Hydration Properties of an Amino Sugar-Containing Synthetic Polymer by DSC and NMR Spectroscopy

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Poly(D-glucopyranosyl-α(1 → 6)-2-deoxy-2-methacrylamido-D-mannitol-co-D-glucopyranosyl-α(1 → 6)-2-deoxy-2-methacrylamido-D-glucitol) (poly(MAGPM-co-MAGPG)) was prepared by radical polymerization from methacrylamide monomers which were synthesized by the methacryloylation of amino sugars. The fraction of a different state of water in poly(MAGPM-co-MAGPG) was determined using DSC. The temperature dependence on the chemical shift of the ^{1}H NMR was different between the low and high water retentions. The ^{17}O spin-lattice relaxation time of water in poly(MAGPM-co-MAGPG) was compared to that of glucose, Palatinit®, and PVA. The interaction between the water and the hydroxy groups in poly(MAGPM-co-MAGPG) is strongly affected not only by the mobility of the sugar side chain, but also by the highly concentrated hydroxy groups along the hydrophobic main chains. The sugar side chain in poly(MAGPM-co-MAGPG) has a considerably higher hydration ability in comparison with glucose, Palatinit®, and PVA, although these substances have a hydroxy group as a hydrophilic group in common.

Sugar chains are found in all forms of life, which exist in glycoproteins, peptidoglycans, and proteoglycans occurring in blood, plasma membranes, intercellular matrices, and connective tissues.[1] It was demonstrated that although these substances contain only a few different monosaccharides, they vary markedly in their carbohydrate content, in the number and degree of branching of their carbohydrate units, and in the distribution of such units along the polypeptide chains. The carbohydrate units of the glycoproteins play important roles in various kinds of biological events,[2] which are recognition phenomena, such as those that regulate cell differentiation and growth, and that dearrangement of this recognition might lead to pathological phenomena, including malignancy.

Poly(vinylsaccharide)s, which exhibit biological functions, have been used in biomedical applications, such as cell-specific culture substrate.[3—5] Oligosaccharide-carrying polystyrene[6] and polypeptide[7] functioned as specific recognition markers for lectins and hepatocytes.[8] These functions are attributable to the biological recognition phenomena induced by lectins and hepatocytes onto the mono- and oligosaccharides.

Poly(vinylsaccharide)s have a synthetic backbone and side chains derived from naturally occurring carbohydrates. These monomers result from the coupling reaction of an unsaturated component with a carbohydrate derivative. The linkage is usually formed by an ether-, ester-, or amido group. The synthesis of unsaturated amides from amino-sugars[9] or sugar-lactones[10] has no use for protecting groups. The use of commercially available amino sugars, such as glucosamine hydrochloride, was possible[11] and via the reductive amination of reducing sugars further interesting components could be prepared. These polymers have attracted more interest as biomaterials for contact lens, artificial lungs, and skin because of their biocompatiblity.[12,13] These materials are usually used in the wet state. The performance of the materials is affected by water.[14—22] The hydration properties are defined as the distribution and mobility of water around the substances. The hydration properties have been studied using differential scanning calorimetry (DSC)[14,15] and NMR.[16—19,21—29] The fraction of a different state of water, meaning whether it freezes at sub-zero temperatures, is determined using DSC. The hydration dynamics of water around the polar groups is evaluated using ^{17}O NMR.[16—18,21—30] The hydration properties of some polymers[16—18,21,22] and low-molecular-weight molecules[22—30] have already been studied using ^{17}O NMR. It has been reported that the mobility of water around sugars is restricted because of the equatorial OH groups existing in the various conformers of sugar.[27—29] Whether the sugar side chains of synthetic polymers retain the specific hydration properties of low-molecular-weight sugars is important. Despite the large number of investigations on the synthesis and characterization of synthetic polymers with sugar chains, there has been no systematic detailed study of the hydration properties for synthetic polymers having sugar chains.

The objective of this study was to establish a clear view of the hydration properties of the sugar side chains of poly(D-glucopyranosyl-α(1 → 6)-2-deoxy-2-methacrylamido-D-mannitol-co-D-glucopyranosyl-α(1 → 6)-2-deoxy-2-methacrylamido-D-glucitol) (poly(MAGPM-co-MAGPG)). The amino sugar used in this study was an equivalent molar mixture of D-glucopyranosyl-α(1 → 6)-2-amino-2-deoxy-D-mannitol (GPM) and D-glucopyranosyl-α(1 → 6)-2-amino-2-deoxy-D-glucitol (GPG). D-Glucopyranosyl-α(1 → 6)-2-deoxy-2-methacrylamido-D-mannitol (MAGPM) and D-

glucopyranosyl-α(1 → 6)-2-deoxy-2-methacrylamido-D-glucitol (MAGPG), a methacrylamide monomer having a sugar side chain, were synthesized by the methacryloylation of GPM and GPG, respectively. Poly(MAGPM-co-MAGPG) was prepared by radical polymerization. The structures of these compounds are shown in Fig. 1. The hydration properties of poly(MAGPM-co-MAGPG) were determined by DSC, the chemical shift of ^{1}H NMR, and the spin-lattice relaxation time of the ^{17}O NMR of water. We assume that the hydration properties of the sugar side chain of MAGPM and MAGPG units in poly(MAGPM-co-MAGPG) are identical because only the configuration of the amino group bound one asymmetric carbon atom (C2) in their sugar side chains is different, while the amount and configuration of the hydroxy groups are the same in the isomers. The mobility of water in poly(MAGPM-co-MAGPG) is compared to that of water in poly(vinyl alcohol) and low-molecular-weight sugars, such as glucose and Palatinit®, whose structures are the same as that of the sugar side chain of poly(MAGPM-co-MAGPG). These substances have a hydroxy group as a hydrophilic group. The effect of their structure on the mobility of water is discussed.

