Synthesis and Hydrophilicity of Multifunctionally Hydroxylated Poly(acrylamides)

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ABSTRACT: This paper reports synthesis and hydrophilicity of hydroxylated poly(acrylamides), in which one, three, and six hydroxyl groups were attached to the terminal of their side chains, respectively: poly(2-hydroxyethyl acrylamide), poly(*N*-[tris(hydroxymethyl)methyl] acrylamide), and poly(*N*,*N*-di(*N*-[tris(hydroxymethyl)methyl]-2-acetamido) acrylamide). The initial hypothesis of molecular design of a very hydrophilic polymer was that hydrophilicity increases with an increase in the number of hydroxyl groups per repeating unit due to the enhanced shielding effect on hydrophobic main chains. Contrary to expectations, the relative hydrophilicity of these polymers, determined from the surface tension of these aqueous solutions, tended to decrease with an increase in the number of hydroxyl groups per repeating unit. As the number of hydroxyl groups per repeating unit increased, the chain mobility of the methylene groups adjacent to the terminal hydroxyl groups (determined from the spin−lattice relaxation time (T_1) measurement) was restricted, the fraction of bound water (determined from the DSC measurement) was decreased, and the proton-deuterium exchange rate of hydroxyl groups in D_2O−DMSO-d_6 (DMSO-d_6 = dimethyl sulfoxide-d_6) mixed solution (determined from ^1H NMR spectroscopic measurement) was decreased. These results indicate that the degree of intramolecular hydrogen bonding of the hydroxyl groups is enhanced with an increase in the number of hydroxyl groups per repeating unit, which eventually reduces the hydrogen bonding with water, resulting in a reduction of the hydrophilicity.

Introduction

The control of adhesion and adsorption of biocolloids, such as lipids, proteins, and living organisms including viruses, bacteria, and mammalian cells, on polymer surfaces has been essentially required in the fields of the coating industry, biomedical engineering, and biotechnology. In particular, there has been growing interest in the use of polymers as biomaterials for artificial organs and drug delivery systems. When a polymer is fabricated for an implant device, the fate of the device in the living body in terms of biocompatibility is largely determined by the nature of blood- or tissue-contacting surfaces. Protein adsorption on a surface is an initial event when implanted surfaces were contacted with living fluids such as blood. The properties and structure of the outermost surface layers determine protein adsorption characteristics.

The principles of protein adsorption have been presented in a number of monographs, review papers, and conference proceedings.[1−3] Of various intermolecular forces operating at an interfacial layer between a polymer and a protein in water, hydrophobic and electrostatic interactions are major driving forces in the adsorption process of proteins. Minimized hydrophobicity and enhanced nonionic character are essential for a nonadsorptive surface design. In fact, hydrophilicity and hydrophobicity of polymer surfaces are determinant factors influencing the protein adsorption and cell adhesion and growth.[4−10] That is, protein adsorption as well as cell adhesion and growth are largely suppressed or inhibited on nonionic hydrophilic surfaces such as cellulose. Hence, hydrophilic surface designs to provide the least adherent or adsorptive nature have been directed toward developing short-term blood contacting surfaces.[8] On the other hand, hydrophobic and polar surfaces impart high cell affinity due to enhanced protein adsorption, which is suitable for a cell adhesive matrix and tissue-contacting surface.

Although a variety of water-soluble vinyl polymers have been used for blood- and tissue-contacting surface design, their hydrophilicities are not sufficient to completely prevent protein adsorption as well as cell adhesion under severe physiological environments. Water-soluble nonionic vinyl polymers commonly used for biomedical applications include poly(vinyl alcohol), poly(acrylamide), and poly(vinyl pyrrolidone). The water solubility of these polymers is apparently attained by the hydroxyl or primary amide group in their side chains. These polymers have one hydratable group per repeating unit. The adverse effect counteracted by the hydrophilicity of the hydratable group in the side chains is the hydrophobicity of their main chains which are totally composed of hydrocarbons. The degree of hydrophilicity of water-soluble vinyl polymers may be controlled by various factors: the type of hydratable functional group, its functionality per repeating unit, and its spatial configuration or distribution along the main chain. Therefore, if we can increase the functionality of hydratable groups in a repeating unit and can well distribute them around the main chain, the main chain should be shielded from an access of water, resulting in enhancement of the hydrophilicity.

Along with this working principle or hypothesis, as a first step toward developing a "superhydrophilic" vinyl polymer, we molecularly designed nonionic water-soluble vinyl polymers which are poly(acrylamides) derivatized with multifunctional hydroxyl groups in their side chains. Figure 1 shows molecular structures of prepared water-soluble polymers, in which one, three, and six hydroxyl groups were attached to the terminals of their side chains, respectively. In this paper, we report the preparation method of multifunctional hy-

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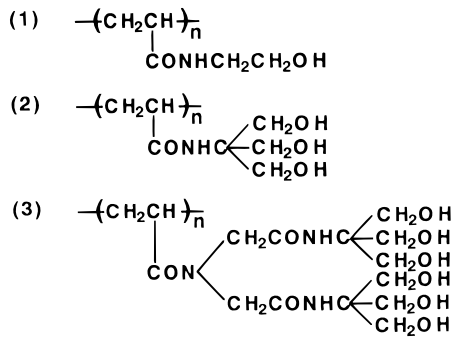


