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# Adenosine-Induced Changes of the Phase Transition of Poly(6-(acryloyloxymethyl)uracil) Aqueous Solution

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ABSTRACT: Poly(6-(acryloyloxymethyl)uracil) (PAU) having uracil moieties as side chains was synthesized by ordinary radical polymerization to investigate its molecular discrimination for soluble nucleic acid bases in terms of phase transition changes of the polymer aqueous solution. PAU itself in distilled water formed a precipitate due to the polymer complexes at lower temperatures and changed drastically to become water-soluble above a characteristic transition temperature, showing an upper critical solution temperature (UCST). The phase transition behavior was shifted to lower temperatures with increasing concentrations of adenosine (Ado), which is the complementary nucleic acid base to uracil moiety. Ado might interact specifically with uracil moieties of the polymers, preventing the formation of the polymer complex at lower temperatures. Such changes were not observed in PAU aqueous solution containing guanosine (Guo). Additions of Ado and Guo exerted different effects to phase transition changes of the polymer. Moreover, addition of poly(adenylic acid) (Poly(A)) lowered remarkably the transition behavior of PAU solution as compared with Ado addition. It was conceivable that PAU and Poly(A) formed stable polymer complexes, assuming soluble states in even cold water. PAU changed its phase-transition temperature in response to species of the additive materials, demonstrating the specific molecular discrimination in aqueous milieu.

KEY WORDS Poly(6-(acryloyloxymethyl)uracil) / Phase Transition / Molecular Recognition / Hydrogen Bond /Upper Critical Solution Temperature /

Poly(*N*-isopropylacrylamide) (PIPAAm),[1] poly(vinyl methyl ether),[2] and poly(*N*-vinylisobutyramide)[3] are thermo-sensitive polymers which show characteristic lower critical solution temperatures (LCSTs) in aqueous media. PIPAAm chains, for example, change drastically from hydrated to dehydrated states near 32 °C. Since the hydration change of the polymeric chains varies the swelling behavior of its chemically crosslinked PIPAAm, there are many reports on the thermally sensitive hydrogels which provide self-regulating or auto feed-back drug release systems.[4] Additionally, the hydration/dehydration of PIPAAm could be applied for hydrophilic/hydrophobic surface alteration of silica gels for reverse-phase liquid chromatography.[5] The PIPAAm-grafted silica surfaces demonstrated hydrophilic/hydrophobic property changes responding to temperatures in only aqueous mobile phase. Resolution of some hydrophobic steroids was achieved by the differences of their hydrophobic affinities onto the PIPAAm-modified silica surfaces above LCST of the polymer. This chromatography system should be noted as a novel reverse-phase chromatography without hydrophobic organic mobile phases. Adhesion of mammalian cells was also controlled on PIPAAm-grafted cell-culture substrata.[6] The cell adhesion is governed generally by hydrophobicity of the substrate surfaces. The cells adhered and proliferated on the hydrophobic surfaces of the polymer-bearing substrate at 37 °C and readily detached from the hydrophilic surfaces below the LCST. The cell detachment with the temperature treatment enabled us to avoid any damage of the cell membranes due to the normal enzymatic treatment. The thermo-sensitive polymers with such phase transition behavior have been studied widely as smart materials for drug delivery systems, chromatography, and cell-culture technologies.

In more recent studies, attention has been directed to environmentally responsive polymers that show phase transition changes in response to the chemical external stimuli as well as to physical ones such as temperature. An IPAAm copolymer having binding sites showed LCST changes in response to biologically active molecules.[7] Biopolymers such as deoxyribonucleic acid, ribonucleic acid, and proteins undergo structural changes by binding specifically with biologically active molecules, expressing the biological functions. Polymers that show their structural transitions in response to foreign molecules are attractive for development of biomimetic materials.