Experimental

Synthesis of MAGPM and MAGPG. An equivalent molar mixture of GPM and GPG, which was prepared from 6-*O*-α-D-glucopyranpsyl-D-fructofuranose (from Mitsui Sugar Co., Ltd.) by reductive amination,[31] was used in this study. The liquid chromatography of a 4.3 wt% amino sugar aqueous solution was performed using a column of Biorad Aminex A-7C (7.8 mm×250 mm) in 0.03 M $Ca(NO_3)_2$ aqueous solution (1 M = 1 mol dm^{-3}) at 35 °C. The only two isomers, GPM and GPG, were confirmed from a chromatogram for this sample, and their ratio was GPM : GPG = 59 : 41. Thirteen grams (3.7 mmol) of GPA, 35 ml of DMSO, and 3.0 g of pyridine were mixed in a 100-ml flask at 10 °C. The reaction was initiated by the addition of 5.8 g (3.7 mmol) of methacrylic anhydride. The mixture was stirred at room temperature for 2 h. The reaction was terminated by precipitating the compound with acetone. This solution was then filtered. The white solids were continuously washed with acetone and dried under vacuum for 24 h at room temperature. A white powder (8.4 g) consisting of a mixture of MAGPM and MAGPG was obtained in approximately 70% yield. Anal. Calcd for $C_{16}H_{29}NO_{11}\cdot 0.47H_2O$: C, 45.77; H, 7.13; N, 3.34%. Found C,45.79; H, 7.16; N, 3.35%. ^{13}C NMR of MAGPM (100 MHz, DMSO-d_6, TMS.) δ = 168.3 (C=O), 139.7 (–C(–CH$_3$)=CH$_2$), 119.5 (=CH$_2$), 18.5 (–CH$_3$), 98.7 (C(1′)), 70.1 (C(4′)), 60.7 (C(6′)), 60.6 (C(1)), 53.8 (C(2)), 70.1 (C(6)), 73.5, 72.5, and 72.2 for the remaining pyranose carbons, δ = 70.5, 68.8, and 67.8 for the methylene carbons in the mannitol part. ^{13}C NMR of MAGPG (100 MHz, DMSO-d_6, TMS) δ = 167.5 (C=O), 140.1 (–C(–CH$_3$)=CH$_2$), 118.9 (=CH$_2$), 18.5 (–CH$_3$), 98.7 (C(1′)), 70.1 (C(4′)), 60.7 (C(6′)), 60.4 (C(1)), 54.4 (C(2)), 69.5 (C(6)), 73.5, 72.5, and 72.2 for the remaining pyranose carbons, δ = 71.9, 69.4, and 67.2 for the methylene carbons in the glucitol part.

Synthesis of Poly(MAGPM-co-MAGPG). A mixture of 9.8 g of MAGPM and MAGPG in 23 ml of DMF and 22 ml of water was prepared in a glass ampoule with 0.049 g of AIBN as the initiator. The glass ampoule was attached to a vacuum line, degassed three times with liquid N_2, and then closed under vacuum. The polymerization proceeded at 60 °C for 48 h under high vacuum. The polymerization was terminated by precipitating the polymer with a large amount of methanol, and the product was dried under high vacuum. To remove the monomer, the product was dissolved in water, and then the aqueous solution was sealed in a dialysis tube of seamless cellophane tubing (Viskase Co., Ltd.). The dialysis tube was washed with pure water. The aqueous solution was subsequently freeze-dried to give a white solid at a yield of 7.2 g of poly(MAGPM-co-MAGPG) (73% yield). ^{13}C NMR of poly(MAGPM-co-MAGPG) (100 MHz, D_2O, TSP-d_4.) δ = 182.4 (C=O), 48.6 (–C(–CH$_3$)–CH$_2$–), 57.3 (–CH$_2$–), 20.3 (–CH$_3$), 101.4 (C(1′)), 72.6 (C(4′)), 63.7 (C(6′)), 63.7 (C(1)), 57.3 (C(2)), 72.6 (C(6)), 74.7, 74.9, and 76.3 for the remaining pyranose carbons, δ = 73.8 for the methylene carbon in the mannitol and glucitol parts. Poly(MAGPM-co-MAGPG) is dissolved only in water and highly polar organic solvents, such as dimethyl sulfoxide and *N*,*N*-dimethylformamide, but not in alcohol and organic solvents, such as benzene and chloroform.

