

## Interaction between Poly(L-lysine) and Poly(N-isopropyl acrylamide-co-acrylic acid) in Aqueous Solution

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**Abstract :** A series of pH/temperature sensitive polymers were synthesized by copolymerizing N-isopropyl acrylamide(NIPAAm) and acrylic acid(AAc). The influence of polyelectrolyte between poly(allyl amine)(PAA) and poly(L-lysine)(PLL) on the lower critical solution temperature(LCST) of pH/temperature sensitive polymer was compared in the range of pH 2~12. The LCST of PNIPAAm/water in aqueous poly(NIPAAm-co-AAc) solution was determined by cloud point measurements. A polyelectrolyte complex was prepared by mixing poly(NIPAAm-co-AAc) with poly(allyl amine)(PAA) or poly(L-lysine)(PLL) solutions as anionic and cationic polyelectrolytes, respectively. The effect of polyelectrolyte complex formation on the conformation of PLL was studied as a function of temperature by means of circular dichroism(CD). The cloud points of PNIPAAm in the aqueous copolymers solutions were strongly affected by pH, the presence of polyelectrolyte solute, AAc content, and charge density. The polyelectrolyte complex was formed at neutral condition. The influence of more hydrophobic PLL as a polyelectrolyte on the cloud point of PNIPAAm in the aqueous copolymer solution was stronger than that of poly(allyl amine)(PAA). Although polymer-polymer complex was formed between poly(NIPAAm-co-AAc) and PLL, the conformational change of PLL did not occur due to steric hinderance of bulky N-isopropyl groups of PNIPAAm.

### Introduction

Most of the intelligent polymers studied previously are responsive to only one kind of stimulus.<sup>1-4</sup> But for some applications, independent response to several factors, such as temperature and pH, may be required.<sup>5</sup> These temperature and pH-sensitive polymers which contain both temperature and pH-sensitive components undergo marked solubility changes in water in response to temperature and pH changes.<sup>6,7</sup>

Poly(N-isopropyl acrylamide)(PNIPAAm) is the most popular polymer among the temperature sensitive polymers since it exhibits a sharp lower critical solution temperature(LCST) close to 32 °C in water.<sup>8,9</sup> Poly(acrylic acid)(PAAc) is one of the

pH-sensitive polymers that contains ionizable groups. PAAc can lead to pH-dependent phase transition.

Poly(N-isopropyl acrylamide-co-acrylic acid)[poly(NIPAAm-co-AAc)] bearing weakly acidic pendent groups is an anionic polyelectrolyte. When this copolymer is mixed with a cationic polyelectrolyte solution, a polyelectrolyte complex is formed through ionic interaction between two oppositely charged polyelectrolytes. Therefore, the LCST of PNIPAAm incorporated with acrylic acid is affected by polyelectrolyte complex according to the various pH values.

In a previous study,<sup>10</sup> we reported the influence of polyelectrolyte complex formation between poly(acrylic acid)(PAAc) and poly(allyl amine)(PAA) on the LCST of PNIPAAm/water in poly(NIPAAm-co-AAc). From the results of this study, the LCST

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of PNIPAAm in poly(NIPAAm-co-AAc) solution with PAA increased with pH value and appeared above the  $pK_a$  value of PAAc, which was not observed in the absence of PAA. But, it was lower than that of PNIPAAm in the absence of PAA due to the polyelectrolyte complex formation. Also, it decreased with an increase of AAc content in the copolymer below the  $pK_a$  value of PAAc whereas it increased with AAc content in the copolymer above the  $pK_a$  value of PAAc.

As another cationic polyelectrolyte solute, poly-(L-lysine)(PLL) is a polypeptide with basic side groups and takes random coil and  $\alpha$ -helical conformations at pHs below and above 9.5 related its  $pK_b$  value, respectively.<sup>11</sup> The length of alkyl side chain of PLL is much longer than that of PAA. Therefore, it is interesting to study the effect of hydrophobicity of alkyl groups of PLL on the phase transition temperature of PNIPAAm in poly(NIPAAm-co-AAc) compared with PAA. The objective of this work was to study the influence of poly(L-lysine)(PLL) on the LCST of PNIPAAm in aqueous poly(NIPAAm-co-AAc) solution and the effect of more hydrophobic side chain of PLL upon the polyelectrolyte complex formation in comparison with the case of PAA. Furthermore, circular dichroic studies have been made on the conformational change of PLL by the formation of polyelectrolyte complex with poly(NIPAAm-co-AAc).

