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NMR analyses of polysaccharide derivatives containing amine groups

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Amylose, amylopectin, hydroxyethylcellulose, methylcellulose, and cellulose were reacted with diethylaminoethyl chloride HCl salt and 3-chloro-2-hydroxypropyltrimethylammonium chloride under aqueous alkaline conditions in order to introduce tertiary amine and quaternary ammonium groups into polysaccharides. Degrees of substitution were obtained from ^{1}H- or ^{13}C-NMR spectra of hydrolyzates, and distributions of diethylaminoethyl groups in polysaccharides were measured by ^{13}C-NMR. Since amylose, amylopectin, and hydroxyethylcellulose were soluble in the reaction media, these three polysaccharides had higher reactivity for etherifications than cellulose. Methylcellulose, which has hydrophobic methyl groups, had as much reactivity as cellulose. Primary hydroxyl groups, C-6, of polysaccharides had the highest reactivity for diethylaminoethylation.

INTRODUCTION

Polysaccharide derivatives containing amine groups have unique properties as cationic polysaccharides. Since most of organic and inorganic compounds have a negative charge in aqueous suspension, cationic polymers have significant roles in controlling the surface charge of solids in aqueous suspension. Especially in the papermaking process, cationic starches are abundantly used as retention aids, strength agents, and sizing chemicals. Diethylaminoethyl starch (DEAE starch) and 2-hydroxy-3-trimethylammoniopropyl starch (HTMAP starch) have mainly been used in papermaking, and the degrees of substitution (DS) are generally within the range 0·01–0·04. Tertiary amine groups are cationized when the amine groups are protonated, whereas quaternary ammonium groups maintain the cationic charge over all pH ranges.

Cationic polysaccharides are generally prepared by etherification of polysaccharides with aqueous alkali and alkyl halides containing amine groups (Hartmann, 1930). Diethylaminoethylation was applied to cellulose, and distributions of DEAE groups were examined by gas chromatography (GC) (Roberts & Rowland, 1967; Rowland *et al.*, 1971; Roberts *et al.*, 1972; Rowland & Wade, 1980; Rowland & Howley, 1985, 1988; Bertoniere & Zeronian, 1987). HTMAP cyclodextrin was prepared as a phase-transfer catalyst, and DS and distribution of HTMAP groups were analyzed by ^{1}H- and ^{13}C-NMR, respectively (Deratani *et al.*, 1989). HTMAP cellulose and HTMAP hydroxyethylcellulose were prepared using a homogeneous LiCl-dimethylacetamide system, and their polyelectrolyte complexes with anionic cellulose derivatives had excellent blood compatibility (Ito *et al.*, 1986*a*,*b*). DS and distributions of substituents of polysaccharide derivatives have been analyzed using elementary analysis, titration of functional groups, GC and others. However, especially in the case of ionic polysaccharides, it is often difficult to obtain correct DS values because of errors due to counter-ions, inaccuracy of colloid titration, and indistinct endpoints of color reactions. Although GC analyses of hydrolyzates of DEAE polysaccharides have been reported (Roberts & Rowland, 1967; Rowland *et al.*, 1971; Roberts *et al.*, 1972; Rowland & Wade, 1980; Rowland & Howley, 1985, 1988; Bertoniere & Zeronian, 1987), partial protonations of DEAE groups even under alkaline conditions cannot be negligible for DS and distribution analyses. Hydrolyzates containing protonated amine groups are no longer volatile even after trimethylsilylation. On the other hand, DS and distributions of substituents have been recently analyzed by NMR (Goodlett *et al.*, 1971; Parfobdry & Perlin, 1977; Ho & Klosiewicz, 1980; Lee & Perlin, 1982; Reuben & Conner, 1983; Isogai *et al.*, 1984; Reuben, 1986; Reuben & Casti, 1987, Tezuka *et al.*, 1987, 1990).

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In this study, therefore, DEAE polysaccharides and HTMAP polysaccharides were prepared from amylose, amylopectin, hydroxyethylcellulose, methylcellulose, and cellulose; and DS and distributions of substituents were analyzed by NMR methods. The relationships between DS and either amine groups were introduced, and the structures of polysaccharide, or preparation conditions, were examined.