On the other hand, there are temperature-responsive materials having upper critical solution temperatures (UCSTs) which show an opposite temperature dependence of phase transition behavior as compared with that of PIPAAm in aqueous media. Klenina *et al.* reported that polyacrylamide (PAAm) and poly(acrylic acid) (PAAc) mixed in aqueous solution underwent phase separation due to cold water, though they dissolved homogeneously in hot water.[8] The solubility change of the solutes was caused by the formation and dissociation of the interpolymer complex via hydrogen bonding.[9] By applying the polymer complex

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system to an interpenetrating polymer networks (IPNs) hydrogel, some of us[10,11] first succeeded in preparing positive temperature-dependent hydrogels. These demonstrated shrunken and swollen states at lower and higher temperatures, respectively. Additionally, the swelling change of the IPN hydrogels occurs with sigmoidal transitions within certain characteristic temperature ranges and is reversible against the temperature fluctuation. Randomly copolymerized poly(AAm-*co*-AAc) hydrogels exhibit continuous swelling changes in response to temperature changes. The discontinuous changes between the shrunken and swollen states in the IPN hydrogel are due to both attractive and repulsive polymer-polymer interactions as well as to polymer-water interactions. In the IPN systems, in order to obtain both hydrogen bonded polymer complexes at lower temperatures and positive formation/dissociation change of the complex as a function of temperature in aqueous media, one is required to utilize the complementary macromolecules that function as hydrogen-bonded donors and acceptors. Furthermore, the IPN hydrogels composed of PAAc and PAAm derivatives such as poly(*N*-acryloyloxyglycineamide)[12] or poly(*N,N*-dimethylacrylamide-*co*-AAm)[13] also demonstrated the drastic swelling changes near body temperature. These hydrogels were developed as temperature-responsive hydrogels that were capable of controlling "off" and "on" states of drug release in response to normal and febriferous body temperatures, respectively.

Recently, we found that poly(6-(acryloyloxymethyl)-uracil) (PAU) itself had a UCST not only in distilled water but also in physiological aqueous solution.[14] PAU in aqueous solution formed a precipitate at lower temperatures and was dissolved drastically and homogeneously above characteristic transition temperatures. Since N-H and C=O groups in a uracil moiety act as hydrogen-bonded donors and acceptors, respectively, polymer complexes might be formed between uracil moieties as side chains of PAU via the hydrogen bonding, resulting in the precipitation in cold water. When the temperature of the solution is raised, the non-covalent bonding between uracil moieties might be broken and the moieties had hydrated states, resulting in the dissolution in hot water. Little is known regarding a nucleic acid base-containing polymer which shows such a dynamic solubility change in response to the external stimuli in water, while there are many attractive research reports concerning the synthetic polymers having nucleic acid bases.[15] The temperature-responsive formation/dissociation of the PAU complexes was observed in phosphate buffered solution at pH 7.4. Poly(AU-*co*-AAm) hydrogels in the physiological condition showed the similar volume-phase transition and drug-release control to those of the IPN hydrogels.[10,13] If PAU is capable of changing its phase transition behavior in response to specific foreign molecules, it will be a biomimetic material that shows dynamic structural changes in company with molecular recognition.

In this study, PAU was synthesized by an ordinary radical polymerization to study the phase transition between the dehydrated and hydrated states of PAU aqueous solution as a function of temperature and the influence of addition of soluble nucleic acid bases on the phase transition behavior of the polymer.

## EXPERIMENTAL

### *Materials*

6-Chrolomethyluracil and 18-crown-6-ether were purchased from Tokyo Chemical Industry Co., Ltd. Poly(adenylic acid) (Poly(A)) was purchased from Aldrich Chemical Company, Inc. Other reagents were purchased from Wako Pure Chemical Industries, Co., Ltd. *N,N*-Dimethylformamide (DMF) (b.p. 37 °C at 9 mmHg) and dimethylsulfoxide (DMSO) (b.p. 60 °C at 4 mmHg) were distilled before use. 2,2'-Azobis(isobutyronitrile) (AIBN) was purified by recrystallization from methanol. Other reagents were used as received.