## Experimental

**Materials.** N-isopropyl acrylamide(NIPAAm, Polysciences Inc.) was recrystallized in n-hexane. Acrylic acid(AAc, Junsei Chem. Co.) was purified by distillation at 40 °C/26 mmHg. N,N'-azobisisobutyronitrile(AIBN, Tokyo Kasei Kogyo Co.) was purified in methanol. 1,4-Dioxane(Duksan Co.) was purified by distillation.

Poly(L-lysine)(PLL, Aldrich, M.W. 65,000), sodium hydroxide standard solution(Katayama Chem.), hydrochloric acid standard solution(Katayama Chem.) and poly(acrylic acid)(PAAc Aldrich, M.W. ca. 250000) were used as received.

**Synthesis of Poly(NIPAAm-co-AAc).** Synthesis of linear poly(NIPAAm-co-AAc), containing 0, 10, 20, 30, 40, 50 mol% of AAc, was carried

**Table I. The Feed Composition of Poly(NIPAAm-co-AAc)**

Code <sup>a</sup>	NIPAAm (g)	AAc (mL)	AIBN <sup>b</sup> (g)	Dioxane <sup>c</sup> (mL)
Poly(NIPAAm)	1.00	0.00	$4.30 \times 10^{-3}$	2.90
Poly (NIPAAm-co-AAc 10)	0.90	0.06	$4.40 \times 10^{-3}$	2.80
Poly (NIPAAm-co-AAc 20)	0.80	0.12	$4.50 \times 10^{-3}$	2.70
Poly (NIPAAm-co-AAc 30)	0.70	0.18	$4.50 \times 10^{-3}$	2.58
Poly (NIPAAm-co-AAc 40)	0.60	0.24	$4.40 \times 10^{-3}$	2.47
Poly (NIPAAm-co-AAc 50)	0.50	0.30	$4.30 \times 10^{-3}$	2.37

<sup>a</sup> mol% of AAc against NIPAAm.

<sup>b</sup> AIBN as an initiator(0.3 mol% of monomers).

<sup>c</sup> Dioxane as a solvent(25 wt% of monomers).

out in 1,4-dioxane(25 wt%) with AIBN as an initiator(0.3 mol% of monomers). Feed composition is listed in Table I. Dried nitrogen was bubbled through the solution for 20 min prior to polymerization. After polymerization at 70 °C for 5 hr, the mixtures were dissolved in methanol and precipitated in diethyl ether. The precipitates were filtered and washed with acetone and deionized water to remove the unreacted monomers and homopolymer, respectively. Then, the precipitates were washed again with acetone to substitute water and dried in vacuum desiccator for 2 days.

**Preparation of Polyelectrolyte Complex.**  $1.7 \times 10^{-1}$  unit mol/L of poly(NIPAAm-co-AAc) solution, containing 0, 10, 20, 30, 40, 50 mol% of AAc, and  $4.2 \times 10^{-1}$  unit mol/L of PLL solution were prepared by dissolving polymers in distilled water, respectively. Before mixing two solutions, the pH of each solution was adjusted identically with HCl or NaOH standard solution in the range of 2~12. To each copolymer solution, PLL solution was added with stirring. Each concentration of poly(NIPAAm-co-AAc) and PLL was  $4.2 \times 10^{-2}$  unit mol/L and  $1.1 \times 10^{-1}$  unit mol/L, respectively, after mixing.

**Cloud Point Temperature Determination.** The turbidity of solutions was determined by

measuring the absorbance at 450 nm of each of the solutions prepared above using Hewlett-Packard 8452A Diode Array UV/VIS spectrophotometer with HP89090A Peltier Temperature Control Accessory. The temperature of the solutions was raised from 15 to 70 °C in 2 °C increments every 10 min. The absorbance was normalized against the absorbance of the polymer solution at temperature where precipitation started to take place. The cloud point temperature was defined as the temperature at the inflection point in the absorbance versus temperature curve and the cloud point of solution which does not exhibit inflection point was determined at 10% absorbance in the curve.<sup>12</sup>

