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Polymer 43 (2002) 7467–7475

polymer

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# Stabilization of hydrophobic domains in hydrogel by intermolecular hydrogen bonds between carboxylic groups at the distal end of alkyl side-chain

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Received 9 April 2002; received in revised form 27 June 2002; accepted 6 August 2002

**Abstract**

The role of intermolecular hydrogen bond on the stability of hydrophobically associated domains of hydrogel, consisting of 12-acryloyloxydodecanoic acid (ADA; hydrophobic) or 6-acryloyloxyhexanoic acid (AHA; hydrophobic) and acrylic acid (AA; hydrophilic), carrying an alkyl side group terminated by carboxylic acid, was studied by swelling behavior in an organic solvent/water mixture. We chose propionic acid (PAc) and 1-propanol (PrOH) as organic solvents whose solubility parameter, log *P*, logarithmic partition equilibrium coefficient for octanol/water, is similar. The equilibrated swelling ratio of poly(ADA-*co*-AA) and poly(AHA-*co*-AA) gels by PAc was higher than by PrOH at a lower composition (ca. 5–15 mol%) and that of homo-polymer poly(AHA) gel by PAc was higher even at a high composition (up to ca. 70 mol%). The variation in swelling with solvent/water composition indicated a cross-over between the two solvent systems and this phenomenon did not depend on the alkyl side-chain length. Using Fourier transfer infrared spectroscopy, we observed the remarkable shift of the wavelength corresponding to the hydrogen bond in PAc aqueous solutions. Non-dissociated short alkanoic acid, which can penetrate hydrophobic domains and form hydrogen bonds with non-dissociated carboxylic groups of the polymer gel, favors the disruption of hydrophobic domains, causing swelling. Intermolecular hydrogen bonds between carboxylic groups of the alkyl side-chain are thus closely involved in the stability of hydrophobic domains in copolymer gel. 

*Keywords:* Hydrogels; Hydrophobic domain structure; Hydrogen bond

## 1. Introduction

Organisms contain biological gels consisting of high-order structures of proteins, e.g. second and third structures. Intermolecular chemical bonding (disulfide bonds) often transforms a water-soluble protein into a mechanically flexible but rigid gel containing hydrophobic domains in structural spaces [1–5]. Hydrophobic protein domains are assumed to be generated by intermolecular hydrogen bonds in amorphous regions or by hydrophobic interaction between rod-like α-helical regions [5–7], giving these gels their mechanical robustness. A simplified model peptide provides information useful for constructing and manipulating peptide conformation, designing a peptide which changes the conformation from a random coil to an α-helix or β-sheet only when intermolecular cross-linking is induced among them [8,9]. The role of hydrophilic amino acid groups on the formation of amorphous and hydrophobic domains near the cross-linking point remained to be clarified. However, hydrophilic groups move from the surface inward and hydrophobic groups are exposed to outer aqueous circumstance by conformation change.

Water-swollen, charged copolymer hydrogel containing a hydrophobic domain has been synthesized by copolymerizing an acrylic acid (AA) with a hydrophobic long alkyl side-chain, 16-acryloyloxyhexandecanoic acid (AHDA) or 12-acryloyloxydodecanoic acid (ADA) [10]. According to the literature, these copolymer gels with molecularly-ordered

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0032-3861/02/$ - see front matter 
PII: S0032-3861(02)00593-1

aggregates structure showed less swelling in water even at pH 10 but swelled in ethanol/water. Swelling in ethanol/water, triggered discussions on the role of hydrophobic interaction between hydrocarbon chains in the stability of hydrophobic domains in the gel. However, it was very difficult to explain the stability of the hydrophobic domain of the amorphous poly(ADA-*co*-AA) gel [10] only resulting from the hydrophobic interaction between alkyl side-chains. So, we noted the role of hydrogen bonds between non-dissociated carboxylic groups at the distal end of the alkyl side-chain in the stabilization of those hydrophobic domains.

The aim of the study was firstly to clarify the role of hydrogen bonds between inter-chains non-dissociated carboxylic groups in the stabilization of hydrophobic domains of copolymer gel, and secondly to provide a chemically synthesized hydrogel as a simple model of hydrophobic domain in proteins induced by intermolecular disulfide bonds. To make the alkyl side-chain length approximately that of the amino acid protein side-chain, we selected 6-acryloyloxyhexanoic acid (AHA) monomer having a shorter alkyl chain (C6) and synthesized poly-(AHA-*co*-AA) gel. We noted especially the swelling behavior due to the disruption of hydrophobic domains in short alkanoic acids/water systems. A short alkanoic acid, e.g. an important constituent distributed widely in the nature, constructs dimer by hydrogen bonding between non-dissociated carboxylic groups [11,12]. We selected propionic acid as swelling agents and compared the swelling with that by 1-propanol whose hydrophobic parameter, log *P* (which is a logarithmic partition coefficient in octanol/water system [13]), is similar to propionic acid. This choice enabled us to suppress hydrophobicity and clarify hydrogen bonding. We evaluated newly-formed hydrogen bonds between the solvent and non-dissociated carboxylic groups of the gel based on the wavelength number shift of stretching vibration of carbonyl group $\nu$(C=O) [14–17] observed in differential infrared spectra.

## 2. Experiments

### 2.1. 12-Acryloyloxydodecanoic acid synthesis [18]

A mixture of 10 g 12-hydroxydodecanoic acid and 7.02 g triethylamine in 40 ml dichloromethane were dropped slowly over 5 h into a solution of 6.27 g acryloylchloride in 24 ml dichloromethane at −30 °C. The mixture was then stirred for 21 h at −30 °C. The solution separated into two phases, a precipitate was filtered and 6 ml water was added to the filtered solution, then 6.67 g NaOH in 15 ml water was added dropwise at 0 °C and the mixture was stirred for 45 min. The mixture was adjusted to pH 1 by cold conc. HCl-solution. It separated into two phases. After a precipitate was filtered, dichloromethane in a liquid phase was removed under reduced pressure in a rotary evaporator, leaving yellowish adhesive solid precipitate. The precipitate was dissolved in a mixture of dichloromethane and acidified by HCl-solution. After dichloromethane was removed under reduced pressure of an oil pump, a white product, 12-acryloyloxydodecanoic acid (Fig. 1a), was collected (yield 53%).

a) $CH_2=CH-C(=O)-O-(CH_2)_{11}-C(=O)-OH$

b) $CH_2=CH-C(=O)-O-(CH_2)_{5}-C(=O)-OH$

Fig. 1. Chemical structure of (a) 12-acryloyloxydodecanoic acid (ADA) and (b) 6-acryloyloxyhexnaoic acid (AHA).

