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Hydration of phosphorylcholine groups attached to highly swollen polymer hydrogels studied by thermal analysis

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ABSTRACT

Hydration of polymer chains plays a key role for determining the extent of protein adsorption on polymeric materials. Here we investigated the hydration of poly(2-methacryloyloxyethyl phosphorylcholine (MPC)) chains, which resist protein adsorption and following cell adhesion effectively. The hydration was compared with that of poly(methoxy oligo(ethylene glycol)-monomethacrylate (Me(EG)$_n$MA)) chains, which also have hydrophilic units. The poly(MPC) and poly(Me(EG)$_n$MA) hydrogels with equilibrium water contents (EWCs) in the range from 86 to 97 wt% were prepared. By differential scanning calorimetric measurements, water in both the hydrogels was classified into two states: freezable and nonfreezable water. The poly(MPC) hydrogels had larger nonfreezable water than the poly(Me(EG)$_n$MA) hydrogels even when their EWCs were similar, which indicated the higher hydrating ability of poly(MPC) chains. We suggested that the difference in the amount of nonfreezable water around polymer chains may influence the degree of protein adsorption resistance after contact with body fluid for a long period.

1. Introduction

According to the rapid advancement in developments of artificial organs, drug delivery systems, biochip-based diagnosis systems, and tissue engineering devices, the importance in the design of material surfaces that resist protein adsorption has been stressed [1–4]. Protein adsorption on material surfaces is the first phenomenon in contact with blood or tissues [5]. The adsorbed proteins are denatured, which is followed by platelet adhesions and cell adhesions for inducing thrombus formation and unfavorable immunoreactions. Thus, protein adsorption-resistant surfaces are essentially needed to obtain safe and stable medical treatment and diagnosis. Especially, cell-based tissue engineered devices and implantable artificial organs should have protein adsorption resistance surface for controlling cell/materials interactions for long period. To date, many protein adsorption-resistant surfaces have been designed. Recently, some research groups have achieved very low protein adsorption levels of <10 ng/cm^2 by controlling the packing density and/or lengths of surface-tethered hydrophilic polymer chains [6–8]. On the other hand, the physico-chemical factors that determine the ability of the surfaces to resist protein adsorption have not been elucidated yet. A satisfactory understanding of such factors allows not only the systematic design of protein adsorption-resistant surfaces but also the elucidation of the mechanism of protein adsorption resistance.

Surface free energy on polymer materials has often been considered to be a key determinant of the extent of protein adsorption [9,10]. However, it has been demonstrated that there is no clear correlation between surface free energy and adsorbed amount of protein [11]. In addition, although the high conformational flexibility of surface-tethered chains has been experimentally and theoretically explained to make sterical inhibition of the access of proteins to surfaces by an excluded volume effect [12,13], it is not an essential requirement for protein adsorption resistance. In fact, self-assembled monolayers (SAMs) with polar functional end groups, such as short-chain poly(ethylene glycol) (PEG), i.e. oligo(ethylene glycol) (OEG), and an equimolar mixture of $-SO_3^-$ and $-N^+(CH_3)_3$ end groups, showed high resistance to protein adsorption [14,15].

The present discussion on the factors that determine the outcome of protein adsorption resistance is centered on the relationship between the hydration structures of material surfaces and protein adsorption [11,16]. The hydration structures of PEG chains have been intensively studied [17–19] because their utilization as surface modifiers is a well-known approach for rendering surfaces highly resistant to protein adsorption [6,9,11,14]. We have developed our original biocompatible polymer, poly(2-methacryloyloxyethyl phosphorylcholine) (poly(MPC)), which is inspired from the

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structure of phosphatidylcholines in cell membrane [4,20–22]. As a criterion of the hydration structures that provide the resistance of protein adsorption, the hydration structures of poly(MPC) chains provide us strong interest for understanding the biocompatibility. The surfaces grafted with the poly(MPC) chains resist platelet adhesion for longer periods than OEG-monomethacrylate polymer surfaces [23]. In addition, no conformation of albumin in poly(MPC) aqueous solutions changes during 72 h incubation, whereas albumin denatured by incubation in PEG aqueous solutions within 24 h [24]. Thus, it can be expected that the hydration of poly(MPC) chains may be different from that of PEG chains.