MATERIALS AND METHODS

Samples

Amylose (potato), amylopectin (potato), hydroxyethylcellulose (DS = 0·7), and methylcellulose (DS = 1·6) were commercially available (Wako Chemicals Inc., Tokyo, Japan). Microcrystalline cellulose powder (Avicel, Asahi Chemicals Inc., Tokyo, Japan) was used as a cellulose sample. The highest grade of reagents (Wako Chemicals Inc.), diethylaminoethyl chloride HCl salt, 50% aqueous solution of 3-chloro-2-hydroxypropyltrimethylammonium chloride, and 3-chloropropyltrimethylammonium chloride were used for etherifications of polysaccharides.

Etherifications of polysaccharides

Polysaccharides were etherified under aqueous alkaline conditions (Bullock & Guthrie, 1964): to one gram of a polysaccharide sample, 7·8 ml of a sodium hydroxide solution was added, and the mixture was stirred at room temperature for 0·5 h. Then an etherification reagent was added to the mixture. Molar ratios, DEAE-Cl/NaOH and HTMAP-Cl/NaOH, were constant as 1 : 25 for all etherifications. The reaction products were obtained by dialysis followed by freeze-drying.

Triphenylmethylamylose was prepared as follows (Roberts, 1963): amylose (2 g), pyridine (33 ml) and triphenylmethyl chloride (8 g) were stirred at 90 °C for 16 h. The reaction product was regenerated by pouring the reaction mixture into methanol, and was isolated as white powder (*c.* 3·5 g).

Analyses

A polysaccharide derivative (100 mg) was dissolved in 72% sulfuric acid (1 ml) at room temperature, and the solution was left standing for 2 h for complete dissolution. Then water (22 ml) was added to the mixture to reduce the concentration of sulfuric acid to 3%, and the solution was heated at 120 °C for 1 h. The solution obtained was neutralized with barium carbonate, and the supernatant was concentrated. The syrup was then dried *in vacuo*. Deuterated dimethylsulfoxide (0·5 ml) was added to the syrup, and the sample was subjected to NMR analyses. One drop of deuterated sulfuric acid was added to some NMR samples. ^{1}H- and ^{13}C-NMR spectra of hydrolyzates of polysaccharide derivatives were recorded on a Brucker AC-300 (magnetic field = 7·05 T, frequencies = 300·1 MHz for ^{1}H and 75·5 MHz for ^{13}C). When DS and distributions of substituents were obtained from ^{13}C-NMR spectra, the inverse-gated decoupling method for elimination of the nuclear Overhauser effect (NOE) was applied with a 10-s pulse delay at the 45° tip angle of the macroscopic magnetization and the pulse width of 7 μs for quantitative measurements (Landucci, 1985). The number of spectral acquisitions of ^{13}C-NMR was about 1000.

The molecular weight of polysaccharides was determined by high performance size exclusion chromatography (HPSEC) as phenylisocyanate derivatives dissolved in tetrahydrofuran (Wood *et al.*, 1986; Isogai & Usuda, 1991).

RESULTS AND DISCUSSION

NMR analyses of hydrolyzates

Figure 1 shows ^{1}H-NMR spectra of hydrolyzates of DEAE amylose (DS = 0·03). When deuterated sulfuric acid was not present in the solution, a broad peak due to hydroxyl groups of water and hydrolyzates was overlapped with resonances due to anomeric protons of glucose and glucose derivatives. Furthermore, since DEAE groups of hydrolyzates were partly protonated in DMSO, methyl protons of protonated DEAE groups appeared at 1·20 ppm, and those of free DEAE groups appeared at 0·97 ppm. When one drop of deuterated sulfuric acid was added to the solution, the resonance due to DOH was shifted at 6–8 ppm, and all the methyl protons of the DEAE groups were protonated and were shifted at 1·22 ppm. Other protons of the DEAE groups were overlapped with those of glucose and DEAE glucose. Thus, DS of DEAE polysaccharides were obtained from peak ratios between anomeric protons (4·27, 4·29, 4·91 and 4·92 ppm) and the methyl protons of DEAE groups by this ^{1}H-NMR method.