### 2.2. 6-Acryloyloxyhexanoic acid synthesis [19]

A mixture of 10 g ε-caprolactone and 10 ml triethylamine in 50 ml methanol was heated under reflux for 6 h, then evaporated to dryness under reduced pressure, producing 12.8 g methyl-6-hydroxy hexanoate (M4HH) (yield 99.6%). 10 g M4HH and 10 g vinyl acrylate were dissolved in 400 ml diisopropyl ether and 1 g lipase PS (Amano Enzyme Inc., Japan) was added to the mixture, which was stirred at 60 °C for 6 h. After removal of diisopropyl ether under reduced pressure of an oil pump, 3.28 g methoxycarbonylpentylacrylate (MCPA) was obtained (yield 31.0%) using chromatographic separation on a silica gel column. The 3.28 g MCPA was suspended in 328 ml of 0.1 M phosphate buffer (pH 7) and 0.033 g lipase OF (Meito Sangyo Inc., Japan) was added to the mixture, which was stirred for 6 h at room temperature. Lipase OF in the mixture was then filtered out. The mixture was acidified with 2 N HCl-solution. The reactant was extracted with diethylether, and the diethylether fraction washed with a saturated sodium chloride aqueous solution and dehydrated by adding anhydrous sodium sulfate, finally producing 2.5 g AHA (Fig. 1b) (yield 82.1%). The pH measured at $2.69 \times 10^{-2}$ M AHA aqueous solution was 2.81. The p$K_a$ was determined to be 4.05, i.e. the degree of dissociation was calculated to be 0.056, indicating that the carboxylic acid at the distal end of the AHA alkyl chain was partially dissociated in the aqueous solution.

### 2.3. Gel preparation

6-Acryloyloxyhexanoic acid or 12-acryloyloxydodecanoic acid and acrylic acid were dissolved in ethanol and 0.24 mol% of α, α′-azobis (isobutyronitrile) (initiator) and 1 mol% of *N*, *N*′-methylenebis (acrylamide) (cross-linker) were added to the total monomer mixture. Copolymerization was conducted for 24 h at 60 °C, and the resulting gels were immersed in a large amount of ethanol to remove monomers and non-cross-linked polymers. poly(AA) gel was also prepared. $^1$H NMR from the ratio of peak intensity

Table 1
Log $P$ and p$K_a$ of solvents and acrylic acid

| | Log $P$ | P$K_a$ |
|---|---|---|
| Formic acid (Fac) | −0.54 | 3.55 |
| Acetic acid (AAc) | −0.17 | 4.56 |
| Propionic acid (PAc) | 0.33 | 4.67 |
| 1-propanol (PrOH) | 0.34 | |
| Acrylic acid (Ac) | | 5.5[a] |
| 6-acryloyloxyhexanoic acid (AHA) | | 4.05[b] |

[a] p$K_a$ of carboxylic acid of AA polymer [20].
[b] p$K_a$ of carboxylic acid of AHA monomer.

determined the molar portion of the AHA unit in poly(AHA-*co*-AA) and the ADA units in poly(ADA-*co*-AA) copolymer gels for AHA α-proton (2.35 ppm), ADA α-proton (2.31 ppm) and AA α-proton (2.44 ppm). The copolymer gel obtained was AHA56, AHA100 and ADA 33. The molar percentage of AHA in copolymer gel are 56 and 100. The molar percentage of ADA in copolymer gel is 33.

### 2.4. Gel swelling and swelling ratio measurement

Solvents were chosen based on hydrophobic parameter, log $P$, the logarithmic partition coefficient in octanol/water [13]. Solvents were formic acid, acetic acid, propionic acid (PAc) and 1-propanol (PrOH) (Table 1). Log $P$ and p$K_a$ of the solvents, p$K_a$ of acrylic acid (Ac) [20] of AA polymer and p$K_a$ of AHA monomer, are shown in Table 1. Swelling was measured by monitoring the lengths of rectangular dried gel and swollen gel at 30 °C using a cathetometer (Nippon Optical Works Co.), and swelling ratio $S_v$ was calculated using the following equation

$$S_v = \frac{\text{volume of swollen gel}}{\text{volume of dried gel}} \quad (1)$$

$$S_v = \left(\frac{l}{l_0}\right)^3 = \frac{v}{v_0} \quad (1a)$$

where $l_0$ and $l$ are the lengths of dry gel and swollen gel. The swelling ratio was measured in a water/solvent mixture in which the pH was adjusted with aqueous HCl or NaOH.

### 2.5. Infrared spectra measurement

Infrared spectra were obtained from a FTIR-500 spectrometer (Jasco, Japan) from 650 to 4600 $cm^{-1}$ at a resolution of 4 $cm^{-1}$ and for 100 scans. The wavelength number shift of stretching vibration of carbonyl group $\nu$(C=O) corresponding to newly formed hydrogen bond formation was estimated in the spectral region 1680–1740 $cm^{-1}$ from differential spectra between swollen gel in water/solvent and water/solvent solution [14–17].