In this study, we investigated the hydration structures of poly(MPC) chains using a chemically cross-linked poly(MPC) hydrogel. The poly(MPC) hydrogels with six different equilibrium water contents (EWCs) were prepared in the range from 86.1 to 96.5 wt%. The different states of water absorbed in the hydrogels were classified and quantified by differential scanning calorimetry (DSC). They were compared with those in chemically cross-linked hydrogels composed of OEG-monomethacrylate polymer chains–poly(ω-methoxy tetra- or octa(ethylene glycol) monomethacrylate (Me(EG)$_n$MA) ($n = 4$ or 8)) chains– with a similar EWC range. The origin of nonfreezable water around poly(MPC) chains was also discussed.

2. Experimental section

2.1. Materials

The detailed synthetic process of MPC (Fig. 1a) has been reported elsewhere [20]. Me(EG)$_n$MA (Fig. 1b) with molecular weights of 286 ($n = 4$) and 469 ($n = 8$) was purchased from Sigma–Aldrich (St. Louis, MO) and used without further purification. Ammonium peroxodisulfate (APS) (>98.0%, Kanto Chemicals, Tokyo, Japan), triethylene glycol dimethacrylate (TEGDMA) (>95%, Tokyo Kasei Kogyo, Tokyo, Japan), and *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TMEDA) (>98.0%, Kanto Chemicals) were also used without further purification. Distilled water was used for all sample preparations.

2.2. Preparation of chemically cross-linked poly(MPC) and poly(Me(EG)$_n$MA) hydrogels

The procedure for preparing chemically cross-linked poly(MPC) hydrogels has already been described [25]. The poly(Me(EG)$_4$MA) and poly(Me(EG)$_8$MA) hydrogels were prepared by the same procedure. In brief, the hydrogels were prepared in an aqueous medium by free radical polymerization. An aqueous monomer solution, TEGDMA (1.0 mol% to a monomer) as a cross-linker, and a 0.22 mol/L APS aqueous solution (0.53 mol% to a monomer) as an initiator were placed in a Petri dish. The concentrations of the monomer solutions were 1.5, 1.75, 2.0, 2.25, 2.5, and 3.0 mol/L for MPC and 0.75, 1.0, 1.25, and 1.5 mol/L for Me(EG)$_4$MA and Me(EG)$_8$MA. The solution in the Petri dish was stirred for 30 min to allow complete mixing. The solution began to make gelation 1 min after the injection of TMEDA (5.3 mol% to a monomer) as a catalyst. After its complete gelation, the obtained hydrogel was removed from the Petri dish, and subsequently, it was immersed in excess distilled water for 48 h to remove any unreacted compounds and to allow complete swelling. The water in the dish was replaced several times. All the fully swollen hydrogels were transparent. All these processes were carried out at room temperature. The fully swollen hydrogels were used for the following EWC and DSC measurements.

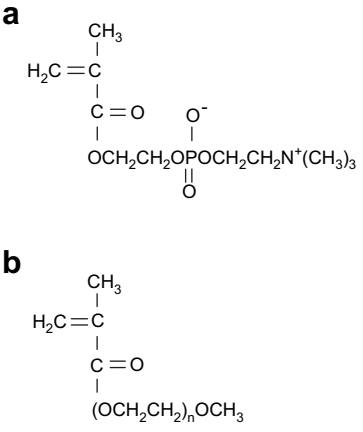

Fig. 1. Chemical structures of (a) MPC and (b) Me(EG)$_n$MA.

