

Biocomplementary Interaction Behavior in DNA-Like and RNA-Like Polymers

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ABSTRACT: A series of nucleobased polymers and copolymers were synthesized through atom transfer radical polymerization (ATRP). Biocomplementary DNA- and RNA-like supramolecular complexes are formed in dilute DMSO solution through nucleobase recognition. ^1H NMR titration studies of these complexes in CDCl_3 indicated that thymine-adenine (T-A) and uracil-adenine (U-A) complexes form rapidly on the NMR time scale with high association constants (up to 534 and 671 M^{-1} , respectively) and result in significant T_g increase. WAXD and differential scanning calorimetry analyzes in the bulk state indicate the presence of highly physical cross-linked structures and provide further details into the nature of the self-assembly of these systems. Furthermore, this study is of discussion on the difference in the hydrogen bond strength between T-A and U-A base pairs within polymer systems, indicating that the strength of hydrogen bonds in RNA U-A pairs is stronger than that in DNA T-A base pairs. © 2009 Wiley Periodicals, Inc. *J Polym Sci Part A: Polym Chem* 47: 6388–6395, 2009

Keywords: adenine; biomaterials blending biopolymers; self-assemble; supramolecular complexes; supramolecular structures; thymine; uracil

INTRODUCTION

Thymine (T) and uracil (U) are both pyrimidine nucleobases and able to form stable hydrogen-bonded base pairs with adenine (A) in DNA and RNA, respectively. Remarkably structural difference between T and U (methyl group) results in significant difference in their physical and chemical properties. Although it is well accepted that hydrogen bonds are essential to the formation of the structure of nucleic acids, the elucidation of these differences existed between DNA and RNA hydrogen bonds has not been clearly established. Only a limited number of reports have appeared

so far to compare the hydrogen bonding interactions between DNA (T-A) and RNA (U-A) base pairs.^{1–7} Recently, Wang and coworkers^{8,9} compared $^{2\text{h}}\Delta^{13}\text{C}_{\text{DNA}}$ and $^{2\text{h}}\Delta^{13}\text{C}_{\text{RNA}}$ and indicated that the RNA (U-A) hydrogen bond is about 3% stronger than the DNA (A-T) hydrogen bond.

Self-assembled polymers are usually formed through covalent linkages in traditional polymer chemistry, including bonds connecting monomer units and attaching functional groups to the polymer backbone. Recently, highly directional and sufficiently strong noncovalent host-guest pairs have substituted for these conventional covalent linkages.^{10–12} Nevertheless, controlling the secondary (and higher) structures of synthetic polymers remains a challenging task.¹³ Syntheses of well-defined polymer architectures exhibiting specific structures are rather difficult comparing with the level of precise structural control in biomaterials at molecular level. Therefore, during past few years,

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several reports by introducing nucleobases functionalization to main- and side-chain polymers mediated by biocomplementary hydrogen bonding have been explored.^{14–26} This concept stemmed from the direct observation of biomaterials where the complexity and molecular functionality can form noncovalent bonds to construct materials exhibiting specific structures such as proteins.²⁷

Recently, we have reported the biocomplementary interactions of a DNA-like side-chain homopolymer with alkylated nucleobases mediated by T-A base pair.²⁸ In this case, we studied polymer blend systems featuring reversible cross-linking networks mediated by nucleobase hydrogen bonding. To further understand the physical cross-linking density of nucleobase-functionalized polymer and copolymer between T-A and U-A base pairs, the differential scanning calorimetry (DSC) was employed to determine the glass transition temperatures of the complexes in these nucleobase-functionalized polymer blends. This study investigates on the difference in the hydrogen bond strength between T-A and U-A base pairs within polymer systems that can provide general fundamental properties of these systems.