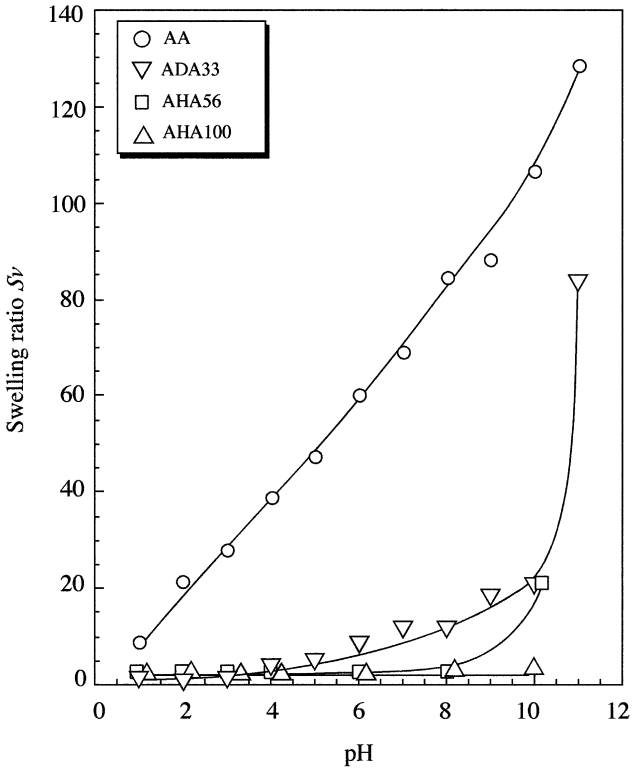



Fig. 2. pH dependence of swelling of AA (○), ADA33(△), AHA56 (□) and AHA100 (▽) gels.

## 3. Results and discussion

### 3.1. pH dependence of degree of swelling

The pH dependence of degree of swelling, $S_v$, of AA, ADA33, AHA56 and AHA100 gel is shown in Fig. 2. AA gel swells well with increasing pH. ADA33 gradually swells till pH 10 and markedly swells at pH 11. AHA56 does not swell until pH 10. AHA100 gel scarcely swells in water even at a pH 10. The ionization of the AA and AHA carboxylic group in the copolymer network influences swelling only negligibly. These results mean the interaction between carboxylic acids at the distal end of the alkyl side-chain of ADA or AHA is strong and hides the hydrophilic AA or AHA moiety in the hydrophobic domain.

### 3.2. Swelling behavior in alkanoic acid solution

Fig. 3 shows $S_v$ of AA, ADA33, AHA56 and AHA100 gels in alkanoic acids/water mixtures. AA gel temporarily shrinks in 3 mol% of alkanoic acids and swells up to 20–30 mol% of alkanoic acids, and then shrink (Fig. 3a). There is no significant difference in swelling potential among alkanoic acids. The actual pH measured at 3 mol% of acid composition was FAc (1.82), AAc (2.14), and PAc (2.25) at 30 °C. p$K_a$ of carboxylic group of AA gel was estimated to be 5.5 (Table 1) [20]. This temporary shrinking may be caused by a marked decrease in electrostatic repulsion

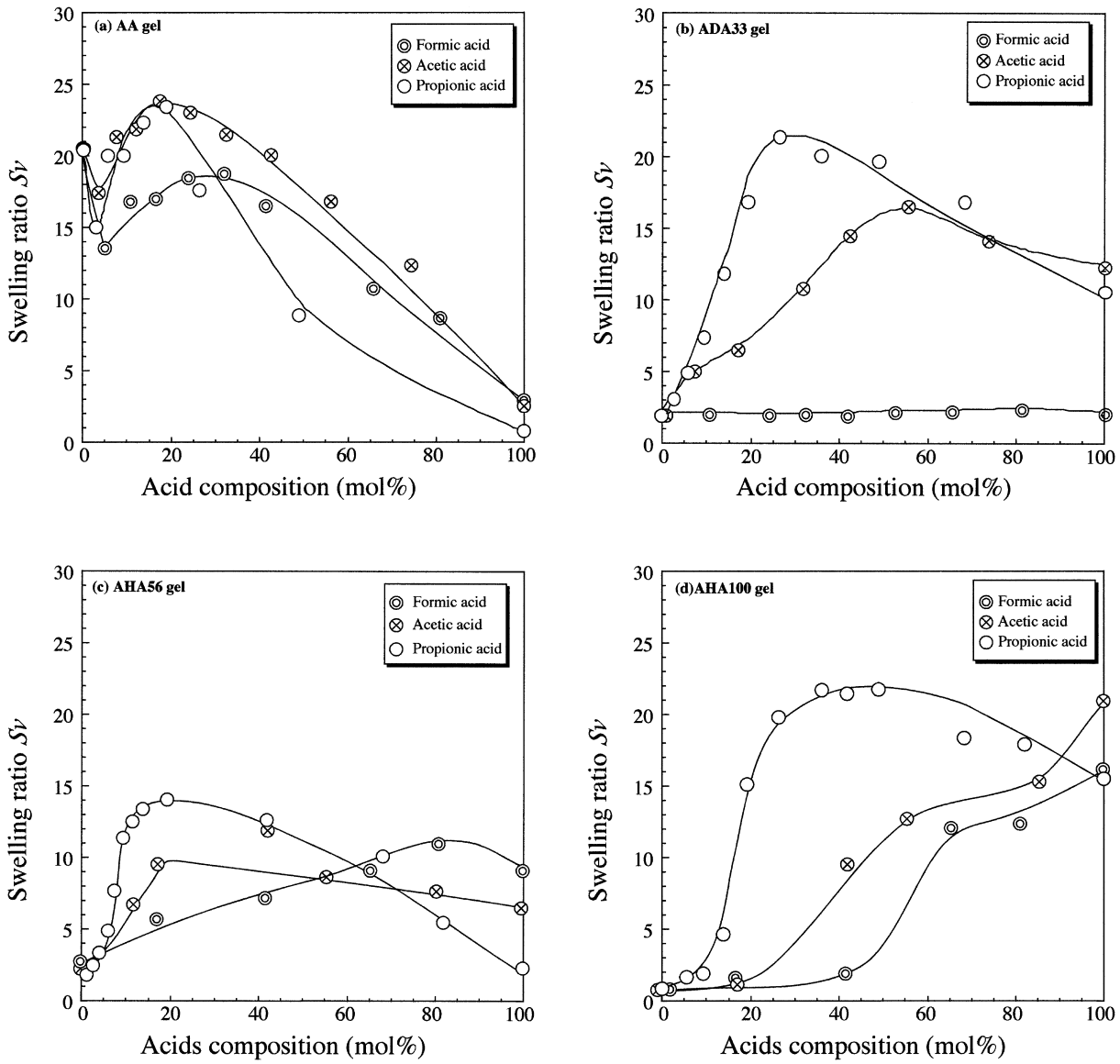



Fig. 3. Swelling behavior of (a) AA, (b) ADA33, (c) AHA56 and (d) AHA100 gels in solvent/water, formic acid (◎), acetic acid (⊗), and propionic acid (○).

