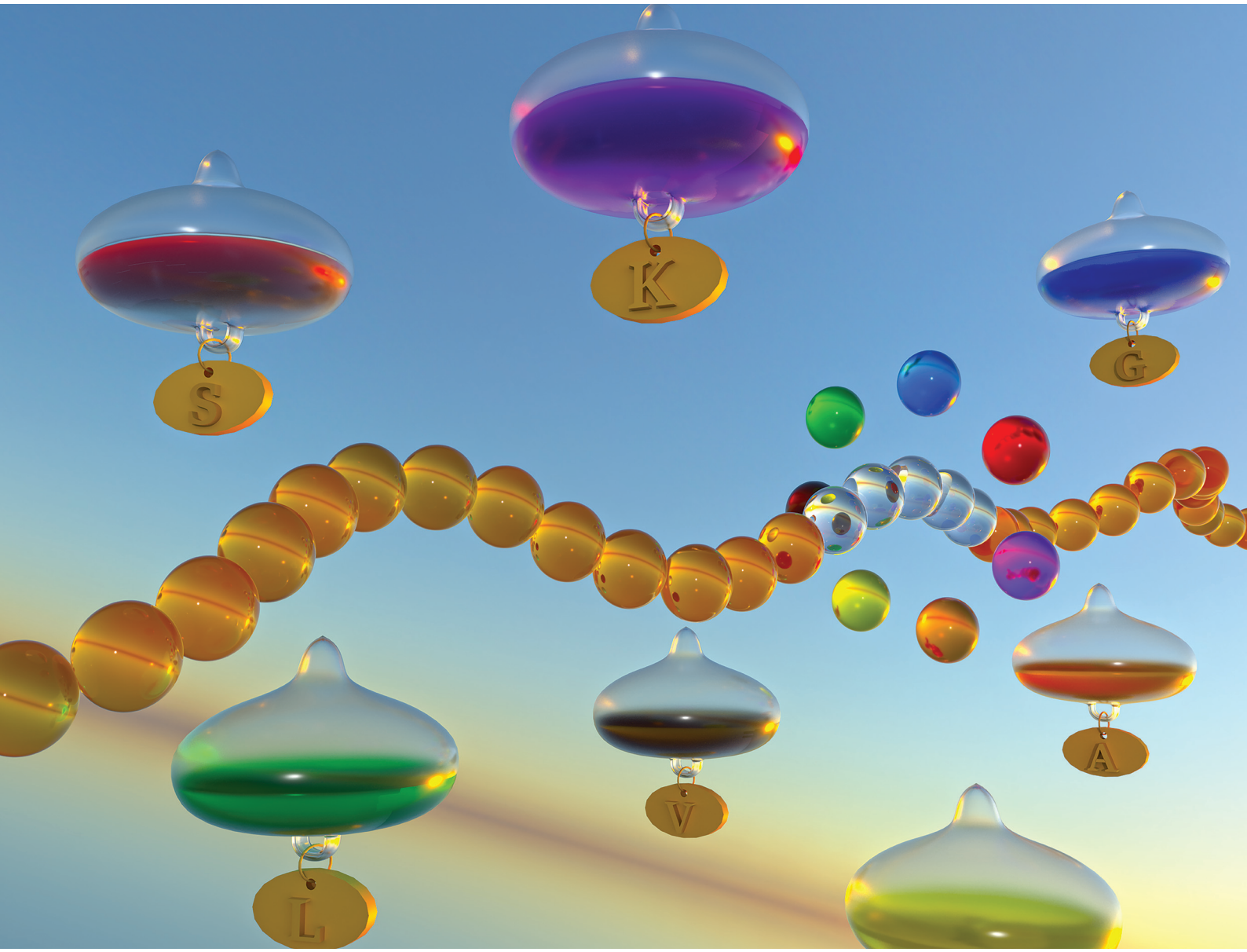
Volume 14
Number 24
28 June 2023
Pages 2851-2942

Polymer Chemistry

rsc.li/polymers



ISSN 1759-9962

ROYAL SOCIETY OF CHEMISTRY

PAPER

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Cite this: *Polym. Chem.*, 2023, **14**, 2857

Synthesis of amino acid-derived vinyl polymers with precisely controlled hydropathy and their thermoresponsive behavior in water†

Atsushi Sawamoto, Shin-nosuke Nishimura, Nobuyuki Higashi and Tomoyuki Koga *

Controlling the thermoresponsive behavior of bio-based synthetic polymers is critical for the development of smart functional materials with various potential applications in biomedical and nanotechnological fields. Although the effects of polymer architectures, such as molecular weight and hydrophilic/hydrophobic balance, are being clarified, the rational design principles are not yet fully understood, particularly in vinyl-polymer systems, because of their structural diversity and complexity. Herein, the synthesis of amino acid-derived vinyl polymers with systematically different hydropathies and their thermal responses in water are reported. Thirteen distinct block and statistical random co/homopolymers are precisely synthesized *via* an ultra-rapid reversible addition–fragmentation chain transfer polymerization of *N*-acryloyl alanine (A) methyl ester (main component) and various *N*-acryloyl amino acid (X) methyl esters (X = G, S, K, L, F, V) (guest monomer). All polymers possess exactly the same total chain length (DP = 35) and composition (A/X = 30/5), and water-soluble polymers exhibit lower critical solution temperature behavior. Interestingly, a clear correlation between the hydropathy indices (HI) of amino acids X and the transition temperature (T_t) is observed; namely, T_t systematically reflects the hydrophobicity of the X units. Furthermore, the specific monomer sequence (*i.e.*, the distribution of X units in the polymer chain) affects the thermal response, particularly when the guest amino acids have extremely high or low HI. These findings are important for the precise design of chemically diverse and complex stimuli-responsive bio-based polymers.