Characterization of Monomer and Polymer. The ^{13}C and ^{1}H NMR spectra were recorded on a JEOL ALPHA 400S spectrometer. Dimethyl sulfoxide-d_6 (Me_2SO-d_6) containing tetramethylsilane (TMS) and deuterium oxide (D_2O) containing 3-(trimethylsilyl)propionic-2,2,3,3-d_4 acid, sodium salt (TSP-d_4) were used as the solvent. IR spectra were measured using KBr disks with a Perkin–Elmer 1800.

DSC Measurement. The glass transition temperature (T_g) and the fractions of the two states of water, which were called freezing

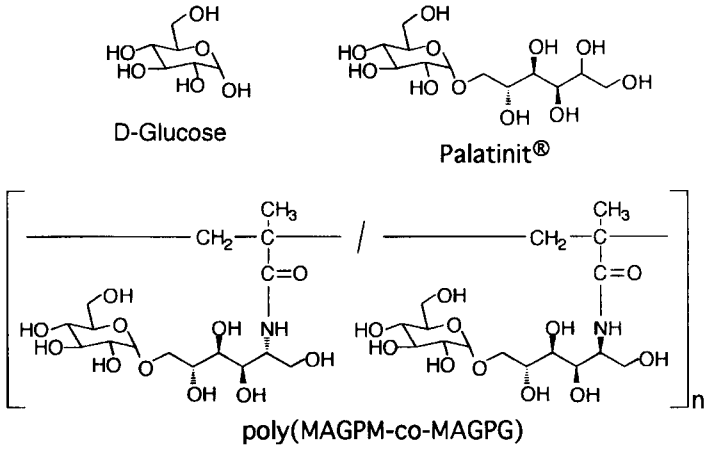

Fig. 1. The structure of poly(MAGPM-co-MAGPG), D-Glucose, and Palatinit®.

water (W_f) and bound non-freezing water (W_{Bnf}), were determined by a Perkin–Elmer DSC 7. The T_g was evaluated by DSC at a heating rate of 20 K min^{-1}. The amount of both states of water was estimated in the temperature range from 123 to 303 K at a heating rate and cooling rate of 10 K min^{-1}.[15,32]

Sealed aluminum pans were used for all of the DSC measurements and were boiled for 1 h in pure water to eliminate any possibility of $Al(OH)_3$ formation during the experiment. The poly(MAGPM-co-MAGPG) sample was used after drying at room temperature under a vacuum for 24 h. The amount of remaining water in this dried sample was determined by Karl Fisher measurement using a CA-05 (Mitsubishi Chemical Industries, Ltd.). The content of the residual water in the poly(MAGPM-co-MAGPG) sample after drying was 5.6 wt%. The dried polymer sample was weighed in the pan and pure water was added to the sample pan. The water content was adjusted by the amount of pure water. The sample pans were sealed and left at room temperature for 24 h to allow the sample to equilibrate. After the measurement, it was confirmed that the sample weight did not change during heating. The water content [g g^{-1}] was calculated using the following equation:

$$\text{Water content} = W_{H_2O}/W_{dry}, \quad (1)$$

where W_{dry} was estimated by subtracting the weight of the remaining water from the weight of the dried polymer and W_{H_2O} is the sum of the weight of the remaining water and the added pure water in the sample. The water retention (H) was calculated using

$$H = W_{H_2O}/(W_{dry} + W_{H_2O}). \quad (2)$$

In this study, two endothermic peaks were observed from −20 to 0 °C during the heating process. One peak appeared at around 0 °C and the other peak below 0 °C. The heat of fusion of water melting at the lower temperature might be different from that of pure water.[33]

There are some different crystal structures of ice under certain conditions.[34] Ices I, Ic, II, and III exist around atmospheric pressure from −150 to 0 °C. The peak around 0 °C corresponds to ice I. The melting enthalpy of water (ΔH) of ice I is 334 [J g^{-1}]. Nakamura et al.[35] suggested that the broadened peak at the lower temperature corresponded to one of these ice structures. The ΔH of ice III (312 [J g^{-1}]) is the smallest of these crystal structures. If the lower peak corresponds to ice III, the difference between employing the ΔH of ice I and ice III is 6.6%. As already mentioned, both endothermic peaks were analyzed by employing the ΔH of water at 0 °C (334 [J g^{-1}]). The amount of freezing water contains an experimental error of 6.6%. The following equation can be applied to the calculation of the amount of freezing water:

$$W_f = Q^h/\Delta H, \quad (3)$$

where Q^h is the heat absorbed during the heating process, which is calculated from the peak area on the DSC chart. W_{Bnf} was estimated by subtracting the weight of freezing water (W_f) from the weight of water in the sample (W_{H_2O}), as denoted by

$$W_{Bnf} = W_{H_2O} - W_f. \quad (4)$$

^{1}H NMR Measurement of Water. ^{1}H NMR spectra were recorded on a JEOL GSX-270 spectrometer operating at 270 MHz between −35 and 35 °C. The dry polymer, pure water, and a capillary enclosing the $CDCl_3$ solution containing TMS as the internal standard were placed in a sample glass tube. Samples with different water retentions were made by adjusting the amount of both the dried polymer and pure water in the ^{1}H and ^{17}O NMR experiments. The sample glass tubes were not spun. All chemical shifts were evaluated relative to TMS set at 0 ppm.