between carboxylic groups of AA in acid/water compared to that in water. ADA33, AHA56 and AHA100 gels, having alkyl side-chains, scarcely swells in water but gradually swell with increasing alkanoic acids composition (Fig. 3b–d). At the lower composition, alkanoic acid swelling potential clearly depends on the order of the hydrophobicity ($\log P$) of non-dissociated form, PAc (0.33) > AAc (−0.17) > FAc (−0.54), not on molecular size. Based on p$K_a$, PAc (p$K_a$ = 4.67), AAc (p$K_a$ = 4.56), and FAc (p$K_a$ = 3.55) (Table 1), more than 99.5% of them dissolve as a non-dissociated form in 3 mol% of short alkanoic acids solutions. Non-dissociated PAc molecules having the highest compatibility with alkyl-chain may be partitioned into hydrophobic regions. On the other hand, at the higher composition (ca. 50–100 mol% for AA gel and ca. 60–100 mol% for AHA56 gel) involving less containing water, ADA33 gel was not swollen by FAc, however, the swelling potential was FAc > AAc > PAc for AA gel and AHA56 gel. These reversed phenomena of alkanoic acid swelling potential may be ascribed to cosolvency of non-dissociated AA moieties of these gels to the alkanoic acids although the mechanism has not been clarified yet.

### *3.3. Comparison of gel swelling between functional groups of solvent*

We chose PAc and PrOH because these solvents have similar $\log P$, where $P$ is the logarithmic partition equilibrium coefficient of the octanol/water mixture. This choice enabled us to suppress hydrophobicity and clarify hydrogen bonding.

Fig. 4 shows AA, ADA33, AHA56 and AHA100 gels swelling behavior in PAc/water and PrOH/water mixtures. $S_v$ values for PrOH are larger than those for PAc in the wider

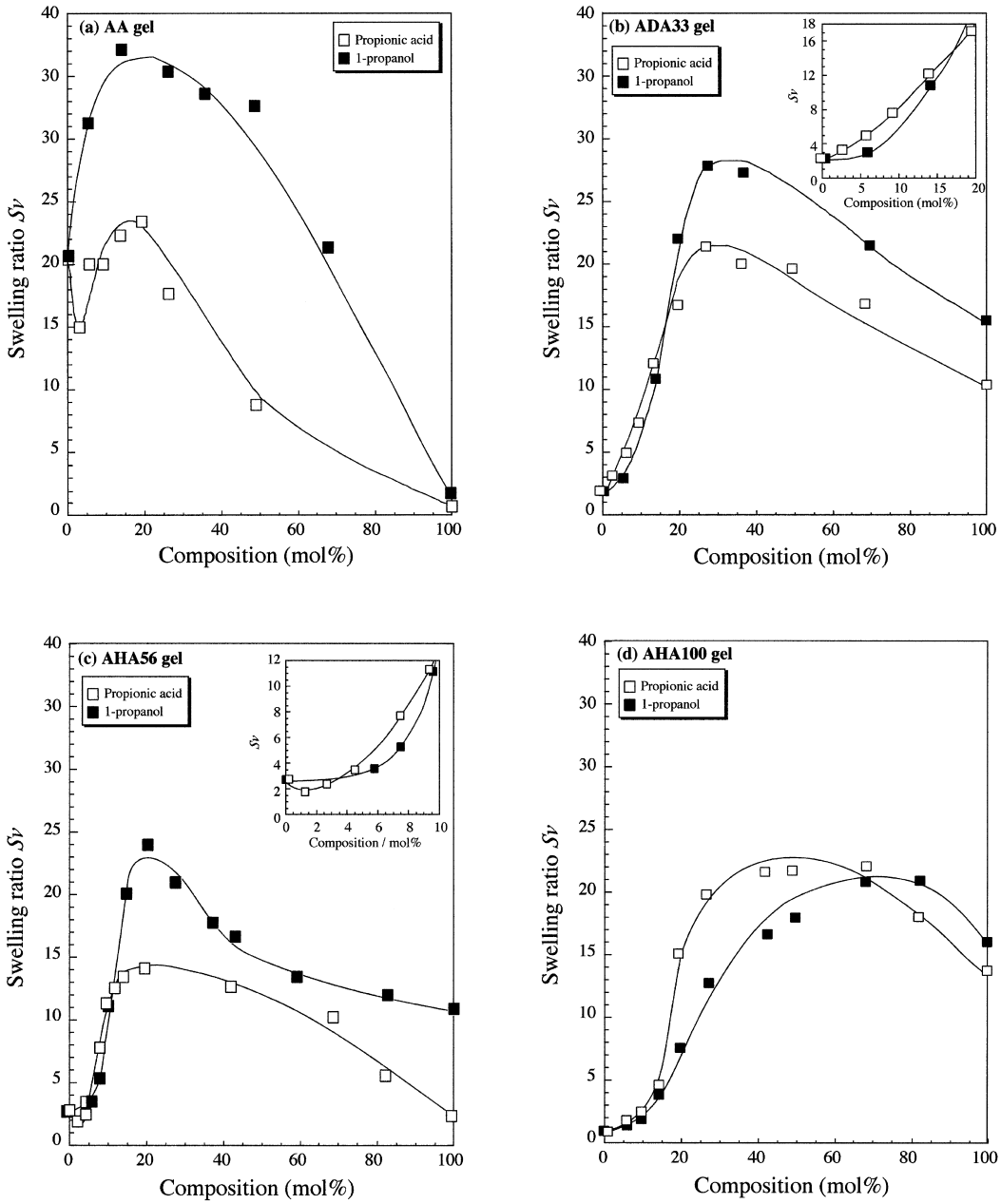
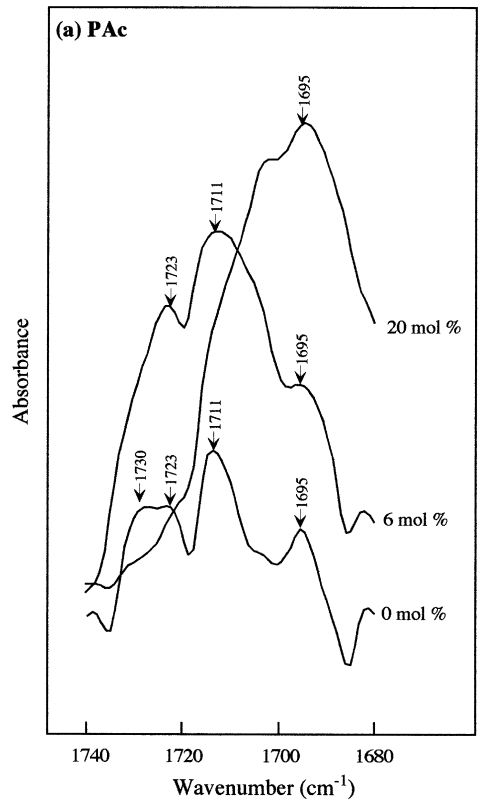