Received 30th March 2023,
Accepted 2nd May 2023

DOI: 10.1039/d3py00353a

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Introduction

Thermoresponsive polymers are fascinating soft materials with great potential in biomedical and nanotechnological fields, including drug carriers, sensors/separations, tissue engineering, actuators, and self-assembling materials.[1–7] Thus far, numerous vinyl polymers exhibit lower critical solution temperature (LCST) or upper critical solution temperature (UCST) behavior; consequently, several applications of these polymers have been developed based on their temperature-induced reversible changes in conformation and water solubility. Focusing on LCST-type phase behavior, poly(*N*-isopropyl acrylamide) (PNIPAM)[8,9] and poly(oligo(ethylene glycol)methacrylate)s (POEGMA)[10,11] are representative vinyl polymers and have been widely used in most previous biomedical studies. The precise and flexible control of the transition temperature while maintaining the high biocompatibility of LCST-type polymers is a crucial factor for their application in the field of biomaterials.

The transition temperature depends on the polymer architecture, including hydrophilicity/hydrophobicity,[12–14] molecular weight,[15,16] shape,[17,18] and monomer sequence.[19–21] Indeed, combining different vinyl monomers affords polymers with various hydrophobic/hydrophilic balances, thus leading to versatile thermal responses. Although numerous studies have been conducted on LCST polymers, the rational design principles of such LCST-type vinyl polymers, including the individual effects of each of the structural factors, are not yet thoroughly understood. This is primarily owing to the difficulty in constructing a library of vinyl monomers with similar polymerizability suitable for copolymerization but systematically different hydrophobicities. In addition, such vinyl monomer libraries must be applicable to reversible deactivation radical polymerization (RDRP) techniques[22–25] that can control the molecular weight, narrow dispersity, and monomer sequence.

Amino acid-based polymers[26–41] are good candidates for this purpose and for applications in various fields, such as

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† Electronic supplementary information (ESI) available: Experimental procedures for monomer synthesis and supplemental figures and tables. See DOI: https://doi.org/10.1039/d3py00353a

stimuli-responsive materials,[27–32] cell scaffolds,[33,34] bio-related,[35,36] and chiral materials.[37] Naturally occurring amino acids (20 types) are the building units of proteins and demonstrate diverse physical and chemical properties based on the structures of side chains, including hydrophobicity/hydrophilicity, ionicity, and hydrogen bond ability. Thus, the introduction of amino acid structures into synthetic vinyl polymers as side chains provides more flexibility for versatile polymer design with excellent biocompatibility, bio-functionality, and stimuli-responsive characteristics. A previous study reported the synthesis of various poly(*N*-acryloyl amino acid)s (homopolymers/random or block copolymers) and their thermoresponsive behaviors.[20,21,27–31,40,41] By varying the type of amino acids and their terminal structures, as well as their molecular architectures, the thermoresponsiveness of the resultant polymers could be tuned successfully (such as LCST–UCST, and transition temperature). Most importantly, the hydrophobicity of amino acids varies widely and systematically and they are well understood as hydropathy indices (HI).[42] Therefore, amino acid-derived vinyl monomers can be useful in accurately understanding the effect of the hydrophilic/hydrophobic balance of vinyl polymers on their thermoresponsiveness, and offer a practical library for constructing a new class of stimuli-responsive synthetic polymers. In addition, the applicability of most *N*-acryloyl amino acids to various RDRP techniques is favorable for versatile and complex polymer designs.

In this study, seven distinct amino-acid-derived vinyl monomers with different hydropathies were synthesized to construct a monomer library for LCST-type vinyl polymers (Fig. 1). From this library, various sequence-controlled block copolymers and random copolymers with the same chain length and monomer composition (A/X) but systematically different hydrophobicity were prepared to precisely determine the parameters affecting their LCST behavior in water. The construction of amino acid-derived[38,39] and amino acid-mimicked[43] vinyl monomer libraries, and the development of a robust and controlled polymerization technique for these monomers is challenging and valuable not only for the design of functional bio-based stimuli-responsive smart polymers but also as an artificial protein model for single-chain folding.[44]

Experimental

Materials

Glycine methyl ester hydrochloride, L-alanine methyl ester hydrochloride, L-serine methyl ester hydrochloride, L-leucine methyl ester hydrochloride, L-valine methyl ester hydrochloride, L-phenylalanine methyl ester hydrochloride, and L-lysine (Z) methyl ester hydrochloride were purchased from Watanabe Chemical Co. Ltd. Acryloyl chloride, HCl (aqueous), triethylamine (TEA), *N*,*N*-diisopropylethylamine (DIPEA), anhydrous magnesium sulfate ($MgSO_4$), sodium sulfate (Na_2SO_4), dimethyl sulfoxide (DMSO), DMSO-d_6, *N*,*N*-dimethylformamide (DMF), methanol (MeOH), ethanol (EtOH), diethyl ether, hexane, dichloromethane (DCM), ethyl acetate, and tetrahydrofuran (THF) were purchased from Nacalai Tesque, Inc. Further, 2,2′-azobis[2-(2-imidazolin-2-yl)propane]dihydrochloride (VA-044), 2-{[(2-carboxyethyl)sulfanylthiocarbonyl]sulfanyl}propanoic acid, 25% hydrogen bromide-acetic acid solution, tetramethylsilane (TMS), and Wakogel® FC-40 were