^{17}O NMR Measurement of Water. ^{17}O NMR spectra were recorded on a JEOL ALPHA 400S spectrometer operating at a 54.21 MHz ^{17}O NMR resonance frequency at 35 °C. Poly(vinyl alcohol) (PVA), supplied by Wako Chemical Co., Ltd., has a degree of polymerization of 2000. D-Glucose, supplied by Junsei Chemical Co., Ltd., and Palatinit®, supplied by Mitsui Sugar Co., Ltd., were used without further purification. A sample and pure water were placed in a capillary NMR tube. The 90 degree ^{17}O pulse width of 16 μs and a recycling time of 0.1 s were used. The number of scans was 1,024. The samples were not spun. The spin-lattice relaxation time, T_1, measurements of water in the samples were performed using the built-in auto-T_1 (180°—τ—90°) routine of the JEOL instrument. The T_1 was calculated by the least-squares method using the accompanying computer. Each experiment was repeated three times.

Theory of ^{17}O Relaxation Time

The spin-lattice relaxation times of ^{17}O in pure water and solutions, T_1° and T_1, respectively, are well correlated with the concentration of the solute by the following empirical equation:[18,23,24,27–30]

$$\frac{T_1^\circ}{T_1} = 1 + Bm + Cm^2, \quad (5)$$

where m is the molality of the sugar for D-glucose and Palatinit®, while for poly(MAGPM-co-MAGPG) and PVA, m is the molality of the repeat units in the polymer.

If the exchange times of water molecules between the hydration water (mole fraction x_h) and pure water (mole fraction $(1-x_h)$) are faster than the relaxation times of both pure water and hydration water (T_1° and T_{1h}), the following relationship is obtained:[36]

$$1/T_1 = (1 - x_h)/T_1^\circ + x_h/T_{1h}, \quad (6)$$

which can be rewritten as

$$T_1^\circ/T_1 = 1 + n_h(T_1^\circ/T_{1h} - 1)m/55.5, \quad (7)$$

where n_h is the number of water molecules adjacent to a solute molecule. From a comparison of Eqs. 5 and 7, the following relationship could be obtained:

$$55.5B = n_h(T_1^\circ/T_{1h} - 1), \quad (8)$$

The $n_h(T_1^\circ/T_{1h} - 1)$ is defined as the dynamic hydration number (n_{DHN}).[24,27] The dynamic hydration number represents significant information on the dynamic state of the hydration water relative to pure water, which increases with increasing n_h and decreasing T_{1h}.

Results and Discussion

Synthesis of Monomer and Polymer. The chemical structures of the white powder of MAGPM and MAGPG were verified by ^{13}C and ^{1}H NMR and IR after drying. The ^{13}C and ^{1}H NMR spectra of MAGPM and MAGPG are shown in Fig. 2. The signals of the N–H proton resonance

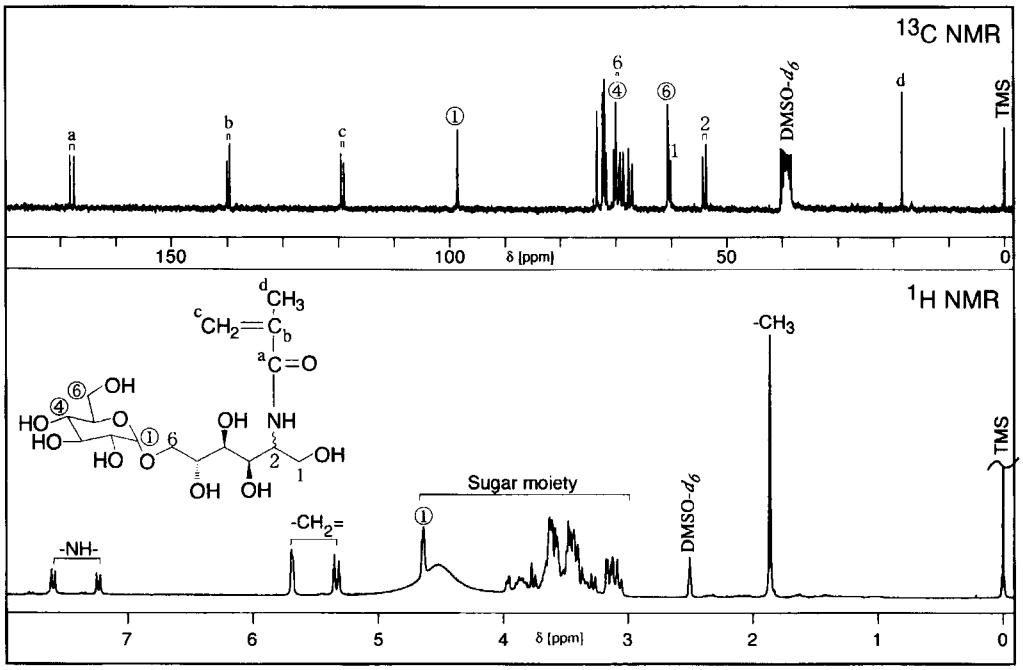

Fig. 2. ^{13}C NMR and ^{1}H NMR of MAGPM and MAGPG at 40 °C. Solvent, DMSO-d_6; TMS standard.

