Studies on Novel Phosphatidylcholine-Modified Acrylamide-Based Hydrogels

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ABSTRACT: Gel formation of a water-soluble zwitterionic phosphatidylcholine analogous acrylamide monomer, 1-(acrylamidomethyl)-2′-(trimethylammonio)ethyl phosphate (**AMP**), was examined using *N,N′*-methylenebisacrylamide as the crosslinking constituent, ammonium persulfate as the initiator, and *N,N,N′,N′*-tetramethyl ethylene diamine as the accelerator at room temperature in water system. The swelling properties of the synthesized hydrogel **AMPG** were investigated in distilled water at different temperatures, in water–acetone medium with different volume ratios, or in different inorganic salts aqueous of various compositions. Furthermore, lysozyme release behavior of the gel was found to depend on the composition of immersed solutions. © 1997 John Wiley & Sons, Inc. J Appl Polym Sci **64**: 1403–1409, 1997

Key words: water-soluble phosphatidylcholine analogous acrylamide monomer; hydrogel; swelling properties; temperature dependence; dependence of solvent composition; dependence of ion kind and intensity; egg lysozyme release behavior

INTRODUCTION

In biological systems, it has been widely observed that water-soluble proteins exhibit an insoluble state or surface adhesion even though in aqueous solutions. These insolubilization and adhesion phenomena of the natural biopolymeric systems have been explained by the chain interpenetrations. Apart from the biological interests, water-containing crosslinked polymers (hydrogels) have long been studied and used in a diverse assortment of applications, such as thickening agents in foods, contact lens, or other pharmaceutical products.[1,2] Hydrogels display high hydrophilicity and can imbibe large quantities of water or aqueous solutions. The three-dimensional network is able to retain the liquids forming a swollen gel phase, and the liquid prevents the polymer network from collapsing into a compact mass.[3,4] Fur-

thermore, swelling properties of polyelectrolyte gels depend strongly on gel composition, temperature, electric fields, and the concentration of electrolyte solutes in the surrounding solution.
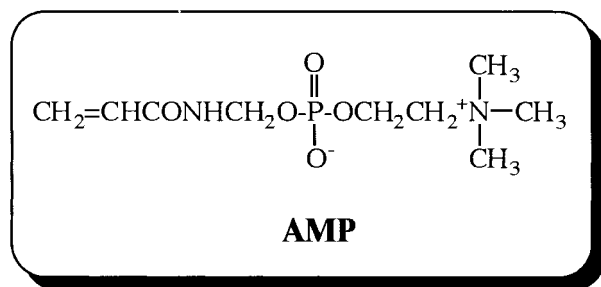
On the other hand, phospholipid analogues have recently attracted much interest in chemistry and biochemistry[5–12] because phospholipids are found in various cellular membranes with the highest concentration.[13] Considerable attention has been paid to polymeric phospholipid analogues containing phosphatidylcholine moieties, which exist on the surface of the phospholipid bilayer, concerning biocompatibility and other properties.[14,15]

Recently, we reported the syntheses and properties of two amphiphilic phospholipid analogous polymer gels with order structures.[16] However, to our knowledge, no study has been reported about high hydrophilic hydrogels prepared from water-soluble vinyl monomers bearing phosphatidylcholine groups or analogues. From this point of view, it seems to be highly important to investigate the

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behavior of hydrogels containing phosphatidyl-choline groups. In this present work, a novel acryl-amidic hydrogel containing phosphatidylcholine groups was synthesized from water-soluble vinyl monomer 1–(acrylamidomethyl)–2′–(trimethyl-ammonio)ethyl phosphate (**AMP**). Furthermore, the swelling properties and lysozyme release be-havior of this gel obtained were investigated in different surrounding solutions.

$$CH_2=CHCONHCH_2O-\overset{\overset{\displaystyle O}{\|}}{\underset{\underset{\displaystyle O^-}{|}}{P}}-OCH_2CH_2{}^+\overset{\overset{\displaystyle CH_3}{|}}{\underset{\underset{\displaystyle CH_3}{|}}{N}}-CH_3$$

