Roles of Hydrophobic Interaction in a Volume Phase Transition of Alkylacrylamide Gel Induced by the Hydrogen-Bond-Driving Alkylphenol Binding

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Received: August 20, 2004; In Final Form: October 23, 2004

It was found that a degree of the binding of alkylphenols to *N*-alkylacrylamide gel increased transitionally to induce the volume phase transition of gel. Binding isotherms of nonylphenol (n-Ph), propylphenol (p-Ph), ethylphenol (e-Ph), methylphenol (m-Ph), and phenol (Ph) to *N*-isopropylacrylamide (NIPA), *N*,*N*-diethylacrylamide (DEA), *N*,*N*-dimethylacrylamide (DMA), and acrylamide (AM) gels were examined. Two types of binding, the site binding at $\beta < 1$ and the multimolecular binding at $\beta > 1$, were observed, where β was a degree of binding to a monomeric unit of the chain. The former binding was analyzed with the Hill equation applicable to the cooperative binding and the latter binding with the Brunauer−Emmett−Teller (BET) equation applicable to the multilayer adsorption. The binding constant, *K*, and the Hill coefficient, *N*, decreased and increased, respectively, in the order of DEA, NIPA, and DMA gels in the case where the binding alkylphenol was the same. The *K* value increased in the order of Ph, m-Ph, e-Ph, p-Ph, and n-Ph that bound to the same type of gel. The *N* value was found to change little with the type of binding alkylphenol. The complexes of *N*-alkylamide with alkylphenol were condensed to form the ordered nanostructures that were observed as broad scattering peaks in small-angle X-ray scattering experiments. The fluorescence excimer emission was observed for the phenol-binding DMA gel, which corresponded to the condensed state of phenol.

1. Introduction

The stabilizing and functionalizing mechanisms of the enzymatic protein molecules have not been fully understood despite the intensive investigation during the past decades. A combination of the hydrogen bonding, the hydrophobic interaction, and the electrostatic interaction[1,2] between the amino acids of the proteins makes the mechanism sophisticated. Some of the hydrophobic residues are distributed on a surface of the protein and might affect the strength and stability of the interamide hydrogen bond, which depends on the degree of exposure to competing bonds from water. The hydrogen bond is strengthened by shielding it from water. Coupling of the hydrophobic interaction with hydrogen bonding often strengthens the hydrogen bond.[3] It has been revealed that hydrogen bonding of the hydroxyl group of tyrosine to another amino acid plays an important role in the enzyme activities.[4] Strength of the hydrogen bonding is sophisticatedly controlled by the hydrophobic interaction between the phenyl group of tyrosine and the hydrophobic residues of the other amino acid groups. Our interest is on the effect of the hydrophobic interaction of the phenyl group of tyrosine and the peptide backbone[5] on hydrogen bonding. A systematic study on this matter, however, has been rarely made. We have found that the binding of phenol as an analogue of tyrosine to the polymer chain of *N*-isopropylacrylamide (NIPA), which could be regarded as an analogue of isoleucine, induces a conformational transition with a change in the concentration of phenol.[6] Hydrogen bonding between the amide group of the NIPA chain and the hydroxyl group of the phenol molecule[6] has been indicated by the IR spectra for the phenol-binding NIPA gel. The present investigation is aimed toward revealing the effect of hydrophobic alkyl chains on the binding behavior of alkylphenol molecules to alkylamide chains.

It is well-known that NIPA gel exhibits the transitional volume change with temperature that is called the volume phase transition. The roles the hydrophobic isopropyl group play in the volume phase transition of NIPA gel have been intensively investigated in the past decades.[7−10] The collapse of the gel chain is induced by the dehydration of the isopropylacrylamide group, which is the result of transfer of the hydrated water molecules to the bulk because of the higher chemical potential of hydrated water molecules compared to that in the bulk. The dehydration is endothermic, as demonstrated by differential scanning calorimetric measurements of the NIPA gel.[11,12] The transition temperature decreases with the addition of salts[13] or organic molecules that are miscible with water.[14] The added small molecules reduce the chemical potential of water in the bulk and decrease the transition temperature at which the chemical potential of the hydrated water molecule exceeds that in the bulk. Addition of a small amount of hydrophobic substance, such as phenol, to the NIPA gel has been found to decrease the volume phase transition temperature,[6] which is explained by the binding of the phenol molecule to the NIPA chain, because the change of the chemical potential of water in the bulk with the addition is too small to explain the reduction of the transition temperature. At the volume phase transition, the degree of phenol binding, β, transitionally increases. The Young's modulus of the gel transitionally increases with the volume phase transition.[15]

In the present study, the binding behavior of nonylphenol (n-Ph), propylphenol (p-Ph), ethylphenol (e-Ph), methylphenol (m-Ph), and phenol (Ph) to *N*,*N*-dimethylacrylamide (DMA), NIPA, *N*,*N*-diethylacrylamide (DEA), and acrylamide (AM) gels were investigated to clarify the hydrophobic interaction effects on the binding driven by the hydrogen bond. Small-angle X-ray

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10.1021/jp046236x CCC: $30.25
Published on Web 12/13/2004

scattering (SAXS) measurements were made to examine the possible nanostructures stabilized by the π/π interaction[16] between phenyl groups. The fluorescent emission spectra of phenol-binding DMA gel were observed to clarify the existence of the excimer of dimeric phenol. Clarifying the binding behavior and the nanostructure of the gel is crucial for developing the application of the transitional change of the elasticity with the binding[15] to the actuator, the drug delivery system, and the chemical switch.[17−19] Studies on the binding mechanism of the notorious n-Ph as the environmental endocrine disrupter[20] will be also helpful to develop good absorbing agents of it.