appear at 7.2 and 7.6 ppm, and the $-CH_2=$ proton resonance is at 5.7 and 5.4 ppm. The peaks at 168.3 and 167.5 ppm were the carbonyl carbon resonance. The formation of the amide linkage was confirmed by infrared absorptions at 1653 ($\nu_{C=O}$) and 1532 cm^{-1} (δ_{N-H}), and the 1604 cm^{-1} band was attributed to the vinyl group. The elementary analysis of the monomer corresponded to that expected (see Experimental section). There was little contamination in the ^{1}H NMR spectrum, which means sufficient purity for the polymerization; therefore, this monomer was used without further purification.

The ^{13}C and ^{1}H NMR spectra of poly(MAGPM-co-MAGPG) are shown in Fig. 3. The broadened peaks of the main chain methylene and α-methyl proton resonance appeared at 1.8 and 1.1 ppm. The peak at 57.3 ppm was the main chain methylene carbon resonance. The peak at 48.6 ppm was attributed to the main chain quaternary carbon resonance. The formation of the amide linkage was confirmed by infrared absorptions at 1651 ($\nu_{C=O}$) and 1564 cm^{-1} (δ_{N-H}). The carbon resonances of the pyranose ring of poly(MAGPM-co-MAGPG) were similar to those of MAGPM and MAGPG (see Experimental section).

DSC measurements. Figure 4 shows the DSC curves of poly(MAGPM-co-MAGPG) for the sample with $H > 0.4$ at temperatures ranging from -20 to 10 °C. The melting peaks of freezing water were observed for samples with $H > 0.40$. Neither the endothermic nor exothermic transition of the dry polymer was observed from -150 to 190 °C, and poly(MAGPM-co-MAGPG) was then decomposed at about 190 °C. Also, the glass transition temperature was not observed under 190 °C. Figure 5 shows the DSC curves of poly(MAGPM-co-MAGPG) for those samples with $H = 0.24$ and 0.25 at temperatures ranging from -150 to 70 °C. Only the glass transition temperatures (T_g) at 5.0 and 2.2 °C were

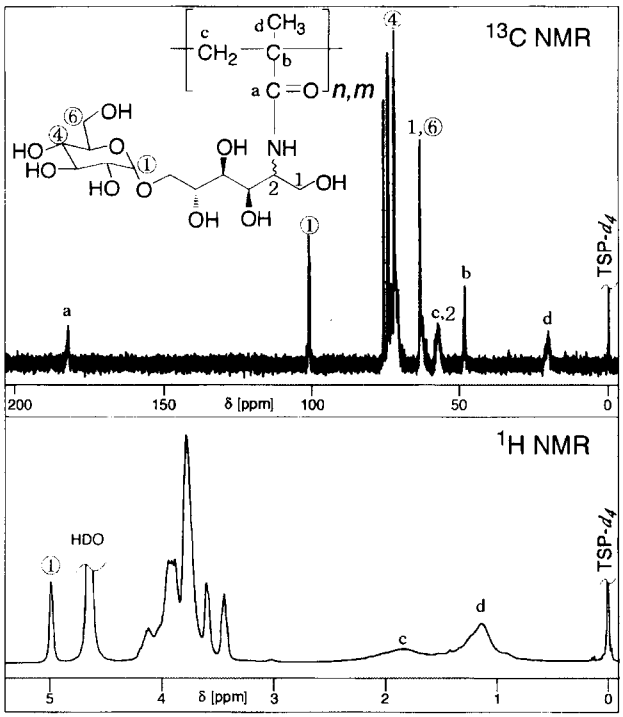

Fig. 3. ^{13}C NMR and ^{1}H NMR spectra of poly(MAGPM-co-MAGPG) at 40 °C. Solvent, D_2O; TSP-d_4 standard.

observed for the samples with $H = 0.24$ and 0.25. The T_g dropped with the increasing amount of bound non-freezing water in these samples. Because the bound non-freezing water could break the intermolecular hydrogen bonds of the polymer; the mobility of the polymer chains was increased by the increasing the amount of bound non-freezing water.