EXPERIMENTAL SECTION

Materials

Vinylbenzyl chloride was purchased from Acros Organics (Dresden, Germany) and distilled before use. Thymine, uracil, adenine, and ethyl 2-bromobutyrate were obtained from Aldrich. 1-Bromohexadecane was purchased from Fluka (Buchs, Switzerland) and used as received. NMP and DMF were purchased from TEDIA and distilled over CaH₂ before use. All other chemicals were used as received without purification. 1-hexadecylthymine (T-C16), 1-hexadecyluracil (U-C16), 9-hexadecyladenine (A-C16) were synthesized through alkylated nucleobases according to procedures described previously.^{28–30}

Nucleobase-functioned monomers [1-(4-vinylbenzyl)thymine (VBT), 1-(4-vinylbenzyl)uracil (VBU), and 9-(4-vinylbenzyl)adenine (VBA)] were synthesized from thymine and vinylbenzyl chloride according previous studies.^{28–30}

Atom Transfer Radical Polymerization (ATRP) of Nucleobase-Functioned Polymers and Copolymers

All these polymers were synthesized according to our previous study.²⁸ The polymerization was per-

formed under argon atmospheres with samples withdrawn at various time intervals. After heating at 180 °C for 30 h, the reaction mixture was passed through an aluminum oxide column to remove the Cu(II) catalyst. The final polymer product was purified by precipitation into methanol. The molecular weight, conversion, and polydispersity index (PDI, M_w/M_n) of these polymers were presented in the Scheme 1 and Table 1.

Supramolecular Complexes

Desired amounts of PVBT (or PVBU) and PVBA (50:50 molar ratios) were dissolved in DMSO, stirring continuously for 24 h at room temperature. The sample was cast onto a Teflon dish and dried under vacuum at 70 °C for 24 h.

Characterizations

Fourier transform infrared (FTIR) Spectroscopy was recorded using a Nicolet Avatar 320 FTIR spectrometer; 32 scans were collected at a spectral resolution of 1 cm⁻¹. The conventional KBr disk method was employed: the sample was dissolved in DMF, then cast onto a KBr disk, and dried under vacuum at 70 °C for 24 h.

Nuclear Magnetic Resonance Spectroscopy

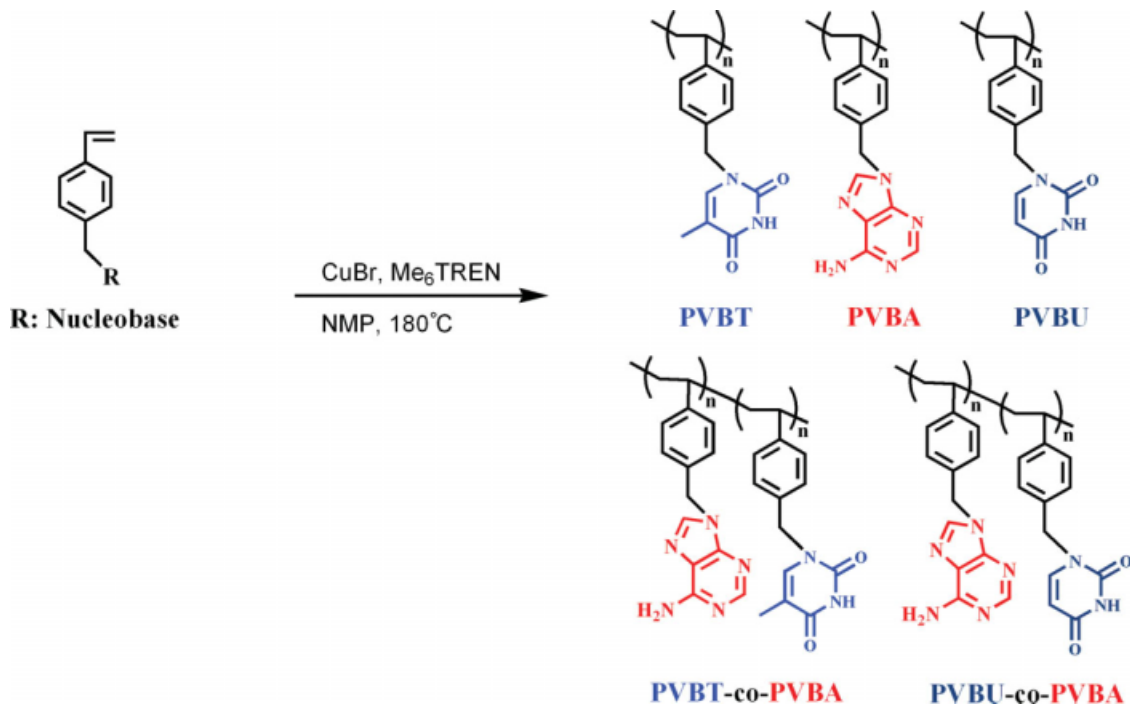
¹H NMR spectra were recorded on a Varian Inova 500 MHz Spectrometer equipped with a 9.395 T Bruker magnet and operated at 500 MHz. Samples (ca. 5 mg for ¹H NMR) in deuterated solvent were analyzed at room temperature.

