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Note

Preparation of partially *N*-succinylated chitosans and their cross-linked gels

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Many polysaccharides have the ability to form gels, which indirectly reflects their function in natural systems[1]. Furthermore, this property is useful for the industrial utilisation of polysaccharides. Recently, we described gels from *N*-acylchitosans[2,3], *N*-alkylidenechitosans[4,5], and chitosan salts[6].

We now report on partially *N*-succinylated derivatives (*cf.* ref. 7) of chitosan and glycolchitosan, and the gels produced therefrom by a cross-linking reaction mediated by a water-soluble carbodi-imide[8].

N-Succinylation of chitosan and glycolchitosan was incompete (see Table I), in contrast to *N*-acylation with monobasic carboxylic anhydrides[3]; an *N*-succinylated chitosan of d.s. 0.79 was obtained by using an excess of succinic anhydride (14.6 mole/amino group). The product showed ν_{max}^{KBr} 3400 (OH, NH), 1650 (C=O of amide), 1550 (NH of amide and CO_2^-), 1390–1400 (CO_2^-), and 1150–1000 cm^{-1} (C-O), and the absence of *O*-acyl absorption indicated that only *N*-succinylation had occurred.

The partially *N*-succinylated chitosans (PSC) were classified (Table I) as (*a*) soluble only in dilute acid (d.s. ~0.2), (*b*) soluble in dilute acid, water, and dilute alkali (d.s. 0.3–0.6), and (*c*) soluble in water and dilute alkali, and insoluble in dilute acid (d.s. >0.65). The PSC of d.s. 0.3 swelled in water and dilute alkali at room temperature, but dissolved in water at ~100°. The solution (>2%) gelled on cooling to room temperature, and the gel exhibited sol–gel–sol transformation on cooling and heating. Partially *N*-succinylated glycolchitosans (PSGC) were readily soluble in water, dilute acid, and dilute alkali.

Partially *N*-succinylated chitosan gels (PSCgel) and partially *N*-succinylated glycolchitosan gel (PSGCgel) were prepared from aqueous solutions (>0.5%) of PSC and PSGC (>1%) by cross-linking[8] mediated by a water-soluble carbodi-imide (>0.5%, Table II). The i.r. spectra of PSC and PSGC and of xerogels of PSCgels and PSGCgels were similar. Absorptions at 1550 (NH of amide and CO_2^-) and 1390–1400 cm^{-1} (C-O) were retained even in the xerogel produced from the PSGC of d.s. 0.09,

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TABLE I

DATA FOR PARTIALLY *N*-SUCCINYLATED CHITOSANS AND GLYCOLCHITOSANS

Anhydride used (mole/amino group)	*Degree of N-succinylation*[a]			$[\alpha]_D^{18}$ *(degrees)*	*Solubility*[b]		
	A	B	C		*Water*	*0.5M AcOH*	*0.5M NaOH*
Partially N-succinylated chitosans							
0.24	0.10	0.15	0.11	−15[d]	—	—	—
Found: C, 40.31; H, 6.97; N, 7.04. $[X^c(H_2)_{0.76}(COC_2H_3CO_2Na)_{0.09} \cdot 1.1\ H_2O]_n$							
Calc.: C, 40.33; H, 7.02; N, 7.06%.							
0.49	0.14	0.18	0.20	−16[d]	S	+	—
Found: C, 39.81; H, 6.83; N, 6.63. $[X^c(H_2)_{0.68}(COC_2H_3CO_2Na)_{0.17} \cdot 1.2\ H_2O]_n$							
Calc.: C, 39.94; H, 6.84; N, 6.67%.							
0.74	0.30	0.29	0.28	n.d.[e]	—	+	S
Found: C, 39.84; H, 6.67; N, 6.64. $[X^c(H_2)_{0.60}(COC_2H_3CO_2Na)_{0.25} \cdot 1.2\ H_2O]_n$							
Calc.: C, 39.92; H, 6.64; N, 6.38%.							
0.98	0.35	0.34	0.35	−16[f]	—	—	—
Found: C, 40.49; H, 6.66; N, 6.30. $[X^c(H_2)_{0.55}(COC_2H_3CO_2Na)_{0.40} \cdot 1.0\ H_2O]_n$							
Calc.: C, 40.55; H, 6.45; N, 6.31%.							
3.67	0.63	0.66	0.61	−15[f]	—	—	—
Found: C, 37.57; H, 5.91; N, 5.22. $[X^c(H_2)_{0.33}(COC_2H_3CO_2Na)_{0.52} \cdot 2.0\ H_2O]_n$							
Calc.: C, 37.82; H, 6.36; N, 5.26%.							
14.6	0.79	0.80	0.80	−15[f]	—	—	+
Found: C, 37.95; H, 5.84; N, 5.06. $[X^c(H_2)_{0.17}(COC_2H_3CO_2Na)_{0.68} \cdot 2.0\ H_2O]_n$							
Calc.: C, 37.81; H, 6.11; N, 4.88%.							
Partially N-succinylated glycolchitosans							
1.2	0.09	0.10	n.d.[e]	−12[f]	—	—	—
2.9	0.21	0.22	n.d.[e]	−21[f]	+	—	—
5.9	0.53	0.53	n.d.[e]	−20[f]	—	—	—
8.8	0.66	0.64	n.d.[e]	−20[f]	—	—	—
14.7	0.79	0.74	n.d.[e]	−22[f]	+	+	+

[a]Determined from *A*, the amount of succinic acid released by saponification; *B*, the reaction of unsubstituted amino groups with ninhydrin; and *C*, elemental analytical data (see Experimental); and expressed as *N*-succinyl groups/amino group. [b]Key: —, insoluble; +, soluble; S, swelled. [c]X connotes a glucosaminide residue: $C_6H_{10}(COCH_3)_{0.15}$. [d]In 5% acetic acid (*c* 1). [e]Not determined. [f]In water (*c* 1).

suggesting that only a few of the *N*-succinyl groups were involved in cross-links. PSCgels and PSGCgels were transparent and colorless, swelled on soaking in water (xerogel), and could be regenerated.