2.3. Determination of EWC

Each fully swollen hydrogel was freeze-dried for 24 h to remove the absorbed water. The weight of the freeze-dried hydrogel was recorded as W_d. The freeze-dried hydrogel was fully swollen again for 48 h. The excess water on the surface of the swollen hydrogel was gently removed with a filter paper before the measurement of its weight, W_s. The EWC of the hydrogel can be calculated by using the following equation.

$$\mathrm{EWC} = \frac{W_s - W_d}{W_s} \times 100 \quad (1)$$

2.4. DSC measurements

A 4–6 mg hydrogel was placed in an aluminum pan after gently wiping off the excess water on its surface, and then the pan was hermetically sealed. An empty aluminum pan was used as the control. Measurements were performed using an SII NanoTechnology (Chiba, Japan) model DSC6100 differential scanning calorimeter interfaced to an EXSTAR 6000 thermal analysis system version 5.8 (SII NanoTechnology). During the cooling and heating experiments, the sample cell was purged with nitrogen gas at a flow rate of 50 mL/min. The melting point peak of indium calibrated the temperature and heat flow of the equipment. The samples were initially cooled from room temperature to −70 °C at a rate of 5 °C/min and then heated to 40 °C at the same rate.

3. Results and discussion

Since the concentrations of water and polymer chains strongly influence their hydration properties, the poly(MPC) hydrogels with different EWCs were prepared. As shown in Fig. 2, the EWC of the poly(MPC) hydrogels could be controlled within the range of 86.1–96.5 wt%. To understand the effect of chemical structures on hydration, poly(Me(EG)$_4$MA) and poly(Me(EG)$_8$MA) hydrogels with a similar EWC range were prepared. The EWC of the poly(Me(EG)$_4$MA) hydrogels and poly(Me(EG)$_8$MA) hydrogels ranged from 87.5 to 95.9 wt% and 89.7 to 95.7 wt%, respectively. In the case of the poly(Me(EG)$_8$MA) hydrogels, the EWC could not control below 89 wt% even when monomer solutions with concentrations higher than 1.5 mol/L were used.

Figs. 3–5 show the typical DSC heating thermograms of the poly(MPC), poly(Me(EG)$_4$MA), and poly(Me(EG)$_8$MA) hydrogels swollen with different EWCs, respectively. For comparison, the thermogram of bulk water is also presented in the respective figures. In the poly(MPC) hydrogels, a single endothermic peak was observed, and the transition occurred over a temperature range similar to that of the ice-to-water transition for bulk water. We

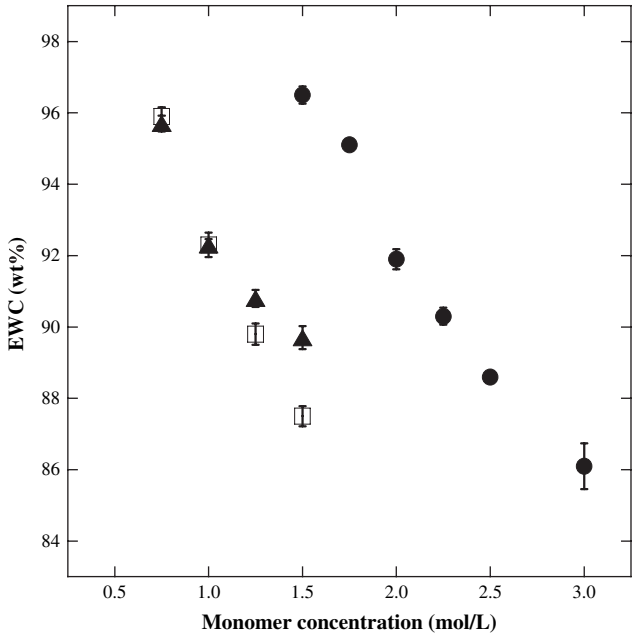

Fig. 2. EWC of the three types of hydrogels at the concentrations of the monomer solutions: poly(MPC) (●), poly(Me(EG)$_4$MA) (□), and poly(Me(EG)$_8$MA) (▲) hydrogels. The plotted values are the average of six measurements, and double the standard deviation is used as the range of errors in the values.