**Titration.** Titrations of the poly(NIPAAm-co-AAc) were performed as follows. 100 mg of each linear copolymer was dissolved in 25 mL of 0.1 M NaCl. In order to increase the solubility of the polymers during the titrations, back titrations were performed on ionized polymers. The polymers were fully ionized by adding 2~6 mL of 0.1 N NaOH. Titrations were performed at 20 °C by adding 0.1 N HCl in small quantities and measuring the pH after a stable value has been reached. The temperature was kept constant ( $\pm 0.3$  °C) for 30 min before and during each titration using a waterbath.<sup>13</sup>

**Circular Dichroism Measurement.** Aqueous solutions of  $8.0 \times 10^{-4}$  unit mol/L of PLL and  $8.0 \times 10^{-4}$  unit mol/L of poly(NIPAAm-co-AAc) solution, which had been adjusted by HCl and NaOH standard solution to have the same pH, were mixed by dropwise addition of the poly(NIPAAm-co-AAc) solution into the PLL solution with stirring. Each concentration of poly(NIPAAm-co-AAc) and PLL was  $4.0 \times 10^{-4}$  unit mol/L after mixing. The mixed solutions of PLL and PAAC were prepared by the same method.

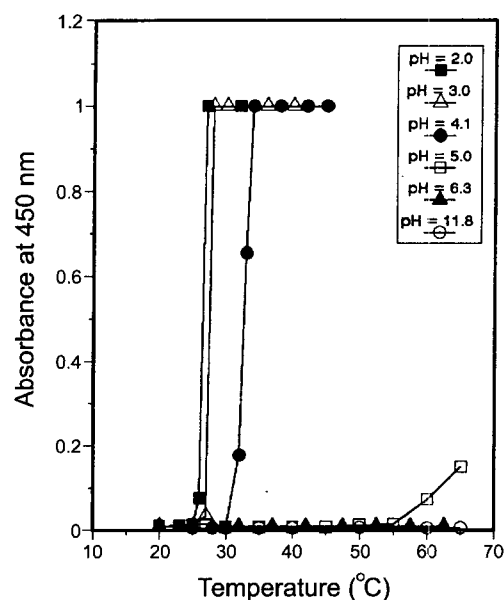
Circular dichroism (CD) spectra of the mixed solutions in thermostated quartz cell were recorded at a path length of 1.0 cm using JASCO J-500A Circular Dichroism Spectropolarimeter with JEIO TECH RBC-31 REF. Bath Circulator.

## Results and Discussion

**Effect of Polyelectrolyte.** Poly(NIPAAm-co-

**Table II. Monomer Ratios in Linear Poly(NIPAAm-co-AAc) Compared to Monomer Feed Ratios**

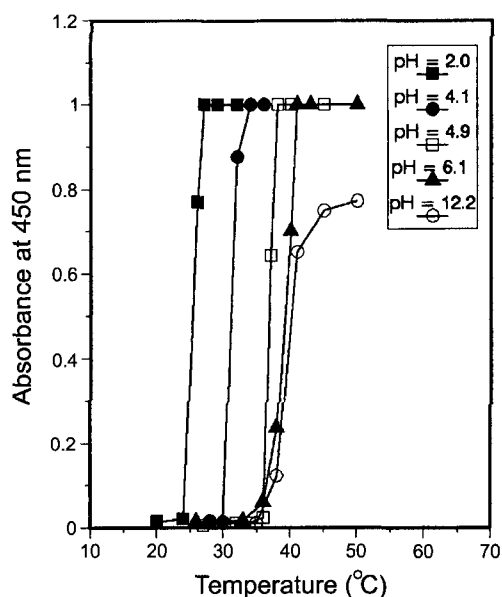
Comonomer AAC Feed Molar Composition (mol%)	Copolymer AAC Feed Molar Composition (mol%)	% Conversion
0	0	84.5
10	13.8	29.6
20	21.8	58.5
30	32.9	89.7
40	35.8	81.9
50	45.8	79.9



**Figure 1.** The absorbance of aqueous poly(NIPAAm-co-AAc-30) solution against pH as a function of temperature.

AAc)s were prepared from comonomer feed compositions ranging from 0 to 50 mol%. The copolymer compositions determined from back titration method are given in Table II. The differences between the AAC content in the copolymers and the feed compositions were less than 6 mol%.