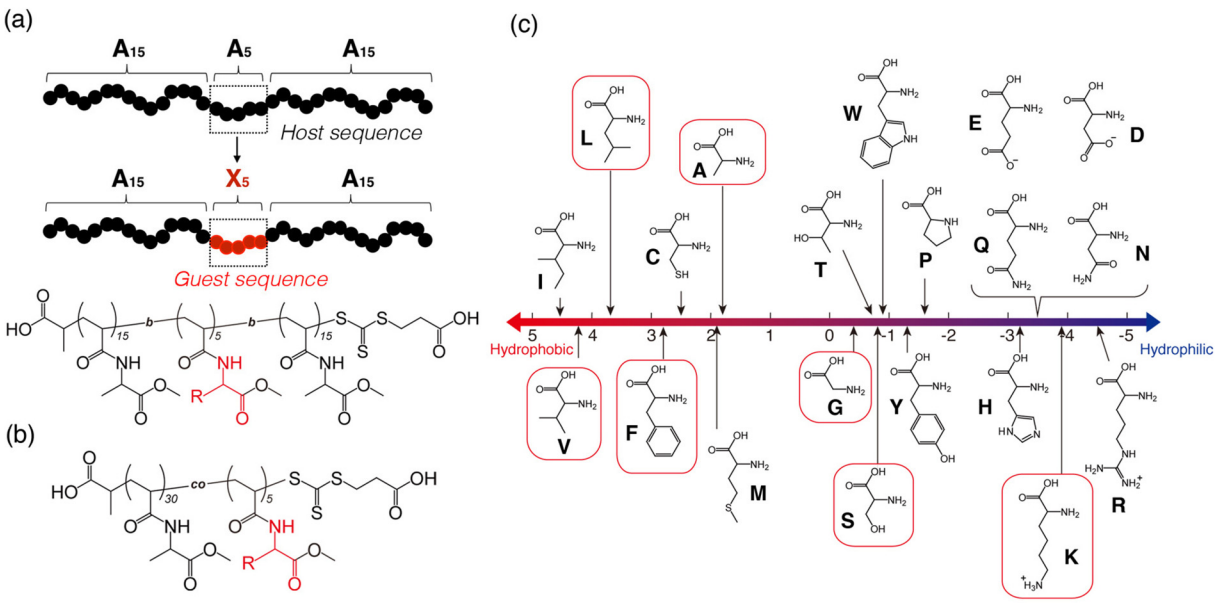

Fig. 1 Chemical structures of thermoresponsive amino acid-derived vinyl polymers (a) A_{15}-*b*-X_5-*b*-A_{15} and (b) A_{30}-*co*-X_5, based on host–guest strategy. (c) Hydropathy indices of constituent amino acids for vinyl monomer library. The amino acids indicated within the red square are the seven components used in this study.

purchased from Wako Pure Chemical Co. Ltd. 2,5-Dihydroxybenzoic acid (DHBA) was purchased from Sigma-Aldrich.

Measurements

^{1}H-NMR spectroscopy was performed on a JEOL JNM-ECA500 (JEOL Resonance) spectrometer (500 MHz) using DMSO-d_6 as the solvent. The number-average molecular weight (M_n (g mol^{-1})) and polydispersity index ($Đ = M_w/M_n$) of the synthesized polymers were evaluated *via* size exclusion chromatography (SEC) using a JASCO LC-net II/ADC (JASCO Ltd) equipped with a refractive index (RI) detector (column, GF-710F). The measurements were conducted in THF at 40 °C (flow rate: 1 mL min^{-1}). Low-dispersity poly(methyl methacrylate)s (GL Sciences Inc., M_w = 2810, 5000, 10 290, 27 600, 60 150, 138 600, and 298 900) were used as calibration standards. Matrix-assisted laser desorption ionization-time-of-flight MS (MALDI-TOF MS) analyses were performed on an Autoflex speed (Bruker Daltonics) using DHBA as a matrix. The transmittance of the aqueous polymer solution (1 wt%) was measured at 650 nm using a V-650 spectrophotometer (JASCO Ltd) equipped with a water-cooled Peltier cell holder ETCS-761 (JASCO Ltd). The pH of the sample solution was adjusted to be 2.2 ± 0.1 with 0.01 M HCl aqueous solution. The transmittance of each polymer solution was measured continuously at a constant heating rate of 1 °C min^{-1} in a quartz cell with a path length of 1 cm. At least, three independent transmittance tests were performed. The transition temperature (T_t) was determined by differentiation of the curve of transmittance changes.