2. Experimental Section

Materials. The polymer gel was prepared by radical copolymerization in anaqueous solution containing 1 M of the monomer, which was DEA, NIPA, DMA, or AM, and 5 mM of the cross-linker *N*,*N*′-methylenebisacrylamide. Copolymerization was initiated by adding 5 mM of ammonium persulfate. The syntheses of DMA and AM gels were carried out at 60 °C. The syntheses of DEA and NIPA gels were carried out at 5 °C with the addition of 1.6 mM of *N*,*N*,*N*′,*N*′-tetramethylendiamine as an accelerator, and the transparent gels were obtained. It should be noted that the DEA and NIPA gels synthesized at a temperature higher than the collapsing temperatures of the gels were opaque and fragmented. The gels were synthesized for 24 h in containers with two glass plates separated by a 1-mm-thick tetrafluoroethylene spacer, rinsed with a large amount of distilled water, cut into small pieces (about 10 mm^2), and dried in a vacuum. The DEA, NIPA, and DMA monomers were kindly supplied by Kohjin Co., Ltd. All chemicals used were of analytical grade.

Binding Isotherm and Gel Volume. The binding isotherm of alkylphenol to the gel chain was obtained as follows: The weight of a dried gel, W_{dgel}, the weight of the solution, W_{sol} (typically 3 g), and the initial concentration of the alkylphenol in the solution, C_0, were measured, and the gel was immersed in the solution. It took more than 2 weeks for the gel to attain a binding equilibrium with the solution at 25 °C.[6] The weight of *N*-alkylacrylamide hydrogels equilibrating with the solutions, W_{gel}, was measured. The gel volume was evaluated as the gel weight per monomeric unit of the chain, W

$$W = \frac{W_{gel}}{W_{dgel}} M_{Ch} \tag{1}$$

where M_{Ch} is the molecular weight of the monomeric unit of the gel chain. The concentration of the alkylphenol at equilibrium, C_{out}, was evaluated from the optical absorbance of the solution measured by a spectrophotometer (Ubest UV/VIS, Jasco Co., Ltd). The wavelengths of light used in measuring the absorbance of Ph, m-Ph, e-Ph, and p-Ph, respectively, were 269.6, 219.6, 221.6, and 221.6 nm. The C_{out} of n-Ph was evaluated from the fluorescence intensity emitted at a wavelength of 307 nm, which was excited at a wavelength of 277 nm. The fluorescence was measured by the fluorescence spectrometer (F2500, HITACHI Co., Ltd) with a 0.5-nm bandwidth of the monochromator. The degree of binding, β, defined as the number of alkylphenol molecules bound to one monomeric unit of the gel chain, is given by

$$\beta = \frac{(C_0 - C_{out}) \cdot W_{sol}}{W_{dgel}} M_{Ch} \tag{2}$$

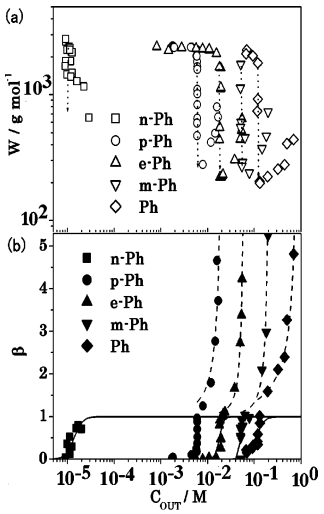

Figure 1. Binding isotherm of alkylphenol to the DMA gel and volume change of the gel with a change in the alkylphenol concentration, C_{out} at 25 °C. (a) Open symbols represent the volume change of the DMA gel. Downward arrows show the transitions of the gel volumes. (b) Closed symbols represent the binding isotherms. Solid and broken lines, respectively, represent the best-fitting curves to eq 4 at $\beta < 1$ and to eq 5 at $\beta > 1$.

It should be noted that the alkylphenol concentration in the solution phase of the gel was assumed equal to C_{out}. For the system of n-Ph, the experiments at $\beta > 1$ were not made because the times to reach equilibrium were very long when the initial concentration of n-Ph was above the saturated concentration.

Small-Angle X-ray Scattering. The measurements of SAXS were made at 25 °C with a SAXS spectrometer of BL45XU installed at SPring8 of Japan Synchrotron Radiation Research Institute, Hyogo, Japan. The intensity of X-rays detected by a position-sensitive proportional counter was corrected for the cell scattering and absorption. The observed scattering vectors, $q = (4\pi/\lambda)\sin\theta/2$, where λ and θ, respectively, were a wavelength of the beam and the scattering angle, ranged from 0.1 to 6 nm^{-1}. The β value of the measured gel was determined by the binding experiments mentioned herein.

Measurement of the Fluorescence. Fluorescence measurements were made for the purpose of observing the excimer emission from the phenol dimers into the phenol-binding DMA gel. The edges of the platelike phenol-binding DMA gel were fixed at the diagonal corners of a cubic quartz cell as the gel was sticky. Fluorescence measurements were made for the suspended gel. It should be mentioned that the phenol-binding NIPA and DEA gels are too opaque for fluorescence measurement. Fluorescence measurement was also made for a 0.1 M phenol aqueous solution. The emission spectra were observed for the samples using an excitation wavelength 270 nm.