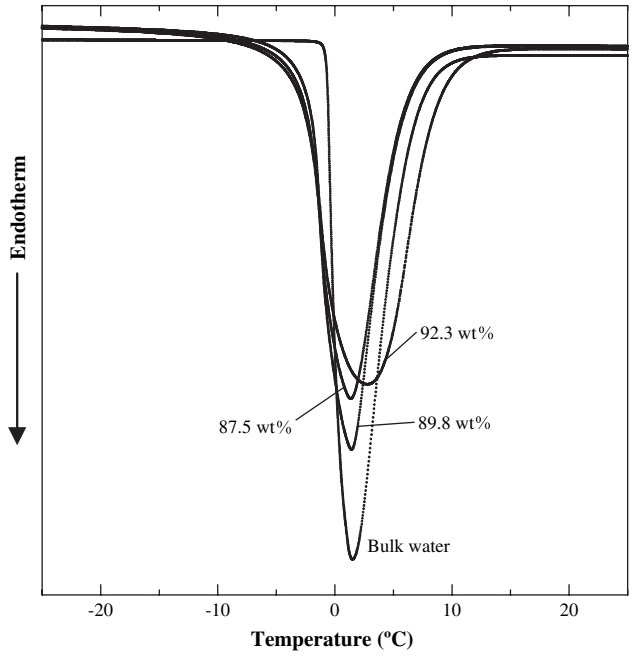

Fig. 4. DSC thermograms at a heating rate of 5 °C/min for the poly(Me(EG)$_4$MA) hydrogels with different EWCs and for bulk water. The values indicated in the figure show the EWCs.

could not observe any enthalpy change for freeze-dried poly(MPC) hydrogels in the temperature range, indicating that the polymer chains have no contribution to the endothermic behavior. Herein, it was concluded that the peak was derived from the melting of freezable water in the poly(MPC) hydrogels. As with the poly(MPC) hydrogels, the thermograms of the poly(Me(EG)$_4$MA) and poly(Me(EG)$_8$MA) hydrogels yielded single endothermic peak due to the melting of freezable water. In all the hydrogels, no other thermal transitions were observed during the heating experiments. In accordance with the earlier studies on hydrated polymer materials [17,26], the single endothermic peak observed for each hydrogel was broad toward the low-temperature side, which was in contrast to that for bulk water. This results from the distribution of the melting temperature of freezable water in the hydrogels [27].

From the area of each single peak, we estimated the enthalpy change (ΔH_f) associated with the melting of freezable water in the hydrogels. The values of ΔH_f for the poly(MPC), poly(Me(EG)$_4$MA), and poly(Me(EG)$_8$MA) hydrogels are plotted as a function of EWC in

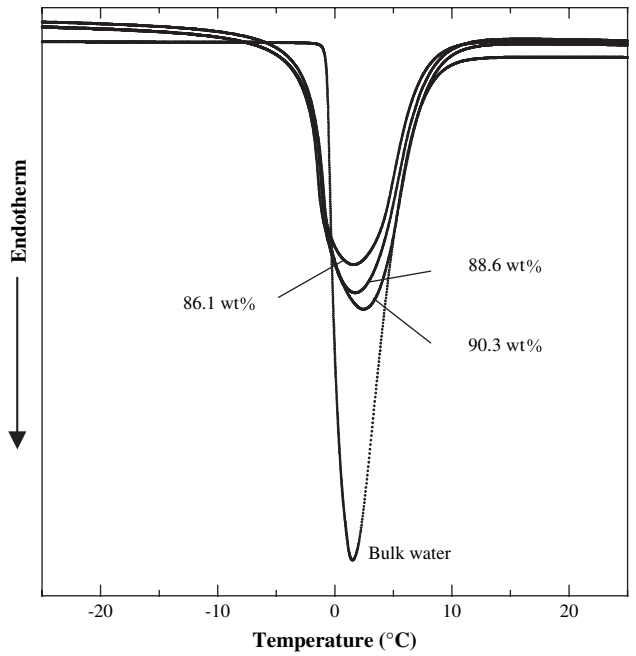

Fig. 3. DSC thermograms at a heating rate of 5 °C/min for the poly(MPC) hydrogels with different EWCs and for bulk water. The values indicated in the figure show the EWCs.