### *Synthesis of 6-(acryloyloxymethyl)uracil (AU)*

Following Brahme *et al.*,[15e] AU was synthesized by a reaction of potassium acryloylate with 6-(chloromethyl)-uracil, and then purified by repeated precipitation from DMSO into distilled water and finally recovered from DMSO into ethyl alcohol as a precipitate.

### *Synthesis of poly(6-(acryloyloxymethyl)uracil) (PAU)*

The structural formula of the PAU is shown in Figure 1. PAU was synthesized by ordinary radical polymerization in DMF solution. 1.3 g (6.8 mmol) of AU, and 0.035 g (0.2 mmol) of AIBN were added to DMF. The reaction mixture was kept at 60 °C for 48 h and then the solvent was removed in vacuo. The viscous liquid obtained was dissolved in 5 mL (1 mL = 1 $cm^3$) of DMSO and poured into acetone. This polymer was purified by repeated precipitation from DMSO into acetone and finally recovered from ethyl alcohol as a precipitate.

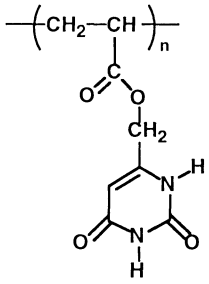

**Figure 1.** Structure of poly(6-acryloyloxymethyl uracil) (PAU).

### *Transmittance Measurements*

Polymers were dissolved in distilled water at 0.1 w/v%. Optical transmittance of the polymer aqueous solutions at various temperatures was monitored at 500 nm by means of a spectrophotometer (JASCO, Ubest V-530). The quartz cell was thermostatted with a jacket equipped with a temperature controller (JASCO, EHC-477S). The transmittance measurement was carried out by cooling the polymer solution from higher to lower temperatures with 1 °C/min of scanning rate. The transmittance value of the polymer solution containing urea was measured until it showed a constant value at one temperature. After the equilibration at that temperature, the solution was re-equilibrated at a higher temperature.

## RESULTS AND DISCUSSION

Figure 2 shows that the temperature dependence of optical transmittance for PAU in distilled water without and with soluble urea. While the transmittance for the polymer aqueous solution was 0 % below 40 °C, it started to increase near that temperature and became 100 % above 70 °C. PAU

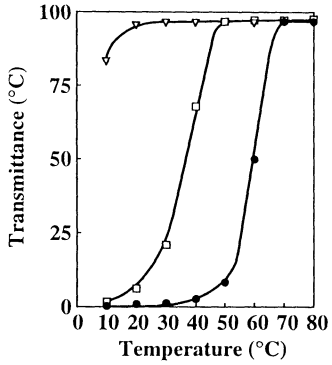


**Figure 2.** Influence of urea addition on temperature dependence of optical transmittance for PAU aqueous solution (0.1 wt%). Urea concentration: (●) 0 M; (□) 1 M; (▽) 3 M.

formed a precipitate below the characteristic transition temperatures and this was dissolved in distilled water at higher temperatures, showing the sigmoidal solubility change as a function of temperature. It was considered that the solubility change was due to the formation and dissociation of the molecular complexes between uracil moieties. Additionally, the soluble urea, which prevents formation of the hydrogen bonding, lowered the transition behavior of the polymer and removed the thermo-sensitivity in 3 M urea solution. It can be assumed that hydrogen bonding between uracil moieties plays a principal role to form the complexes. In other words, since the uracil moieties had N-H and C=O groups as hydrogen-bonded donors and acceptors, respectively, they might form the molecular complexes via hydrogen bonding at lower temperatures, resulting in dehydrated states and the phase separation from water. Indeed, poly(uridylic acid) in water exhibited the structural transition between the helical form due to the formation of polymer complex at low temperatures and the coiled form at high temperatures.[16] In the case of the synthetic polymers with hydrophobic main chains, the formation and dissociation of the polymer complexes in water brings about the precipitation and the dissolution, respectively.