Figure 2 shows ^{13}C-NMR spectra of hydrolyzates of DEAE amylose (DS = 0·22) with and without one drop of deuterated sulfuric acid. As described previously, DEAE groups were partly protonated when D_2SO_4 was not present in the NMR solutions. Furthermore, chemical shifts of carbons due to DEAE groups were influenced by the substituted positions C-2, C-3, and C-6 of glucose. Thus, carbons of DEAE groups showed complex patterns, and different patterns were often obtained even from the same DEAE polysaccharide samples. When D_2SO_4 was present in NMR solutions, all DEAE groups were protonated and only one NMR pattern was obtained from one sample. Methyl carbons (C-10) of DEAE groups had a single resonance at 8·39 ppm, and C-9 and C-8 carbons were

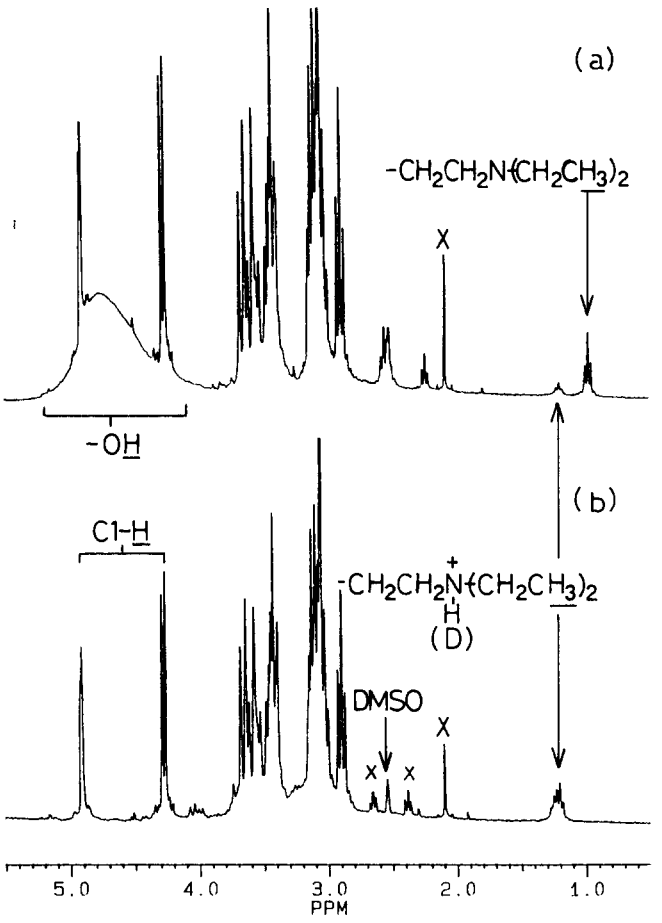

Fig. 1. ^{1}H-NMR spectra of hydrolyzates of diethylaminoethyl amylose (DS = 0·03): (a) without D_2SO_4; (b) with D_2SO_4.

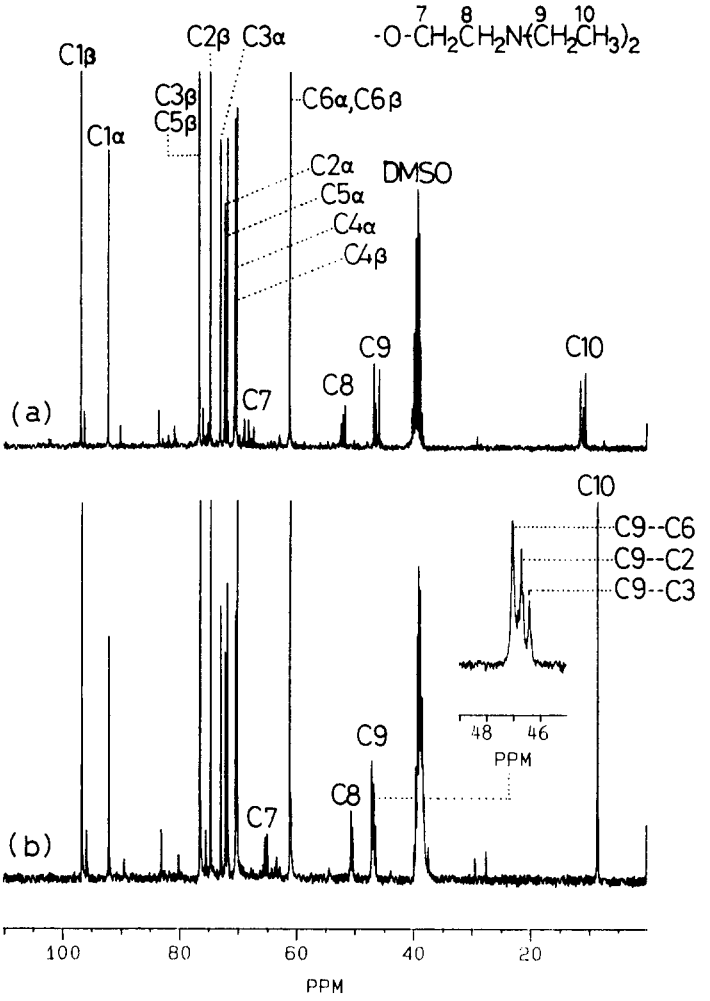

Fig. 2. ^{13}C-NMR spectra of hydrolyzates of diethylaminoethyl amylose (DS = 0·22): (a) without D_2SO_4; (b) with D_2SO_4.