Gel Permeation Chromatography

The weight-average molecular weight (M_w), number-average molecular weight (M_n), and PDI (M_w/M_n) were measured using a Waters 410 gel permeation chromatography system equipped with a refractive index detector and three Ultrastaygel columns (100, 500, and 1000 Å) connected in series. DMF was the eluent and the flow rate was 0.6 mL/min. The system was calibrated using polystyrene standards.

Differential Scanning Calorimetry

DSC was performed using a DuPont 910 DSC-9000 controller operated under an atmosphere of dry N₂. Samples weighed (ca. 5–10 mg) and sealed in an aluminum pan were cooled to room



Scheme 1. Synthetic procedures of nucleobase-functionalized polymers. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

temperature, and then scanned from 30 to 250 °C at a scan rate of 20 °C/min. The glass transition temperature was taken as the midpoint of the heat capacity transition between the upper and lower points of the deviation from the extrapolated glass and liquid lines.

Wide-Angle X-Ray Diffraction

WAXD spectra of powders were obtained using a Rigaku D/max-2500 X-ray Diffractometer. The radiation source was Ni-filtered Cu K α radiation at a wavelength of 0.154 nm. The voltage and current were set at 30 kV and 20 Ma, respectively. The sample was mounted on a circular sample holder; the data were collected using a proportional counter detector over the 2θ range from 3 to 60° scanned at a rate of 5° min⁻¹. Bragg's law ($\lambda = 2d \sin \theta$) was used to compute the d -spacing corresponding to the complementary behavior.

Molecular Dynamic Simulation

The modeling work presented here was performed using ChemOffice 2008. The structures of VBT/VBA complex (as a specific interaction between complementary monomers) were initially drawn by ChemDraw. Each 2D structure was converted into 3D structure by Chem3D, followed by molecu-

lar mechanics minimization using MM2 force field where these MM2 parameters were obtained from the "MM2 (1991) Parameter Set" provided by Allinger (University of Georgia) and implemented in Chem3D. Molecular simulation was carried out to simulate the d -spacing of VBT/VBA complex.

RESULTS AND DISCUSSION

ATRP of Nucleobase-Functionalized Homopolymers and Copolymers

The nucleobase-functionalized polymers were synthesized through atom transfer radical

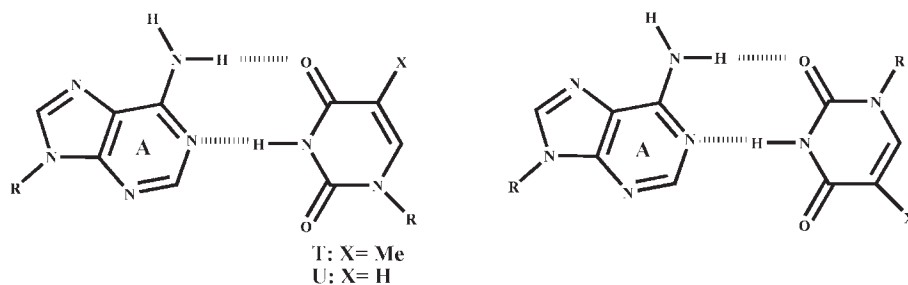
Table 1. Summary of Molecular Weights (M_w), Polydispersity Index (PDI), and Conversions for the Nucleobase-Functionalized Polymers

| Polymers | M_w | PDI | Conv. (%) ^b |
|---------------------------|-------|------|------------------------|
| PVBT | 7,527 | 1.38 | 91 |
| PVBA | 3,503 | 1.17 | 75 |
| PVBU | 6,693 | 1.39 | 88 |
| PVBT-co-PVBA ^a | 4,246 | 1.24 | 84 |
| PVBU-co-PVBA ^a | 4,730 | 1.32 | 81 |