Figure 1 illustrates absorbance at 450 nm versus temperature for  $4.2 \times 10^{-2}$  unit mol/L of poly(NIPAAm-co-AAc-30) solution according to the various pH values. Distinct cloud points were observed at pH 2.0~4.1 and change of absorbance against temperature became small with an



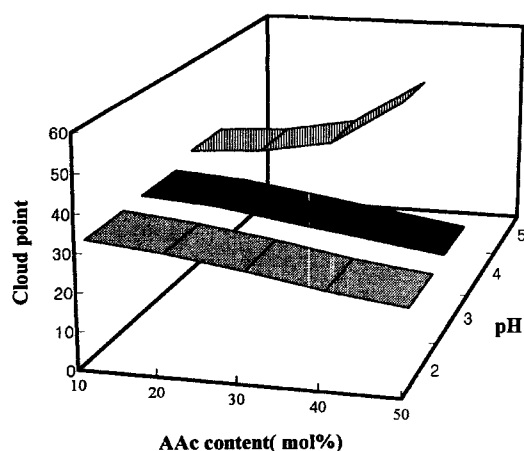
**Figure 2.** The absorbance of aqueous poly(NIPAAm-co-AAc-30) solution with PLL against pH as a function of temperature.

increase of pH. Particularly, drastic change of absorbance of the solution versus temperature was observed around pH 4.3~4.7, which is related to the  $pK_a$  value of PAAc.<sup>14</sup> Cloud points of PNIPAAm in poly(NIPAAm-co-AAc-30), which are defined as the transition point of absorbance versus temperature, were 26.5, 27.5 and 39.0 °C for the pH value of 2.0, 3.0 and 4.1, respectively. From these results, it was found that the cloud point of PNIPAAm in the poly(NIPAAm-co-AAc-30) solution decreased in the nonionized state of PAAc whereas it increased in the partially ionized state of PAAc in comparison with the cloud point of PNIPAAm itself, 31~33 °C.<sup>8,9</sup> It is thought that the cloud point of PNIPAAm decreases owing to inter- or intra-hydrogen bonding between amide group of PNIPAAm and carboxylic acid group of PAAc below  $pK_a$  value of PAAc. Above the  $pK_a$  value of PAAc, the cloud point of PNIPAAm in the poly(NIPAAm-co-AAc-30) solution was not observed up to 70 °C, since carboxylic acid group of PAAc was completely ionized and resulted in electrostatic repulsion of ionized groups of PAAc.

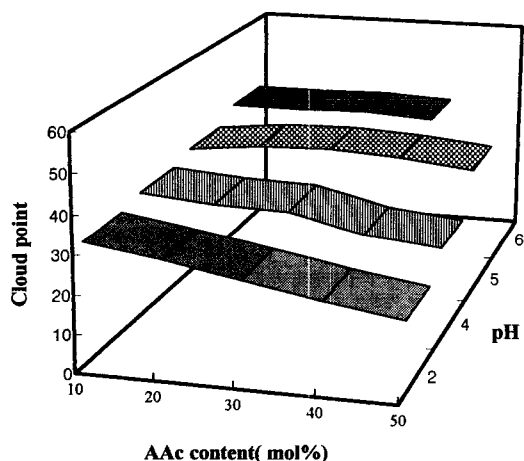
Figure 2 illustrates absorbance at 450 nm versus temperature for  $4.2 \times 10^{-2}$  unit mol/L of aqueous

poly(NIPAAm-co-AAc-30) solution according to the various pH values in the presence of PLL as a polyelectrolyte solute. Distinct cloud points were observed at all pHs studied. Drastic change of absorbance of the solution against temperature was observed even above the  $pK_a$  value of PAAc in the presence of PLL. The cloud point of PNIPAAm in poly(NIPAAm-co-AAc-30) solution in the presence of PLL was 25.0, 31.0, 37.5 and 39.0 °C for the pH value of 2.0, 4.1, 4.9 and 6.1, respectively. From these results, it was found that the cloud point increased with pH value because the degree of ionization of carboxylic acid groups increased with pH. Also, it was found that the cloud point of PNIPAAm in the solution appeared above the  $pK_a$  value of PAAc, which was not observed in the absence of PLL. The cloud point of PNIPAAm in the copolymer solution in the presence of PLL decreased due to the polyelectrolyte complex formation between carboxylic acid group of PAAc and amine group of PLL, indicating that dehydration occurred around PNIPAAm. At pH value of 7.0, the cloud point of PNIPAAm in the solution was not observed due to the insoluble polyelectrolyte complex formation between them, indicating compact form of the complex structure. Also, drastic change of absorbance of the solution against temperature was observed at the pH value of 12.2, which is above the  $pK_a$  value of PLL. This phenomenon is not a result by polyelectrolyte complex formation but the conformational transition of PLL itself by heating. It was already reported that PLL undergoes a conformational transition from the  $\alpha$ -helix to both the random coil and  $\beta$ -form by heating above pH 11, the latter partly giving rise to precipitation.<sup>15</sup>