shifted at 46·37–47·00 and 50·23–50·55 ppm, respectively. C-7 carbons were shifted at probably 65·24–64·85 ppm. When triphenylmethylamylose was diethylaminoethylated and its hydrolyzates were analyzed by ^{13}C-NMR, the resonance at 47·00 ppm disappeared. Therefore, this resonance is due to C-9 carbons of DEAE groups linked to C-6. Since it is well known that hydroxyl groups of C-2 are more reactive than C-3 for almost all etherifications under aqueous alkaline conditions (Isogai *et al.*, 1984), the resonances at 46·65 and 46·37 ppm must be due to C-9 carbons of DEAE groups linked to C-2 and C-3, respectively. Thus, distributions of DEAE groups can be measured from ^{13}C-NMR spectra of hydrolyzates of DEAE polysaccharides by the addition of D_2SO_4. Although split resonances due to C-7 and C-8 carbons of DEAE groups reflected distribution of the substituent, distribution ratios were not clear when DS was lower than 0·03.

Figures 3 and 4 show ^{1}H- and ^{13}C-NMR spectra, respectively, of hydrolyzates of HTMAP-amylose (DS = 0·03). In the case of HTMAP cyclodextrin, methyl and methine protons of HTMAP groups appeared at 3·04 and 4·24 ppm, respectively (Deratani *et al.*, 1989). However, all protons of HTMAP groups of

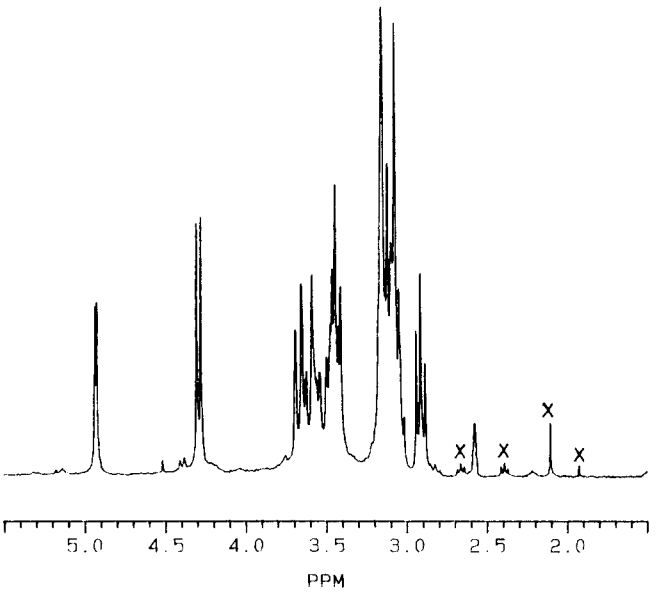

Fig. 3. ^{1}H-NMR spectrum of hydrolyzates of 2-hydroxy-3-trimethylammoniopropyl amylose (DS = 0·03).