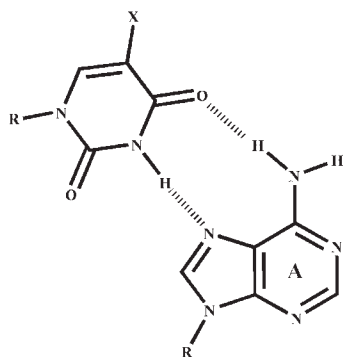
^a Repeating unit of ¹H NMR analysis showed a 1:1 ratio.

^b Conversion, as determined through a kinetic experiments.

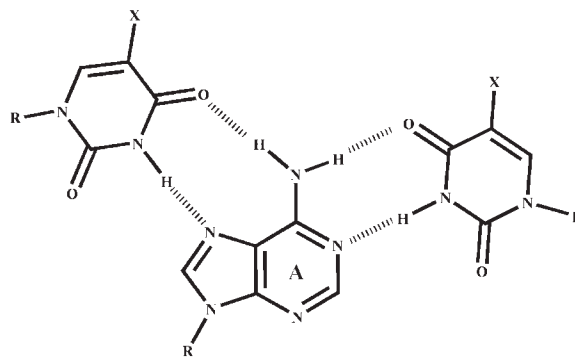
(A) Watson-Crick Base-pair



(B) Hoogsteen Base-pair



(C) Base-triples

**Scheme 2.** Some different hydrogen-bonding modes of T-A and U-A interactions.

polymerization (ATRP) (Scheme 1) where the controls of the molecular weight and polydispersity index (PDI) were described previously.^{28–30} However, both T-A and U-A blends possess two major types of hydrogen bonding interactions through the Watson–Crick and the Hoogsteen sites which

can form aggregates or base-triples with an appropriate partner and molar ratio of 2:1 (Scheme 2).¹⁶ Thus, the ratio of the molecular weight between Poly[1-(4-vinylbenzyl)thymine] (PVBT) or Poly[1-(4-vinylbenzyl)uracil] (PVBU) to Poly[9-(4-vinylbenzyl)adenine] (PVBA) was

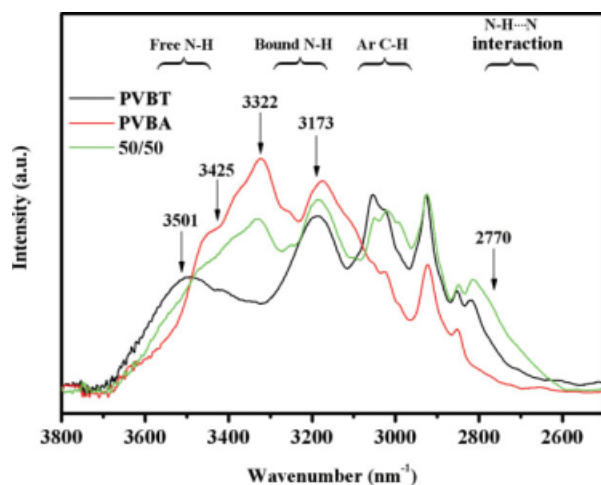


Figure 1. FTIR spectra recorded at room temperature in the range 2500–3800 cm^{-1} for 50/50 PVBT/PVBA in the bulk state. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