3. Results

Binding and Volume Change Behavior. Figure 1 shows the C_{out} dependence of the volume change behavior of the DMA gel on binding of Ph, m-Ph, e-Ph, p-Ph, and n-Ph to the gel. There exists two binding regimes: one exhibits a transitional β increase with an increase in C_{out} and the other a gradual β increase. The former exists in the range of $\beta < 1$ and the latter in the range of $\beta > 1$. It should be noted that β seems to increase to infinity at C_{out} values close to the saturation concentration of the alkylphenol. The gel volume sharply decreases in the former binding regime, whereas it gradually increases in the latter binding regime with the increases of C_{out}, as seen in Figure

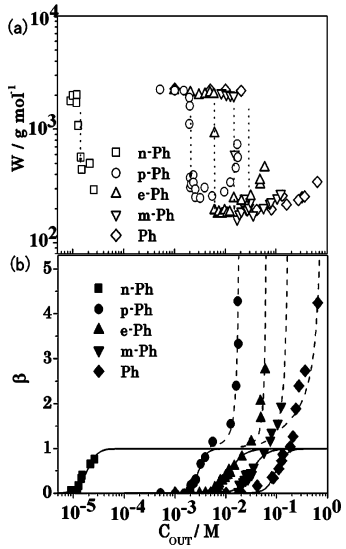

Figure 2. Binding isotherm of alkylphenol to the NIPA gel and volume change of the gel with a change in the alkylphenol concentration, C_{out} at 25 °C. (a) Open symbols represent the volume change of the NIPA gel. Downward arrows show the transitions of the gel volumes. (b) Closed symbols represent the binding isotherms. Solid and broken lines, respectively, represent the best-fitting curves to eq 4 at $\beta < 1$ and to eq 5 at $\beta > 1$.

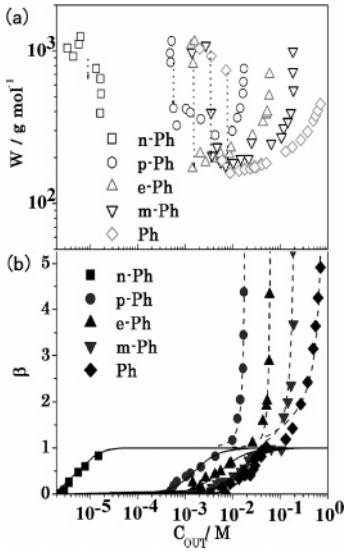

Figure 3. Binding isotherm of alkylphenol to the DEA gel and volume change of the gel with a change in the alkylphenol concentration, C_{out} at 25 °C. (a) Open symbols represent the volume change of the DEA gel. Downward arrows show the transitions of the gel volumes. (b) Closed symbols represent the binding isotherms. Solid and broken lines, respectively, represent the best-fitting curves to eq 4 at $\beta < 1$ and to eq 5 at $\beta > 1$.

1a. The former is a site-binding to the amide group of the monomeric unit of the gel chain, and the latter is a multimolecular adsorption on the site occupied by the alkylphenol. The behavior mentioned herein is observed irrespective of the types of alkylphenol and gel. The alkylphenol concentration at which the site binding occurs increases with a decrease in the alkyl chain length of alkylphenol, as shown in Figure 1b.

The alkylphenol binding behavior of the NIPA and the DEA gels shown in Figures 2 and 3 is similar to that of the DMA gel except for the existence of the gradually increasing β region with C_{out} in the site-binding regime. The transitional increases in β with C_{out} are observed for the NIPA and the DEA gels at $\beta < 0.2$. It is seen in Figure 4 that the volumes of the NIPA

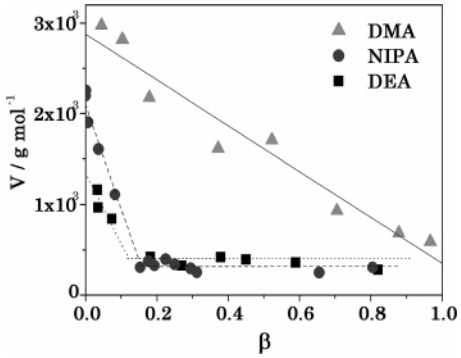

Figure 4. Volumes of the p-Ph binding DEA (dotted line), NIPA (broken line), and DMA (solid line) gels as functions of β. Dotted, broken, and solid lines represent approximate linear relations between the volume and β.

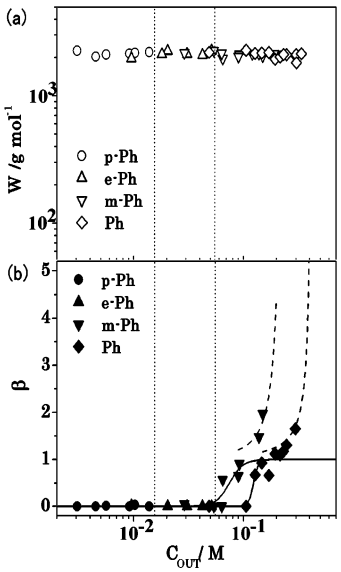

Figure 5. Binding isotherm of alkylphenol (p-Ph, e-Ph, m-Ph, and Ph) to the AM gel and volume change of the gel with a change in the alkylphenol concentration, C_{out} at 25 °C. (a) Open symbols represent the volume change of the AM gel. (b) Closed symbols represent the binding isotherms. Solid and broken lines, respectively, represent the best-fitting curves to eq 4 at $\beta < 1$ and to eq 5 at $\beta > 1$. Two vertical dotted lines represent the saturation concentrations of p-Ph ($C_{sat} = 0.155$) and e-Ph ($C_{sat} = 0.55$).

and DEA gels linearly decrease with β at $0.15 > \beta > 0$, remain constant at $1 > \beta > 0.15$, and increase at $\beta > 1$. The NIPA and DEA gels are in the collapsed state at $1 > \beta > 0.15$. The volume change behavior of the NIPA and DEA gels with the site binding is different from that of the DMA gel, which linearly decreases with β in the range 0−1. The side groups of the NIPA and DEA gel chains being more hydrophobic than that of the DMA gel chain induces the differences in the site binding and the volume change behavior with β as described already.

