.6°$ (*c* 0.8, methanesulfonic acid), d.s. 0.10 for NAc$\}$[16]. The d.s. for the *N*-acetyl group was calculated from C/N value in the elemental analysis.

Chitosan oxalate gel. — Chitosan (0.97 g, d.s. 0.10 for NAc) was dissolved in aqueous oxalic acid solution (30 mL, 1.13 g, 1.5 mol·GlcN^{-1}) on a boiling water bath, and a transparent gel was obtained by cooling for 18–72 h. The gel was dialyzed against distilled water at room temperature for three days, and a transparent chitosan oxalate gel was obtained. The gel was composed of ~5% chitosan oxalate (1.0 for oxalic acid/GlcN) and about 95% water. A portion of the gel was homogenized, washed with distilled water, and dried to give the xerogel (1.3 g): $\nu_{\mathrm{KBr}}^{\max}$ 1740 (COOH), 2600, 1620, and 1400 cm^{-1} ($NH_3^+COO^-$); ^{13}C-c.p.-m.a.s.-n.m.r. data: δ 167 and 164 (C=O of COOH), 101 (C-1), 83 (C-4), 72 (C-3), 76 (C-5), 64 (C-5), and 56 (C-2).

Anal. Calc. for $[C_6H_{10.9}NO_4(C_2H_3O)_{0.10}\cdot 0.90\ C_2H_2O_4\cdot 0.56\ H_2O]n$: C, 37.46; H, 5.50; N, 5.46. Found: C, 37.61; H, 5.51; N, 5.48.

Chitosan gel. — Chitosan oxalate gel (20 g by wet weight) was treated with 1 N NaOH solution (500 mL) at room temperature for five days, and then dialyzed in distilled water (500 mL) for three days by changing with fresh distilled water several times to give an opaque chitosan gel (18 g by wet weight). A portion of the gel was homogenized, washed with distilled water, and dried to give an amorphous powder. $\nu_{\mathrm{kBr}}^{\max}$ 1600 cm^{-1} (NH_2); ^{13}C · c.p.-m.a.s.-n.m.r. data: δ 107 and 105 (C-1), 86 and 82 (C-4), 76 (C-3), 76 (C-5), 60 (C-6), and 58 (C-2). No acetyl or oxalate carbon signals were detected.

Anal. Calc. for $[C_6H_{10}NO_4(C_2H_3O)_{0.12}\ (H)_{0.88}H_2O_4\cdot 0.43\ H_2O]n$: C, 37.46; H, 5.50; N, 5.46. Found: C, 37.61; H, 5.51; N, 5.48.

N-*Acetylchitosan gel.* — The chitosan gel obtained above was treated with acetic anhydride (about 10 mol·GlcN^{-1}) in a 70% aqueous methanol solution at room temperature for 18 h, and a transparent *N*-acetylchitosan (d.s. 0.96 for NAc) gel was obtained. A portion of the gel was homogenized, washed well with distilled water, and dried to give an amorphous powder. $\nu_{\text{KBr}}^{\text{max}}$ 1650 (C=O of NAc) and 1560 cm^{-1} (C=O of NAc); ^{13}C-c.p.-m.a.s.-n.m.r. data: δ 174 (C=O), 104 (C-1), 84 (C-4), 75 (C-5), 75 (C-3), 56 (C-2), 61 (C-6), and 61 (Me).

Anal. Calc. for $[C_6H_{10}NO_4(C_2H_3O)_{0.96}(H)_{0.04}H_2O_4 \cdot 0.47H_2O]n$: C, 37.46; H, 5.50; N, 5.46. Found: C, 37.61; H, 5.51; N, 5.48.

Hydrolysis rate by lysozyme. — A portion (15 mg, >80 mesh) of the *N*-acetylchitosan xerogel obtained above was mixed with a solution of hen egg-white lysozyme (1 mL, 10 mg·mL^{-1}, Sigma, 57 200 U·mg^{-1}) in 0.05M McIlvain buffer solution (2 mL, pH 6.2). The suspension mixture was incubated for 1 h at 37° with mechanical shaking. Aliquots were withdrawn from the supernatant solution, and increases in the reducing sugar value were analyzed by a modification[16] of the method of Schales and Schales and calculated in μmol of *N*-acetyl-D-glucosamine.

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