Syntheses of amino acid-derived vinyl monomers

N-Acryloyl alanine (A) methyl ester (NAAMe) and *N*-acryloyl glycine (G) methyl ester were prepared as described previously.[20] For other amino acid-derived vinyl monomers, *N*-acryloyl leucine (L) methyl ester, *N*-acryloyl phenylalanine (F) methyl ester, *N*-acryloyl valine (V) methyl ester, *N*-acryloyl serine (S) methyl ester, and *N*-acryloyl lysine (Z) (K(Z)) methyl ester, detailed synthetic procedures and structural characterizations are described in ESI and Fig. S1.†

General method for one-pot syntheses of block-type amino acid-derived vinyl polymers *via* ultra-rapid RAFT polymerization

For the synthesis of block-type amino-acid-derived vinyl polymers, A_{15}-*b*-X_5-*b*-A_{15}, an initiator (VA-044), Ala-based vinyl monomer (NAAMe), and chain transfer agent (CTA) (2-{[(2-carboxyethyl)sulfanylthiocarbonyl]sulfanyl}propanoic acid) were dissolved in a DMSO/water mixed solution, the feed compositions of which are presented in Tables S1–S6,† and placed in a glass tube sealed with a silicon W cap. Subsequently, freeze–vacuum–thaw deoxygenation *via* N_2 purging was performed by inserting a syringe needle through the seal. Reversible addition fragmentation chain transfer (RAFT) polymerizations were conducted at 80 °C for 15 min per each step. Subsequently, the deoxygenated mixed solution of the next amino acid-based monomer (X; X = Ala, Gly, Ser, Leu, Phe, Val, Lys (Z)) and initiator were added to the solution using a syringe through the silicon W cap, and the procedure was repeated for the final Ala block extension. For the stepwise addition of the monomer and initiator, solutions were prepared by dissolving the initiator in water and monomer in DMSO. After each step of polymerization, a small amount of solution (100 μL) was sampled by syringe for SEC and MALDI-TOF MS analysis. The obtained polymers were analyzed directly without any purification. All the target polymers were finally purified by dialysis using a Spectra/Por®7 Dialysis Membrane Pre-treated RC Tubing with a molecular weight cut-off (MWCO) of 1 kDa in ultra-pure water and lyophilized. The chemical structures were confirmed by ^{1}H-NMR spectroscopy (Fig. S2†).

Syntheses of amino acid-derived random copolymers

In a typical synthesis of statistical random copolymers, A_{30}-*co*-X_5, two types of amino acid-based monomers were dissolved in DMSO/water with an initiator and CTA, as described above. The feed compositions of the polymerizations are listed in Tables S7 and S8,† respectively. The resulting solutions were deoxygenated by N_2 purging. RAFT polymerization were performed at 80 °C for 15 min. The copolymers were purified and characterized in a manner similar to that described above.

Deprotection of Z groups in Lys residues

An appropriate amount of the polymer (A_{30}-*co*-K$(Z)_5$) was dissolved in 5% HBr/acetic acid solution and stirred at approximately 25 °C (room temperature) for 4 h. The resultant solution was concentrated *in vacuo* followed by reprecipitation with acetone to obtain pure deprotected A_{30}-*co*-K_5 as an orange-colored solid. Deprotection was confirmed by MALDI-TOF MS and ^{1}H-NMR analyses. Deprotection of the Z groups in A_{15}-*b*-K$(Z)_5$-*b*-A_{15} was performed in the same manner.

Results and discussion

Construction of an amino acid-derived vinyl monomer library

The aim of this study was to fabricate a bio-based vinyl polymer system with a tailor-made thermoresponsiveness. To construct such a polymer system, focus was placed on an amino-acid-derived monomer library. As described above, natural amino acids can be fascinating vinyl monomer units for this purpose because they exhibit diverse chemical/physical properties based on their side chain structures and can be easily converted into vinyl monomers using the same synthetic strategy, regardless of the type of amino acid. In fact, acrylamide monomers, *N*-acryloyl amino acid methyl esters (amino acids: alanine, glycine, and β-alanine), have been previously synthesized for LCST-type vinyl polymers by the simple condensation of commercially available amino acid methyl ester hydrochlorides with acryloyl chloride.[20,21,31] The homopolymers composed of these monomers exhibited LCST behavior in water over a wide temperature range (18 °C (A), 45 °C (βA),

and 72 °C (G)), respectively.[21,40] In addition, widely tunable LCST behaviors (T_t = 18–72 °C) were achieved by varying the copolymer composition of two different monomers (A and G).[31] Furthermore, these amino acid-based acrylamides were applicable to RDRP, including RAFT,[20,21] nitroxide-mediated polymerization (NMP),[44] and atom transfer radical polymerization (ATRP).[33]