The changes in the bound non-freezing water content (W_{Bnf}/W_{dry}) and freezing water content (W_f/W_{dry}) are shown

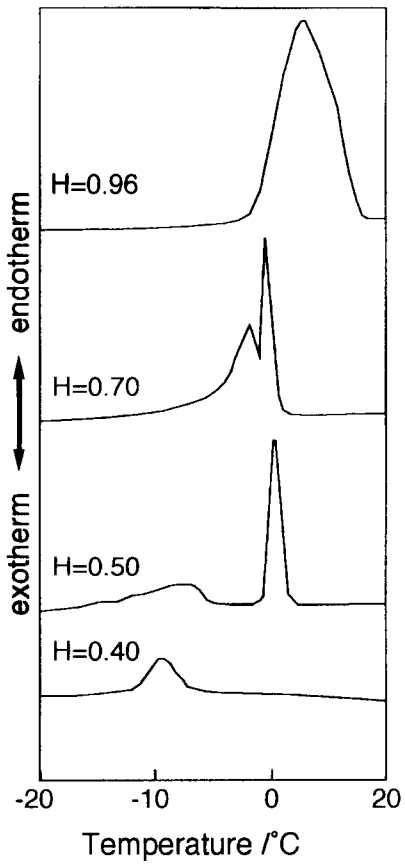

Fig. 4. DSC curves of poly(MAGPM-co-MAGPG) for various water retentions at a heating rate 10 K min^{-1}.

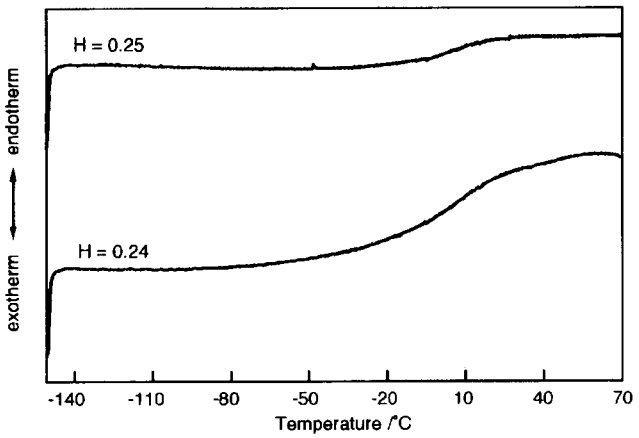

Fig. 5. DSC curves of poly(MAGPM-co-MAGPG) for the samples with $H = 0.24$ and 0.25 at a heating rate 20 K min^{-1}.

in Fig. 6 as a function of the water content for poly(MAGPM-co-MAGPG). From the intercept of W_f/W_{dry} and the x-axis, the amount of bound non-freezing water is evaluated. The value of W_{Bnf}/W_{dry} was 0.68 [g/g-polym], which corresponded to $H = 0.40$. At least, it is considered that all the water molecules in the samples cannot freeze at a subzero temperature when the water content is less than 0.68 [g/g-polym]. The W_{Bnf}/W_{dry} approached a constant value (0.73 [g/g-polym]) at a water content above 2.3 [g/g-polym] ($H > 0.70$).

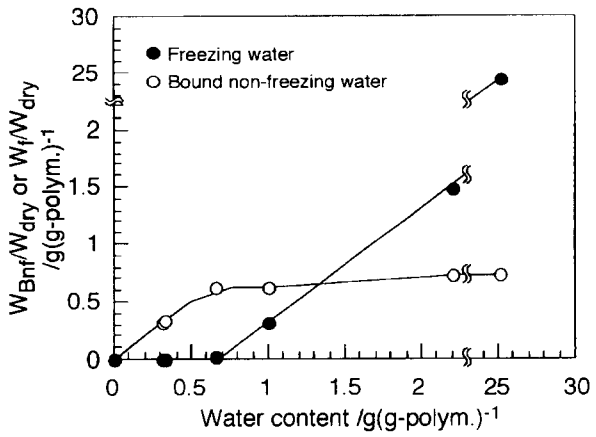

Fig. 6. Changes in bound non-freezing water content (W_{Bnf}/W_{dry}) and freezing water content (W_f/W_{dry}) as a function of water content for poly(MAGPM-co-MAGPG).

^{1}H NMR Measurement of Water. Figure 7 shows the ^{1}H NMR spectra for the sample with $H = 0.50$ over the temperature range from -15 to 35 °C. A weak peak of the sugar side chains of poly(MAGPM-co-MAGPG) was observed at around 3.5 ppm at 35 °C. The line width of the peak broadened, and the intensity decreased with decreasing temperature. The peak disappeared at -15 °C. The mobility of the sugar side chains significantly decreased with decreasing temperature from 35 to -15 °C.

Figure 8 shows the temperature dependence of the chemical shift of water for different water retentions. It has been reported by Carles et al.[19] that the signal of the water in moist cellulose moves to higher magnetic fields compared to pure water. In terms of the flicking cluster of water,[37] these highfield shifts are believed to be due to a displacement of the equilibrium between the strongly hydrogen-bonded clusters and the monomeric water molecules.[38] They pointed out that the monomeric water molecules are bound to the polar groups by a hydrogen bond that is weaker than the hydrogen bonding system in the clusters, and then there is a shift toward higher fields upon increasing the amount of the monomeric water molecules. It is thought that the signals demonstrated the highfield chemical shifts with increasing amount of water interacting with polar groups in the sample.