Figure 1. Molecular structures of prepared hydroxylated poly(acrylamides): (1) poly(*N*-(2-hydroxyethyl)acrylamide) (PHEAAm); (2) poly(*N*-tris[hydroxymethyl)methyl]acrylamide) (PTrisAAm); (3) poly(*N*,*N*-bis(*N*-[tris(hydroxymethyl)methyl]-acetamido)-2-acrylamide) (PTIAAm).

droxylated poly(acrylamides) and physicochemical measurements of hydrophilicity estimated from surface tension in aqueous solutions, the fraction of bound water determined by differential scanning calorimetry (DSC), the chain mobility estimated from spin−lattice relaxation time (T_1), and the proton−deuterium exchange rate determined by ^{1}H NMR spectroscopy.

Experimental Section

Materials. Acryloyl chloride and 2-amino-2-(hydroxymethyl)-1,3-propanediol, purchased from Wako Pure Chemical Industries Ltd., Osaka, Japan, were used without further purification. Acetonitrile, ethyl acetate, and tetrahydrofuran (Wako) were used after drying over molecular sieves 3A. *N*,*N*-Dimethylformamide and 2-aminoethanol (Wako) were purified by distillation. Iminodiacetic acid diethyl ester and triethylamine (Tokyo Chemical Industry Co., Ltd. Tokyo, Japan) were used as received. Silica gel (70−230 mesh ASTM) was purchased from Wako.

General Procedures. Proton nuclear magnetic resonance spectra (^{1}H NMR) were recorded on a JEOL GSX-270 (270 MHz) NMR spectrometer (Tokyo, Japan). Gel filtration chromatography data were obtained with a TOSOH CCP&8010 system (Tokyo, Japan) that was connected to a TOSOH RI-8012 refractive index detector. Phosphate buffer solution (0.1 M) was employed as an eluent. The molecular weights were reported with poly(ethylene glycol) as calibration standard. The surface tension of aqueous polymer solutions was measured by the Wilhelmy plate method at 25 °C on a Shimadzu ST-1 tensiometer (Kyoto, Japan).

Preparation of *N*-(2-Hydroxyethyl)acrylamide. Acryloyl chloride (5.32 g, 58 mmol) in dry ethyl acetate (40 mL) was added dropwise into a cold solution of 2-aminoethanol (2.99 g, 49 mmol) and NaOH (2.32 g, 58 mmol) in 10 mL of water. The reaction was carried out for 4 h at 0−5 °C under stirring. Then, the ethyl acetate layer was separated and dried over anhydrous Na_2SO_4. Ethyl acetate was evaporated, and the residue was fractionated, using a silica gel column with methanol as an eluent. The eluted fractions, after being examined by thin layer chromatography (TLC), were collected. The initial fractions were collected and evaporated *in vacuo*. The product was a light yellow oil. (4.4 g; yield, 78%). ^{1}H NMR (270 MHz, DMSO-d_6, ppm from Si(CH$_3$)$_4$): δ 8.2 (s, 1H, N*H*), 6.2 (m, 2H, vinyl), 5.5 (dd, 1H, vinyl), 4.0 (d, 1H, CH$_2$O*H*) 3.5 (t, 2H, CH$_2$C*H*$_2$OH), 3.2 (q, 2H, CONHC*H*$_2$CH$_2$OH).

Preparation of *N*-[Tris(hydroxymethyl)methyl]acrylamide. Acryloyl chloride (4.5 g, 50 mmol) was added dropwise into a cold suspension of 2-amino-2-(hydroxymethyl)-1,3-propanediol (12.1 g, 100 mmol) in 250 mL of acetonitrile. The reaction was allowed to proceed under stirring at room temperature overnight. The suspension was heated at 70 °C and filtered while hot. After the filtrate was stored at room temperature overnight, the white solid formed was finely ground into powders in acetonitrile and was isolated by filtration. Repeated recrystallizations produced white crystals (7.0 g; yield, 80%). ^{1}H NMR (270 MHz, DMSO-d_6, ppm from Si(CH$_3$)$_4$): δ 7.5 (s, 1H, N*H*), 6.4 (m, 1H, vinyl), 5.8 (dd, 2H, vinyl), 3.6 (t, 6H, CONHC(C*H*$_2$OH)$_3$), 3.3 (s, 3H, CONHC-(CH$_2$O*H*)$_3$).

Preparation of *N*,*N*-(Diethylacetato)acrylamide. Acryloyl chloride (4.9 g, 50 mmol) was added dropwise into a cold solution of iminodiacetic acid diethyl ester (7.94 g, 42 mmol) and triethylamine (5.09 g, 50 mmol) in 80 mL of tetrahydrofuran under stirring. The reaction was carried out at 0−5 °C for 4 h. Subsequently, the precipitate was filtered off, and the filtrate was collected. It was concentrated under vacuum. The residue was passed through a silica gel column and eluted with methanol. Various fractions were collected after the TLC examination. The initial fractions were evaporated *in vacuo*. The product was a light yellow oil (7.99 g; yield, 78%). ^{1}H NMR (270 MHz, DMSO-d_6, ppm from Si(CH$_3$)$_4$): δ 6.7 (m, 1H, vinyl), 6.0 (dd, 2H, vinyl), 4.1 (q, 4H, CH$_2$COOC*H*$_2$CH$_3$), 3.2 (d, 4H, CON(C*H*$_2$COOCH$_2$CH$_3$)$_2$), 1.2 (m, 6H, CON(CH$_2$-COOCH$_2$C*H*$_3$)$_2$).