Figure 5 shows the volume change behavior of the AM gel with binding of m-Ph and Ph to the gel. No volume change with the m-Ph and Ph binding was observed. This suggests that the hydrophilic nature of the AM gel chain is maintained in the m-Ph and Ph binding states of the acrylamide group. It should be mentioned that no binding of p-Ph and e-Ph to the AM gel was observed even at their saturating concentrations.

Small-Angle X-ray Scattering Spectra. Figure 6 shows the SAXS spectra for the p-Ph binding DMA, NIPA, and DEA gels. Broad peaks are observed around $q = 3.1$ nm^{-1} for the DMA gel, $q = 3.3$ nm^{-1} for the NIPA gel, and $q = 3.7$ nm^{-1} for the DEA gel. Peak positions of the spectra for the e-Ph-, m-Ph-,

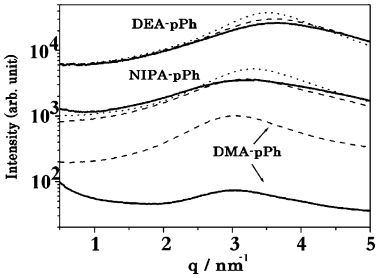

Figure 6. SAXS spectra for the p-Ph binding DMA, NIPA, and DEA gels. β values of the DMA gel represented by the solid and broken lines, respectively, are 0.52 and 0.97. β values of the NIPA gel represented by the solid, broken, and dotted lines, respectively, are 0.66, 0.81, and 1.16. β values of the DEA gel represented by the solid, broken, and dotted lines, respectively, are 0.45, 0.59, and 0.82.

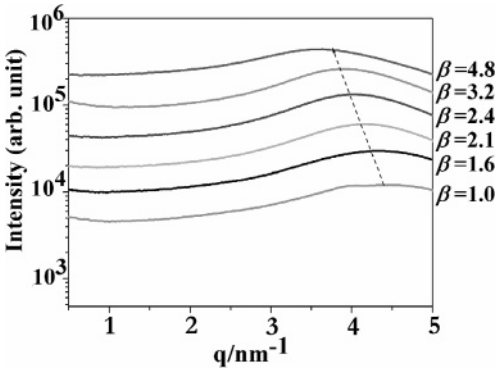

Figure 7. SAXS spectra for the Ph binding DMA gel at $\beta > 1$. The peak position shifts to a low q with an increase in β.

and Ph-binding DMA gels in the multimolecular adsorption state ($\beta > 1$) tend to shift to low q with an increase in β, the typical spectra of which are shown in Figure 7. The peak positions of the spectra for systems in the site-binding regime are substantially β independent, although some of them seem to decrease with β because of the broadness of the peaks. The peaks of the SAXS spectra indicate the regular distribution of particles. A mean characteristic distance between particles, ξ, defined as $\xi = 2\pi/q_{max}$, where q_{max} is a q value of the intensity maximum position of the peak,[21] and a half-width of the peak, Δq, are tabulated in Table 1. The ξ value increases with the alkyl chain length of alkylphenol and increases in the order of DEA, NIPA, and DMA gels. The peak broadening indicates a large fluctuated distribution of the characteristic distance.

Fluorescence Emission Spectra of Phenol-Binding DMA Gel. Figure 8 shows fluorescence emission spectra of phenol-binding DMA gels and 0.1 M phenol solution when the wavelength of exciting light is 270 nm. The spectra are assumed to have an isosbestic point at 312 nm.[22] Peaks with strong intensities at the emission wavelength, λ, of about 300 nm are mainly due to the fluorescence of monomeric phenol. The shoulders around $\lambda = 340$ nm could be identified as the excimer emission of phenol dimer.[22] It has been demonstrated[22] that the peak intensity near 300 nm decreases and the emission intensities near 340 nm increase with an increase in the phenol dimer. The intensity of fluorescence emission spectra of the phenol-binding DMA gel in the λ range from 320 to 400 nm increases with an increase in β, as shown in Figure 8. This suggests an increase of the phenol dimer in the DMA gel with β, which corresponds to the SAXS result that the scattering peak shifts to low q values with an increase in β at $\beta > 1$. The existence of the monomeric state of phenol in the collapsed DMA gel is indicated by the emission peak around $\lambda = 300$ nm shown in Figure 8.