The PSCgels and PSGCgels may be useful model systems for biochemical reactions, and their use for immobilising enzymes will be reported elsewhere.

EXPERIMENTAL

Materials and methods. — Chitosan, $[\alpha]_D^{18}$ −15° (*c* 1, 10% acetic acid), was prepared[10] from chitin. Glycolchitosan was a commercial product (Wako Pure Chemical Industries, Ltd.), and the equivalent molecular weight per amino group

TABLE II

GELATION OF PARTIALLY *N*-SUCCINYLATED CHITOSANS AND GLYCOLCHITOSANS

Chitosans					*Glycolchitosans*				
Degree of[a] N-*succinylation*	(%)[c]	*Gelation*[d] *Water-soluble carbodi-imide* (%) 1.8	0.9	0.45	*Degree of*[b] N-*succinylation*	(%)[c]	*Gelation*[d] *Water-soluble carbodi-imide* (%) 1.8	0.9	0.45
0.20	0.5	—	—	—	0.09	0.5	—	—	—
	1.0	+	—	—		1.0	—	—	—
	2.0	+	+	+		2.0	+	+	—
0.35	0.5	+	+[e]	+[e]	0.53	0.5	—	—	—
	1.0	+	+	+[e]		1.0	+	+	+[e]
	2.0	+	+	+		2.0	+	+	+
0.61	0.5	—	—	—	0.79	0.5	—	—	—
	1.0	+	+[e]	—		1.0	+	+[e]	+[e]
	2.0	+	+	+		2.0	+	+	+
0.80	0.5	—	—	—					
	1.0	—	—	—					
	2.0	+	+	+					

[a]Determined from the elemental analytical data. [b]Determined by g.l.c. [c]Concentration of partially *N*-succinylated chitosans and glycolchitosans. [d]Key: —, no gels formed; +, gels formed. [e]Solubilised by the addition of water.

was 300.8 as determined by colloid titration[9,10] with 2.5mM potassium poly(vinyl-sulfate) (Wako). 1-Ethyl-3-(3-dimethylaminopropyl)carbodi-imide · HCl was purchased from the Peptide Institute, Protein Research Foundation, Osaka (Japan). I.r. spectra were recorded with a Jasco IRA-1 diffraction-grating spectrometer, and specific rotations with a Jasco DIP-SL automatic polarimeter. Elemental analyses were performed at the Chemical Analysis Center of this University. G.l.c. was performed at 140° with a Shimadzu gas chromatograph (GC-5A) equipped with a flame-ionisation detector and with N_2 as the carrier gas at 60 ml/min.

Partial N-*succinylation of chitosan and glycolchitosan.* — Samples (2 g) of chitosan and glycolchitosan were separately dissolved in 5% acetic acid (40 ml), and the solutions diluted with methanol (160 ml). Succinic anhydride (0.24–14.6 mole/amino group, 0.25–15 g) dissolved in the minimum volume of acetone was added with vigorous stirring, and each mixture was stored at room temperature overnight. Gelling occurred after a few hours, except when the reaction was performed with 0.24 mole of anhydride. Each product was dissolved in, or diluted with, water (200 ml), and converted into the sodium salt by adjustment of the pH to 10 with 2M NaOH. Each solution was dialysed against distilled water for 5 days, concentrated to ~100 ml, and lyophilised, and the residue was dried over P_2O_5 at 60° for 5 h *in vacuo* (yields, 2.2–1.8 g).

Determination of degree of N-*succinylation.* — Samples (10 mg) of PSC and

PSGC were *N*-desuccinylated with 6M NaOH (0.5 ml) for 14 h at 110°, and the mixture was diluted with 2.5% acetic acid (8.5 ml). A portion (1.5 ml) of the solution was applied to a column (2 ml) of Dowex 1-X8 (H^+) resin and eluted with water (8.0 ml). The eluate was concentrated to dryness, and the liberated succinic acid was trimethylsilylated with *N*-methyl-*N*-trimethylsilylacetamide and analysed by g.l.c. with pentaerythritol as the internal standard. Alternatively, the color generated by the ninhydrin reaction was compared, using chitosan and glycolchitosan as standards. The degree of succinylation was calculated from elemental analytical data.

Preparation of PSCgel and PSGCgel. — Samples (5–40 mg; final concentration, 0.5–4.0%) of PSC and PSGC were separately dissolved in distilled water or 1.0% acetic acid (0.5 ml). To each solution was added water-soluble carbodi-imide (4.5–36 mg; final concentration, 0.45–3.6%), and the mixture was stored at 30° for 5 h. Each resulting gel was homogenised and suspended in distilled water (15 ml) for 2 days (with three changes of water to remove any urea derivatives), and then suspended in ethanol (10 ml) at room temperature overnight, collected, washed with ether, and dried over P_2O_5 at 100° for 5 h *in vacuo*, to give a colorless powder.

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