Polymerization Procedures. Polymerization was carried out in *N*,*N*-dimethylformamide with 2,2′-azobis(isobutyronitrile) as an initiator in a sealed glass tube, which was degassed under a few freeze−thaw cycles, at 60 °C for 20 h. The polymerization mixture was added to a large volume of diethyl ether or methanol to precipitate the polymer. The precipitated polymer was filtered, washed thoroughly with diethyl ether or methanol, and dried under vacuum at room temperature. The polymer thus obtained was further purified by dialysis in deionized water. The dialyzed solution was freeze-dried to obtain colorless polymer.

Preparation of Poly(*N*,*N*-bis(*N*-[tris(hydroxymethyl)-methyl]acetamido)-2-acrylamide). A mixture of poly(*N*,*N*-(diethylacetato)acrylamide) (1.0 g, 4.1 unit-mmol) and 2-amino-2-(hydroxymethyl)-1,3-propanediol (4.9 g, 41.1 mmol) was refluxed for 20 h in 10 mL of dimethyl sulfoxide. The solution was evaporated to dryness. The solid product obtained was dissolved in water (20 mL) and was purified by dialysis, followed by freeze drying. (1.4 g; yield, 87%). ^{1}H NMR (270 MHz, DMSO-d_6, ppm from Si(CH$_3$)$_4$): δ 7.2 (br, 2H, NH), 4.6 (br, 6H, CONHC(CH$_2$O*H*)$_3$), 3.5 (br, 4H, CON(C*H*$_2$CONHC-(CH$_2$OH)$_3$)$_2$, 3.2 (br, 12H, CONHC(C*H*$_2$OH)$_3$), 1.7 (br, 1H, −(CH$_2$C*H*)$_n$−), 1.2 (b, 2H, −(C*H*$_2$CH)$_n$−).

Differential Scanning Calorimetry. The heat of fusion of water associated with these polymers was determined using a differential scanning calorimeter (DSC; (SEIKO I&E SSC-580 DSC-10, Tokyo, Japan). A 17 mg amount of sample, dried under reduced pressure for 24 h, was weighed in an aluminum pan which was pretreated with boiling water to eliminate any reaction between the aluminum surface and water. A 10−30 mg amount of water was added to each sample with a microsyringe, and the sample pans were sealed. The sample pans were left for 24 h at room temperature in order to facilitate the diffusion of water into these samples. The sample pans were put on the sample holder, on which the Dewar flask filled with liquid nitrogen was placed. Samples were cooled to −80 °C at the rate of 5 °C/min and held for 5 min at −80 °C. DSC was run to +30 °C at the heating rate of 5 °C/min.

Spin−Lattice Relaxation Times (T_1) Measurements. Samples were dissolved in deuterium oxide (D_2O) (99.75%; concentration, 1 wt %). Samples, contained in 5 mm od glass tubes, were replaced with N_2 since the relaxation times are affected by the presence of dissolved oxygen. Measurements were carried out at 60 °C. The T_1s for each proton were obtained by means of the conventional 180°−t−90° pulse sequence, where t is the time interval between 180 and 90° pulses. The intensity $M(t)$ of peaks was used to determine T_1 according to the following expression:[11]

$$M(t) = M_0[1 - 2\exp(-t/T_1)] \quad (1)$$

where M_0 is the equilibrium intensity of the spectrum. The assumption of exponential decay in eq 1 was justified by a good linearity in the plots of $\log[M_0 - M(t)]$ vs t. The T_1s were calculated from six t values by the least-squares method.

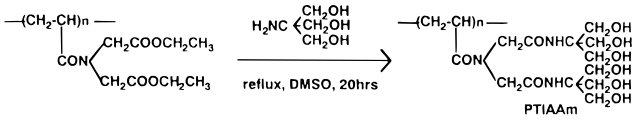
Proton−Deuterium Exchange Measurements. A 10 mg amount of hydroxylated poly(acrylamides) was dissolved

Table 1. Synthesis and Characterization of Poly(*N*-substituted acrylamides)

polymer	yield (%)		elem analysis found	elem analysis calcd	$\overline{M_n}$	$\overline{M_w}/\overline{M_n}$
		C	52.0	52.1		
PHEAAm	78.2	H	8.0	7.8	8 900	2.48
		N	12.3	12.1		
		C	48.1	48.0		
PTrisAAm	80.0	H	8.4	6.9	8 500	2.41
		N	9.2	8.0		
		C	53.1	54.3		
PIEAAm	78.3	H	6.9	6.9	ND[a]	ND[a]
		N	5.4	5.8		
		C	45.9	46.0		
PTIAAm	87.0	H	6.0	6.4	13 100	3.73
		N	9.3	10.7		

[a] ND = Not determined.