Figure 3 shows the influence of addition of soluble Ado and Guo on the temperature dependence of optical transmittance for PAU aqueous solution. As shown in Figure 3a, the phase transition behavior of PAU in water was clearly observed at lower temperatures with increasing Ado concentrations. Adenine in Ado is known to be a complementary base to uracil. It was suggested that the uracil moieties of the polymer side chains interacted specifically with adenine via hydrogen bonding and that PAU failed to form the self-association between the uracil moieties. Therefore, PAU tended to dissolve in water, exhibiting the phase transition at lower temperatures. On the contrary, the transition of PAU solution responding to Guo concentrations was quite different from that of PAU-Ado-water ternary system. PAU solution containing Guo showed less change of the transition temperature as compared with that for the PAU-Ado solution, as shown in Figure 3b. Moreover, the solution already had the turbidity at 70 °C. Guanine in Guo is not a complementary base to uracil. It was conceivable that there was hydrophobic aggregation via non-specific interaction between PAU and Guo, and that PAU did not show the fully hydrated states at high temperature. The addition of Ado and Guo induced different phase transition changes of PAU solution.

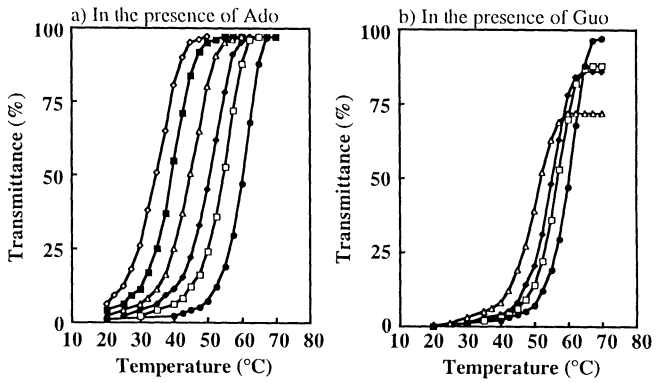


**Figure 3.** Temperature dependence of optical transmittance for PAU (0.1 wt%) in distilled water in the absence and presence of Ado (a) and Guo (b). Ado concentration: (●) 0 mM; (□) 3.7 mM; (◆) 7.5 mM; (△) 18.7 mM; (■) 37.4 mM; (◇) 56.1 mM. Guo concentration: (●) 0 mM; (□) 3.5 mM; (◆) 7.1 mM; (△) 17.7 mM.

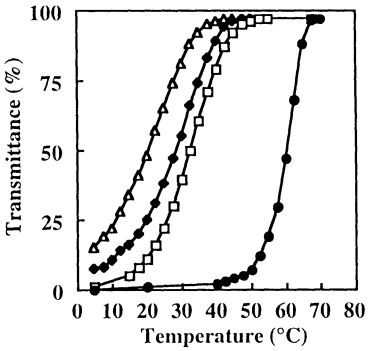


**Figure 4.** Temperature dependence of optical transmittance for PAU (0.1 wt%) in distilled water in the absence and presence of Poly(A). Poly(A) concentration: 0 wt% (●); 0.1 wt% (□); 0.2 wt% (◆); 0.5 wt% (Δ).

The influence of addition of Poly(A) on the transmittance change of PAU solution was investigated. As shown in Figure 4, the phase transition behavior was shifted remarkably to lower temperatures with increasing Poly(A) concentrations. Poly(A) is a homopolymer which has adenine moieties. The main chains of Poly(A) have the hydrophilic phosphate groups with the negative ionic charges. It was considered that the self-association of PAU was blocked by forming the complexes between PAU and poly(A), and that the complexes maintained the hydrated states by the anionic main chains of Poly(A). Since selective complexation of PAU to Poly(A) happened in aqueous milieu, the phase transition behavior of PAU solution was shifted remarkably to lower temperatures.

