^{1}H-NMR Studies on Water in Methacrylate Hydrogels. II

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SYNOPSIS

Properties of water in the wet membranes of a random copolymer of methyl methacrylate and methoxy poly(ethylene glycol)-monomethacrylate P(MMA-co-M100G) and of a graft copolymer of vinyl chloride and methoxy poly(ethylene glycol)-monomethacrylate P(VC-g-M100G) membranes were studied by proton NMR spectroscopy. Both polymers contain flexible polyoxyethylene side chains. Temperature and frequency dependences of relaxation times, changes of signal intensity of water protons on freezing and thawing, and saturation transfer were measured for both copolymers. These experimental results revealed that most of the bound water in these copolymers does not exist in a rigid state as suggested for the other hydrogels. The introduction of flexible hydrophilic side chains resulted in high mobility of the water molecules bound to the side chains (the apparent correlation time $\tau_c \sim 10^{-10}$ s). For the graft copolymer P(VC-g-M100G), hydrophilic polyoxyethylene side chains are contiguous to each other. Because of the interaction between the side chains, the mobility became more complex than that for the random copolymer P(MMA-co-M100G). The difference in the mode of mobility of water on the membrane surface may be related to the difference of the membrane characteristics of these copolymers.

INTRODUCTION

Various kinds of hydrogels made from polymer materials have been recently developed and utilized extensively in medical fields. However, the correlation among their function, polymer materials, and membrane design has not been yet well understood.

Polymers with hydrophilic polyoxyethylene side chains are known to exhibit the characteristics of antithrombus. Nagaoka et al. have suggested that the adhesion of blood platelet and adsorption of plasma protein to the P(VC-g-MnG) graft copolymer, which is made by grafting methoxy poly(ethylene glycol)-monomethacrylate to poly(vinyl chloride), are more restricted than are those to P(MMA-co-MnG), which is the random copolymer of methoxy poly(ethylene glycol)-monomethacrylate and methyl methacrylate with the same length of polyoxyethylene chains and the same water content.[1,2]

It has been suggested that the water properties in some kinds of hydrogels are correlated with film functions such as permeability and selective adsorbancy.[3-5] In the previous paper we reported the properties of water contained in the two kinds of hydrogels of different characteristics, that is, a hydrophilic hydrogel of poly(hydroxyethyl methacrylate) and a hydrophobic hydrogel of poly(methyl methacrylate). The significant differences of these two hydrogels in the behavior on freezing procedures, mobility, and the amount of the bound water explained their difference in the permeability to water and urea.[6] In the present paper, as the second step, the properties of water contained in the more complicated hydrogels [a random copolymer P(MMA-co-M100G) and a graft copolymer P(VC-g-M100G)], which are interesting in terms of their antithrombus characteristics, were studied by proton NMR spectroscopy.

EXPERIMENTAL

Materials

Methoxy poly(ethylene glycol)-monomethacrylates (M100G) with the polyoxyethylene chain (100; de-

gree of polymerization of the polyoxyethylene side chain) used in the present study were obtained by the esterification of methacrylic acid with the methoxy poly(ethylene glycol) and kindly supplied by Yushi Seihin Co., Ltd. A mixture of M100G, methyl methacrylate (MMA) and a small amount (0.05%) of diethylene glycol dimethacrylate (a cross-linking agent) was polymerized at 50°C for 20 h using azobisisobutyronitrile as an initiator in a space between two glass plates. A cross-linked copolymer was obtained in the form of a thin sheet and swollen in water. The cross-linked copolymers of M100G and MMA are referred to as P(MMA-co-M100G).

Poly(vinyl chloride) (PVC) was treated with sodium N,N-diethyl dithiocarbamate in N,N-dimethylformamide (DMF) at 55°C for 2 h to introduce the dithiocarbamate (DTC) groups that are necessary for the photo-induced graft polymerization. Then, M100G was graft copolymerized with the PVC containing the DTC groups in tetrahydrofuran (THF) at 30°C for 8–10 h using a 100 W high-pressure mercury lamp. The reaction mixture was poured into methanol. The precipitate was soaked in methanol for 2 days to remove the unreacted monomers and then dried under vacuum at room temperature. The resultant polymers are referred to as P(VC-g-M100G). A sheet of the P(VC-g-M100G) was prepared by repeatedly spreading the 5 wt % polymer solution in THF onto a glass plate and drying at 35°C in a nitrogen atmosphere.