AMP

EXPERIMENTAL

Materials

The synthesis and characterization of 1–(acrylami-domethyl)–2′–(trimethyl ammonio)ethyl phos-phate (**AMP**) have been described in detail pre-viously.[17] *N,N′*-Methylenebisacrylamide (**MBAA**) and *N,N,N′,N′*-tetramethyl ethylene diamine (**TMEDA**) were purchased from Tokyo Kasei Co., Japan. Lysozyme (M_w = 14300, isoelectric point pI = 11) was obtained from Biochemical Industries, Co., Japan. Organic solvents were purified by dis-tillation. All other chemicals were reagent quality and used without further purification. All reagents were purchased from Wako Pure Chemical Indus-tries, Ltd., Japan, unless otherwise noted.

Preparations of Solid and Swelling Gels

The synthesized monomer **AMP** was used to pre-pare hydrogel by the following method. Into a 20 mL sealable glass bottle, 15.0 mL of distilled, de-gassed, and nitrogen-saturated water was added. Then 6.0×10^{-3} g of **MBAA**, the crosslinking constituent, was placed into the bottle and com-pletely dissolved. After the solution was cooled to 4°C, 1.0×10^{-2} g of ammonium persulfate (**AP**) as the initiator, and 60 μL of **TMEDA** as the accelerator were dissolved in this solution. Five tubes, which were opened at both ends (1.0 cm length and 3.5 mm internal diameter), were im-

mersed in the resulting solution in the bottle with-out bubble. The bottle was placed in a nitrogen-filled golvebox for 30 min and then it was sealed. After they were remained in the nitrogen atmo-sphere at 25°C for 24 h, transparent gel was ob-tained in the bottle and tubes. The gel lumps were removed clearly from the test tubes, stored in a bottle at about 4°C, and rinsed several times with water to remove initiator residues and unreacted monomers trapped in the network.

These gels were cut into 5.0 mm length with 3.5 mm diameter and were used as solid gels. The solid gels were immersed 3 days in distilled water at different temperatures, in water–acetone me-dium with different volume ratios, or in different inorganic salts aqueous with various compositions where they were allowed to swell to equilibrium. The solutions were changed one time per day to let gels achieve swelling equilibrium thoroughly. For each kind of gel and solution, a beaker with five gels and about 50 mL solution were prepared. To prevent evaporative losses, which could appreci-ably increase the ionic strength of the solution, the beakers containing gels and solution were sealed with wrap film. In the experiment for investigating the temperature dependence, the gels were placed in sealable bottles instead of in beakers. After equi-librium was reached, the gels were separated from the equilibrated solutions and thoroughly washed several times with distilled pure water to essen-tially remove all probe solutes and salts. These pure gels that attained swelling equilibrium were used as swelled gels. Then the swelling ratios in various surrounding solutions were determined as the aver-age value of five hydrogels in the same condition.

Measurements of Lysozyme Release for Synthesized Hydrogels

Into a 100 mL beaker, 0.3 g of egg lysozyme (M_w = 14,300) was dissolved onto 50 mL distilled wa-ter to give lysozyme solution. The solid gels (length: 5.0 mm, internal diameter: 3.5 mm) were placed in the lysozyme solution at room tempera-ture for 3 days, then saturated intercontained ly-sozyme gels (**L-AMPG**) were obtained. After these gels **L-AMPG** were taken out of the lyso-zyme solutions, they were washed quickly with distilled water and then immersed in different in-organic salts aqueous with different ion intensity or different pH at room temperature. The concen-tration of released lysozyme was monitored by taking 3 mL aliquots of medium at specific time points, determining the concentration at 280 nm

on a UV spectrophotometer, and then returning the solution to the original medium to keep the same concentration standard.

Characterization Methods

The degree of swelling was determined as $(W_s - W_d)/W_d$, where W_s is the weight of the swollen gel and W_d is the weight of the solid gel. The weight measurements were carried out by using an electronic balance with uncertainty ± 0.0001 g, Hansen HR-60, Japan. The pH of the surrounding solutions in distilled water was brought of pH 1–14 by the addition of NaOH or HCl ($0.01-0.1M$), and was measured with a Beckman Φ 34 pH Meter. The lysozyme content released from swollen gels were judged from the absorbance at 280 nm and 25°C (range: 200–400 nm, scan speed: 200 nm/min), which was measured using a Hitachi 320 spectrophotometer.

