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Thermoresponsive oligoprolines†

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Monodispersed oligoprolines decorated covalently with hydrophobic units show characteristic thermoresponsive behavior with fast and sharp phase transitions at certain concentrations. The phase transition temperatures are dependent on the shape and location of the hydrophobic units, and can be also tuned *via* supramolecular host–guest interactions.

Thermoresponsive polymers are of great interest in sensors and biomedical applications.[1] These polymers show a lower critical solution temperature (LCST) in aqueous solutions. Based on entropy-driven processes, the polymer chains start to dehydrate right below this temperature, followed by chain collapse and forming mesoglobules or aggregates upon heating. The tunability of the LCST is critical,[2] and normally can be mediated by the overall hydrophobicity–hydrophilicity balance of the polymers. Incorporation of hydrophilic or hydrophobic units,[3] end-group modifications,[4] or even controlling particle size[5] have been employed to tune the phase transition temperatures of polymers. Recently, polymer architecture has also been proved to show significant influence on the phase transitions. On changing polymer architecture from linear shape into cyclic[6] or dendritic ones,[7] the thermoresponsive properties vary. Furthermore, the phase transition temperature can also be influenced by supramolecular interactions. With introduction of hydrophilic host molecules such as β-cyclodextrin, the thermoresponsiveness of hydrophobic guest molecules can be tuned.[8]

Oligo- or polypeptides showing thermoresponsive behavior may form a promising class of thermoresponsive polymers due to their improved biofunctions and bioactivities together with the ordered secondary structures. The most intensively investigated thermoresponsive polypeptides are elastine-like peptides (ELPs) with VPGXG as the main amino acid sequence, where X can be any amino acid except proline. It was found that either long or short ELPs showed phase transition behavior and were followed by the conformation changing from random coil to β-spiral.[9] Inspired by these, different kinds of homopolymers and block and random copolymers carrying VPGXG units were reported and their LCST behavior investigated.[10] Though the LCSTs of ELP-based polymers could be finely tuned by several parameters including the X units, the peptide chain length, solution pH value and the salt concentration, most of the reported ELP-based polymers exhibit broad phase transitions (temperature range >3 °C), and the hysteresis during the heating and cooling processes is less addressed. Therefore, other peptide-based polymers were developed to show improved thermoresponsiveness. Recently, poly(glutamate)s carrying oligo(ethylene glycol) (OEG) side-units were reported by Li *et al.*[11] Depending on the length of OEG units, these polypeptides could adopt ordered secondary structures which, in turn, afford the polypeptides with thermoresponsiveness. By varying OEG length and the ratios of the comonomers, the LCSTs of the (co)polypeptides can be tuned. Though thermoresponsive polypeptides have been paid some attention, only few examples were reported due to their relatively harsh synthesis and structural complexities. Developing novel (poly)peptides with unique thermoresponsive properties remains a challenge. To date, most thermoresponsive macromolecules reported are polydispersed.

Oligoprolines are an intriguing class of peptides which are capable of adopting two different helical conformations: the compact, right-handed polyproline I (PPI) in less polar solvents like aliphatic alcohols, and the stretched, left-handed polyproline II (PPII) in polar solvents like water.[12] PPII has been found to be a common secondary structure in natural proteins, and plays important roles in many biological processes. Oligo- or polyprolines themselves are fully water-soluble and not thermoresponsive. To extend the idea to combine advantages from oligoprolines and the thermoresponsive behavior in one matter, and to explore the effects of structure and architecture on the thermoresponsiveness of the monodispersed substances, here we report on the synthesis and characterization of novel thermoresponsive oligoprolines covalently decorated with hydrophobic units (Fig. 1). Both ball-shaped adamantyl (**Ada**) and linear *n*-decyl (**Dec**) groups were selected to investigate the possible

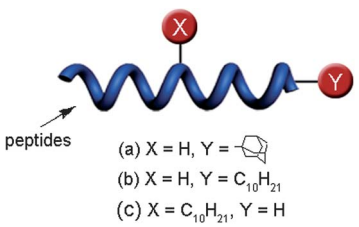

Fig. 1 Cartoon presentation of the oligoprolines modified with different hydrophobic moieties at different positions.