Fig. 4. Swelling behavior of (a) AA, (b) ADA33, (c) AHA56 and (d) AHA100 gels in solvent/water, propionic acid/water (□), and 1-propanol/water (■). Swelling ratio ($S_v$) of ADA33 and AHA56 in the lower solvent composition are displayed in the insert.

region of composition except for the AHA100 gel which does not contain AA moieties. The restraint of the dissociation of the AA moieties in alkanoic acids solutions surely influences the swelling behavior of these gels. Especially, AA gel not having alkyl side-chain swells easily in a lower composition of PrOH mixture (Fig. 4a). On the other hand, adding a small amount of PrOH to the ADA33, AHA56 and AHA100 gels, however, results in a lower increase in $S_v$ (Fig. 4b–d). We found, interestingly enough, that PAc swells ADA33 (Fig. 4b) more than PrOH up to ca. 15 mol% and AHA56 (Fig. 4c) gels more than PrOH ca. 4–15 mol% even though PAc hydrophobicity (log $P = 0.33$) is similar to that of PrOH (log $P = 0.34$). A short alkanoic acid, e.g. an important constituent distributed widely in nature, constructs dimers by hydrogen bonding between non-dissociated carboxylic groups [11,12]. The variation in $S_v$ with organic solvent composition indicates a cross-over between the 2 solvent systems in composition exceeding ca. 15 mol% for ADA33 and AHA56 gels, suggesting that a non-dissociated PAc favors the disruption of hydrogen bonds in these gels and causes greater swelling than in PrOH even at a lower organic solvent composition. AA moieties buried in the hydrophobic domain gradually may come into contact with water by the decomposition of hydrophobic domain with increasing solvent composition. The increase in dissociated carboxylic acids consequently swells gels. As



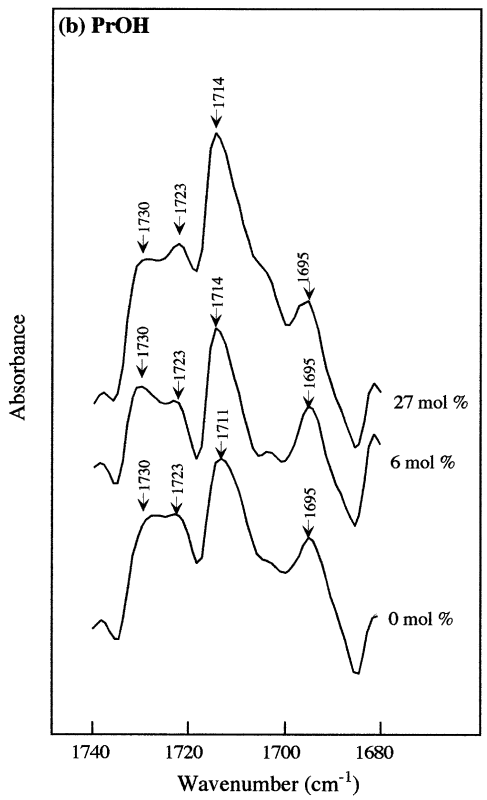
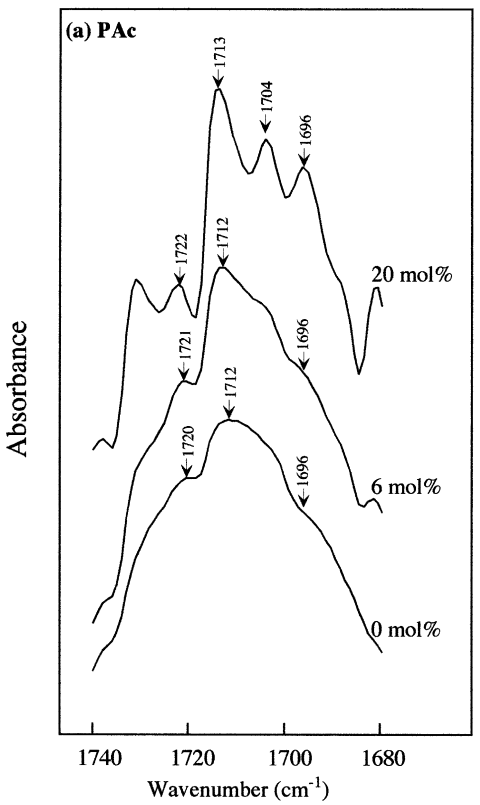




Fig. 5. Differential IR spectra in the $\nu$(C=O) stretching region of AA gel in solvent/water (a) propionic acid (PAc) and (b)1-propanol (PrOH) between 1740 and 1680 ($cm^{-1}$).