**Effect of AAc Content in Poly(NIPAAm-co-AAc).** Figure 3 exhibits the cloud point temperatures of PNIPAAm against AAc mol% in the poly(NIPAAm-co-AAc) copolymer solutions according to the pH values. It was found that the cloud point temperatures of PNIPAAm in the copolymers were affected by pH and AAc content in the copolymer. The cloud point temperatures of PNIPAAm in the solution decreased with an increase of AAc content in the copolymer around pH value of 2.0~3.0 because more hydrogen



**Figure 3.** The plot of cloud point of aqueous poly-(NIPAAm-co-AAc) solution against content of AAc as a function of pH.



**Figure 4.** The plot of cloud point of aqueous poly-(NIPAAm-co-AAc) solution with PLL against content of AAc as a function of pH.

bonding between amide group of PNIPAAm and carboxylic acid one of PAAc occurs with an increase of AAc content in the copolymer. The hydrogen bonding interferes with the access of water molecules to the NIPAAm amide groups.<sup>12</sup> On the other hand, the cloud point temperatures of PNIPAAm in the solution increased with AAc content in the copolymer around pH value of 4.0 because of increased hydrophilicity with an increase of ionized AAc components.

In Figure 4, the cloud point temperatures of

PNIPAAm in the presence of PLL according to the various pH values are plotted against AAc mol% in the poly(NIPAAm-co-AAc) copolymer solution. Not much differences in cloud point temperatures of PNIPAAm between the copolymer solutions without PLL and those with PLL was found around pH value of 2.0 due to the hydrogen bonding between PNIPAAm and PAAc. However, at around pH value of 4.0, the cloud point temperature of PNIPAAm in the poly(NIPAAm-co-AAc) with PLL decreased with an increasing AAc content in the copolymer whereas those of PNIPAAm in the solution without PLL increased with AAc content in the copolymer. This phenomenon can be explained as follows: When the copolymer of NIPAAm containing partially ionized AAc is intermacromolecularly complexed with an oppositely ionized PLL, the cloud point temperature of PNIPAAm is affected by the density of complexing points.<sup>16</sup> The density of complexing points on poly(NIPAAm-co-AAc) increases with AAc content in the copolymer because the complexing points are a carboxylic acid group of AAc in the copolymer and amine group of PLL. An increase of the density of complexing points make form more polyelectrolyte complex between partially ionized PAAc and PLL. This may lead to a decreased cloud point temperature due to the reduced accessibility and mobility of water surrounding the copolymer and dehydration of the copolymer chain by polyelectrolyte complex formation. Therefore, the cloud point temperature of PNIPAAm in the poly(NIPAAm-co-AAc) with PLL decreased with an increasing AAc content in the copolymer. On the other hand, the cloud points of PNIPAAm in the copolymer solutions with PLL did not show obvious tendency against AAc content around pH value of 5.0~6.0. This is supposed as follows: With increasing pH or AAc content, the  $-\text{COO}^-$  groups in the copolymer chain increase. The presence of  $-\text{COO}^-$  groups in the polymer chain increases its rigidity because of electrostatic interchain repulsions and makes difficult the efficient polyelectrolyte complex formation between the  $-\text{COO}^-$  group of copolymer and  $-\text{NH}_3^+$  one of PLL.<sup>17</sup> But, the formation of polyelectrolyte complex is not hindered completely because a long side chain of PLL makes easily the