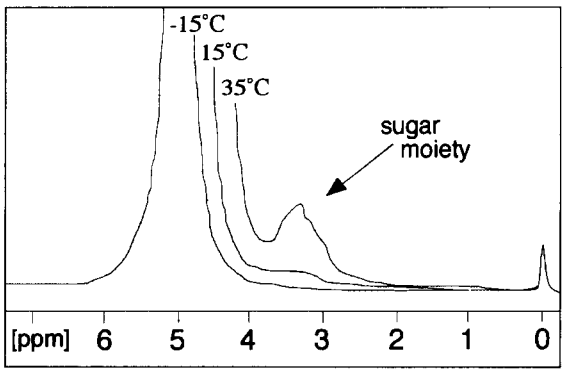

Fig. 7. ^{1}H NMR spectra of poly(MAGPM-co-MAGPG) for the sample with $H = 0.50$ at 35, and 15, -15 °C.

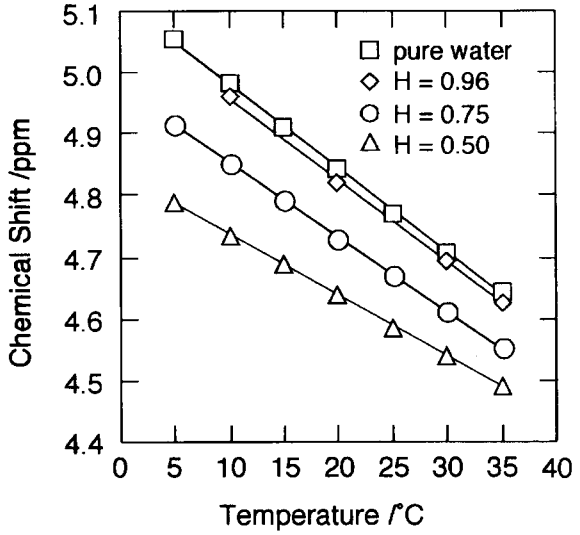

Fig. 8. Temperature dependence of chemical shift on various water retentions.

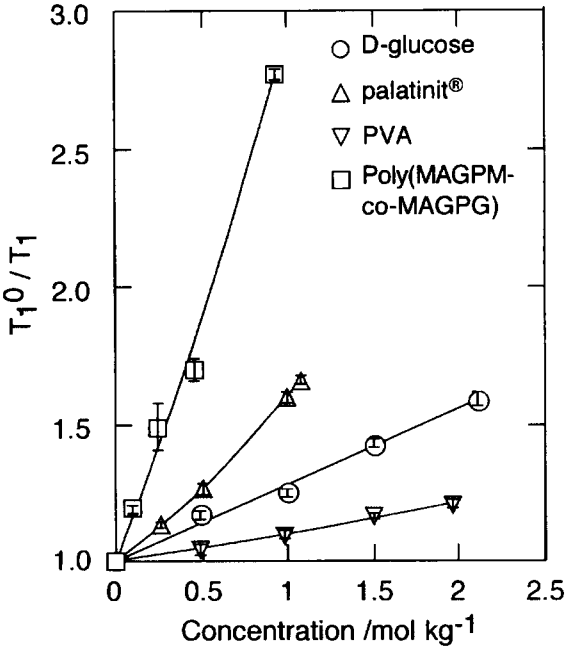

Fig. 9. T_1°/T_1 of H_2 ^{17}O in a solutions of various saccharides as a function of the molality at 35 °C.

At the lower temperature, the highfield chemical shifts became larger with decreasing water retention in Fig. 8. This observation suggests that the chemical shift of water is influenced not only by the concentration of the polar groups, but also by the interaction between water and the polar groups. The interaction between water and the hydroxy groups of poly(MAGPM-co-MAGPG) may be affected by the mobility of poly(MAGPM-co-MAGPG), as shown in Fig. 7, which became depressed with dropping temperature and decreasing water retention.

^{17}O NMR Measurement of Water. The dependence of the T_1°/T_1 of water in glucose, Palatinit®, PVA, and the poly(MAGPM-co-MAGPG) solution on the concentration of the solutes is shown in Fig. 9. The error bars represent the standard deviations. The solid line was obtained from Eq. 6. The correlation coefficients were in the range 0.999—0.995. The T_1°/T_1 of glucose was represented by the linear function of molarity, while those of Palatinit®, poly(MAGPM-co-MAGPG), and PVA were represented by the quadric. The applicability of Eq. 6 is limited to dilute polymer solutions. It has been reported for poly(ethylene oxide)[21] solutions and polyelectrolyte solutions[18] that both x_h and T_{1h} depend on the polymer concentration in concentrated solutions.

As the DSC results show, the amount of bound non-freezing water was constant for the samples with $H > 0.70$. The T_1°/T_1 of water in poly(MAGPM-co-MAGPG) is plotted for the samples with $H > 0.73$, which are dilute solutions. In a concentrated solution, T_1°/T_1 was 37 at $H = 0.32$. This point deviated significantly from the solid line of poly(MAGPM-co-MAGPG).