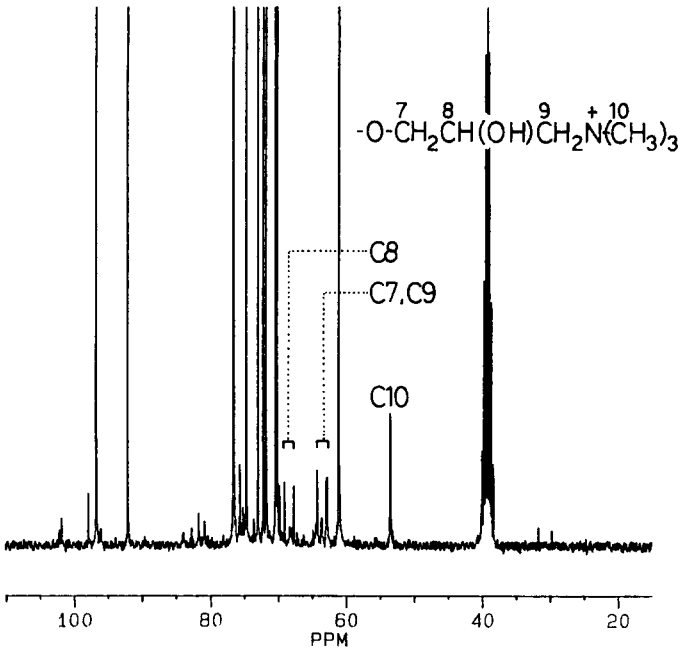

Fig. 4. ^{13}C-NMR spectrum of hydrolyzates of 2-hydroxy-3-trimethylammoniopropyl amylose (DS = 0·03).

hydrolyzates were overlapped with those of glucose and HTMAP glucose. Thus, neither DS nor distributions of HTMAP groups could be obtained from ^{1}H-NMR spectra of hydrolyzates. On the other hand, methyl carbons of HTMAP groups appeared at 53·44 ppm as a single resonance in ^{13}C-NMR spectra of hydrolyzates of HTMAP amylose. Since the resonances at 92·02–101·86 ppm were due to C-1 carbons of glucose and HTMAP glucose, the DS values were calculated from area ratios of C-1 and methyl carbons using the inverse-gated decoupling method. These DS values were almost identical to those obtained by the usual power-gated decoupling method, and differences between them were within 5%. Four resonances at 62·72–64·22 ppm were due to methyl carbons of C-7 and C-9 positions of HTMAP groups, and three resonances at 67·67–69·00 ppm were due to β-carbons. These patterns must reflect distributions of HTMAP groups linked to C-2, C-3 and C-6 of glucose. However, since triphenylmethylamylose did not react with HTMAP-Cl, a definite distribution of HTMAP groups could not be measured at this point. Four resonances at 80·77–84·41 ppm may be due to C-2 and C-3 carbons linked to HTMAP groups, and two resonances at 97·84 and 101·86 ppm may be due to C-1 carbons of glucose, whose C-2 position was linked to HTMAP groups. Nevertheless, an indirect calculation method using resonances due to C-1, C-2 and C-3 carbons and DS of HTMAP did not give correct distributions, probably because of the low DS of HTMAP groups: DS_2 = shifted C-1 carbons; DS_3 = substituted C-2 and C-3 carbons − DS_2; and $DS_6 = DS - DS_2 - DS_3$.

Thus, DS values of DEAE polysaccharides and HTMAP polysaccharides were obtained from NMR spectra of hydrolyzates. Since effects of counter-ions of amine groups can be excluded by NMR methods, correct DS values can be obtained. The NMR methods gave slightly higher DS values than the nitrogen content method, probably because of the effects of counter-ions and/or incomplete drying of hygroscopic amino polysaccharides.

Reactivity of polysaccharides and distribution of substituents

Amylose, amylopectin, hydroxyethylcellulose, methylcellulose, and microcrystalline cellulose powder were etherified with DEAE-Cl and HTMAP-Cl, and DS values were obtained from NMR spectra. Even though hydroxyethylcellulose and methylcellulose had hydroxyethyl and methyl groups, respectively, DS values were obtained by the same NMR methods. Figure 5 shows the relationship between the degree of substitution per hydroxy group of original polysaccharides (DS′) and the molar ratios of DEAE-Cl/hydroxyl group of the original polysaccharides for diethylaminoethylation. Since hydroxyethylcellulose and methylcellulose had substituents, the reactivity for diethylaminoethylation among polysaccharides was compared using the degree of substitution per free hydroxyl group of the original polysaccharides (DS′). Here DS′ is obtained from DS divided by number of hydroxy groups of an anhydroglucose residue of the original polysaccharides. Although amylopectin is actually a branched polysaccharide, it is regarded as a linear polysaccharide for convenience. From this figure, the reactivity of polysaccharides for diethylaminoethylation was in the following order: amylose = amylopectin = hydroxyethyl-cellulose > methylcellulose ⩾ cellulose. The