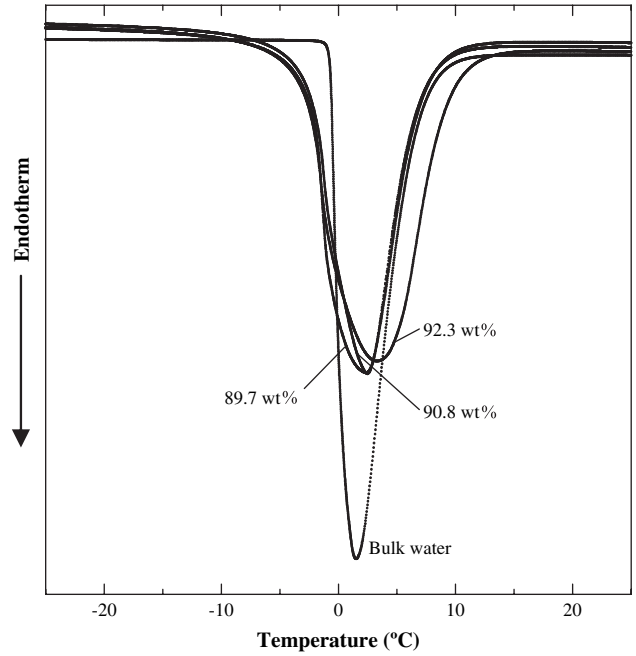

Fig. 5. DSC thermograms at a heating rate of 5 °C/min for the poly(Me(EG)$_8$MA) hydrogels with different EWCs and for bulk water. The values indicated in the figure show the EWCs.

Fig. 6. The values were less than those estimated on the supposition that all the water contained in the hydrogels behave as freezable water, which indicated that a certain amount of water in the hydrogels was unable to freeze. From the ΔH_f values, the amounts of freezable and nonfreezable water in the hydrogels can be calculated. The weight ($W_{freezable}$) of freezable water relative to that of the polymer in a hydrogel is expressed by

$$W_{freezable} = \frac{w_{freezable}}{w_{polymer}} \quad (2)$$

where $w_{freezable}$ and $w_{polymer}$ are the weight percents of freezable water and polymer in the hydrogel, respectively. Since the weight percent ($w_{nonfreezable}$) of nonfreezable water in a hydrogel is the difference between the total water content, namely EWC, and $w_{freezable}$, the weight ($W_{nonfreezable}$) of nonfreezable water relative to that of the polymer in a hydrogel is given by

$$W_{nonfreezable} = \frac{w_{nonfreezable}}{w_{polymer}} = \frac{EWC - w_{freezable}}{w_{polymer}} \quad (3)$$

Here, $w_{freezable}$ can be experimentally obtained by using ΔH_f and can be expressed by the following equation:

$$w_{freezable} = \frac{\Delta H_f}{\Delta H_w} \times 100 \quad (4)$$

where ΔH_f is the enthalpy change associated with the melting of freezable water per weight of a hydrogel and ΔH_w is the enthalpy change for the melting of bulk water. On the basis of Eq. (4), Eqs. (2) and (3) can be rewritten as

$$W_{freezable} = \frac{1}{w_{polymer}}\left(\frac{\Delta H_f}{\Delta H_w} \times 100\right) \quad (5)$$

$$W_{nonfreezable} = \frac{1}{w_{polymer}}\left(EWC - \frac{\Delta H_f}{\Delta H_w} \times 100\right) \quad (6)$$