Figure 5 summarizes the changes of the transition temperatures for PAU solution as a function of Ado concentrations. The transition temperatures were defined as temperatures at 50 % optical transmittances, which are shown in Figures 3a and 4. Figure 5 reveals that the transition temperatures of PAU solution were decreased with increasing Ado concentrations and that the addition of soluble Poly(A) induced a drastic lowering of the temperature values. Calculating the molar ratio of Ado monomer unit to the uracil monomeric unit manifested that Poly(A) shifted the phase transition behavior for PAU

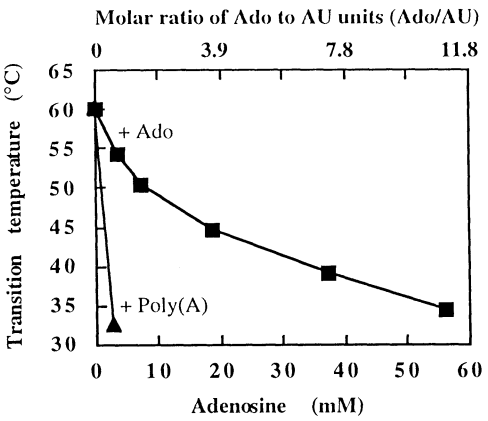


**Figure 5.** Change in the transition temperature of PAU aqueous solution containing various concentrations of adenosine. The transition temperatures are defined as the temperature at 50 % optical transmittance.

solution to a lower temperature as compared with Ado. When the temperature was cooled, the initial hydrogen-bonded complexes between adenine and uracil moieties as spendant groups of each polymeric chain started to be formed. Subsequently, the complex formation triggered the cooperative association of the adjacent complexes between the neighboring adenine and uracil units. Therefore, it was considered that PAU could form more stable molecular complexes with Poly(A) than with Ado in water, showing hydration states and lowering the transition temperatures drastically. It means that the polymer-polymer interaction overcomes the competitive interaction with water molecules in aqueous media.

Nucleic acid bases and their derivatives are studied widely as hydrogen-bonded recognizable moieties in organic solvents[17] or at air-water interfaces.[18] Furthermore, the molecular recognition of the nucleic acid base derivatives in water has recently attracted much attention,[19] because there are competitive interactions between the nucleic acid components and many water molecules through many hydrogen-bonded bridges. PAU was "a new polymer" which showed the UCST changes in response to foreign specific molecules. Our future work will be directed at clarifying in detail the mechanisms for the phase-transition behavior of PAU itself and for the interactions between PAU and nucleic acid bases in water.

## CONCLUSION

PAU was obtained by ordinary radical polymerization. In distilled water, it formed a precipitate and showed dissolution at lower and higher temperatures, respectively. The solubility change occurred drastically at its UCST. Additionally, the transition temperature was shifted to lower temperatures with increasing Ado concentrations, while Guo addition did not result in such a shift as Ado addition induced. The phase transition of PAU itself in water might be due to formation/dissociation of the hydrogen-bonded polymer complexes between uracil moieties of the polymers. Ado, which has a complementary adenine to uracil, interacted specifically with uracil moieties and functioned to prevent the self-association between the polymer chains, causing the lower UCST. Addition of Poly(A) enhanced the phase transition changes as compared with that of Ado. The interpolymer complexes between PAU and Poly(A) were believed to result in larger changes of the transition behavior by its polymer effect.