TABLE 1: Structural Parameters of the Alkylphenol-Binding Alkylacrylamide Gels Obtained from Peaks of the SAXS Curves

DMA				NIPA				DEA			
β	q nm^{-1}	ξ nm	Δq nm^{-1}	β	q nm^{-1}	ξ nm	Δq nm^{-1}	β	q nm^{-1}	ξ nm	Δq nm^{-1}
					n-Ph						
0.53	2.8	2.3	0.8	0.50	2.8	2.2	0.5	0.49	3.0	2.1	0.5
0.71	2.8	2.3	0.7	0.70	2.8	2.2	0.5	0.69	3.0	2.1	0.5
0.73	2.9	2.2	0.4	1.0	2.8	2.2	0.5	1.0	3.0	2.1	0.4
0.80	2.8	2.2	0.6								
					p-Ph						
1.8	3.2	2.0	0.8	0.50	3.3	1.9	1.0	0.45	3.7	1.7	0.8
2.8	3.1	2.0	0.8	0.70	3.2	2.0	0.8	0.60	3.7	1.7	0.7
3.7	3.1	2.0	0.6	1.0	3.3	1.9	0.7	0.82	3.5	1.8	0.6
4.7	3.1	2.0	0.7								
					e-Ph						
0.92	3.6	1.7	1.0	0.28	4.1	1.5	1.6	0.42	4.1	1.6	0.8
1.0	3.6	1.7	0.9	0.39	3.8	1.6	1.4	0.49	4.0	1.6	0.8
1.1	3.6	1.7	0.8	0.51	3.7	1.7	1.5	0.67	3.9	1.6	0.7
1.7	3.6	1.8	0.9								
2.7	3.4	1.9	0.7								
3.4	3.3	1.9	0.7								
4.2	3.3	1.9	0.8								
					m-Ph						
0.73	3.7	1.7	0.9	0.29	4.3	1.5	2.4	0.26	4.6	1.4	0.8
0.97	3.7	1.7	0.8	0.30	4.3	1.5	1.4	0.40	4.5	1.4	0.8
2.1	3.5	1.8	0.6	0.42	4.3	1.5	1.2	0.50	4.3	1.5	0.8
3.0	3.3	1.9	0.7								
7.8	3.1	2.0	1.6								
					Ph						
1.0	4.4	1.4	0.9	0.38	5.2	1.2	1.1	0.23	5.5	1.3	0.9
1.6	4.3	1.5	0.8	0.43	5.1	1.2	1.2	0.33	5.4	1.2	0.9
2.1	4.1	1.5	0.7					0.40	5.3	1.2	0.8
2.4	4.0	1.6	0.7					0.61	5.2	1.2	0.7
3.3	3.9	1.6	0.7					1.0	5.1	1.2	0.7
4.8	3.6	1.8	0.7								

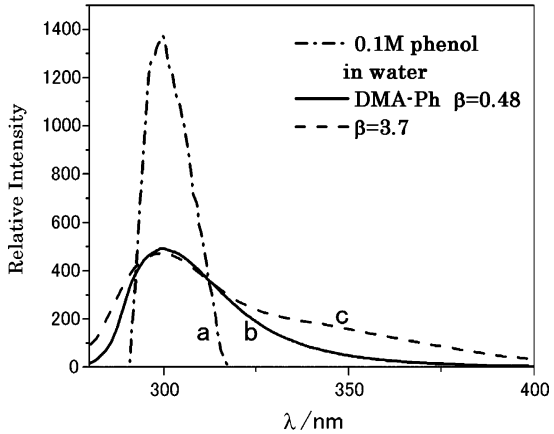

Figure 8. Fluorescence emission spectra of 0.1 M phenol solution (a) and the phenol binding DMA gels (b, $\beta = 0.48$; c, $\beta = 3.7$).

4. Discussion

It should be mentioned that the square shape of the DMA gel was distorted in the region of the linear decrease of the DMA gel volume with β, as shown in Figure 4, and that the macroscopically collapsed domain was separated from the macroscopically swollen domain in the gel, as exhibited in Figure 9. The degrees of alkylphenol-binding of cross-linked chains in the former and latter domains of the gel, respectively, are inferred close to 1 and 0. That is, the collapsed domain is in the binding state and the swollen domain in the unbinding state. The distorted shape was kept for more than 6 months, which indicated the thermodynamic equilibrium between the

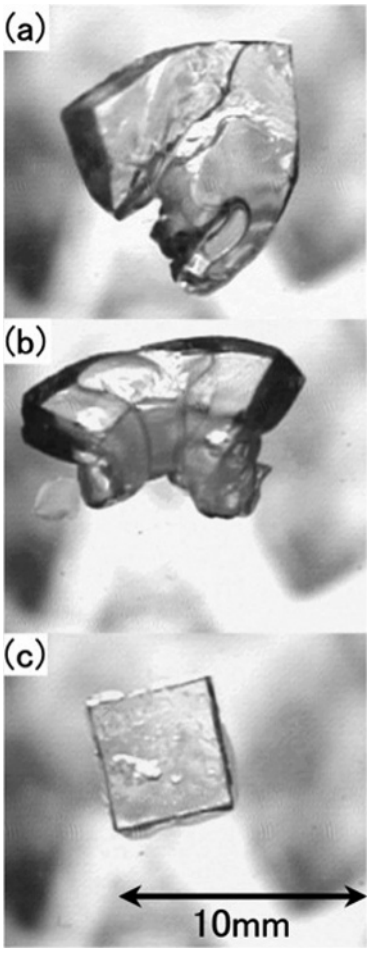

Figure 9. Macroscopic appearances of the Ph binding DMA gels at $\beta = 0.30$ (a), $\beta = 0.45$ (b), and $\beta = 0.85$ (c). The gels shown in (a) and (b) are distorted from the square. The gel shown in (c) is almost in the collapsed state.