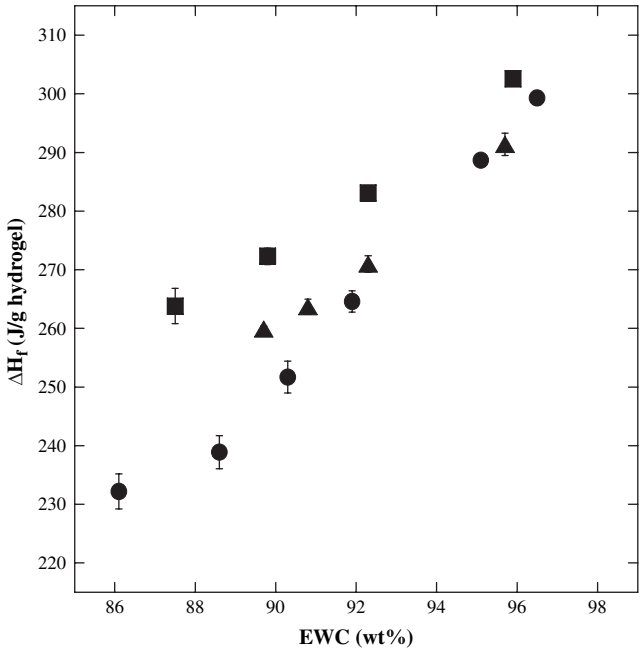

Fig. 6. Enthalpy changes associated with the melting of freezable water in the hydrogels as a function of EWC: poly(MPC) (●), poly(Me(EG)$_4$MA) (■), and poly(Me(EG)$_8$MA) (▲) hydrogels. The plotted values are relative to the weight of each hydrogel and were the average of four measurements. The standard deviation is used as the range of errors in the values.

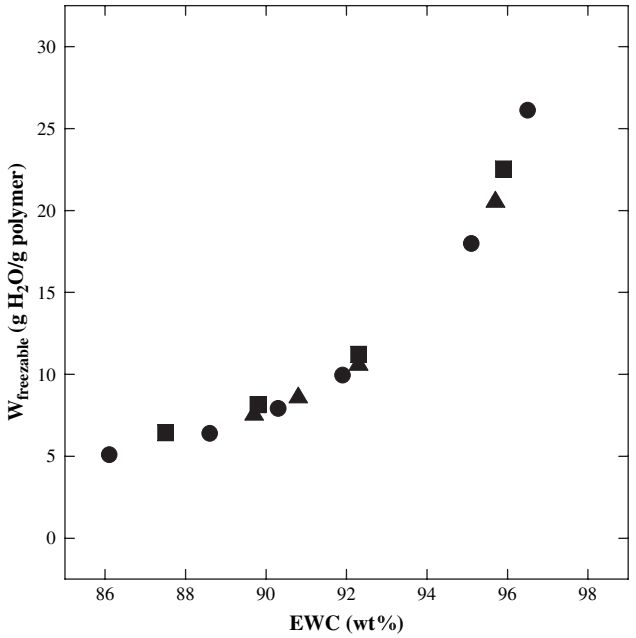

Fig. 7. Weight of freezable water relative to that of the polymer in the hydrogels as a function of EWC: poly(MPC) (●), poly(Me(EG)$_4$MA) (■), and poly(Me(EG)$_8$MA) (▲) hydrogels. The plotted values are the average of four measurements, and the standard deviation is used as the range of errors in the values.

ΔH_w measured for the distilled water used in this study was 327.5 ± 1.4 J/g (mean $\pm$ S.D., $n = 4$), which is almost the same value of bulk water (333.5 J/g). The measured ΔH_w value was used in Eqs. (5) and (6).

The $W_{freezable}$ and $W_{nonfreezable}$ values for the poly(MPC), poly(Me(EG)$_4$MA), and poly(Me(EG)$_8$MA) hydrogels are plotted as a function of EWC in Figs. 7 and 8, respectively. In Fig. 7, no significant differences were observed in the comparison of the $W_{freezable}$ values for the given values of EWC between the three