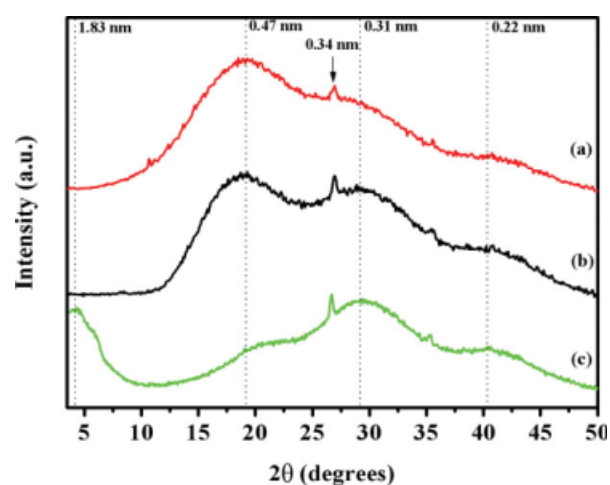
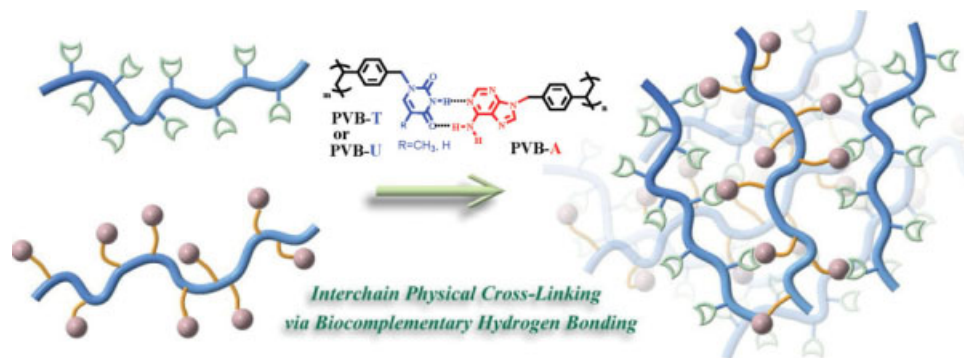


Figure 2. WXR D data for DNA-like polymer complexes: (a) 50/50 PVBT/PVBA, (b) PVBT-co-PVBA, and (c) PVBT. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]



Scheme 3. Graphical representation of the physical cross-linked structure formed from PVB-T/PVBA or PVB-U/PVBA complexes in the bulk state.

designed to be 2:1. The polymerizations were controlled at 180 °C, and the resulting polymers exhibited reasonable molecular weights, narrow PDIs (1.17–1.38), and high conversions (75–91%) (See Supporting Information Table 1). Furthermore, these two monomers possess equal stoichiometric ratio toward both propagating species and ^1H NMR results indicated that both PVB-T-co-PVBA and PVB-U-co-PVBA copolymers possess random repeat units of about 1:1 ratio after 30 h with conversions $\geq 80\%$. These copolymers synthesized through ATRP resulted in close reactivity for each monomer. Although the required (co)polymers have been successfully synthesized via aforementioned method, the control of suitable reaction temperature during the polymerization and the interactions between the monomer and the ligand catalyst species must play a critical role. Great efforts have been in progress in our laboratory to elucidate further insight to the synthetic mechanism.²⁸

Molecular Recognition in Nucleobase Poly[1-(4-vinylbenzyl)thymine]/Poly[9-(4-vinylbenzyl)adenine] (PVB-T/PVBA) Complexes

Biological macromolecules (e.g., nucleic acids) possess ability to selectively binding from molecular recognition or the association of complementary partners via biocomplementary hydrogen bonds.³¹ To investigate the cross-linked structures through bottom-up assembly of PVB-T/PVBA complexes, both FTIR spectroscopy and wide-angle X-ray diffraction (WXR) measurements were employed.