The hydrophobic and hydrophilic properties of the side chains in natural amino acids are well understood by the hydropathy index (HI), which was proposed in 1982 by Kyte and Doolittle.[42] As shown in Fig. 1c, higher positive and negative values indicate more hydrophobic and hydrophilic amino acids, respectively. The most hydrophobic amino acids were isoleucine (HI, 4.5) and valine (HI, 4.2), whereas the most hydrophilic were arginine (HI, −4.5) and lysine (HI, −3.9). These values are particularly important in protein folding; essentially, hydrophilic groups are positioned predominantly on the protein surface, whereas the hydrophobic elements tend to be sequestered into the interior of the three-dimensional (3D) shape.[45] Thus, the wide range of hydrophilicity/hydrophobicity of the constituent amino acids contributes to the formation of unique 3D structures and protein functions. Further, this renders ease in systematically tuning the hydrophobicity of synthetic thermoresponsive polymers, as in the case of this study. Herein, seven amino acids were selected based on their HI values, Val (HI, 4.2), Leu (HI, 3.8), Phe (HI, 2.8), Ala (HI, 1.8), Gly (HI, −0.4), Ser (HI, −0.8), and Lys (HI, −3.9) (Fig. 1c), as vinyl monomer library. Note that serine- and lysine-derived vinyl monomers contain the reactive side groups of –OH and $–NH_2$, respectively, which can be used to further functionalize the polymers. Considering the possibility of side reactions during monomer synthesis and aminolysis of trithiocarbonate (essential for CTA) used in the subsequent RAFT polymerization, the amino group of the lysine unit was protected with a benzyloxycarbonyl (Z) group. The Z group can be easily deprotected after polymerization *via* treatment with an HBr/acetic acid solution. Protection of the serine side chain was not required during monomer synthesis or subsequent RAFT polymerization under the reaction conditions of this study. All amino acid-derived vinyl monomers were successfully obtained and identified by ^{1}H-NMR spectroscopy (Fig. S1†).

Molecular design and precise synthesis of amino acid-derived vinyl polymers *via* one-pot ultra-rapid RAFT polymerization

Recent remarkable progress in RDRP techniques has enabled the precise synthesis of vinyl polymers with well-controlled molecular weight, polydispersity, molecular shape, and monomer sequence. Ultra-rapid RAFT polymerization, which was developed by Perrier *et al.* for the synthesis of well-defined multiblock copolymers,[46,47] was employed in this study because of its applicability to acrylamide monomers and high functional group tolerance. Previously, we successfully prepared amino acid-derived acrylamide-type vinyl polymers with well-controlled primary structures, including molecular weights and monomer sequences, by one-pot ultra-rapid RAFT polymerization.[20,21]

Herein, to enable the investigation of a more versatile polymer design exhibiting tailor-made thermoresponsiveness, various amino acid-derived vinyl polymers with systematically different hydrophobicities were prepared using the seven monomers in the library. Seven distinct block copolymers, poly(*N*-acryloyl amino acid methyl ester)s, containing different amino acid units were designed. Generally, homopolymers composed of highly hydrophobic amino acid-based vinyl monomers are insoluble in water, thus rendering difficulty in elucidating the effects of differences in the hydropathy of amino acid units on LCST behavior. Therefore, the host–guest design concept of peptides and proteins,[48,49] whereby a single amino acid residue is replaced with another in a constant framework to manipulate structural and functional properties, was employed in this study. Guest sequences in the form of $(X)_5$ were introduced in the five central positions in the context host framework, A_{15}-*b*-$(X)_5$-*b*-A_{15} (guest amino acid X = A, G, S, L, F, V, K) (Fig. 1a). The host framework used as a standard, A_{35}, is water soluble and exhibits LCST behavior at approximately 18 °C.[20,31] The total chain length and monomer composition were fixed as DP = 35 and A/X = 30/5, respectively. In addition, statistical random copolymers with the same chain length and composition as the block type were synthesized to gain insight into the effect of the sequence, particularly a consecutive sequence of the same units.

The objective block copolymers were synthesized *via* one-pot ultra-rapid RAFT polymerization using 2-{[(2-carboxyethyl) sulfanylthiocarbonyl]sulfanyl}propanoic acid as chain transfer agent (CTA) and VA-044 as radical initiator (half-life of 10 h at 44 °C, 3.5 min at 90 °C)[43] through the stepwise addition of the corresponding amino acid-based monomers (Fig. S3†), in accordance with our previous study. A mixture of DMSO and water was used as the polymerization solvent in varying ratios depending on the monomer polarity (Tables S1–S6†). Based on our previous study, the reaction was conducted at 80 °C for 15 min, which is sufficient time for the monomer conversion of 100%.[20] Therefore, the chain length (DP) of each block was controlled using the [monomer]/[CTA] ratio. The structural features of the block copolymers were characterized at each polymerization step *via* SEC and MALDI-TOF MS analyses by sampling trace amounts of the solution. Fig. 2 shows the SEC chromatograms and MALDI-TOF MS spectra during the synthesis of A_{15}-*b*-G_5-*b*-A_{15} as an example. The SEC traces demonstrate a symmetrical narrow molecular weight distribution ($Đ \leq 1.13$) at each step and a clear shift to a high molecular weight with the progress of block polymerization (Fig. 2a). The MALDI-TOF MS spectra also support the results of the SEC analysis (Fig. 2b). All the polymers obtained at each step exhibited unimodal and narrow distributions, and the peak top molecular weights were m/z = 2636 (1st step), 3366 (2nd step), and 5555 (3rd step), respectively. These values are in good agreement with the theoretical mass values of A_{15} (2612), A_{15}-*b*-G_5 (3328), and A_{15}-*b*-G_5-*b*-A_{15} (5685) with CTA end groups, respectively. Thus, a well-regulated living polymeriz-