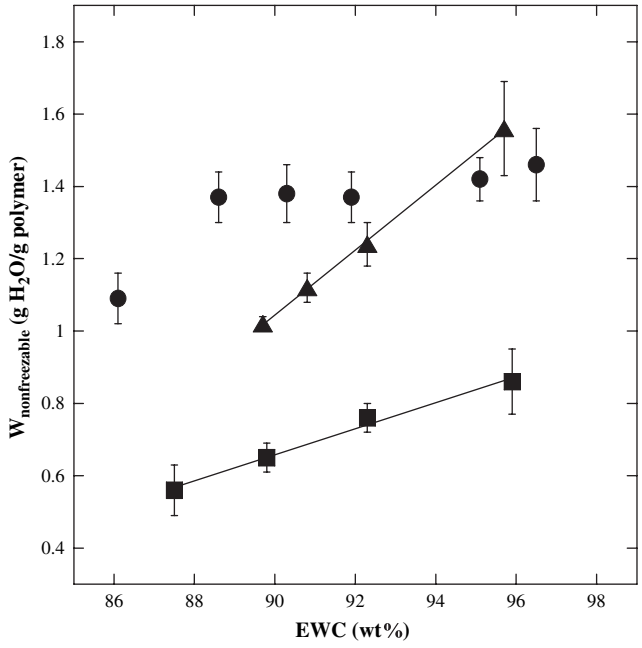

Fig. 8. Weight of nonfreezable water relative to that of the polymer in the hydrogels as a function of EWC: poly(MPC) (●), poly(Me(EG)$_4$MA) (■), and poly(Me(EG)$_8$MA) (▲) hydrogels. The plotted values are the average of four measurements, and the standard deviation is used as the range of errors in the values.

types of hydrogels. However, this result does not indicate that the amount of freezable water in the three types of hydrogels depends on the EWC. As seen in Fig. 6, the ΔH_f values for the given values of EWC differed between the hydrogels, especially in the EWC range from 86 to 92 wt%. According to Eq. (4), this difference remarkably influences the $w_{freezable}$ values. Among the hydrogels, the $w_{freezable}$ values for the given values of EWC clearly decreased in the order corresponding to poly(Me(EG)$_4$MA), poly(Me(EG)$_8$MA), and poly(MPC) hydrogels. By transforming the $w_{freezable}$ values into $W_{freezable}$ values using Eq. (5), little differences in the $W_{freezable}$ values among the hydrogels with similar EWC could be seen.

In contrast, the $W_{nonfreezable}$ values showed the large differences between the hydrogels. As shown in Fig. 8, the $W_{nonfreezable}$ values for the poly(MPC) hydrogels increased from 1.09 to 1.37 g H_2O/g polymer when the EWC was increased from 86.1 to 88.6 wt%. Moreover, the values did not change significantly (1.37–1.46 g H_2O/g polymer) with the increase in the EWC. On the other hand, the $W_{nonfreezable}$ values for the poly(Me(EG)$_4$MA) and poly(Me(EG)$_8$MA) hydrogels linearly increased with the EWC. For the poly(Me(EG)$_4$MA) hydrogels, the $W_{nonfreezable}$ values changed from 0.56 to 0.86 g H_2O/g polymer, whereas the changes in the $W_{nonfreezable}$ values for the poly(Me(EG)$_8$MA) hydrogels were from 1.02 to 1.56 g H_2O/g polymer. The poly(MPC) hydrogels showed higher $W_{nonfreezable}$ values as compared with the poly(Me(EG)$_4$MA) and poly(Me(EG)$_8$MA) hydrogels, except for the values comparable with the poly(Me(EG)$_8$MA) hydrogels when the EWC was above 95 wt%. This shows the higher hydrating ability of the poly(MPC) chains than that of the poly(Me(EG)$_4$MA) and poly(Me(EG)$_8$MA) chains. In addition, the $W_{nonfreezable}$ values for the poly(MPC) hydrogels were higher than those for protein- or polysaccharide-based materials, which have often been used as biomaterials, for similar total water contents [28–30]. It should be noted that the $W_{nonfreezable}$ values for the poly(MPC) hydrogels were constant with regard to the EWC, while those for the poly(Me(EG)$_4$MA) and poly(Me(EG)$_8$MA) hydrogels linearly increased with the EWC. We think that this feature may be related to the overlap of the hydration shells of polymer chains. In general, as the EWC of polymer hydrogels is decreased, the entanglement of polymer chains is enhanced. This leads to a decrease in the space among polymer chains. Here, the hydration shells of polymer chains overlap when the chains are mutually at some distance [31]. The constant $W_{nonfreezable}$ values for the poly(MPC) hydrogels might be caused by the absence of the overlap of the hydration shells.