P(MMA-co-M100G) and P(VC-g-M100G) gels were obtained by washing the polymer sheets with methanol to remove the solvent and the unreacted monomers and then replacing methanol with water. The hydrogels were preserved in distilled water. The water contents of P(MMA-co-M100G) and P(VC-g-M100G) were determined to be 43.0 ± 1.0% and 43.6 ± 1.0%, respectively.[6] The samples were prepared for NMR measurements according to the procedure described previously.[6]

Methods

The NMR measurements were performed at 90 and 400 MHz with JEOL FX90Q and GX400 instruments, respectively. A single sheet of the sample was set in the NMR sample tube of 10 mm o.d. so that the plane of the polymer sheet becomes parallel to the external magnetic field to minimize dipolar splittings.[7,8] The sample was put at the probe temperature for 30 min before NMR measurements. A solution of 1% tetramethylsilane (TMS) in chloroform was used as an external standard of signal intensity. Intensities were determined from spectral

areas with a planimeter. Spin–lattice and spin–spin relaxation times were measured by the inversion recovery and Carr–Purcell–Meiboom–Gill methods, respectively. [7]Li external lock mode was used for FX90Q, whereas the measurements were performed without a lock for GX400.

RESULTS AND DISCUSSION

Frequency and Temperature Dependencies of Relaxation Times

When relaxation is determined by dipole–dipole interaction, relaxation times are described as a function of correlation time τ_c and the distance between interacting protons r[9]

$$\frac{1}{T_1} = \frac{3\gamma^4\hbar^2}{10r^6}\left(\frac{\tau_c}{1 + \omega^2\tau_c^2} + \frac{4\tau_c}{1 + 4\omega^2\tau_c^2}\right) \quad (1)$$

$$\frac{1}{T_2} = \frac{3\gamma^4\hbar^2}{20r^6}\left(3\tau_c + \frac{5\tau_c}{1 + \omega^2\tau_c^2} + \frac{2\tau_c}{1 + 4\omega^2\tau_c^2}\right) \quad (2)$$

where γ and ω are the gyromagnetic ratio of proton and the Larmor frequency, respectively.

Provided that the distance between interacting water protons remains constant within the temperature region considered, T_1 goes through a minimum at $\omega\tau_c \simeq 1$ for 90 MHz. In the region of short τ_c, T_1 decreases with increasing τ_c, whereas in the region of long τ_c, it increases with increasing τ_c. On the other hand, T_2 decreases with increasing τ_c in the whole region. T_1 shows little frequency dependence in the extreme narrowing region ($\omega\tau_c \ll 1$), and it shows frequency dependence in the spin diffusion region ($\omega\tau_c \gg 1$).

Figure 1 shows the temperature dependencies of T_1 and T_2 of water proton in the random copolymer P(MMA-co-M100G) and the graft copolymer P(VC-g-M100G) measured at 90 MHz, with those in poly(methyl methacrylate) for comparison.[6] The value of τ_c is known to increase with decreasing temperature.[9] Then, within the temperature region measured (28°C to −30°C), T_1's of water proton in these three hydrogels are in the short τ_c region of T_1 minimum ($\tau_c < 10^{-9}$ s). The T_1 value at 28°C for P(MMA-co-M100G) is about twice as long as that for poly(methyl methacrylate). This indicates that the mobility of water contained in the random copolymer is higher than that of poly(methyl methacrylate), provided that the distance of interacting water protons is the same for the both hydrogels.