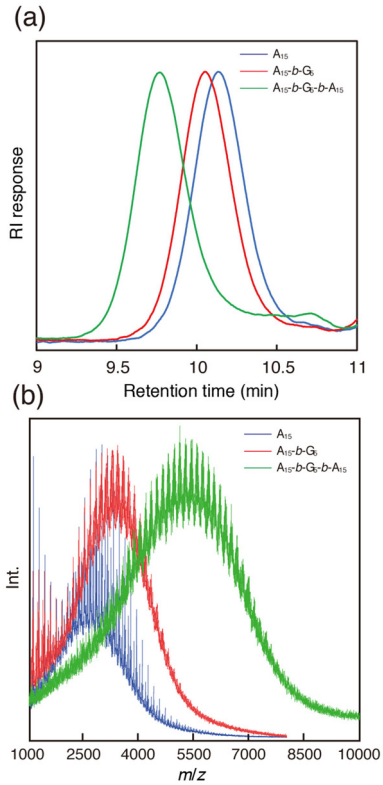

Fig. 2 (a) SEC charts (eluent: THF, 40 °C) and (b) MALDI TOF MS spectra (matrix: DHBA) for consecutive steps during the synthesis of A_{15}-*b*-G_5-*b*-A_{15}.

ation process was confirmed for this system. In all cases, successful block polymerization was confirmed in the same manner (Fig. S4 and S5†), and the results are summarized in Table 1. Note that in all cases, each block length was well controlled with an accuracy target of DP ± 1. Similar reaction conditions were used for the syntheses of the corresponding random copolymers. As presented in Fig. 3 and Table 1, good agreement between the experimental and theoretical molecular weights and narrow distributions ($Đ \leq 1.16$) were observed, thus demonstrating the successful preparation of the target copolymers. The Z groups protecting the amino groups of Lys units in A_{15}-*b*-K_5-*b*-A_{15} and A_{30}-*co*-K_5 could be easily removed *via* treatment with 5% HBr/CH_3COOH; their removal was characterized by the disappearance of the signals of Z protection group at 5.0 ppm and 7.3 ppm in ^{1}H-NMR, and the decrease in molecular weight of Z groups for MALDI TOF MS analysis (Fig. S6†). The final block and random copolymers were purified *via* dialysis against water. Thus, 13 distinct amino acid-derived vinyl polymers with the same total chain length (DP = 35) and monomer composition (A/X = 30/5) but systematic variations in hydrophobicity and sequence were successfully obtained.

Thermoresponsive behaviors of amino acid-derived vinyl polymers with systematically different hydropathy in aqueous solutions

The obtained vinyl polymers, except A_{15}-*b*-F_5-*b*-A_{15}, A_{30}-*co*-F_5, and A_{15}-*b*-L_5-*b*-A_{15}, were soluble in water, even under acidic

Table 1 Summary of amino acid-derived vinyl polymers synthesized in this study

Polymer	Step	$M_{n,SEC}$	$Đ$	MW_{theory} [a]	$M_{p,MS}$ [b]	DP_{feed}	DP_{obsd} [c]
A_{15}-*b*-G_5-*b*-A_{15}	1	2540	1.13	2611.9	2636	15	15.2
	2	3040	1.11	3327.6	3366	5	5.1
	3	5020	1.12	5685.2	5555	15	14.0
A_{15}-*b*-S_5-*b*-A_{15}	1	2660	1.10	2611.9	2636	15	15.2
	2	2970	1.10	3477.8	3487	5	4.9
	3	4400	1.16	5835.3	5844	15	15.0
A_{15}-*b*-L_5-*b*-A_{15}	1	2620	1.12	2611.9	2636	15	15.2
	2	3690	1.09	3608.2	3720	5	5.4
	3	5010	1.14	5965.7	6007	15	14.6
A_{15}-*b*-F_5-*b*-A_{15}	1	2680	1.10	2611.9	2567	15	14.7
	2	3230	1.10	3778.2	3656	5	4.7
	3	5090	1.13	6135.8	6006	15	15.0
A_{15}-*b*-V_5-*b*-A_{15}	1	3120	1.10	2611.9	2478	15	14.1
	2	3630	1.10	3537.5	3209	5	4.0
	3	5610	1.12	5895.0	5680	15	15.7
A_{15}-*b*-$K(Z)_5$-*b*-A_{15}	1	2760	1.12	2611.9	2635	15	15.1
	2	3860	1.11	4353.9	4152	5	4.4
	3	6200	1.14	6711.4	6367	15	14.1
A_{35}		5400	1.12	5755.3	5781		
A_{30}-*co*-G_5		5420	1.10	5685.2	5515		
A_{30}-*co*-S_5		4260	1.16	5835.3	5863		
A_{30}-*co*-L_5		5060	1.10	5965.7	5568		
A_{30}-*co*-F_5		5890	1.11	6135.8	6241		
A_{30}-*co*-V_5		6250	1.12	5895.0	5923		
A_{30}-*co*-$K(Z)_5$		6100	1.12	6711.4	6342		

[a] Theoretical molecular weight was calculated by using the following equation: MW_{theory} = MW of CTA + MW of NAAMe × $DP(A)_{feed}$ + MW of NAXMe × $DP(X)_{feed}$. [b] *m*/*z* value at peak top. [c] Degree of polymerization per block calculated from the peak top shift ($\Delta m/z$) in MALDI TOF MS spectra.