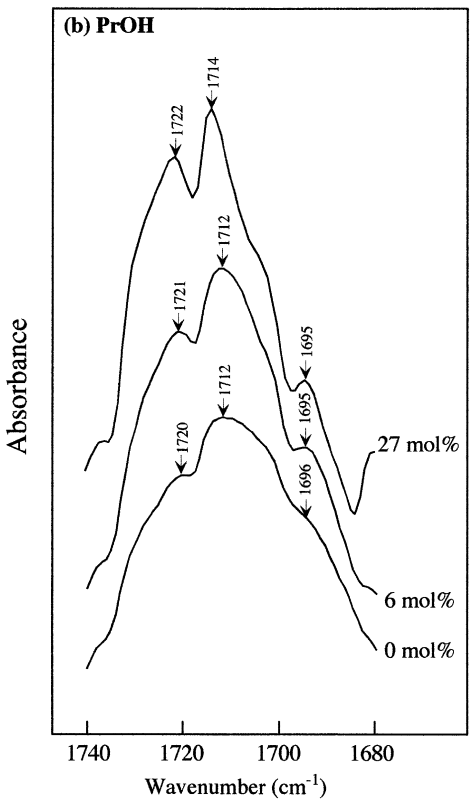
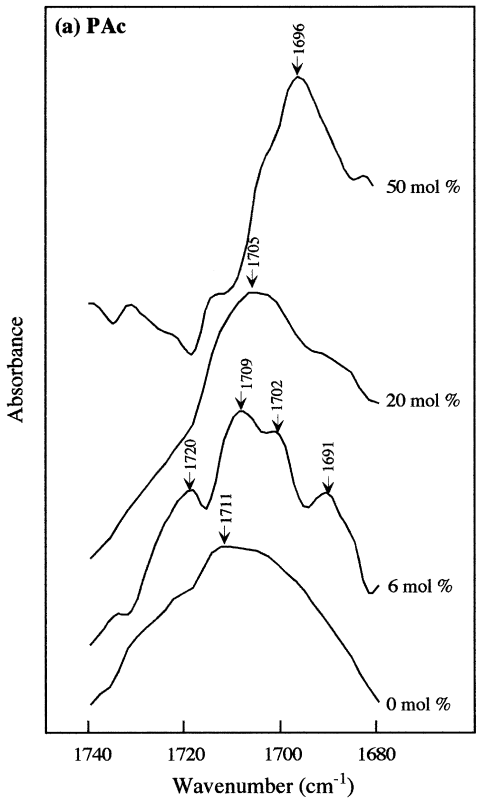




Fig. 6. Differential IR spectra in the $\nu$(C=O) stretching region of ADA33 gel in solvent/water (a) propionic acid (PAc) and (b)1-propanol (PrOH) between 1740 and 1680 ($cm^{-1}$).



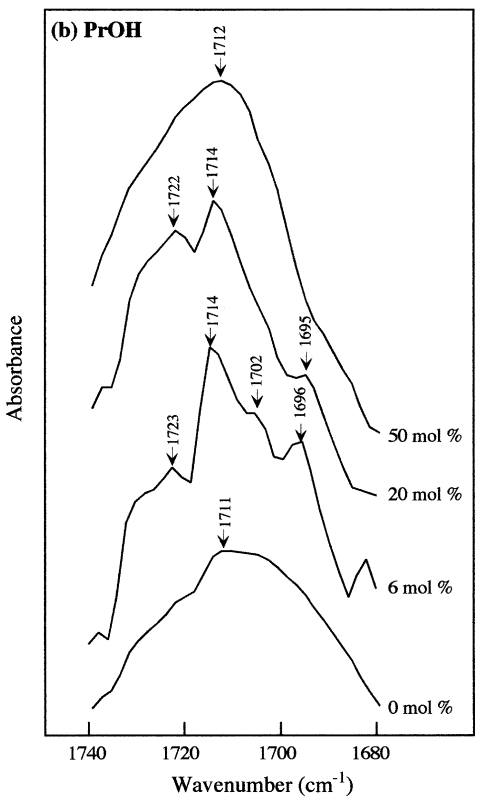
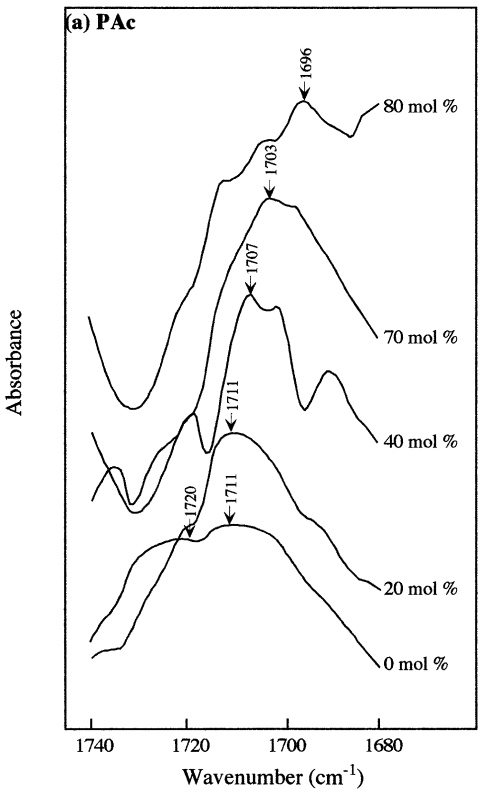




Fig. 7. Differential IR spectra in the $\nu$(C=O) stretching region of AHA56 gel in solvent/water (a) propionic acid (PAc) and (b)1-propanol (PrOH) between 1740 and 1680 ($cm^{-1}$).