RESULTS AND DISCUSSION

A new hydrogel was produced by homopolymerization with a type of phospholipid–analogous acrylamide monomer. The synthesis and characterization of this monomer 1-(acrylamidomethyl)-2′-(trimethylammonio)ethyl phosphate (**AMP**) have been described in detail previously.[17] In the gelation, N,N'-methylenebisacrylamide (**MBAA**) was used as the crosslinking constituent, ammonium persulfate (**AP**) was used as the initiator, and N,N,N',N'-tetramethyl ethylene diamine (**TMEDA**) was used as the accelerator. After dissolving these chemicals in distilled, degassed, and nitrogen-saturated water with certain rates at 4°C, the solution was transferred into 1.0 cm length and 3.5 mm internal diameter glass tubes and left in the tubes for 24 h at 25°C to give crosslinked polymer gels. These gels were removed and stored at 4°C, and rinsed several times to remove impurities and unreacted chemicals trapped in the network. These pure gels obtained are called as solid gels in this article.

The swelling properties of synthesized hydrogels were investigated after the solid gels were immersed for 3 days in different surrounding solutions at different temperatures. The degree of swelling was determined as $(W_s - W_d)/W_d$, where W_s is the weight of the swollen gel and W_d is the weight of the solid gel.

A number of synthetic polymer gels are known to undergo discontinuous volume phase transi-
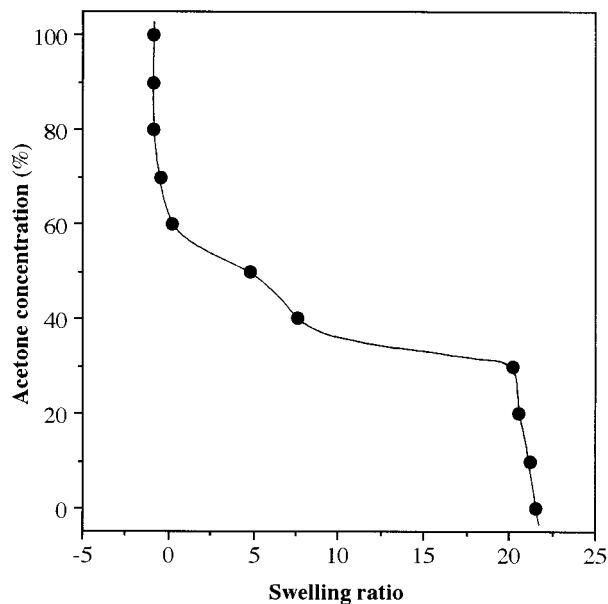
Figure 1 Swelling ratios of hydrogel **AMPG** as a function of solvent composition in the mixture of acetone and water at 25°C.

tions in response to infinitesimal changes in temperature,[19] solvent composition,[20–22] pH,[23] or by application of a small electric field across the gel.[24] For a gel to undergo a discontinuous volume phase transition, the gel should be subject to a sufficiently large internal osmotic pressure and that the solvent in which the gel is immersed should be sufficiently poor. An adequately poor solvent can be readily found for any polymer network. A positive internal osmotic pressure can be created by ionizing the polymer network; the pressure originates from the translational degrees of freedom of the counterions trapped inside the gel surrounded by the Donnan potential wall. The gels that have been found to exhibit discontinuous volume transitions so far are almost synthetic acrylamidic polymer gels. In this work, we present investigations of swelling properties of this new acrylamidic hydrogel containing biocompatible phosphatidylcholine groups.