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## REFERENCES

1. M. Heskins, and J. E. Guillet, *J. Macromol. Sci. Chem.*, **A2**, 1441 (1968).
2. R. A. Horne, J. P. Almeida, A. F. Day, and N-T. Yu, *J. Colloid Interface Sci.*, **35**, 77 (1971)
3. M. Akashi, S. Nakano, and A. Kishida, *J. Polym. Sci., Part A: Polym. Chem.*, **34**, 301 (1996)
4. a) A. S. Hoffman, A. Afrassiabi, and L. C. Dong, *J. Contr. Rel.* **4**, 213 (1986). b) Y. H. Bae, T. Okano, R. Hsu, and S. W. Kim, *Makromol. Chem., Rapid Commun.*, **8**, 481 (1987).
5. H. Kanazawa, K. Yamamoto, Y. Matsushima, N. Takai, A. Kikuchi, Y. Sakurai, and T. Okano, *Anal. Chem.*, **68**, 100 (1996).
6. T. Okano, N. Yamada, H. Sakai, and Y. Sakurai, *J. Biomed. Mater. Res.*, **27**, 1243 (1993).
7. a) T. Aoki, Y. Nagao, K. Sanui, N. Ogata, A. Kikuchi, Y. Sakurai, K. Kataoka, and T. Okano, *Polym. J.*, **28**, 371 (1996). b) T. Aoki, T. Nishimura, K. Sanui, and N. Ogata, *React. & Functional Polymers*, **37**, 299 (1998). c) M. Watanabe, T. Akayoshi, Y. Tabata, and D. Nakayama, *J. Am. Chem. Soc.*, **120**, 5577 (1998).
8. O. V. Klenina and E. G. Fain, *Polym. Sci. U.S.S.R.*, **23**, 1439 (1981).
9. a) D. J. Eustace, D. B. Siano, and E. N. Drake, *J. Applied Polym. Sci.*, **35**, 707 (1988). b) K. Abe, M. Koide, and E. Tsuchida, *Macromolecules*, **10**, 1259 (1977).
10. H. Katono, A. Maruyama, K. Sanui, N. Ogata, T. Okano, and Y. Sakurai, *J. Control. Rel.*, **16**, 215 (1991).
11. H. Katono, K. Sanui, N. Ogata, T. Okano, and Y. Sakurai, *Polym. J.*, **23**, 1179 (1991).
12. H. Sasase, T. Aoki, H. Katono, K. Sanui, N. Ogata, T. Okano, and Y. Sakurai, *Makromol. Chem., Rapid Commun.*, **13**, 577 (1992).
13. T. Aoki, M. Kawashima, H. Katono, K. Sanui, N. Ogata, T. Okano, and Y. Sakurai, *Macromolecules*, **27**, 947 (1994).
14. T. Aoki, K. Nakamura, K. Sanui, N. Ogata, A. Kikuchi, T. Okano, and Y. Sakurai, *Proceed. Intern. Symp. Control. Rel. Bioact. Mater.*, **23**, 767 (1996).
15. a) H. Kaye, *J. Polym. Sci., Polym. Lett.*, **7**, 1 (1969). b) J. Pitha, P. M. Pitha, and E. Stuart, *Biochemistry*, **10**, 4595 (1971). c) M. Akashi, Y. Inaki, and K. Takemoto, *Makromol. Chem.*, **178**, 353 (1977). d) C. G. Overberger and W. B. Ferng, *J. Polym. Sci., Polym. Chem.*, **24**, 3335 (1986). e) N. M. Brahme and W. T. Smith, *J. Polym. Sci., Polym. Chem. Edn.*, **22**, 813 (1984).
16. J. C. Thrierr, M. Dourlent, and M. Leng, *J. Mol. Biol.*, **58**, 815 (1971).
17. J. Rebek Jr., *Angew. Chem. Int. Ed. Engl.*, **19**, 245 (1990).
18. a) H. Kitano and H. Ringsdorf, *Bull. Chem. Soc. Jpn.*, **58**, 2826 (1985). b) K. Kurihara, K. Ohto, Y. Honda, and T. Kunitake, *J. Am. Chem. Soc.*, **113**, 5077 (1991).
19. a) V. M. Rotello, E. A. Viani, G. Deslongchamps, B. A. Murry, and J. Rebek Jr., *J. Am. Chem. Soc.*, **115**, 797 (1993). b) Y. Kato, M. M. Conn, and J. Rebek Jr., *Proc. Natl. Acad. Sci., USA*, **92**, 1208 (1995). c) H. Asanuma, T. Ban, S. Gotoh, T. Hishiya, and M. Komiyama, *Macromolecules*, **31**, 371 (1998).