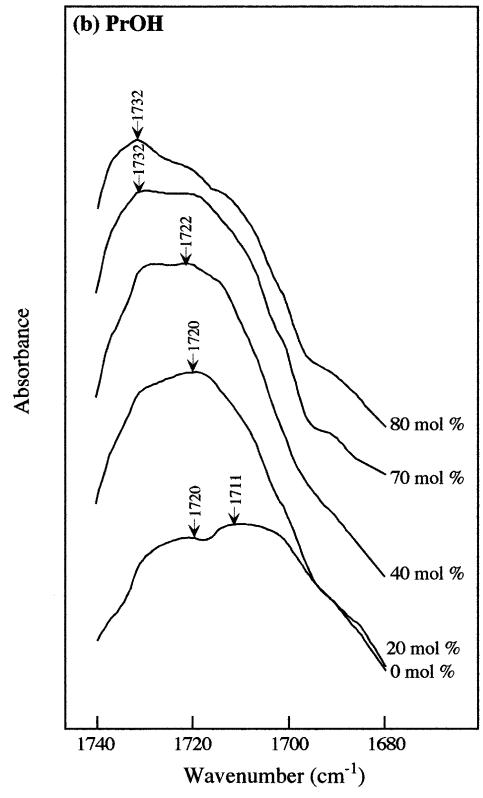




Fig. 8. Differential IR spectra in the $\nu$(C=O) stretching region of AHA100 gel in solvent/water (a) propionic acid (PAc) and (b)1-propanol (PrOH) between 1740 and 1680 ($cm^{-1}$).

seen in the AHA100 gel which does not contain AA moieties (Fig. 4d), the effect of PAc against gel swelling becomes more dominant. PAc swells AHA100 gel more than PrOH up to ca. 70 mol%.

### 3.4. Differential infrared spectra

We calculated the wavelength number shift of stretching vibration of carbonyl group $\nu$(C=O) in the spectral region 1680–1740 $cm^{-1}$ from differential spectra between gel swollen in solvent/water and solvent/water solution. We were interested in the disorganization of hydrogel via exchange reaction of intermolecular hydrogen bonds between non-dissociated carboxylic acids of gel and that of PAc at the lower composition.

The differential infrared spectra of AA gel in PAc/water and in PrOH/water mixtures are shown in Fig. 5a and b. 4 broad shoulders are seen at 1695, 1711, 1723 and 1730 $cm^{-1}$ on AA gel in water. This spectral profile becomes ambiguous with the increase of PAc composition and one shoulder seen at 1695 $cm^{-1}$ remained in 20 mol% of PAc, whereas this spectral profile scarcely changes till 20 mol% of PrOH. These facts suggest that the swelling of the AA gel in a PAc aqueous solution entails the change of hydrogen bond form.

As seen on ADA33 gel (Fig. 6), 3 ambiguous broad shoulders at 1696, 1712, and 1720 $cm^{-1}$ occurring in water, tend to sharpen gradually, with a new peak appearing at 1704 $cm^{-1}$ during ADA33 gel swelling in PAc/water (Fig. 6a), whereas these spectra scarcely change in PrOH/water (Fig. 6b). The carbonyl stretching $\nu$(C=O) band originating from the intramolecular structures and from the intermolecular ones were assigned at ca. 1730 and 1708 $cm^{-1}$, respectively, in ADA homo-polymer [15]. In this solvent composition range, the gel volume increases with the increase of PAc composition, so, the newly peak at 1704 $cm^{-1}$ is considered to result from the disruption of hydrogen bond between non-dissociated carboxylic acid group at the distal end of the alkyl side-chain of ADA.

Fig. 7 shows the differential infrared spectra of AHA56 gel in PAc/water (Fig. 7a) and PrOH/water (Fig. 7b) mixtures. No sharp peak except an ambiguous broad shoulder at ca. 1711 $cm^{-1}$ is observed in un-swollen gel in water. At 50 mol% solvents composition, only one peak at 1696 $cm^{-1}$ is observed in PAc/water, whereas an ambiguous broad shoulder at 1712 $cm^{-1}$ in PrOH/water. It is very difficult to discuss which hydrogen bonds of AA or AHA moieties are changed by PAc.

As seen in AHA100 gel (Fig. 8), no sharp peak except two ambiguous broad shoulders at ca. 1711 and 1720 $cm^{-1}$ is observed in water. The broad shoulder at ca. 1711 $cm^{-1}$ tends to sharpen and shifts gradually to 1697 $cm^{-1}$ with increasing PAc (Fig. 8a). The peak at ca. 1697 $cm^{-1}$ is observed in common among AA, AHA56 gel and ADA33 gel swollen by PAc. Therefore, the shift suggests that it depends on the generation of a short aliphatic acid by the