Scheme 1. Synthesis Scheme of Poly(*N*,*N*-bis(*N*-[tris(hydroxymethyl)methyl]-2-acetamido)acrylamide)



in DMSO-d_6 (0.5 mL). ^{1}H NMR measurements were carried out at 30 °C. After adding D_2O in polymer solutions, in which concentrations were 3-fold of the molar quantity of hydroxyl and amide groups in the polymer, the spectra were recorded at different time intervals. The resonance intensities (determined as a peak area) were calibrated relative to the proton resonance of $Si(CH_3)_4$ as an external standard at 0 ppm. The degrees of proton exchange of hydroxyl groups in the polymer were determined by the time-dependent decrease of resonance intensities.

Results

Synthesis of Hydroxylated Poly(acrylamides). The structures of hydroxylated poly(acrylamides) prepared are shown in Figure 1. Poly(*N*-(2-(hydroxyethyl)-acrylamide) (PHEAAm) and poly(*N*-[tris(hydroxymethyl)-methyl]acrylamide) (PTrisAAm) were obtained by corresponding monomers which were prepared by acryloyl chloride and an amino alkanol. The preparation of the former polymer was reported previously[20] and the later was recently commercially available.[21] Diesterified acrylamide derivative (IEAAm) used for synthesis of hexahydroxylated poly(acrylamide) (PTIAAm) was prepared from acryloyl chloride and iminodiacetic acid diethyl ether. The degree of conversion, the elemental analysis, and the molecular weight of the polymers obtained are tabulated in Table 1. PTIAAm was synthesized by the aminolysis of PIEAAm with 2-amino-2-(hydroxymethyl)-1,3-propanediol according to the reaction in Scheme 1. The proton NMR spectrum of PIEAAm (spectrum A) and that of its aminolysis product (spectrum B) are shown in Figure 2. Upon aminolysis of PIEAAm, the signals corresponding to the $-CH_2$ (d in spectrum A; δ 4.1) and $-CH_3$ (e in spectrum A; δ 1.2) of the terminal ester ($-COOCH_2CH_3$) of PIEAAm completely disappeared and a new peak (e in spectrum B; δ 3.2) assignable to the $-CH_2$ group adjacent to the hydroxyl group appeared. Markedly reduced intensities of protons in the main chain may indicate that the chain mobility of PTIAAm is considerably restricted in DMSO. In addition, the absorption peak of the ester group in the FT-IR spectrum completely disappeared, but the peaks ascribed to the secondary amide groups at 1640 and 1580 cm^{-1} were observed. These results indicate

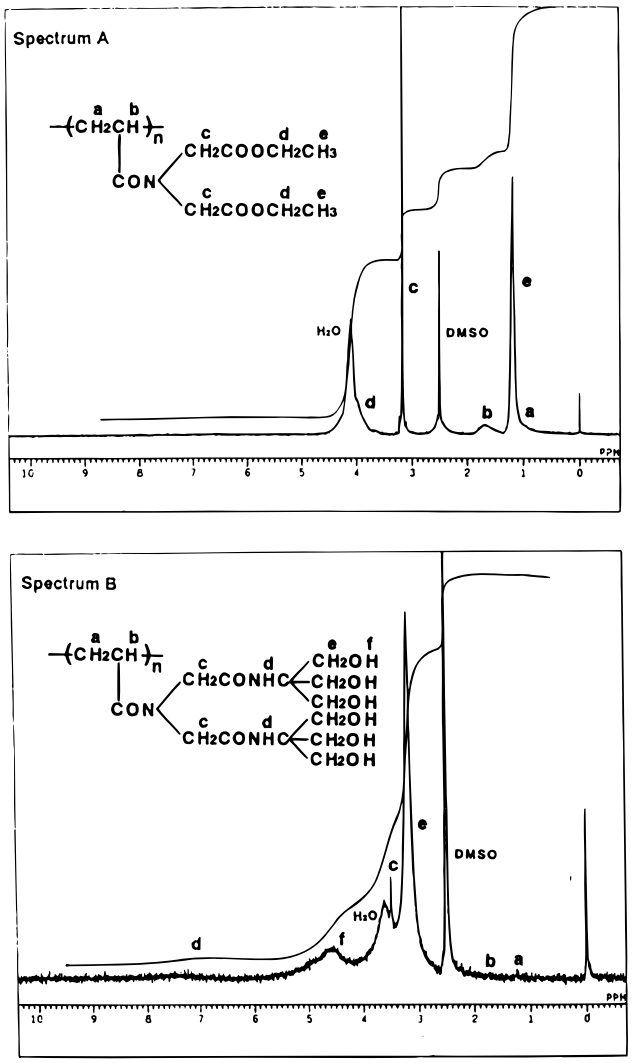


Figure 2. ^{1}H NMR spectra of PIEAAm (spectrum A) and its aminolysis product (spectrum B).

that aminolysis of PIEAAm was completed to produce the hexahydroxylated poly(acrylamide) derivative. A marked difference in solubility was noted upon complete aminolysis. PIEAAm was insoluble in water, but PTIAAm was very soluble.

Surface Tension of Aqueous Polymer Solutions. Irrespective of functionality of hydroxylated poly(acrylamides), these polymers were very soluble in water at room temperature. Since a reduction in surface tension may reflect the degree of the hydrophobicity of the polymer, the surface tensions of these aqueous solutions were determined to estimate the degree of hydrophilicity of these polymers. The higher the hydrophobicity is, the more will be the decrease in surface tension due to surface enrichment at the air−water interface. The surface tensions measured for PHEAAm, PTrisAAm, and PTIAAm are plotted against polymer concentration (Figure 3). As the polymer concentration increased, the surface tension decreased. The concentration-dependent reduction of the surface tension was found to be more profound for higher functionality. The surface tension decreased in the order PTIAAm > PTrisAAm > PHEAAm.