binding and unbinding domains. The coexistence of the collapsed and swollen states of the gel chains in the macroscopic scale might be allowed by much smaller interface energy between them than the thermal energy.[23] The gel volume, V, and the degree of binding, β, transitionally change from (V_S, β_S) to (V_C, β_C) at the transition concentration, C_{out}*, where the subscripts S and C denote the swollen and collapsed states. The gel volume at β between β_S and β_C can be described by a linear function of β such as

$$V = -\frac{V_S - V_C}{\beta_C - \beta_S}\beta + \frac{\beta_C V_S - \beta_S V_C}{\beta_C - \beta_S} \qquad (3)$$

The values of β_S and β_C for the p-Ph binding to the DMA gel can be inferred to be about 0.1 and 1 from Figure 4. The approximately linear decreases in the gel volume with β are also observed for the DEA and NIPA gels at $\beta < 0.15$, as shown in Figure 4. The β_C values for the p-Ph binding to DEA and NIPA gels are inferred to be about 0.15. The β_S value of the corresponding system can be inferred to be about 0.02 from the previous experiment.[6] The gel volume decreases with β at a substantially constant C_{out}*, the volume phase transition concentration of alkylphenol. The C_{out}* values for the various combinations of alkylphenol and *N*-alkylacrylamide gel are tabulated in Table 2.

The volume phase transition of the Ph−NIPA gel system has been previously reported in a detailed manner.[6] The phenol binding induced by the hydrogen bonding between the amide group and the hydroxyl group of phenol divests the alkylamide groups of hydrated water molecules. The dehydration due to the poor affinity of the binding alkylphenol and the alkyl chains of the side groups of DEA and NIPA to the water molecules shrinks the gel chains to reduce the gel volume, as shown in Figures 4 and 10a. The chain entropy increases with the shrinking chains, and the binding and shrunken state of the chain is more stabilized than the binding and swollen state. The highly cooperative binding coupled with the entropy gain of the collapsed chains makes the volume change transitional, as schematically depicted in Figure 10.[10,24] The transitional volume change is cooperatively induced with the β jump from the β_S to the β_C, as observed in the present experiment described already.

The poor affinity of the side groups of DEA and NIPA chains to the water molecules yields the domino effect of the alkylphenol binding on the hydration to dehydrate the chain. The fact that $\beta_C = 0.15$ suggests that one site binding of alkylphenol promotes the dehydration of about five neighboring amide groups of the DEA and NIPA groups. The nontransitional binding becomes transitional due to the entropy gain in collapsing chains, as schematically depicted in Figure 10. The C_{out}*, at which the volume phase transition occurs, increases in the order of n-Ph, p-Ph, e-Ph, m-Ph, and Ph, as shown in Table 2. This fact indicates that the hydrophobic interaction between the alkylphenol and the alkylamide and the low affinity of the alkylphenol to water promote the hydrogen bond formation between the amide and the hydroxyl groups. The transition C_{out}* increases in the order of DEA, NIPA, and DMA gels for binding the same type of alkylphenol, which is shown in Figure 11. The hydrophobicity of the side group of the chain can be said to increase in the order of DMA, NIPA, and DEA gels. It is plausible from their chemical structures that the DMA gel is the most hydrophilic among them. The binding behavior of the alkylphenols reveals that the DEA gel is more hydrophobic than the NIPA gel.

The cooperative binding isotherms in the site-binding regime ($\beta < 1$) shown in Figures 1−3 cannot be explained by the binding mechanism of the Langmuir type.[25] For analyzing the binding isotherm at β from 0.2 to 0.9, we adopt the Hill model[26] widely used as an aid in the study of cooperative binding.[27] The average degree of binding β is represented as follows[26,28]

$$\beta = \frac{(KC_{out})^N}{1 + (KC_{out})^N} \qquad (4)$$

where N is the Hill coefficient, which is a measure of the cooperativity of binding, and K is a binding constant. The N and K values in eq 4 fitted best to the binding isotherms in the site-binding regime represented by solid lines in Figures 1−3 and are tabulated in Table 2. The K values increase with an increase in the alkyl chain length of alkylphenol, whereas the dependence of the N values on the alkyl chain length of alkylphenol is weak. The K value for any alkylphenol increases in the order of DMA, NIPA, and DEA gels, whereas the N value increases in the opposite order. It should be recalled that the alkylphenol binding isotherms obtained in the present experiment are for the collapsed NIPA and DEA gels and the swollen DMA gel. From a comparison between the NIPA and DEA gels, it can be said that the more hydrophobic side group of the gel chain gives the larger K and smaller N values. The decrease of cooperativity with the alkyl carbon number of the side group of the gel chain has been also observed for the ionic surfactant

TABLE 2: Best-Fitting Values of K and N in the Hill Equation to the Binding Isotherms in the Region of $\beta < 1$

	n-Ph	p-Ph	e-Ph	m-Ph	Ph
			DEA		
C_{out}* (mol kg^{-1})	4.0×10^{-6}	5.5×10^{-4}	2.0×10^{-3}	3.7×10^{-3}	7.0×10^{-3}
K (kg mol^{-1})	1.5×10^{5}	6.3×10^{2}	1.8×10^{2}	7.7×10	4.9×10
N	2.6	1.7	1.5	1.7	2.3
			NIPA		
C_{out}* (mol kg^{-1})	1.0×10^{-5}	2.0×10^{-3}	6.0×10^{-3}	1.8×10^{-2}	4.0×10^{-2}
K (kg mol^{-1})	5.4×10^{4}	3.4×10^{2}	8.2×10	3.2×10	1.0×10
N	4.4	5.6	3.1	3.1	4.4
			DMA		
C_{out}* (mol kg^{-1})	1.3×10^{-5}	5.3×10^{-3}	1.3×10^{-2}	5.5×10^{-2}	1.0×10^{-1}
K (kg mol^{-1})	7.1×10^{4}	1.7×10^{2}	5.6×10	1.9×10	9.1
N	4.9	43	12	20	5.3
			AM		
K (kg mol^{-1})				1.3×10	7.0
N				8.1	2.0×10