**Table III. The Cloud Points of Aqueous Poly(NIPAAm-co-AAc) Solutions with PAA or PLL Against Content of AAc as a Function of pH**

pH	Solute	AAc Content (mol%)				
		10	20	30	40	50
2	none	31.5 °C	29.5 °C	26.5 °C	23.5 °C	20.5 °C
	PAA	30.5 °C	29.0 °C	24.5 °C	22.5 °C	18.5 °C
	PLL	31.5 °C	28.5 °C	25.0 °C	21.5 °C	18.5 °C
4	none	34.5 °C	35.5 °C	39.0 °C	51.5 °C	*
	PAA	31.0 °C	30.0 °C	29.5 °C	31.0 °C	35.0 °C
	PLL	33.0 °C	31.5 °C	31.0 °C	26.5 °C	24.5 °C
5	none	44.5 °C	*	*	*	*
	PAA	33.0 °C	34.5 °C	37.0 °C	50.0 °C	54.0 °C
	PLL	35.0 °C	37.0 °C	37.5 °C	36.5 °C	34.5 °C
6	none	*	*	*	*	*
	PAA	**	37.5 °C	46.0 °C	56.5 °C	*
	PLL	38.5 °C	38.5 °C	39.0 °C	38.0 °C	*
12	none	*	*	*	*	*
	PAA	*	*	*	*	*
	PLL	*	*	*	*	*

\* The CP was not observed up to 70 °C.

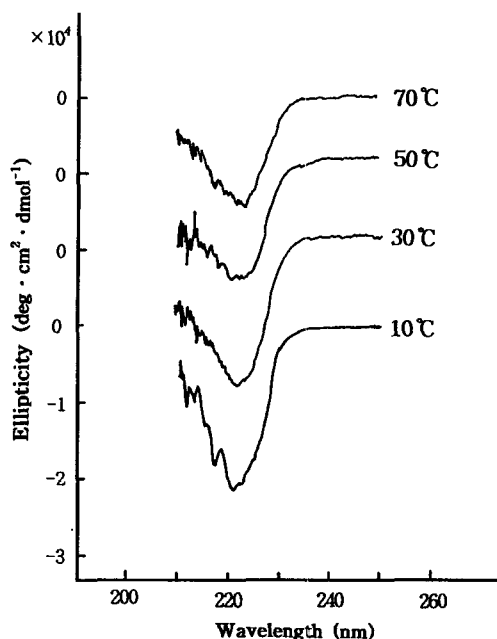
\*\* The CP was not measured due to the occurrence of precipitate.

approach of  $-\text{NH}_3^+$  group to the  $-\text{COO}^-$  one. Hence, we guess that a clear tendency on the cloud points of PNIPAAm according to AAc content is not appeared due to the balance of hydrophilicity of  $-\text{COO}^-$  groups not participated in the polymer complex formation and hydrophobicity of alkyl side chain of PLL participated in the polymer complex formation.

**Effect of Hydrophobicity.** Table III summarizes the effect of hydrophobic side chain of PLL on the cloud point of PNIPAAm in the poly(NIPAAm-co-AAc) as compared with PAA in the range of pH 2~12. The influence of PLL on the cloud point of PNIPAAm in the poly(NIPAAm-co-AAc) was much stronger than that of PAA above 20~30 mol% of AAc content. It is thought that as the side chain length increases from  $\text{C}_1$  for PAA to  $\text{C}_4$  for PLL, the hydrophobicity of polyelectrolyte complex increases, indicating that much stronger

dehydration occurs around PNIPAAm. That is, the  $-(\text{CH}_2)_4$  group for PLL is more hydrophobic than  $-\text{CH}_2$  for PAA, contributing to an increased hydrophobicity of copolymer chain and subsequently to the stabilization of the formed interpolymer aggregate by a synergistic hydrophobic effect.