States of Water in Hydroxylated Poly(acrylamides). DSC melting endotherms of distilled water and water associated with hydroxylated poly(acryalmides) are shown in Figure 4. Distilled water showed a sharp

Table 2. Number of Bound Water per Repeating Unit of Hydroxylated Poly(acrylamides) (W_{bound}, Amount of Bound Water per Dry Polymer; N_{bound}, Number of Bound Water Molecules per Repeating Unit)

		bound water	
		W_{bound}	N_{bound}
polymer	side chain	(g of H_2O/(g of dry polymer))	(mol/repeating unit)
PHEAAm	$CONHCH_2CH_2OH$	0.62	4.0
PTrisAAm	$CONHC(CH_2OH)_3$	0.34	3.3
PTIAAm	$CON(CH_2CONHC(CH_2OH)_3)_2$	0.04	0.9

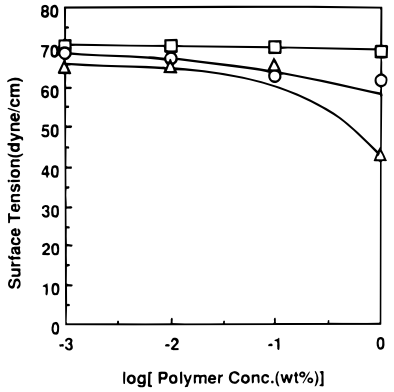


Figure 3. Surface tensions (dyn/cm) of hydroxylated poly(acrylamides): □, PHEAAm; ○, PTrisAAm; △, PTIAAm.

endothermic peak which appeared over 0 °C. This indicates that the endothermic melting of distilled water was initiated very near 0 °C in this experimental condition. For water associated with hydroxylated poly(acrylamides), one endothermic peak at a temperature lower than 0 °C was observed at low water content, while two endothermic peaks appeared on the DSC curves with increasing water content: one is a small shoulder peak in the temperature near −30 °C for PHEAAm, −15 °C for PTrisAAM, and −5 °C for PTIAAm, and the other is an intense peak whose peak maximum corresponds to the temperature of melting near 0 °C. This peak maximum shifted to the higher temperature region with increasing water content. It has been discussed that water generally exists in different states in polymer−water mixed systems:[12,13] nonfreezing water, bound water, and free water. In this paper, we attempted to divide it into two different states of water as follows: (I) For bound water, these water molecules do not freeze below 0 °C due to strong interaction with the polymer, and (II) for free water, these water molecules are unbound to polymers. Therefore, its melting temperature is over 0 °C in our experimental condition as is found for distilled water (Figure 4). The amount of free water was roughly approximated from the peak area present over 0 °C, using the melting enthalpy of 334 J/g reported for free water, because distilled water began to melt very near 0 °C.[14,15] The amount of the calculated free water is plotted against the amount of fed water in Figure 5. The amount of bound water per g of dry polymer (W_{bound}) was calculated by extrapolating the weight of the calculated free water to zero in Figure 5. The number of bound water per repeating unit per dry polymer (N_{bound}) was obtained from W_{bound} and the molecular weight of the polymer. The W_{bound} and N_{bound} thus obtained are given in Table 2. The values for N_{bound} (mol/repeating unit) were 4.0 for PHEAAm, 3.3 for PTrisAAm, and 0.9 for PTIAAm.

Spin−Lattice Relaxation Time (T_1) Measurements. Partially relaxed ^1H NMR spectra of PHEAAm were recorded in deuterium oxide at 60 °C at different time intervals between 180 and 90° pulses (Figure 6). The spectrum consists of four main peaks as expected from the polymer structure. Each peak intensity obtained from sets of such spectra was used to determine T_1 according to eq 1 described in the Experimental Section (Figure 6). T_1s of hydrogens of the main chain and side chain was obtained for PHEAAm and PTrisAAm (Table 3). A striking difference was observed for T_1 of methylene groups adjacent to the terminal hydroxyl groups for both polymers: the T_1 value for PTrisAAm is almost two-thirds of that for PHEAAm. The T_1 measurement for PTIAAm could not be obtained in this experimental condition, since the spectrum of PTIAAm gave broad peaks which could not be separately resolved.

Proton Exchange Measurements. Figure 7 shows the time-dependent changes in the peak area of the protons of the hydroxyl groups of hydroxylated poly(acrylamides) in D_2O−DMSO-d_6 mixed solution (note that the amount of added D_2O is fixed to be 3-fold of the molar quantity of the hydroxyl and amide groups