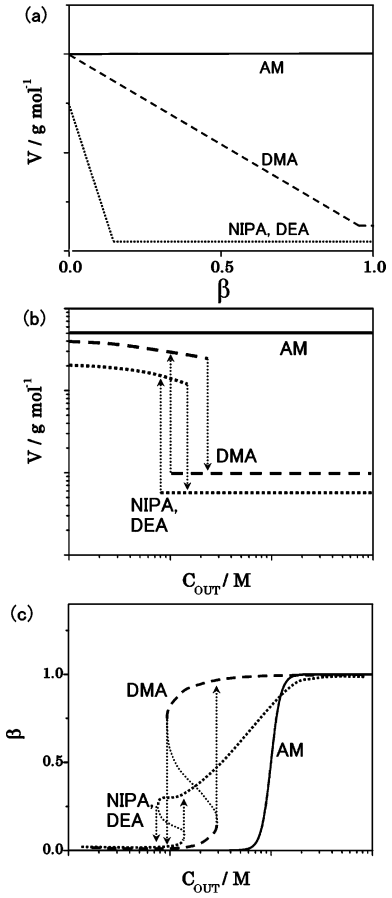

Figure 10. Schematic relations of the gel volume as a function of β (a), the gel volume as a function of C_{out} (b), and β as a function of C_{out} (c) in the volume phase transition process. The solid line represents the case of the AM gel that exhibits no volume change with binding. The broken line schematically represents the case of the DMA gel, and the dotted line represents the case of the NIPA or the DEA gel. The arrows directing upper and lower in (b) and (c) indicate the transition of gel volume.

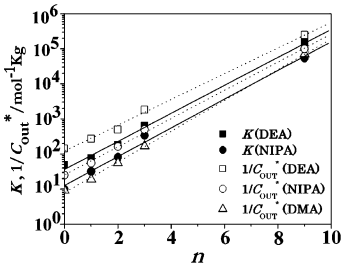

Figure 11. K (solid line) and $1/C_{out}$* (broken line) as functions of the carbon number of the alkyl group of alkylphenol, n, that binds to the DMA, DEA, and NIPA gels. K values are obtained from the analysis of the binding isotherm.

binding to the copolymer gels of alkylacrylate and acrylic acid.[29] The low stability of the hydration around hydrophobic side groups of the chain might make the binding of small molecules less cooperative. It is noted that $1/C_{out}$* of the DMA gel approximately agrees with the K value of the DMA gel. This indicates the synchronized transitions of the gel volume and β of the DMA. Here, it should be mentioned that the binding of alkylphenol coupling with the conformational change of the alkylacrylamide gel chain is different from the mechanism proposed by Hill.[26] In this sense, the N value should be regarded as conventional, although the free energy difference between the binding and unbinding states reflects on the K value.

Figure 11 shows the linearly increasing logarithmic K with an alkyl carbon number of the alkylphenol binding to the DEA and NIPA gels. This indicates that the free energy difference, $\Delta g = -kT \ln K$, between the binding and unbinding states of the system proportionally increases with the alkyl chain length. The increase of Δg values with the alkyl chain length of alkylphenol is considered to reflect on the increase of free energy to transfer the alkyl group from water to the collapsed gel. Incremental rates of Δg values per one methylene or methyl group of the alkyl chain calculated from the slope seen in Figure 11 are 1.1 ± 0.1 kT for the DMA gel, 0.95 ± 0.05 kT for the NIPA gel, and 0.92 ± 0.03 kT for the DEA gel. These values are comparable with the reported value of the environmental energy change at micelle formation in water per a methylene group, 1.1 kT.[30] Figure 11 also demonstrates that the differences between ln K and ln($1/C_{out}$*) values are 0.7 ± 0.3 for the DEA and NIPA gels and substantially null for the DMA gel.

It is worthwhile to mention that the entropy gain of the chain yielded by no volume change of the AM gel with the Ph and m-Ph binding to the gel contributes little to Δg. The differences between Δg values of the DMA and AM gels, 0.3 kT for the Ph binding and 0.4 kT for the m-Ph binding, reflect on the entropy gain of the chains in collapsing. These values are smaller than, but comparable to, the contributions of the hydrophobic methyl group to Δg, 0.7 kT for the DMA gel and 0.6 kT for the AM gel. The very small hydrophobic interaction between the alkylphenol and the AM gel can be inferred from the fact that no binding of p-Ph and e-Ph to the AM gel is observed even at their saturating concentrations. If the hydrophobic interaction is as significant as the case of the alkylphenol binding to the DMA gel, then K values for the e-Ph and p-Ph binding to the

TABLE 3: Parameters *Y* and *H* in Eq 5 Fitting to the Binding Isotherms of Alkylphenol to Alkylacrylamide Gel in the Region of $\beta > 1$

	p-Ph	e-Ph	m-Ph	Ph
		DEA		
Y (mol^{-1} kg)	50	15	4.5	1.1
H	0.1	0.1	0.5	5.1
		NIPA		
Y (mol^{-1} kg)	46	12	5.3	1.1
H	0.3	0.3	0.3	4.0
		DMA		
Y (mol^{-1} kg)	46	15	4.5	1.1
H	0.9	0.2	0.3	3.6
		AM		
Y (mol^{-1} kg)			3.9	2.0
H			0.3	0.1

AM gel, respectively, should be about 40 and 120, which are large enough for e-Ph and p-Ph to bind at their saturating concentrations. It is noticeable that a Δg value of Ph binding to the DMA gel is -1.9 *kT*, which is considered to be the difference between the free energies of amide−Ph and amide−water hydrogen bonds.