The synthesized phosphatidylcholine analogous acrylamide monomer is easily soluble in water but almost insoluble in acetone. Therefore, the swelling behavior of hydrogel **AMPG** was investigated in water–acetone medium with different volume ratios. In Figure 1 the swelling ratio is plotted as a function of acetone composition. From 0 to about 30 vol % of acetone composition, gel **AMPG** swelled largely compared to the original solid gel. The swelling ratios are very close in this composi-
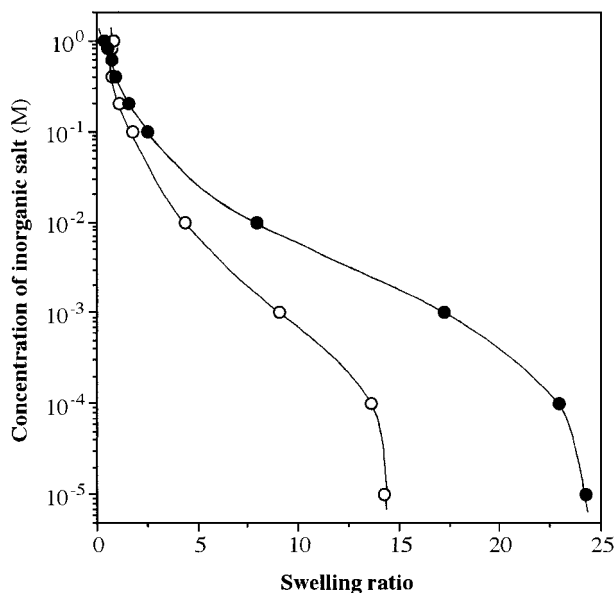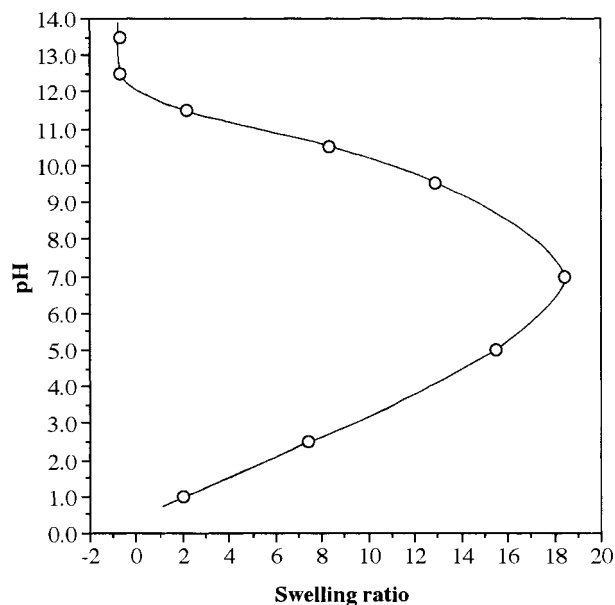
Figure 2 Swelling ratios of hydrogel **AMPG** as a function of ionic strength in NaCl ($\bullet$) and CaCl$_2$ ($\circ$) aqueous solution at 25°C.

tion range. With the increasing of acetone composition from 30 to 60%, the swelling degree decreased obviously. Especially, a nearly discontinuous phase transition was shown in the range of 30–40%. Above 60% acetone this gel collapsed into a compact state and displayed similar swelling ratios. This may be explained by the fact that the immediate shrinking of the outer layer of the gel restricted the bulk water to outflow from the interior. In addition, it is noted that this gel is transparent in the range of 0–50% acetone, while it turns opaque when acetone composition rose to 60% and exists as a white gel in 60–100% acetone.
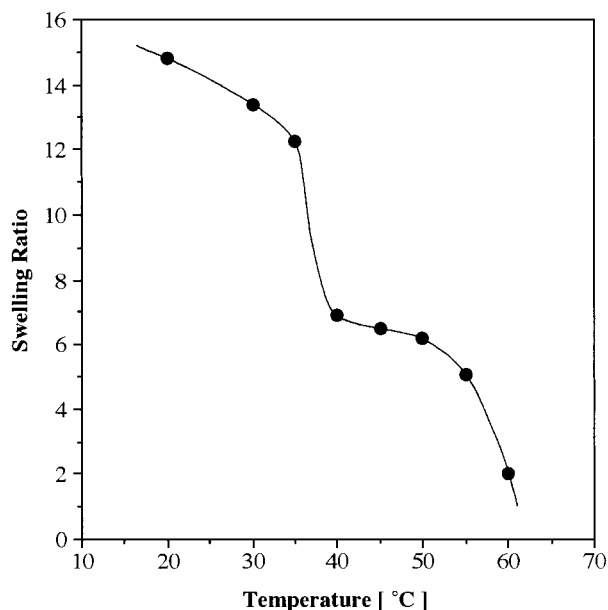
Figure 2 shows the swelling ratios of hydrogel **AMPG** measured in NaCl and CaCl$_2$ aqueous solutions ranging from $1 \times 10^{-5} M$ to $1.0 M$. Gel **AMPG** was observed to show larger swelling ratios at low salt concentration and smaller swelling ratios at high salt concentration both in NaCl and CaCl$_2$ solutions. The swelling ratios decrease with increasing salt concentration because the counterions in salt solution neutralize the bound charges within the gel, reducing the internal osmotic force. It is also easy to explain the phenomenon that the gel **AMPG** shows close swelling degree in the two salt aqueous in high concentration (1.0–0.1M). However, under $1 \times 10^{-2} M$ of NaCl and CaCl$_2$ solutions, the swelling ratio of gel **AMPG** immersed in NaCl aqueous is obviously larger than that in CaCl$_2$ aqueous even though with the