The observed T_1 values measured at 90 and 400 MHz for the four different kinds of hydrogels, that
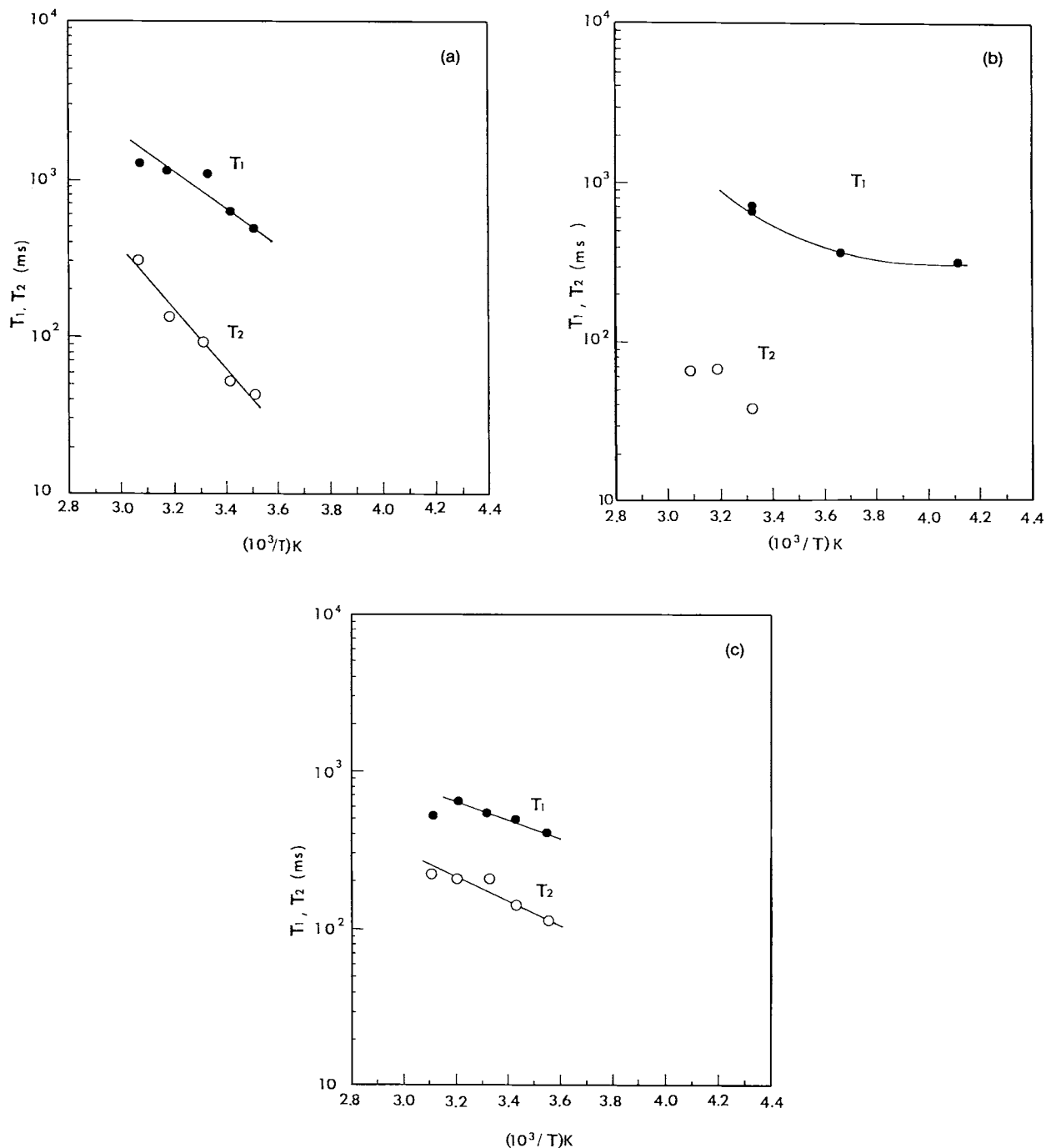
Figure 1   Semilogarithmic plots of T_1 and T_2 against the reciprocal temperature (measured at 90 MHz at 28°C) for (a) P(MMA-co-M100G), (b) poly(methyl methacrylate), and (c) P(VC-g-M100G).

is, poly(methyl methacrylate), poly(hydroxyethyl methacrylate), P(VC-g-M100G), and P(MMA-co-M100G) are shown in Table I. The lower frequency dependence of T_1 of water in the P(MMA-co-M100G) than that in poly(methyl methacrylate) could be also attributed to the higher water mobility

or a broader distribution of relaxation times in the random copolymer.