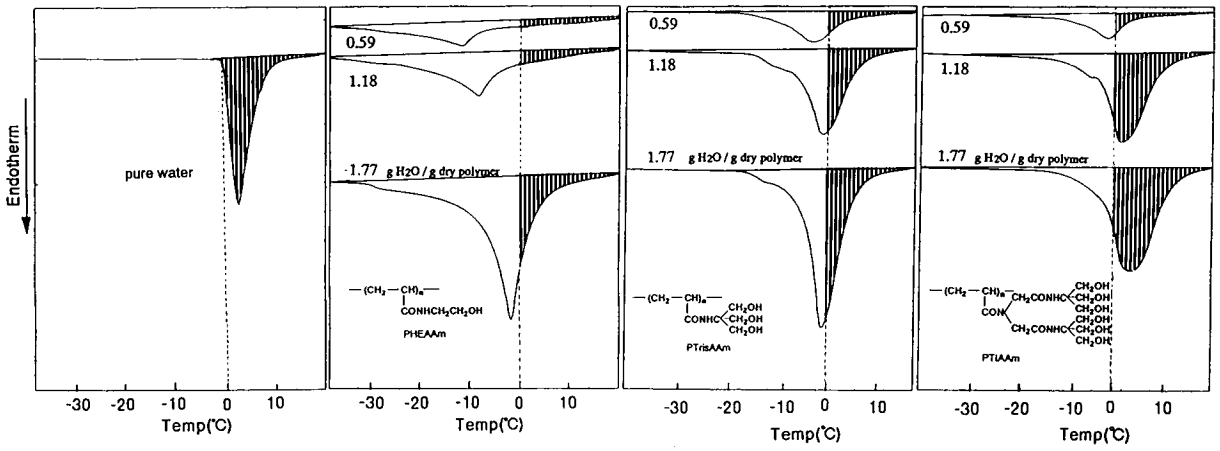


Figure 4. DSC melting endotherms of distilled water and water in hydroxylated poly(acrylamides): (A) distilled water; (B) water in PHEAAm; (C) water in PTrisAAm; (D) water in PTIAAm. Shaded area was defined as a fraction of free water.

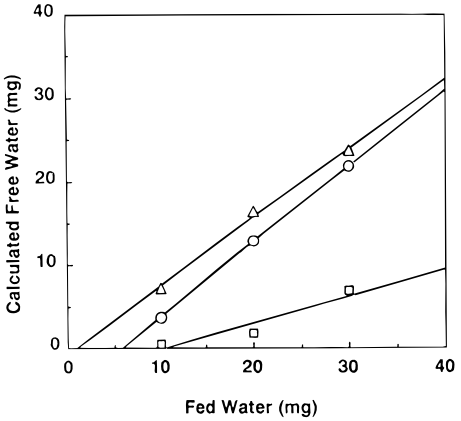


Figure 5. Amount of the calculated free water vs the amount of fed water (polymers, 17 mg): □, PHEAAm; ○, PTrisAAm; △, PTIAAm.

Table 3. Spin−Lattice Relaxation Time (T_1, ms) of Hydroxylated Poly(acrylamides)

	main chain		side chain	
polymer	CH_2	CH	CH_2	CH_2
PHEAAm	235	386	460	485
PTrisAAm	281	474		310

in the polymer). The exchange rate observed in the early period after mixing with D_2O and the equilibrated degree of exchange are in the order PHEAAm > PTrisAAm > PTIAAm.

Discussion

Nonionic hydrophilic polymers such as polyacrylamide and poly(ethylene glycol) have been enjoyed in many different industrial applications. However, it is quite difficult to rank water-soluble polymers in aqueous solutions since there is no estimation method of hydrophilic scale. Therefore, molecular design criteria directing toward a superhydrophilic polymer is not clear. Water solubility must be first as a measure of hydrophilic/hydrophobic balance. The lower critical solution temperature (LCST) and upper critical solution temperature (UCST) must be indicators in a particular system.[22] Flory−Huggins interaction parameters,[23] χ_{12} and χ_{11}, where 1 and 2 denote polymer and water, respectively, must be theoretically best-suited for hydrophilic scale, although determination of these parameters is a quite difficult task. We have tentatively adapted pragmatic approaches to estimation of the hydrophilicity, as will be discussed later.

If we can increase the hydrophilic group density per repeating unit of a vinyl polymer, such a polymer may exhibit an enhanced hydrophilicity because the hydrophobic main chain may be shielded with hydrated hydrophilic groups. On the basis of the above-mentioned working principle, we prepared poly(*N*-substituted acrylamide) derivatives with one, three, and six functionalities of hydroxyl groups (monohydroxylated and trihydroxylated polyacrylamide derivatives[20,21] were previously reported). Elemental analysis and ^{1}H NMR measurements showed that the polymers prepared are hydroxylated poly(acrylamides) with molecular structures, as shown in Figure 1. Before a detailed discussion is given, preliminary information on structures of hydroxylated poly(acrylamides) obtained by means of computer simulation will be described below. The modeling software employed was the Insight II (Biosym Technologies, San Diego, CA). Figure 8 shows space filling models of hydroxylated poly(acrylamides), which were energy-minimized by a force-field method (20 monomer units; isotactic configuration). A rough approximation drawn is as follows. Irrespective of the polymers, models provides cylindrical shapes where hydrophobic main chains are located at the core which is surrounded with hydroxyl groups. Average diameters of these polymer chains, determined from a space filling model, were 1.28 nm for PHEAAm, 1.38 nm for PTrisAAm, and 1.80 nm for PTIAAm. As the number of hydroxyl groups per repeating unit increases, the bulkiness of the side chain increases, which results in an increase in the polymer diameter. Assuming that the unit space per repeating unit is a cylinder in which the center is occupied with the C−C bond of the main chain (length, 0.215 nm), an average density of the hydratable group (−OH and −CONH−) in units were calculated as 7.4 groups/nm^3 for PHEAAm, 12.4 groups/nm^3 for PTrisAAm, and 16.6 groups/nm^3 for PTIAAm. With an increase in the number of hydratable groups per repeating unit, the space density of the hydratable group increased. Thus, it is highly expected that the hydrophobic main chain must be shielded with hydrophilic groups to avoid an access of water, which seems to indicate that our hypothesis is valid.