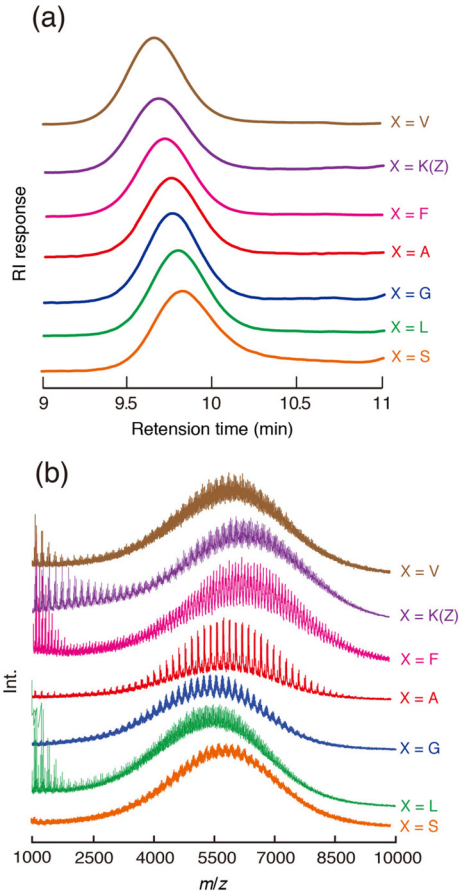

Fig. 3 (a) SEC charts in THF at 40 °C and (b) MALDI TOF MS spectra (matrix: DHBA) of A_{30}-*co*-X_5 copolymers (X = S, L, G, A, F, K(Z), and V).

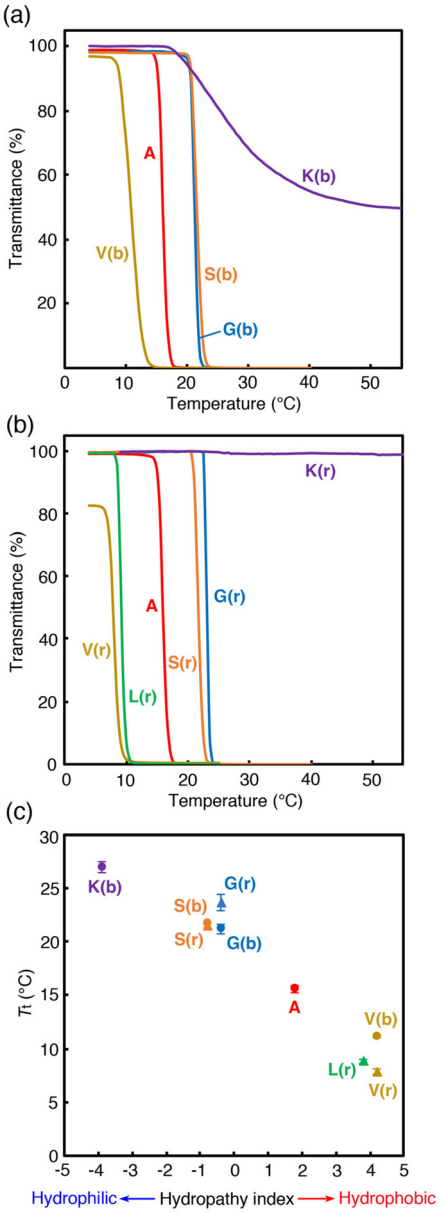

Fig. 4 Temperature dependence of transmittance at 650 nm for: (a) block type A_{15}-*b*-X_5-*b*-A_{15} (X(b)) and (b) corresponding random copolymers, A_{30}-*co*-X_5 (X(r)), in water at pH 2.2 ± 0.1 (1 wt%). (c) Relationship between phase transition temperature (T_t) and hydropathy indices of constituent guest amino acid (X). In this figure, block and random copolymers are marked as X(b) and X(r), respectively. Error bars represent the standard deviation.

conditions (pH 2.2). Under these pH conditions, the terminal carboxylic acid groups derived from CTA are completely protonated, and thus, the end groups did not contribute to the difference in thermal behavior among the polymers. Thermoresponsive behaviors of host–guest type block copolymers were first studied by measuring transmittance at 650 nm in water (1 wt%, pH = 2.2 ± 0.1). As shown in Fig. 4a, all water-soluble polymers exhibited LCST behavior; namely, the polymer solutions were homogeneous and transparent in the low-temperature region, and the transmittance drastically decreased with increasing temperature owing to the dehydration of the polymer chains (phase separation). The host Ala-based polymer (A_{35} (X = A)) exhibited a sharp LCST phase separation with a transition temperature (T_t) of 16 °C. This result corresponds well with a previous report for similar homopolymers with a slightly shorter DP (A_{15}).[20] Replacing Ala units in the guest X positions with other hydrophilic amino acid residues (Gly, Ser, Lys) increased the T_t, with the highest T_t = 27 °C for X = Lys. In addition, the transmittance of the A_{15}-*b*-K_5-*b*-A_{15} aqueous solution at high temperatures was significantly higher than that of the host polymer, and the curve exhibited a gradual slope. Under acidic conditions, under which Lys residues were protonated, the ionized hydrophilic groups stabilized the polymer/water hydration state and suppressed aggregation among polymer chains due to electrostatic repulsion. However, for the X = Val polymer, which contained nonpolar residue with the highest HI, T_t dropped by 3 °C compared with host polymer (X = Ala). Fig. 4c shows the relationship between T_t (marked as X(b)) and the HI values of the constituent amino acids. Evidently, a clear correlation between them can be observed, that is, T_t shifts to a lower temperature with increasing hydrophobicity of the polymers. As is com-