By measuring ¹³C relaxation times, Yokota has estimated the correlation time τ_c of the polyoxyethylene side chain to be less than 10^{-9} s.[10,11] He reported also that the ¹³C-T_1 value of the polyoxyeth-

**Table I   The Frequency Dependence of
Relaxation Times T_1's of Water Contained in
Hydrogels $[T_{1obs}(s)]^a$**

Frequency	90 MHz	400 MHz
Poly(methyl methacrylate)	0.64	1.40
Poly(hydroxyethyl methacrylate)	0.19	0.60
P(VC-g-M100G)	0.53	0.91
P(MMA-co-M100G)	1.13	1.42

Measured at 28°C.
[a] Inversion recovery method was used.

ylene side chain of the membrane is close to that of the poly(ethylene oxide) solution and concluded that its mobility is almost the same as that of liquid poly(ethylene oxide) since the side chains in the membrane are swollen thoroughly with water. Therefore, water molecules bound to the mobile polyoxyethylene side chains must move also as fast as the chains or rather faster. Thus, the mobility of free and bound water around the hydrophilic side chains is considerably high. Although the poly(hydroxyethyl methacrylate) membrane also contains hydrophilic groups, the apparent mobility of water is much lower than that in the poly(methyl methacrylate).[6] Since the hydrophilic groups of poly(hydroxyethyl methacrylate) are immobile, the mobility of the water bound to the groups is much more restricted.

As reported in the previous paper, the water signal intensities of poly(methyl methacrylate) and poly(hydroxyethyl methacrylate) hydrogels decreased when the position to 20 ppm up-field of the observed water signal was irradiated. This indicates that a saturation transfer occurred by irradiating a part of the very broad water region where actually no signal is observed. This phenomenon was attributed to the exchange between observable water and unobservable water with a considerably rigid structure (bound water).[6] Thus, the existence of bound water in a rigid state was suggested by saturation transfer phenomena. On the contrary, little signal intensity change was observed for P(MMA-co-M100G) and P(VC-g-M100G) under the same experimental condition. This suggests that the amount of the bound water in a rigid state should be negligible or quite small for these copolymers.

As shown in Figure 1(c), T_1 values of water proton in the graft copolymer P(VC-g-M100G) are smaller as compared with those for the random copolymer, indicating that the mobility of water con-

tained in the graft copolymer is lower. The temperature dependencies of T_1 and T_2 of water proton in the graft copolymer are lower than those in the random copolymer. This indicates that the distribution of τ_c values is more significant for the graft copolymer. Although the length of the side chains is the same for both copolymers, polyoxyethylene side chains of the random copolymer are apart, whereas those of the graft copolymer are contiguous to each other as illustrated in Figure 2. Therefore, the interaction between side chains would be more significant. The distribution of the correlation times of water bound to the side chains could be attributed to the heterogeneous mobility resulting from the complex chain interaction.

From the ratio of T_1's measured at 90 and 400 MHz, the values of apparent correlation time τ_c of water protons in the random copolymer and the graft copolymer were roughly estimated to be 10^{-10} s and 2×10^{-10} s (28°C), respectively, under the first approximation that the relaxation is determined only by the dipole–dipole interaction of protons on the same water molecule and that the correlation time is isotropic without taking the distribution of τ_c values into account. These values are larger than that of free water ($\sim 10^{-11}$ s) but smaller than that of intermediate water ($\sim 10^{-9}$ s) obtained for poly(methyl methacrylate) and poly(hydroxyethyl methacrylate).[6]

SYNTHETIC HYDROGELS

A   PMMA

B   PHEMA

C   P(MMA-co-M100G)

D   P(VC-g-M100G)

○ -O-CH2CH2OH

□ -(O-CH2CH2)100 -OCH3
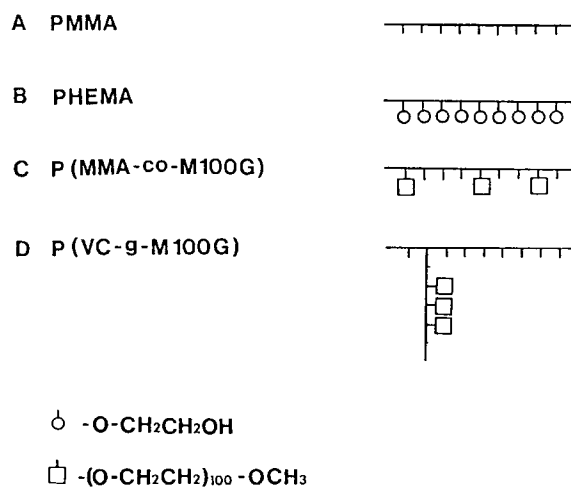
Figure 2   The four synthetic hydrogels. (A) Poly(methyl methacrylate) (PMMA); (B) poly(hydroxyethyl methacrylate) (PHEMA); (C) P(MMA-co-M100G); (D) P(VC-g-M100G).