same salt concentration. It may correspond to the number of electric charge of cations in the solutions. Namely, Na$^+$ is a one-valence ion, and Ca^{2+} is a two-valence ion. Therefore, one Na$^+$ ion in salt aqueous can osmose into the network of hydrogel, combine with one counterion (Na$^+ \cdots$ PO$_4^-$), and then neutralize the fixed-charge within the gel. In the case for Ca^{2+} ion, one ion can combine with two counterions (PO$_4^- \cdots$ Ca$^{2+} \cdots$ PO$_4^-$) and form a intramolecular or intermolecular ionic bonding that limits the gel to expand. Furthermore, the neutralized negative charge by Ca^{2+} is the twice of those by Na$^+$ at the same concentration of salt aqueous. Therefore, the ion swelling pressure comes from these remaining charges within gel immersed in CaCl$_2$ aqueous is smaller than that in NaCl aqueous. It also causes a smaller swelling ratio of the hydrogel **AMPG** immersed in CaCl$_2$ solution.

The pH dependence of the swelling property of hydrogel **AMPG** is shown in Figure 3. The swelling ratio was measured in distilled water as the surrounding solution whose pH was controlled in the range of 1.0–13.5. It can be clearly seen that the gel **AMPG** shows smaller swelling ratios in the high acidic range or in the high basic range, while it shows larger swelling ratios in the neutral area. Similar to the ion strength dependence discussed above, the bound charges on the gel networks are neutralized partly in the range of high concentration of H$^+$ (acidity) or OH$^-$ ions (basic-

Figure 3 Swelling ratios of hydrogel **AMPG** as a function of pH in distilled water at 25°C.

Figure 4 Swelling ratios of hydrogel **AMPG** as a function of temperature in distilled water (pH = 5.6).

ity). At the neutral point (pH = 7.0) the charge density within the gel is the largest so that the largest ion swelling pressure is given to cause the largest swelling degree.

We also studied the swelling behavior of hydrogel **AMPG** at different temperatures. Figure 4 shows the swelling ratios measured from 20 to 60°C in distilled water (pH = 5.6). The degree of swelling for gel **AMPG** was found to decrease gradually with the increasing of the temperature. Moreover, an obvious shrinking was observed in the range of 30–40°C. The temperature effect may come from the rubber elasticity of polymer networks that show expanding force in the bound state at low temperature, and show shrinking force in the extended state at high temperature.

The temperature sensitivity of hydrogel **AMPG** was further investigated at different temperature (20–60°C) with NaCl and CaCl$_2$ aqueous as the surrounding solutions ($1 \times 10^{-2} M$). The swelling ratio as a function of temperature is shown in Figure 5. It can be seen that the swelling degree of gel **AMPG** in NaCl is obvious larger than that in CaCl$_2$ aqueous when they were measured at 20°C. In this case, both the rubber elasticity of polymer networks and unneutralized bound charges within polymer networks display the expanding force to cause the gel swelling. The difference of swelling ratios in NaCl and CaCl$_2$ solutions may mainly come from the different effect of Na$^+$ and Ca^{2+} ions discussed above. In the