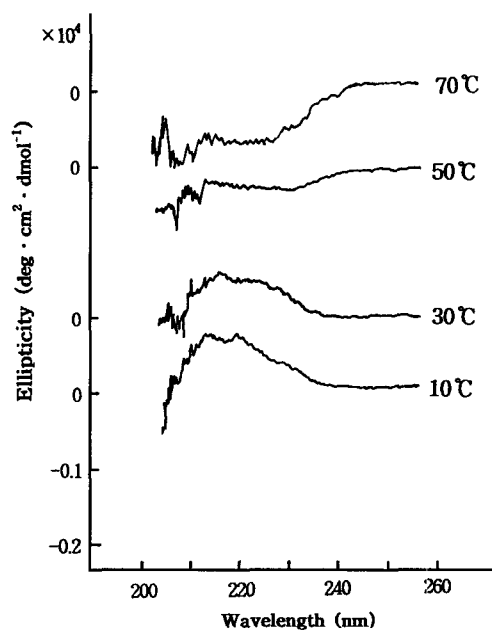
**Effect of Polymer Complex on Conformation of PLL.** As is well known, PLL takes  $\alpha$ -helical conformation at pHs above 9.5 but exits in an irregular random coil form at pHs below 9.5,<sup>11</sup> in which the flexible backbone undergoes continuous change as a result of thermal motion. This is because at pHs below  $\text{pK}_a$ , the all amine groups of PLL have a positive charge and repel each other so strongly that they overcome the tendency for intrachain hydrogen bonds to form. Ellipticity  $[\theta]$  at 222 nm is frequently used to estimate the  $\alpha$ -helical content, because  $[\theta]_{222 \text{ nm}}$  in  $\text{deg.cm}^2 \cdot \text{dmol}^{-1}$



**Figure 5.** The circular dichroism spectra of aqueous PLL/PAAc solution against temperature as a function of wavelength at pH 6.

is nearly zero for the random coil and  $-4 \times 10^4$  for the  $\alpha$ -helical conformation, respectively. It is also known that PLL undergoes a conformational transition from the  $\alpha$ -helical to  $\beta$ -form above  $50^\circ\text{C}$  in aqueous solution.<sup>18</sup> It is worthy to examine the temperature dependence of the conformation of PLL in the polyelectrolyte complex with poly(NIPAAm-co-AAc) as a function of pH.

Figure 5 exhibits the CD spectra of the PLL/PAAc mixture with equimolar amounts of the L-lysyl group(LL) and the carboxyl group(AAc) in aqueous solutions of various temperatures at pH 6. It was found that ellipticities at 222 nm were large negative values at all temperatures, indicating the conformational change of PLL from random-coil to  $\alpha$ -helix. It is thought that the positive charges on PLL are blocked by negative charges on PAAc through the polymer-polymer complex and then the intrachain hydrogen bonds are formed. It was also found that  $[\theta]_{222}$  increased from  $-2.20 \times 10^4$  to  $-1.36 \times 10^4 \text{ deg} \cdot \text{cm}^2 \cdot \text{dmol}^{-1}$  with increasing temperature, the apparent helicity being calculated as 55 and 34%, respectively. This decrease in the helicity may be due to possible



**Figure 6.** The circular dichroism spectra of aqueous PLL/poly(NIPAAm-co-AAc-50) solution against temperature as a function of wavelength at pH 6.

dissociation of the complex.

Figure 6 shows the CD spectra of the PLL/poly(NIPAAm-co-AAc-50) mixture with equimolar amounts of the L-lysyl group(LL) and the carboxyl group(AAc) in aqueous solutions of various temperatures at pH 6. In the case of PLL/poly(NIPAAm-co-AAc-50), the random-coil conformation of PLL did not essentially change at all temperatures. It is thought that bulky N-isopropyl groups of PNIPAAm bound in PLL through ionic polymer-polymer interaction inhibit the intrachain hydrogen bonding due to steric hindrance. Ellipticity at 222 nm changed from a small positive value(ca. 750) to a negative one(ca. -750) with increasing temperature, probably due to the formation of a trace of the  $\beta$ -structure.<sup>15</sup>

In conclusion, the cloud points of PNIPAAm in aqueous poly(NIPAAm-co-AAc) solution were strongly influenced by the pH, AAc content and the type of polyelectrolyte solute. Polyelectrolyte complex in the poly(NIPAAm-co-AAc) system with PLL was formed in neutral condition. The influence of PLL on the LCST of PNIPAAm in the poly(NIPAAm-co-AAc) was much stronger than

that of PAA above 20~30 mol% of AAc content due to hydrophobicity of side alkyl chain of PLL. Although polymer-polymer complex was formed between poly(NIPAAm-co-AAc) and PLL, the conformational change of PLL did not occur due to steric hinderance of bulky N-isopropyl groups of PNIPAAm.

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