Freezing and Thawing

Figure 3 shows the change of proton signal intensities of water on decreasing temperature from 28°C to −60°C and increasing temperature from −60°C to 28°C. As the mobility of water decreases with decreasing temperature, the proton signal of water in the frozen state was too broad to be observed. Therefore, a decrease in the signal intensity corresponds to an increase in the amount of the frozen water.

For the graft copolymer, the signal intensity decreases monotonically from 0°C to −60°C. On the other hand, the random copolymer showed two-step freezing procedures. At −25°C, the intensity decreased relatively abruptly by about 60% of that at 28°C. Then it decreased gradually until −60°C. For comparison, the procedure observed for poly(methyl methacrylate)[6] was shown in Figure 3(c). For poly(methyl methacrylate), more than 95% of the observed water was frozen at −25°C.

The freezing procedure for the random copolymer P(MMA-co-M100G) is the same as that for poly(methyl methacrylate) from 28°C to −25°C, whereas below −25°C, it is the same as that of the graft copolymer P(VC-g-M100G). Almost all the observable water around the main chain of the random copolymer [poly(methyl methacrylate)] has frozen at −25°C, but a part of the water around hydrophilic side chains remains unfrozen and freezes gradually from −25°C to −60°C. The two-step freezing procedure was not observed for the graft copolymer P(VC-g-M100G). Since the trunk and the graft parts of P(VC-g-M100G) have no water content, the freezing procedure would include mainly only the freezing behavior of water around the hydrophilic side chains.

As shown in Figure 3, the freezing and thawing procedures are not superimposable. For the graft copolymer, the hysteresis is quite small as compared with that observed for poly(methyl methacrylate).[6] One plausible explanation for this hysteresis would be that during freezing water molecules in the network of the gel are pushed out of it, but that on thawing, the gel network would not be hydrated readily.[12] Therefore, the smaller hysteresis would indicate the easier hydration of the very flexible hydrophilic side chains of P(VC-g-M100G) on thawing. For the random copolymer, the freezing and thawing procedures below −25°C include only the behavior of the water molecules around the mobile hydrophilic side chains. Therefore, the hysteresis was also small, whereas it was relatively large above −25°C since it includes that around the main chain
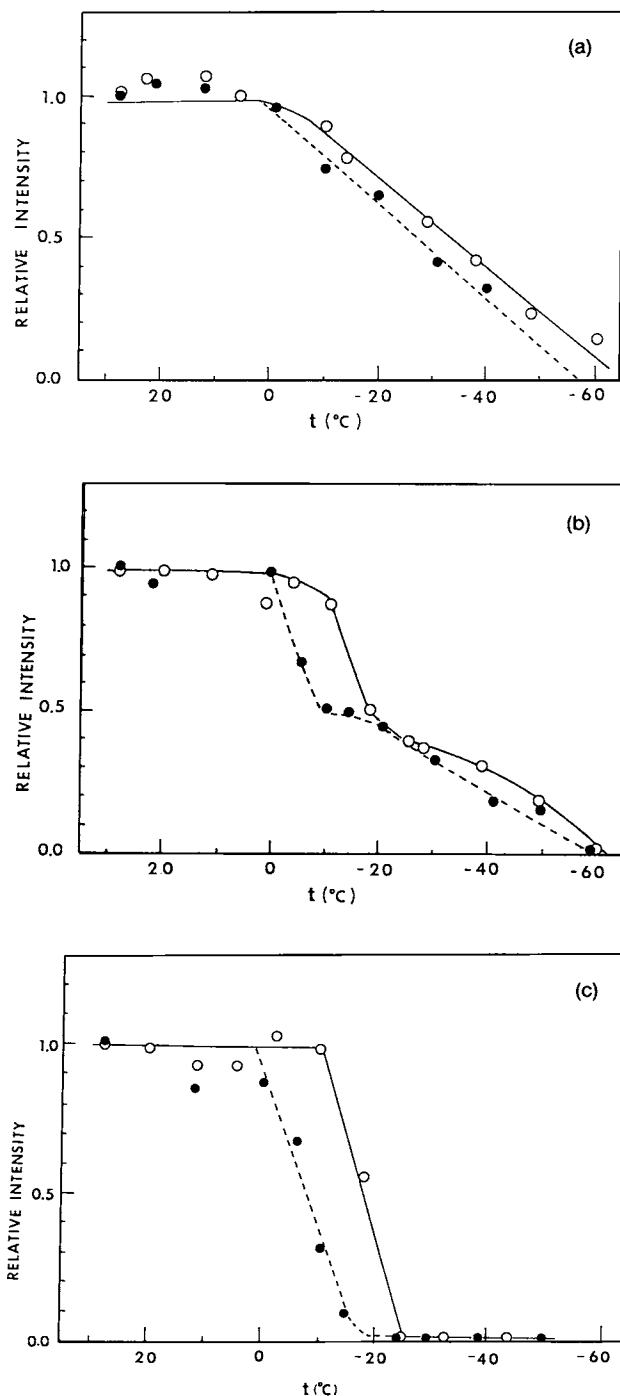