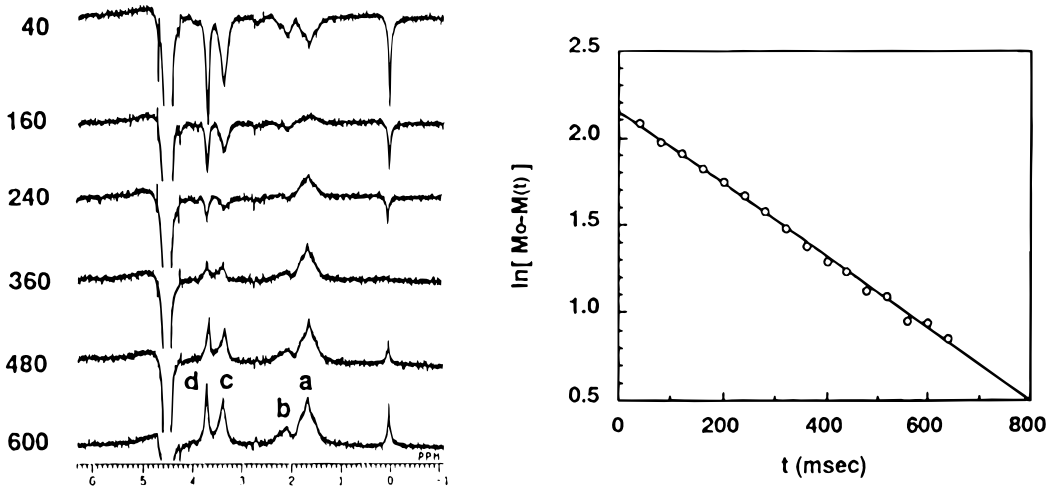


Figure 6. ^{1}H NMR spectra of PHEAAm in D_2O at 60 °C (left) and plots of ln[$M_0-M(t)$] for terminal proton vs t (right). The number given to the left hand side of each spectrum denotes t which is the time interval between the 180° pulse and 90° pulse, in milliseconds: a, $-(CH_2CH)_n-$; b, $-(CH_2CH)_n-$; c, $CONHCH_2CH_2OH$; d, $CONHCH_2CH_2OH$.

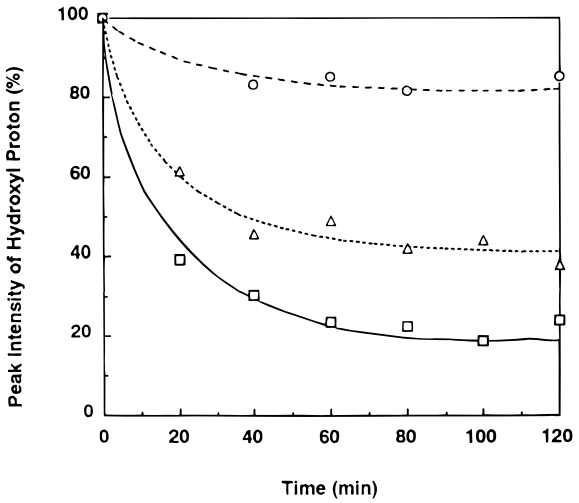


Figure 7. Time-dependent decrease of protons of hydroxyl groups of hydroxylated poly(acrylamides) in D_2O−DMSO-d_6 mixed solution (D_2O-hydratable groups = 3:1 by molar ratio): □, PHEAAm; △, PTrisAAm; ○, PTIAAm.

Although the hydrophobic scale of polymer surfaces has been obtained by contact angle measurement, which can be translated to surface free energy, the hydrophilic scale of water-soluble polymers has not been established yet. A simple method of estimating the hydrophilicity of water-soluble polymer is to measure the interfacial tension between hydrophobic media and aqueous polymer solution. If a polymer has both hydrophilic and hydrophobic groups or segments in a molecule, hydrophobic portions are predominantly arranged at the air−water interface to minimize the surface tension. Since a reduction in surface tension should reflect the degree of the hydrophobicity of the polymer, relative hydrophilicity of hydroxylated poly(acrylamides) can be estimated from the surface tension of aqueous solutions. The surface tension decreased in the order PTIAAm > PTrisAAm > PHEAAm (Figure 3). This tendency was profound at high concentrations. This means that the hydrophobicity of these polymers increases in the same order as above. The result indicates that the relative hydrophilicity of the polymer decreases with an increase in the number of hydroxyl groups per repeating unit, which is contrary to our expectations. This brought us to the question of why the hydrophilicity of hydroxylated polymer decreases with its multifunctionality, in spite of the increase in the hydrophilic group density and the spatial distribution which is favored for shielding of the hydrophobic main chain from an access of water.