Finally, we discussed the origin of nonfreezable water around poly(MPC) chains. The $W_{nonfreezable}$ values for poly(MPC) chains were transformed into the number (N_w) of nonfreezable water molecules per poly(MPC) repeating unit by the following equation:

$$N_w = W_{nonfreezable} \times \frac{M_p}{M_w} \quad (7)$$

where M_p is the molecular weight per polymer repeating unit ($M_p = 295$ for poly(MPC)) and M_w is the molecular weight of water. The results are summarized in Table 1. The N_w value per poly(MPC) repeating unit was 23–24. The phosphorylcholine groups in poly(MPC) chains are bulky and hydrophilic, so they have the large hydration capacity. Also, the value was consistent with the number of water molecules associated with each phosphorylcholine group in dodecylphosphorylcholine surfactants below the critical micelle concentration, that is, 24–25 [32]. As represented by the interaction with PEG chains of water, the formation of nonfreezable water in polymer–water systems has frequently been explained as a result of the hydrogen bonds between water molecules and polymer chains [17,26,28]. However, poly(MPC) chains have difficulty in the formation of the 23–24 nonfreezable water molecules by only the hydrogen bonds with water molecules because the primary atoms that can form hydrogen bonds with water molecules are one carbonyl oxygen and two non-ester phosphate oxygens per repeating unit. A possible explanation for the origin of nonfreezable water molecules around poly(MPC) chains may be the weak electrostatic interaction of water molecules with zwitterionic groups in phosphorylcholine groups. Kitano et al. showed that poly(MPC) chains did not significantly disturb the hydrogen bonds between their surrounding water molecules, suggesting that the phosphorylcholine groups may counteract the electrostatic hydration [33,34]. It is clear that further study is needed to characterize the origin of the nonfreezable water molecules. We believe that it can be achieved by NMR relaxation time measurements or vibrational spectroscopy such as infrared and Raman, since these methods can probe the faster motion of water networks than thermal analysis and especially, vibrational spectroscopy can provide information on local water networks.

Table 1
Number (N_w) of nonfreezable water molecules per poly(MPC) repeating unit

EWC (wt%)	96.5	95.1	91.9	90.3	88.6	86.1
N_w[a]	24 ± 2	23 ± 1	23 ± 1	23 ± 1	23 ± 1	18 ± 1

[a] The values were obtained by averaging the results of four measurements, and the standard deviation was used as the range of errors in these values.

4. Conclusion

Hydration of poly(MPC) chains was investigated by using chemically cross-linked poly(MPC) hydrogels in the EWC range from 86.1 to 96.5 wt%. It was compared with that of poly(Me(EG)$_4$MA) and poly(Me(EG)$_8$MA) chains with a similar hydration level. From the results of the enthalpy change associated with the ice-to-water transitions in the hydrogels obtained by DSC measurements, poly(MPC) chains had a higher amount of nonfreezable water than poly(Me(EG)$_4$MA) and poly(Me(EG)$_8$MA) chains. The high hydrating ability of poly(MPC) chains was kept at a high level in the EWC range. In addition, it was suggested that nonfreezable water around poly(MPC) chains was derived from electrostatic interaction as well as hydrogen bonds. It has been observed that poly(MPC) chains resist platelet adhesion and protein denaturalization for longer periods than OEG-monomethacrylate polymer and PEG chains. Thus, the results in this study may indicate that the nonfreezable water around polymer chains detected by thermal analysis may be one of the promising parameters for considering a longer duration of resistance against the protein adsorption. This study is our starting point for the establishment of hydration parameters that can characterize the relationship between the outcome of protein adsorption resistance and hydration of materials.

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