range of high temperature, the ion swelling pressure still shows an expanding force but the rubber elasticity of polymer networks contrariously shows a shrinking force. As a result of the two functions, the swelling ratios both for NaCl and CaCl$_2$ solutions were found to decrease with the increasing of temperature. Furthermore, the degree of reducing is larger for NaCl aqueous than that for CaCl$_2$ aqueous, due to the formation of ionic bondings in CaCl$_2$ aqueous. After it is heated to 60°C, the swelling ratios are found nearly equal for the two different solutions. It may be because that the rubber elastic shrinking force is enough strong to control the swelling properties for the hydrogel **AMPG**. Moreover, in contrast to the temperature dependence in distilled water, no obvious difference was observed in NaCl and CaCl$_2$ aqueous. It is to say that the temperature sensitivity for gel **AMPG** is higher than the ion effect.

Currently, there is great interest in stimuli-sensitive polymers used as the drug delivery systems.[25] It is because that these polymers can change their structure and physical properties in response to external signals. From this perspective, the synthesized stimuli-sensitive hydrogel **AMPG** was studied for application as a controlled drug release system. In this experiment, egg lysozyme was used as an indicator of drug release. The synthesized hydrogel **AMPG** was equilibrated in a lysozyme aqueous 3 days at 25°C to give a saturated intercontained lysozyme gel (**L-**
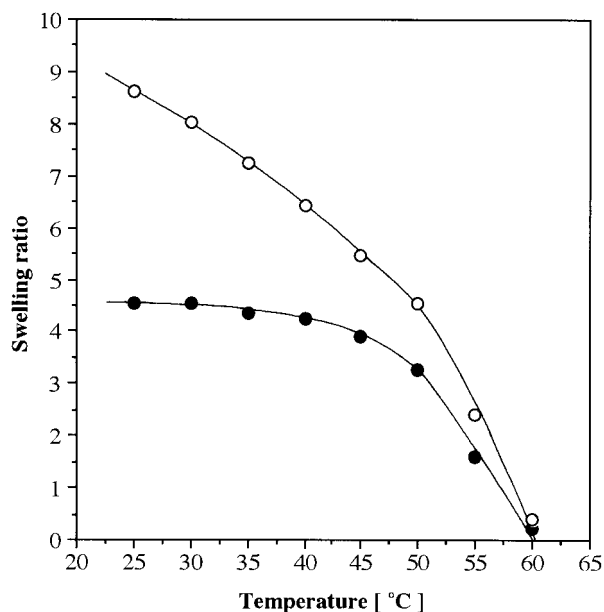
Figure 5 Temperature sensitivity of hydrogel **AMPG** in NaCl (○) and CaCl$_2$ (●) aqueous solutions.
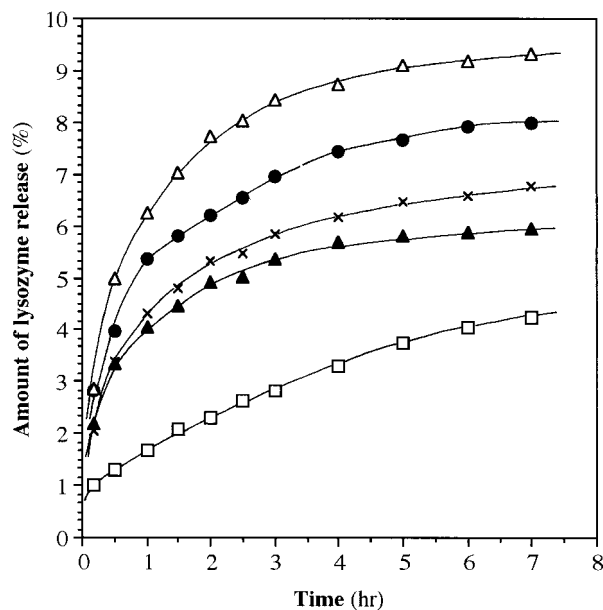
Figure 6 Release behavior of saturated intercontained lysozyme gel **L-AMPG** in different immersed solutions at 25°C. ($\triangle$) in $1M$ KCl solution; ($\bullet$) in $1M$ NaCl solution; ($\times$) in $0.1M$ NaCl solution; ($\blacktriangle$) in $1M$ CaCl$_2$ solution; ($\square$) in distilled water.