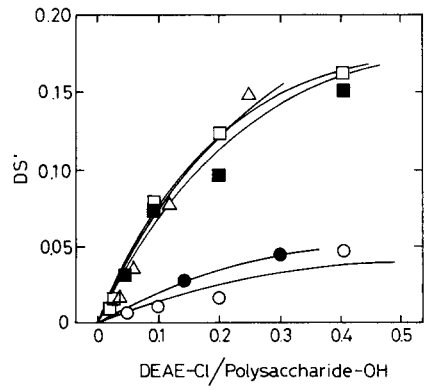

Fig. 5. Relationship between DS′ of diethylaminoethyl polysaccharides and molar ratios of diethylaminoethyl chloride HCl salt/hydroxy group of the original polysaccharides: amylose (□), amylopectin (■), hydroxyethylcellulose (△), methylcellulose (●), and cellulose (○). DS′ is the degree of substitution per hydroxyl group of the original polysaccharides. The molar ratio of diethylaminoethyl chloride HCl salt/NaOH is 1 : 25.

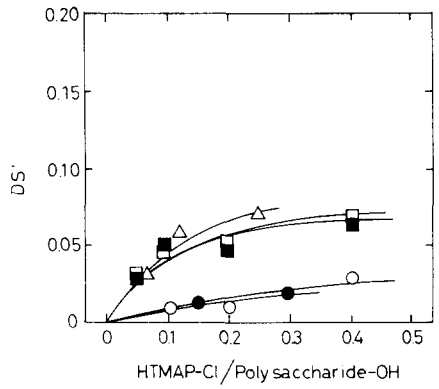

Fig. 6. Relationship between DS' of 2-hydroxy-3-trimethylammoniopropyl polysaccharides and molar ratios of 3-chloro-2-hydroxypropyltrimethylammonium chloride/hydroxy group of original polysaccharides: amylose (□), amylopectin (■), hydroxyethylcellulose (△), methylcellulose (●), and cellulose (○). See caption to Fig. 5.

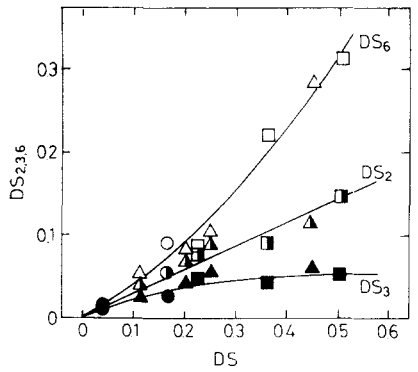

Fig. 7. Distribution of diethylaminoethyl groups of polysaccharides between C-2, C-3 and C-6 of anhydroglucose residue: amylose (□, ◨ and ■), amylopectin (△, ◭, and ▲), and cellulose (○, ◑ and ●). DS_6: □, △ and ○; DS_2: ◨, ◭ and ◑; DS_3: ■, ▲ and ●.

former three polysaccharides were soluble in the reaction media, and thus resulted in high reactivity owing to homogeneous reactions. Although methylcellulose was water-soluble, it showed low reactivity, probably because of the hydrophobic methyl groups. Since cellulose was insoluble but only swollen in the medium, it had the lowest reactivity.

The relationship between DS' and HTMAP-Cl/hydroxy group of original polysaccharides is shown in Fig. 6. The reactivity of the hydroxyl groups of polysaccharides for the introduction of HTMAP groups was in the following order: amylose = amylopectin = hydroxyethylcellulose > methylcellulose ⩾ cellulose. This reactivity order was almost equal to that for diethylaminoethylation. However, DS' values were about one-third of those for DEAE polysaccharides. Since no reactions occurred on polysaccharides when 3-chloropropyltrimethylammonium chloride was used as an etherification reagent, HTMAP-Cl must react with polysaccharides through epoxide form under alkaline conditions.

Distributions of DEAE groups between C-2, C-3 and C-6 of an anhydroglucose residue of amylose, amylopectin and cellulose is shown in Fig. 7. The primary hydroxyl group, C-6, showed the highest reactivity for all the DS range. When the DS values were lower than 0·3, the differences between DS_6 and DS_2 were not so clear. When the DS values were higher than 0·3, the relative reactivity had the following evident order: C-6 > C-2 > C-3.

Degrees of polymerization (DP_w) of amino polysaccharides were roughly calculated from HPSEC patterns using polystyrene standards (Wood *et al.*, 1986; Isogai & Usuda, 1991). When the DS values of amine groups were lower than 0·2, depolymerization was negligible for all polysaccharides. However, the DP_w values of DEAE polysaccharides were reduced to about half of the original ones when the DS values were about 0·5.

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