The n_{DHN} values of sugars in aqueous solution at 35 °C are presented in Table 1. The n_{DHN} value of PVA was the lowest in this study. The value of poly(MAGPM-co-MAGPG) was the highest for all of the samples. As shown in Eq. 8, it is thought that the value of n_{DHN} increases with the increasing value of n_h. The n_h value significantly changes in relation to the methods, such as DSC, NMR, IR, and the dielectric relaxation.[21] It may not be suitable for the assumption that the amount of bound non-freezing water obtained by the DSC measurement is equal to that of the hydration water. However, we considered that the value of n_h was proportional to the amount of the polar groups hydrogen-bonding to water molecules because the substances used in this study had a hydrophilic group such as a hydroxy group in common.

Table 1. The Value of n_{DHN} of Sugars, PVA, and Poly(MAGPM-co-MAGPG) in Aqueous Solution at 35 °C

	Glucose	Palatinit®	PVA	Poly(MAGPM-co-MAGPG)
n_{DHN}	15.5	25.0	4.88	88.1

The value of n_{DHN} of Palatinit® is about 1.6-times larger than that of glucose. The amount of the polar groups in Palatinit® is 11, while that of glucose is 6. The difference between the values of Palatinit® and glucose approximately corresponds to the amount of the polar groups in them. It has been reported by Uedaira et al.[27] that the mobility of the hydration water of the di- and trisaccharides is nearly equal to those of the constituent monosaccharides in some sugar solutions. Also, Richardson et al.[26] suggested that the mobility of the water "bound" to sucrose is not drastically different from that bound to glucose. It was indicated that the hydration behavior of Palatinit® was the same as that of other low-molecular-weight sugars.

It should be noted that the n_{DHN} value of poly(MAGPM-co-MAGPG) was much greater than that of Palatinit® and the oligosaccharides reported by Kawai et al.[29] These values were 48.3 for trehalose and 71.0 for maltopentaose though the amount of the polar group of poly(MAGPM-co-MAGPG) is equal to that of Palatinit®. Because the sugar side chains in poly(MAGPM-co-MAGPG) are fixed to the backbone

chain, while those of glucose and Palatinit® are able to move freely in an aqueous solution, it is thought that the mobility of the sugar side chains in poly(MAGPM-co-MAGPG) is lower than that of glucose and Palatinit®. We suggest that the mobility of the hydration water in poly(MAGPM-co-MAGPG) is depressed by the low mobility of the sugar side chains in poly(MAGPM-co-MAGPG).

Moreover, the n_{DHN} for poly(MAGPM-co-MAGPG) was much greater than that of the other water-soluble polymer such as PVA and poly(ethylene oxide) which is about 6.7 as reported by Breen et al.[21] Even though both poly(MAGPM-co-MAGPG) and PVA have a hydrophilic group such as a hydroxy group in common, there is one hydroxy group in PVA while there are a number of hydroxy groups in the repeat unit of poly(MAGPM-co-MAGPG) which contains 4 equatorial hydroxy groups in the glucose unit. Uedaira et al. states that the mobility of hydration water around glucose is the most restricted due to the more equatorial hydroxy groups in the monosaccharides.[27] In the case of synthesized glycoconjugate polymers such as a lactose-carrying polystyrene, the backbone is thought to assume a pseudo helical conformation to cover its surface with hydrophilic side chains.[39] Highly concentrated multiantennary glyco signals along the hydrophobic main chains are formed by this conformation, which enhances the interaction with various types of carbohydrate-binding proteins.[40] Similarly, it was suggested that the backbone in poly(MAGPM-co-MAGPG) covered its surface with the hydroxy groups by means of bonding the amino sugar having a number of hydroxy groups containing equatorial hydroxy group to the polymer chain. The solid observations of the ^{17}O spin-lattice relaxation time show that the mobility of the hydration water in poly(MAGPM-co-MAGPG) is restricted by the highly concentrated hydroxy groups along the hydrophobic main chains as well as by the lower mobility of the sugar side chain. Therefore, it is concluded that the sugar side chain in poly(MAGPM-co-MAGPG) has a significantly higher hydration ability in comparison with glucose, Palatinit®, and the other water-soluble polymers.

Conclusions

The hydration properties of poly(MAGPM-co-MAGPG) have been examined by comparison of the DSC and NMR spectroscopy results. The mobility of the polymer chains was increased by the increasing amount of bound non-freezing water. The water molecules in poly(MAGPM-co-MAGPG) were the bound non-freezing water when the water content was less than 0.68 [g/g-polym]. The chemical shift of the ^1H NMR of water was influenced not only by the concentration of the hydroxy groups, but also by the interaction between water and the hydroxy groups. The dependence of the T_1°/T_1 of water in glucose, Palatinit®, PVA, and poly(MAGPM-co-MAGPG) solution on the concentration of the solutes was evaluated using ^{17}O NMR. The dynamic hydration number of poly(MAGPM-co-MAGPG) was the greatest of these substances. The interaction between water and the hydroxy groups in poly(MAGPM-co-MAGPG) is strongly affected not only by the mobility of the sugar side chain, but also by the highly concentrated hydroxy groups along the hydrophobic main chains. The sugar side chain in poly(MAGPM-co-MAGPG) has a significantly higher hydration ability in comparison with glucose, Palatinit®, and PVA, although these substances had a hydroxy group as a hydrophilic group in common.

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