AMPG). After the gel **L-AMPG** was transferred into various immersed solutions, the content of lysozyme that released from the saturated intercontained lysozyme gel into the solution was measured by UV spectrophotometer, respectively.

Figure 6 shows the results of the lysozyme release in distilled water, KCl, NaCl, and CaCl$_2$ aqueous, receptively. It is noted that the amount of lysozyme release is larger and release speed is faster for gel **L-AMPG** in inorganic salt solutions than that in distilled water. Especially, the difference was obviously observed in the initial stage, perhaps relating to the different release process in initial stage and later stages. Namely, the initial release was predominated by the lysozyme molecules that kept simply in the gel, rather than those molecules combined static electricity with gel **AMPG**. As the isoelectric point of lysozyme used is 11, the lysozyme molecule exists as a cation in the distilled water. When the saturated intercontained lysozyme gel **L-AMPG** was immersed in inorganic salt solutions, ions K$^+$, Na$^+$, and Ca^{2+} replace the lysozyme cation and then enter into the gel, giving lager release of lysozyme. Especially, among these inorganic salts, the fastest release speed was observed for KCl aqueous, while the slowest release speed was shown in CaCl$_2$ solution. The ion K$^+$ holds only one positive

charge as well as ion Na$^+$, but shows a larger radius than that of Na$^+$ ion to afford a smaller number of hydrate water molecules. Therefore, the combination of K$^+$ ion with fixed negative charge is easy to form, giving a faster release speed of lysozyme.

On other hand, there are two reasons that lead to the slowest lysozyme release speed in CaCl$_2$ aqueous solution. First, with the permeating of Ca^{2+} ions, some new like-crosslinkings, intramolecular or intermolecular ionic bondings, can be formed among Ca^{2+} and PO$_4^-$ ions (PO$_4^-$ $\cdots$ Ca^{2+} $\cdots$ PO$_4^-$) because of bearing two positive charges in one Ca^{2+} ion. The result of increasing crosslinking density is that the release of lysozyme from hydrogel **L-AMPG** is restrained. Second, it is well known that the solubility of water-soluble protein lysozyme in water is strongly depended on the ion intensity of the aqueous solution. The concentrations of these inorganic salt solutions (NaCl, KCl, and CaCl$_2$) are the same ($1.0M$), but the ion intensities are different due to the different charges of Na$^+$, K$^+$, and Ca^{2+} ions. The CaCl$_2$ aqueous solution shows a strong ion intensity to give a slow release speed and a little release amount of lysozyme. In addition, the released amounts for all immersed solutions are under 10% of adsorbed lysozyme in gel **L-AMPG**.

The synthesized zwitterionic hydrogel containing biocompatible phosphatidylcholine groups shows different swelling and drug release behaviors in different external surroundings. Moreover, only under 10% adsorbed lysozyme could be release after the intercontained lysozyme gel was immersed in various solutions for 3 days. This gel will be applied widely in the field of biomaterial such as carrying and delivering of drugs.

CONCLUSION

The hydrogel **AMPG** containing phosphatidylcholine groups was synthesized from water-soluble vinyl monomer 1–(acrylamidomethyl)–2′–(trimethylammonio)ethyl phosphate (**AMP**) at room temperature in water system by using the water-soluble crosslinking constituent and initiator. The degree of swelling for gel **AMPG** was found to decrease gradually with the increasing of temperature, coming from the effect of rubber elasticity of polymer networks. In water–acetone medium, a nearly discontinuous phase transition was observed in the range of acetone composition 30–40 vol %. Furthermore, it was revealed that the

swelling degree increased with the increasing of charge density within the gel and with the decreasing of salt concentrations of external solutions, by investigating the swelling behavior in different pH and different inorganic salts aqueous solutions. On the other hand, the released amounts and speed of lysozyme that adsorbed in the synthesized hydrogel were noted to depend on the kind and intensity of ions in inorganic salt solutions.

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