Figure 3 Change in intensity of the water proton signal on freezing and thawing measured at 90 MHz. TMS was used as an external reference. (a) P(VC-g-M100G); (b) P(MMA-co-M100G); (c) poly(methyl methacrylate) on cooling (O) and on heating (●).

of poly(methyl methacrylate) as well as the side chains. Thus, the water contained in these graft and random copolymers does not completely freeze until

−60°C and shows small hysteresis on freezing and thawing, suggesting that some characteristics of the polymers should still be preserved at very low temperature for these two membranes.

The results obtained for these four synthetic polymers, poly(methyl methacrylate), poly(hydroxy ethyl methacrylate), P(MMA-co-M100G), and P(VC-g-M100G), are illustrated in Figure 2. The water contained in the membranes of poly(methyl methacrylate) and poly(hydroxyethyl methacrylate) was classified into three states: that is, bound, intermediate, and free states.[6] The bound water takes a very rigid structure, and the proton NMR signal was too broad to be observed. The intermediate water with relatively higher flexibility is observable and involved in the fast exchange with the bound water. Because of the immobile hydrophilic groups, the amounts of the bound and the intermediate water are much higher and the mobility of water is lower in a hydrophilic hydrogel of poly(hydroxyethyl methacrylate) than they are in a hydrophobic hydrogel of poly(methyl methacrylate). Water is considered to be unable to permeate into the bound water region, whereas urea can break the hydrogen bond of the bound water and permeate into the bound water region. Therefore, for the hydrogel that has more bound water, the permeability to water is expected to be lower. In fact, it has been found that the permeability of hydrophobic poly(methyl methacrylate) membrane to water is much higher than that of hydrophilic membrane at the same water content but that the permeability to urea is barely affected by the polymer materials.[3]

On the other hand, since P(MMA-co-M100G) and P(VC-g-M100G) possess long and flexible hydrophilic side chains, water could not be described simply as a three-state model. The water bound to the flexible side chain also has high or rather higher mobility than that of the side chains themselves. Thus, the mobility of most of the bound water of these copolymers is very high and the amount of the bound water in a rigid state is considered to be negligible or quite small.

As illustrated in Figure 2, the side chains of a random copolymer P(MMA-co-M100G) are located apart and are unlikely to affect each other. On the other hand, those of a graft copolymer P(VC-g-

M100G) are located contiguously and the interaction among the side chains must be more significant. Therefore, their motional mode would become more complicated and heterogeneous than that of the random copolymer. The difference in the heterogeneity of the mobility of side chains and water molecules on the membrane surface between the random copolymer and the graft copolymer might be related to the difference in their antithrombus characteristics.

Thus, the difference of the properties of water contained in these four synthetic membranes suggests that the differences of the length and the mobility of the hydrophilic side chains should be deeply correlated with their membrane functions.

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