As clearly shown in Table 3, the estimated number of bound water molecules per monomer unit (N_{bound}) significantly decreased as the functionality of the hydroxyl group increased. Since a hydroxyl group which has a strong hydrogen bonding ability acts as both hydrogen donor and acceptor, hydroxyl groups interact with adjacent hydroxyl groups as both donors and acceptors for each other. Therefore, an increase of intramolecular hydrogen bonding may cause a decline in the ability of hydrogen bonding with water, resulting in the loss of affinity for water. Additional evidences in support of enhanced intramolecular hydrogen bonding were given from viewpoints of side chain mobility and proton−deuterium exchange. Since T_1 of a polymer is determined mainly by intramolecular segmental motion, the reduction in the T_1 value means that intramolecular segmental motion is restricted,[16,17] which is caused by hydrogen bonding or steric hindrance. For example, the formation of intramolecular hydrogen bonding reduces the chain mobility of the poly(vinyl alcohol).[17] The spin−lattice relaxation time (T_1) measurement shows that the side chain mobility for PTrisAAm was restricted as compared with PHEAAm. The proton−deuterium exchange experiment also differentiated the degree of intramolecular hydrogen bondings between hydroxyl groups since the concentration of D_2O is limited to be 3-fold of the molar quantity of hydroxyl and amide groups in polymer. The proton−deuterium exchange rates and equilibrium ratios of hydroxyl groups of the polymers in D_2O were in the order PHEAAm > PTrisAAm ≫ PTIAAm (Figure 7). These results indicate that the exchange rate was markedly reduced as the number of hydroxyl groups per repeating unit increased. The reduction in the proton−deuterium exchange rate and the degree of exchange at equilibrium means that the intramolecular hydrogen bonding formation is enhanced.[18]

To summarize, as the number of hydroxyl groups per repeating unit increased (1) the chain mobility of methylene groups adjacent to the terminal hydroxyl groups (determined from T_1 measurement) was restricted, (2) the fraction of bound water (determined from DSC measurement) was decreased, and (3) the proton−deuterium exchange rate (determined from ^1H NMR spectroscopic measurement) was decreased. All results indicate that intramolecular hydrogen bonding of hydroxyl groups is enhanced with an increase in

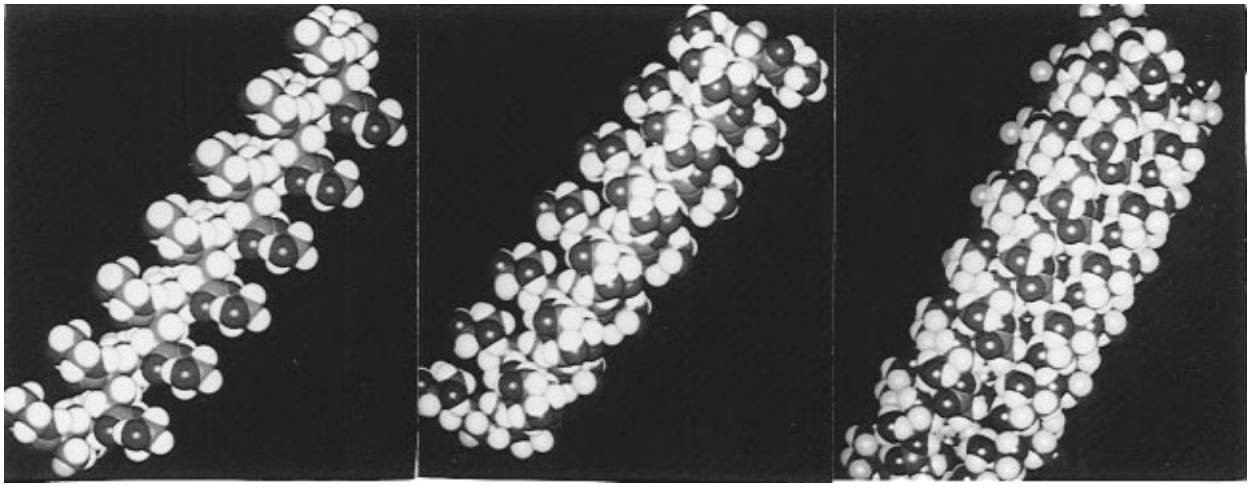


Figure 8. Space filling models of hydroxylated poly(acrylamides): left, PHEAAm; center, PTrisAAm; right, PTIAAm.

hydroxyl group per repeating unit. Although the main chain may be shielded with spatially well-distributed hydroxyl groups, it can be said that intramolecular hydrogen bonding eventually reduces the hydrogen bonding with water, resulting a reduction of the hydrophilicity. For example, fully hydrolyzed poly(vinyl alcohol) and agarose are only soluble in hot water, and poly(hydroxymethylene) and cellulose are insoluble even in hot water. Thus, it is critical how adding hydrogen bonding hydroxyl groups to a polymer reduces polymer−water interaction. This paper may conclude that intramolecular hydrogen bonding, which induces the formation of thermodynamically stable six membered rings which are derived from 1,3-glycol units, could reduce polymer−water interaction. Therefore, the molecular design of a superhydrophilic vinyl polymer should be directed toward minimizing the degree of intramolecular hydrogen bonding. This must be realized by density control and spatial distribution of water hydratable groups in polymer. A study on the preparation of poly(acrylamides) with multifunctional primary amide groups will be reported in the near future.[19]

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