Figure 1 illustrates the FTIR spectra in the N–H stretching region of the PVB-T/PVBA complexes. The characteristic peak at 3501 cm^{-1} was attributed to the free amide NH group of PVB-T

and those peaks at 3173 cm^{-1} and 3173 cm^{-1} involved in T–T and A–A interactions, respectively.³² The presence of the band at 3501 cm^{-1} indicates that fraction of these thymine side groups of the PVB-T polymer were not involved in T–T interactions.²⁸ The intensity of the free amide N–H stretching vibration at 3501 cm^{-1} decreased upon increasing the amount of added PVBA, indicating that PVB-T associated strongly with PVBA and that A–T interactions were more favorable than either T–T or A–A interactions. FTIR spectra revealed the presence of bands at 3322 and 3425 cm^{-1} corresponded to hydrogen-bonded amide N–H groups at 50/50 PVB-T/PVBA complex, indicating that A–T interaction are frequencies generally observed for medium strength hydrogen-bonded N–H.³³ Furthermore, an absorption peak at about 2600–2800 cm^{-1} is observed, indicative for N–H \cdots N interassociation,³⁴ implying that PVB-T and PVBA formed instant hydrogen-bonded complexes in the bulk state.

Further investigation on the self-assembly of these complexes was carried out using WXR measurements. The WAXD pattern of the pure PVB-T (Fig. 2) displays several amorphous halos, centered at 2θ of 4.34° ($d = 1.83$ nm), 19.0° ($d = 0.41$ nm), 29.3° ($d = 0.37$ nm), and 40.49° ($d = 0.22$ nm) which correspond to distances between the main chains and phenyl rings.^{35,36} By comparing these observed amorphous halos with the interchain distances between PVB-T and poly(styrene) (PS),³⁶ the introduction of T–T interaction expands the interchain spacing and reduces the intramolecular distance between phenyl rings. The intermolecular distance is increased because of the larger size thymine units attached to the phenyl rings, while the intramolecular distance is reduced as a result of the repulsion caused by T–T

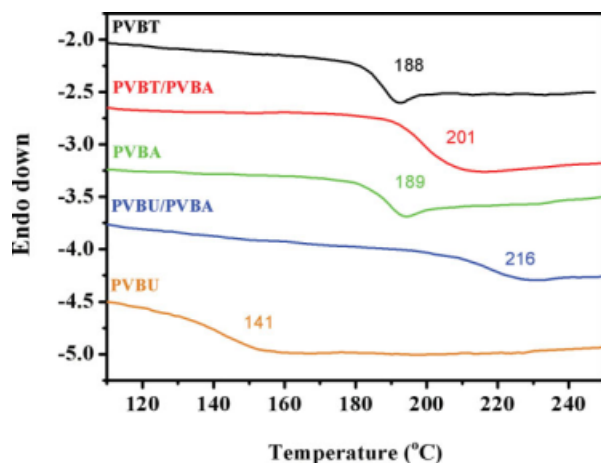


Figure 3. DSC data for DNA-like and RNA-like polymer complexes. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

interactions.^{37,38} In addition, the 2θ peak at of 26.8° ($d = 0.34$ nm) is consistent with the presence of π - π stacking interactions between T-T layers which generally occurs in stabilized sheets.^{19,39}

These amorphous halos of both 50/50 PVBT/PVBA complex and PVBT-co-PVBA copolymer are obviously shifted to lower values of 2θ as compared with PVBT and PVBA, indicating the formation of unspecified structure with relatively larger size scale. In addition, the disappearance of the interchain spacing (1.83 nm) reflects the occurrence of the T-A interaction. The simulated intermolecular VBT-chain-to-VBA-chain distance based on a molecular dynamic simulation (by ChemOffice 2008) shows that the d -spacing is 2.6 nm. On the other hand, these results were observed in the WXR pattern of the complex PVBT/PVBA and PVBT-co-PVBA. Based on the previous results, these nucleobase units in the complexes form physical cross-linked structure via biocomplementary hydrogen bonds (Scheme 3). In addition, the change in d -spacing and size of aggregate of U-A base pair within PVBU/PVBA blend and PVBU-co-PVBA copolymer are similar to the system comprising PVBT and PVBA.