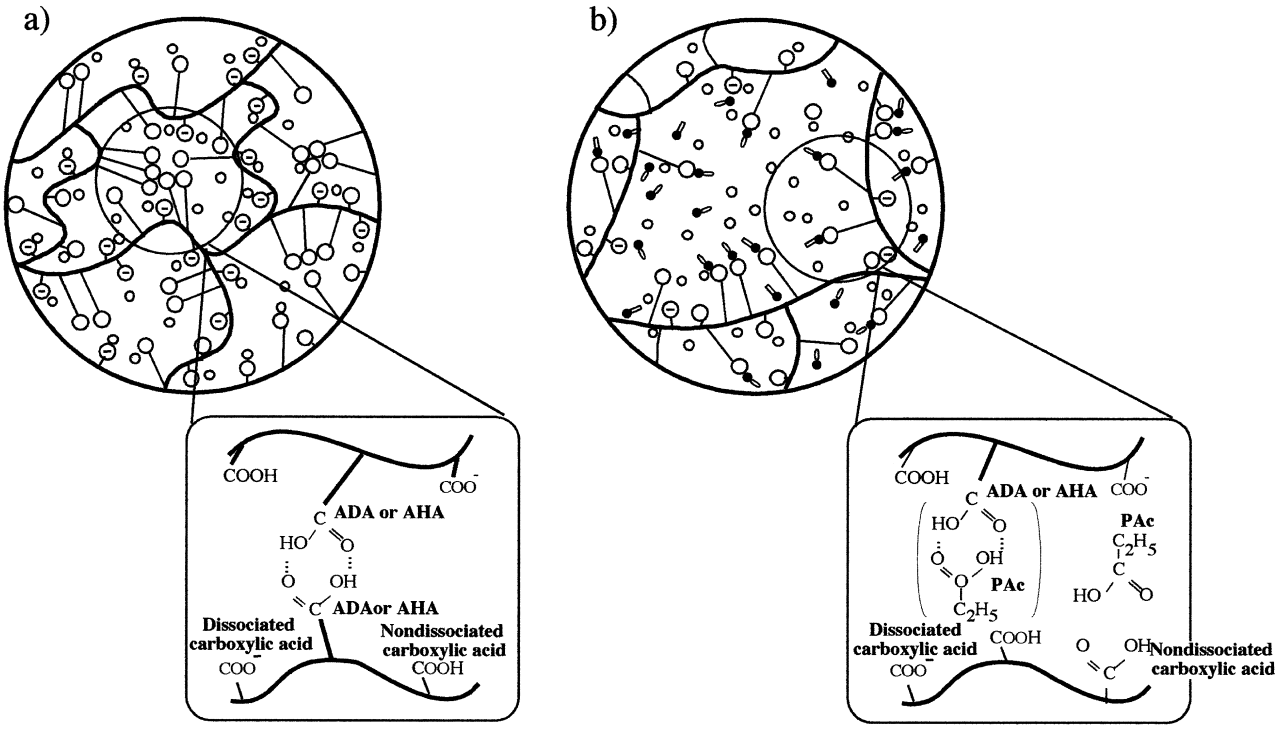



Fig. 9. Microscopic structure of copolymer gel, (a) in water, (b) in aqueous solution of a short alkanoic acid at equilibrated swelling, water molecule (○), dissociated AA (–⊖), non-dissociated AA (–○), alkyl side-chain of AHA or ADA molecule having non-dissociated carboxylic acid at the distal end (—○), non-dissociated acid molecule (□●). Temporary formation of intermolecular hydrogen bond between non-dissociated carboxylic acid of Pac and ADA or AHA and the decomposition of hydrophobic domain are designated in the parenthesis.

decomposition of the hydrogen bond between the AHA molecules. The weak and ambiguous band at 1711 shifts to 1732 $cm^{-1}$ without changing the sharpness with increasing PrOH (Fig. 8b). Hydrogen bonds in AHA100 gel may be weakened gradually with increasing PrOH, but remain present to a lesser extent, although gel swells.

These results show that both AHA56 containing ionizable AA moieties and AHA100 gel partially containing ionizable AHA ones, swell in the volume with the disruption of hydrogen bonds in PAc/water. The original hydrogen bonds remaining in the hydrophobic domain are easily interchanged to newly formed hydrogen bonds temporarily, i.e. PAc–AHA, which disrupts the hydrophobic domains and promotes swelling.

### *3.5. Role of intermolecular hydrogen bonds in hydrophobic amorphous domains in hydro gel*

Based on the above finding, we discuss the role of intermolecular hydrogen bonds between non-dissociated carboxylic acid at the distal end of polymer alkyl-chain on the stability of hydrophobic amorphous domains in copolymer gel (Fig. 9). The hydrophobic interaction between alkyl groups in the side-chain and hydrogen bonds between carboxylic groups at the distal end of the side-chain forms amorphous hydrophobic domains within the gel network (Fig. 9a). Since dissociation of carboxylic groups is severely restricted with hydrophobic domains, water molecules cannot easily penetrate. When small amounts of organic solvents having compatibility with alkyl-chains are added to water, these molecules penetrate equally into hydrophobic domains in copolymer gel, regardless the alkyl length (ADA or AHA). A short alkanoic acid favors the formation of temporary intermolecular hydrogen bonds with non-dissociated carboxylic groups at the distal end of the alkyl side-chain of copolymer gel (Fig. 9b). Original hydrogen bonds between non-dissociated carboxylic groups are then disrupted and weaken the hydrophobic interaction between alkyl-chains. These effects allow water molecules to enter hydrophobic domains, then disrupted them. As the result gel volume increases. Intermolecular hydrogen bonds between carboxylic groups at the distal end of the alkyl side-chain are thus closely involved in hydrophobic domain stability in copolymer gel. The subsequent decrease in $S_v$ for both solvent systems may be ascribed to cosolvency of PAc/water and PrOH/water although the mechanism has not been clarified yet.

## 4. Conclusion

We determined the role of intermolecular hydrogen bonds in the stability of hydrophobic domains in hydrogel by studying the solvent effect in the disruption of hydrophobic domain via swelling experiment and infrared spectra measurement. We chose two types of solvents having nearly equal hydrophobicity ($\log P$), i.e. propionic acid (PAc; $\log P = 0.33$) and 1-propanol (PrOH; $\log P = 0.34$) to suppress hydrophobicity and clarify hydrogen bonding. PAc swelled poly(ADA-*co*-AA) and poly(AHA-*co*-AA) gels more than PrOH at a lower composition (ca. 5–15 mol%) and more hydrophobic poly(AHA) gel even at a high composition (up to ca. 70 mol%). This was explained by the role of PAc as hydrogen bond scissors, originating PAc as a non-dissociated carboxylic acid group and temporarily forming a dimer by intermolecular hydrogen bonds. In other words, the stability of amorphous hydrophobic domains containing hydrophilic molecules cannot be uniquely determined by $\log P$, but involves other types of molecular interaction such as the hydrogen bond formation of the solvent.

AHA100 hydrogel synthesized by ionizable AHA was usefully applicable to a cross-linked protein model. This is because, in the same way as with polar amino acids of polypeptide chains, AHA molecule can be altered to either a non-dissociated form or a dissociated form, depending on the circumferential situation (hydrophobic or hydrophilic).

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