The binding at $\beta > 1$ is the alkylphenol condensation in the hydrophobic field of the collapsed chain. The attractive force to induce the condensation is due to the π/π interaction between the phenyl groups of the alkylphenol molecules,[16] as well as the hydrogen bonding between their hydroxyl groups. The molecular condensation in the field can be regarded as the multimolecular adsorption on the site that can be analyzed with the following equation

$$\beta = \frac{H\cdot Y\cdot C_{out}}{(1 - Y\cdot C_{out} + H\cdot Y\cdot C_{out})(1 - Y\cdot C_{out})} + 1; \quad H = q_1/q_2; \quad Y = q_2 e^{\mu_0/kT} \quad (5)$$

The first term in eq 5 is derived by BET for the multilayer adsorption of gas molecules on the solid.[31] Here, the binding site is assumed to be the alkylphenol bound to the amide group of the chain, and many alkylphenol molecules can adsorb on the site to form a multiple layer of alkylphenol. The BET equation can describe well the condensation equilibrium of molecules on adsorbent at an adsorbate concentration close to the saturation concentration, which is given by Y^{-1} in eq 5. The best-fitting *H* and *Y* values to the binding experiments represented by broken lines ($\beta > 1$) in Figures 1−3 and 5 are tabulated in Table 3. The obtained Y^{-1} value is very close to the saturation concentrations of the alkylphenol (p-Ph, 0.0155 ± 0.001 M; e-Ph, 0.055 ± 0.01 M; m-Ph, 0.19 ± 0.01 M; Ph, 0.6 ± 0.1 M), as expected. The *H* value being less than 1 reflects on the preferential piling-up adsorption of the alkylphenol molecules, and the *H* value being greater than 1 reflects on the preferential first-layer adsorption of the phenol molecules. The steric hindrance of the alkyl chains of the alkylphenol reduces the attractive π/π force and makes *H* values less than 1, except for the case of binding to the AM gel, which is swollen in the binding state.

The regularly distributed particles indicated by the SAXS spectra shown in Figures 6 and 7 are composed of the alkylphenol-bound gel chain segments. The sizes of the particles should be less than the correlation lengths. This suggests that several monomeric units in the binding state segregate to compose the particles. The segregation forces are considered to be the π/π interaction between the phenyl groups of the alkylphenol molecules[16] and the hydrophobic interaction between the alkyl chains surrounded by water molecules. The water molecules remaining in the collapsed gel chains should be taken into account to explain the sizes of the segregated particles shown in Table 1. The complex of the alkylphenol molecule with the amide group cannot be miscible with the water molecules and is segregated from the water to form a self-assembled particle. The segregation number of the alkylphenol, n_{seg}, is given by the minimizing condition of the segregation free energy $\gamma n_{seg}^{2/3} - n_{seg}\Delta\epsilon$, that is, $n_{seg} \approx (\gamma/\Delta\epsilon)^3$, where γ and $\Delta\epsilon$, respectively, are the interface tension (the free energy needed for increasing one unit of the interface area) between the water and the segregating materials in the gel and the free energy decrement in transferring one binding alkylphenol molecule from water into the segregating microdomain of the alkylphenol. The affinity of the side group of the gel chain to water molecules increases in the order of the DEA, NIPA, and DMA gels according to the results of the binding experiment described before. The amount of water remaining in the collapsed chain increases in the same order of gel type, along with the γ value, whereas a $\Delta\epsilon$ change with a change of the gel type is not as much as γ because of a small hydrophobicity difference among the complexes with the same type of alkylphenol. Thus, n_{seg} and the segregation size increase in the order of DEA, NIPA, and DMA gels, as shown in Table 1. The hydrophobicity of alkylphenol increases in the order of Ph, m-Ph, e-Ph, p-Ph, and n-Ph. The γ value increases in the same order of the alkylphenol types, because γ increases with an increase in the hydrophobicity of the complex. Thus, the segregation size increases in the order Ph, m-Ph, e-Ph, p-Ph, and n-Ph because the $\Delta\epsilon$ change with a change of the alkylphenol type is not as much as γ. It should be noted that the segregation size increases with β at $\beta > 1$, as shown in Table 1. Little contribution of the multimolecular adsorption energy to the energy decrement in transferring the bound alkylphenol from the water-rich microdomain into the segregating microdomain results in a decrease in the decrement energy per each bound alkylphenol molecule, $\Delta\epsilon$. Therefore, the $\Delta\epsilon$ value decreases with β at $\beta > 1$. Thus, the segregation size increases with β at $\beta > 1$. There is a decreasing tendency of Δq with an increase in β, except for the case of the m-Ph binding DMA gel at $\beta = 7.8$. The small Δq value indicates a narrow distribution of the size of the particles, which is induced by the suppression of the spatial fluctuation of the microdomain size of the binding portion. It is plausible that the segregation of adsorbed molecules at extremely high β values melts to homogenize, and no SAXS peaks emerge. In the melting process, Δq increases with β, as is observed in the exceptional case just mentioned.

Acknowledgment. The SAXS experiments were performed at SPring-8 with the approval of Japan Synchrotron Radiation Research Institute.

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