Comparison of Hydrogen Bonds Between the T-A and U-A Base Pairs

Self-assembly in nucleobase systems is formed through biocomplementary hydrogen bonds. If these hydrogen bonds are strong enough, macro-phase separation between two incompatible poly-

mers can be prevented.⁴⁰ To further understand the physical cross-linking density of nucleobase-functionalized polymer and copolymer comprising T-A and U-A base pairs, ^1H NMR spectroscopy and DSC were employed to determine their association constants (K_a) and related glass transition temperatures. Through ^1H NMR spectroscopic titration, the equilibrium constant (K_a) for the complexes of PVBT/PVBA and PVBU/PVBA can be obtained.⁴¹ However, all these polymers were insoluble in nonpolar organic solvents, such as CDCl_3 (often used in ^1H NMR titration experiments). In addition, the K_a obtained using polar solvent such as d_6 -DMSO was below the determination limit of the NMR method, indicative of almost no formation of hydrogen bonding between T-A and U-A base pairs within polymer systems because the K_a estimation using d_6 -DMSO would be deviated as compared with nonpolar solvent from the strong polarity of d_6 -DMSO.^{20,42} Therefore, 9-hexadecyladenine (A-C16)²⁸ was chosen as a model compound for PVBA. The A-C16 was blended with 1-hexadecylthymine (T-C16) and 1-hexadecyluracil (U-C16), model compounds for PVBT and PVBU using CDCl_3 as solvent at 25°C to obtain the values of K_a for the T-A and U-A complexes. The chemical shifts of these amide protons of thymine and uracil in the complexes of T-C16/A-C16 and U-C16/T-C16 were used to obtain values of K_a of 534 and 671 M^{-1} from Benesi-Hildebrand plots, respectively (see Supporting Information). The ^1H NMR titration experiments reveal that the hydrogen bonding strength of the

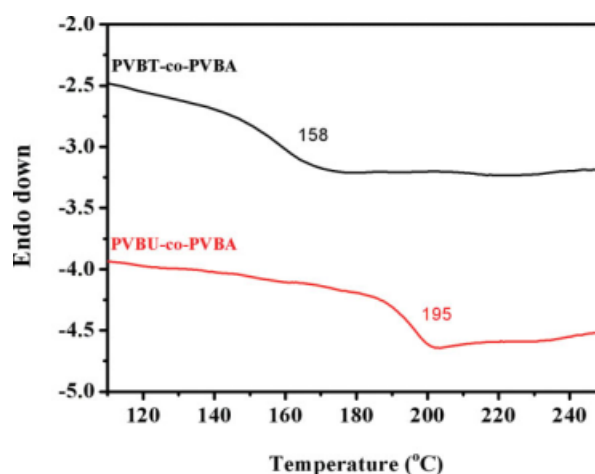


Figure 4. DSC data of PVBT-co-PVBA and PVBU-co-PVBA. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

U-A base pair of RNA is stronger than that of T-A base pair of DNA as previously described,^{3,8,9} indirect representing the relatively stronger strength of hydrogen bonding interaction within PVBU/PVBT complex.

Figure 3 displays DSC traces of PVBT/PVBA and PVBU/PVBA complexes, indicating that the glass transition temperatures (T_g) of pure PVBT and PVBU are about 188 and 141 °C, respectively. The T_g of the 50/50 PVBU/PVBA blend at 216 °C is higher than T_g of the 50/50 PVBT/PVBA blend at 201 °C, implying that the highly complementary hydrogen bonding strength between PVBU and PVBA is greater than that between PVBT and PVBA. This observation is consistent with the difference in K_a described earlier. In addition, the T_g behavior of copolymers also show similar trend as the blend system, the T_g of PVBU-co-PVBA copolymer (195 °C) is higher than T_g of PVBT-co-PVBA (158 °C) (Fig. 4).

CONCLUSIONS

In summary, these nucleobase-functionalized polymers were successfully synthesized through living controlled ATRP at 180 °C. In the bulk state, highly cross-linked structures through biocomplementary hydrogen bonding and result in substantial increase in T_g relative to their original components. Furthermore, the hydrogen bonding strength of the styrenic polymer (and copolymer) in RNA (U-A) complex is stronger than that in DNA (T-A) base pair. This study is the first discussion attempting to construct nucleobase pairs in polymer matrix as a means of manipulating the physical propensities of polymers. The study of these systems on elucidating the factors affecting supramolecular polymer control and the development in materials with practical application are continuing.

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