monly known from past research, the LCST of vinyl polymers varies with the hydrophilic–hydrophobic balance. However, a study recently reported that both the hydrophobicity of the polymer and the difference in the mobility of the side chains affect the LCST behavior of poly(*N*-(*ω*-acryloyloxy-*n*-alkyl)-2-pyrrolidone)s (PNARPs: R = methyl (Me), ethyl (Et), propyl (Pr)) aqueous systems.[50] In this case, PNAMeP exhibited a lower LCST than PNAEtP and PNAPrP, although PNAMeP was less hydrophobic than the others, owing to the restricted mobility of the polymer side chain, thus resulting in a change in the entropy of the hydrated water. Although the structures of the amino acid units are different in amino acid-derived vinyl polymers, they are linked to the backbone of the main C–C chain in the same manner at N-terminus *via* amide bond. Therefore, the mobility of the polymer side chain units does not differ significantly with the polymers; consequently, the temperature responsiveness seems to be governed by the hydropathy of the amino acid units.

Interestingly, even for amino acid units with reactive functional groups, the resulting polymers exhibited similar T_t values when the HI values were nearly equal (*e.g.*, Ser and Gly). These features of this polymer system enable the precise design of a wide variety of thermoresponsive polymers. Note that the block polymers with guest sequences of X = Phe and Leu were insoluble in water, although their HI values were slightly lower than that of Val. This is probably owing to the high associative propensity of isobutyl and phenyl groups in the side chain of amino acid units, as is observed in native protein/peptide aggregations, such as the leucine-zipper motif[51,52] and β-sheet aggregation.[53,54]

To evaluate the effect of consecutive sequences of the same amino acid units, another set of seven polymers with the same total chain lengths (DP = 35) and A/X ratios (30/5) but with a statistically random sequence was characterized by transmittance test (Fig. 4b). Overall, a similar trend was observed for the block types, and T_t correlated well with the hydropathy of the constituent amino acids (Fig. 4c) (marked as X(r)). However, for guest amino acids with extremely high hydrophilicity (X = Lys) and hydrophobicity (X = Leu, Val), clear differences in their thermal behaviors were observed depending on the sequences. For random copolymer with X = Lys, phase separation did not occur, even when the temperature was increased to 80 °C (*i.e.*, T_t > 80 °C). This is probably because the cationic amino groups are dispersed throughout the polymer chain, thus rendering the continuous length of Ala units (PNAAMe) shorter than that in the block type (15 mer), as well as because electrostatic repulsion works more efficiently between the polymers. By contrast, in the case of hydrophobic X = Val, the random distribution leads to an increase in hydrophobicity of the entire polymer chain, rather than locally, thus enhancing the aggregation compared with the block type. These results highlight that hydrophobic and hydrophilic amino acid units have opposite effects on T_t when dispersed into the polymer chain. Regarding the highly aggregative X = Leu, the continuous clustering of Leu units seems to significantly reduce water solubility, as observed in A_{15}-*b*-L_5-*b*-A_{15}. These results clearly demonstrate that even a slight difference in both the hydrophobic/hydrophilic balance and monomer sequence has a significant influence on the thermal response, which can be easily tuned by specifying the constituent amino acids in this aqueous bio-based vinyl polymer system.

Conclusions

Herein, a new family of bio-based thermoresponsive vinyl polymers with precisely controlled hydropathies was successfully synthesized. These polymers can be easily obtained without changing the total chain length or monomer composition through one-pot ultra-rapid RAFT polymerization from an amino acid-derived vinyl monomer library, including alanine, glycine, serine, phenylalanine, leucine, valine, and lysine derivatives. By employing an amino acid unit as a monomer component, the effect of the hydropathy of the polymers, even with a small difference, on their LCST behavior could be accurately evaluated. A comparison of the block-type polymers to statistical random copolymers with the same monomer composition revealed a stark difference in the monomer sequence, which affected the LCST behavior. Notably, such monomer sequence dependence was observed when the HI value of the guest comonomer unit (X) was significantly different from that of the host main-chain unit. These results can provide important insights for understanding the structure–property relationship in thermoresponsive vinyl polymer systems and macromolecular engineering. Additionally, the findings of this study can help design a novel concept for structurally and functionally diverse amino acid-derived vinyl polymers with significant potential as nanobiomaterials, such as smart drug carriers, cell scaffolds, and self-assembling/folding building blocks.

Author contributions

T. K. and N. H. conceived the research. A. S. and S. N. conducted the experiments. T. K. obtained funding for the project and supervised the study. A. S., S. N., N. H., and T. K. co-wrote the manuscript. All authors discussed the results and commented on the manuscript.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

This work was partly supported by grants-in-aid for Scientific Research (KAKENHI) (no. 22K05232) from the Japan Society for the Promotion of Science (JSPS), by grants-in-aid from the Harris Science Research Institute of Doshisha University, and by grants-in-aid for SDGs studies of Doshisha University.

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