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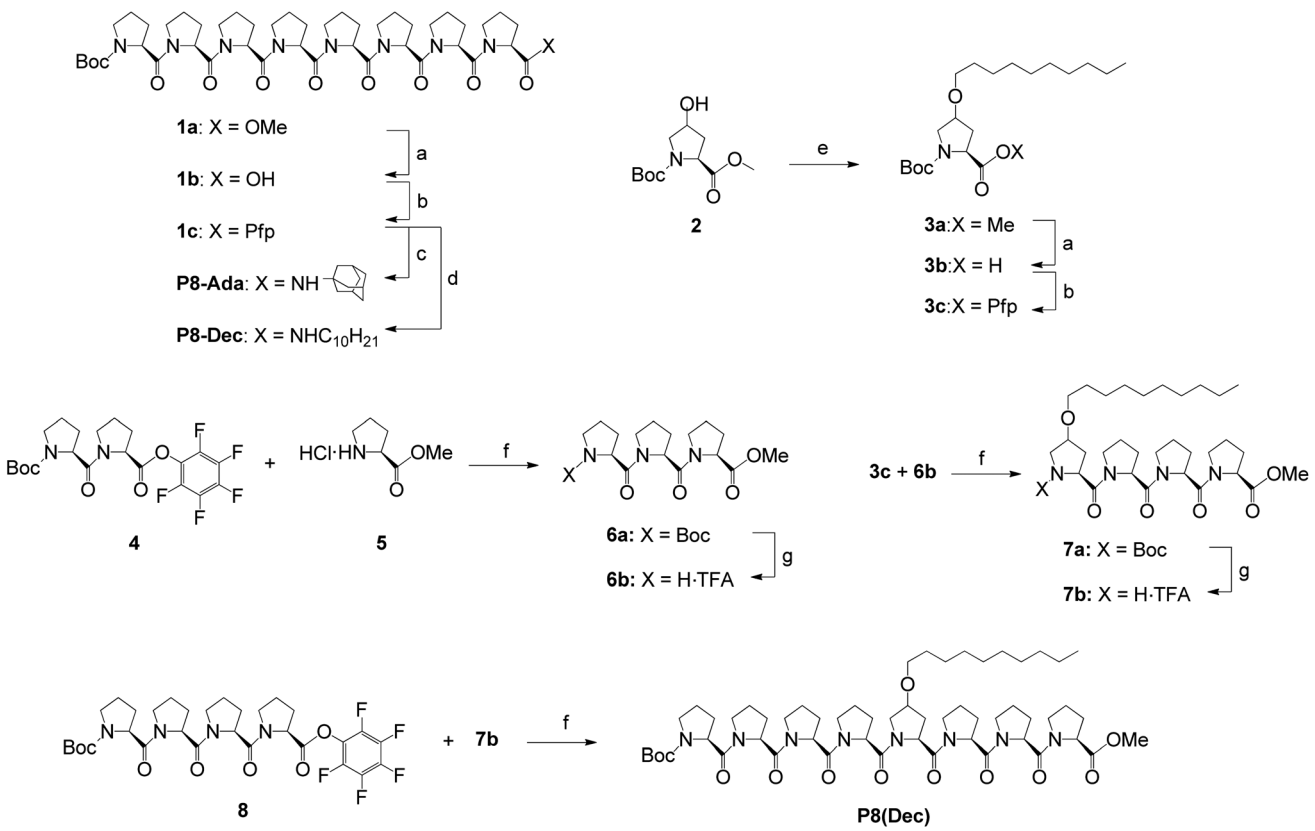
geometry effects of hydrophobic units on their thermoresponsive behavior. Moreover, the linear hydrophobic unit **Dec** is located either at chain-end or in the center of the oligopeptides to examine the possible architecture effects on this property. Furthermore, their thermoresponsive behavior was also examined through host–guest interaction.

The monodispersed oligoprolines were synthesized by a similar procedure according to the previous report *via* solution peptide synthesis strategy as shown in Scheme 1. Starting from the proline octamer ester **1a**,[13] saponification with LiOH afforded the acid **1b**, which was then reacted with pentafluorophenol (Pfp-OH) to form the active ester **1c**. Amidation of this active ester with either adamantylamine or *n*-decylamine furnished, respectively, the peptides **P8-Ada** and **P8-Dec** with hydrophobic units at the chain end. The proline octamer with alkyl chain located in the middle of peptide chain [**P8(Dec)**] was synthesized through multiple steps. Williamson etherification between hydroxyproline **2** and 1-bromo-decane afforded the ester **3a**. After hydrolysis and reacting with Pfp-OH, the active ester **3c** was obtained. Amide coupling between compound **4** and **5** afforded the tripeptide **6a**, which was deprotected with TFA to furnish the ammonium **6b**. Amidation of **3c** with **6b** afforded the tetrapeptide **7a**, which was deprotected with TFA to form the ammonium **7b**. Reaction with the tetrapeptide active ester **8**[13] furnished the octamer **P8(Dec)**. All key compounds were characterized *via* ^{1}H NMR and high resolution mass spectroscopy to prove their high purity (for spectra, see ESI†).

All modified oligoprolines **P8-Ada**, **P8-Dec** and **P8(Dec)** are water-soluble at room temperature, but quickly collapse from water and their aqueous solutions turn turbid when heated up to certain temperatures. Their thermoresponsive behavior was thus investigated and their cloud points (T_cs) were determined by turbidity measurements using UV/Vis spectroscopy. Typical phase transition curves from **P8-Dec** and **P8(Dec)** are shown in Fig. 2a. The T_cs for **P8-Dec** and **P8(Dec)** are 25.6 °C and 33.5 °C, respectively, while the T_c from **P8-Ada** is higher than 90 °C. These results indicate: (1) although both **Ada** and **Dec** units contain 10 carbons, the linear hydrophobic decyl unit contributes more hydrophobicity than that from the ball-shaped adamantyl group, resulting a much lower T_c (compare **P8-Dec** with **P8-Ada**); (2) location of the linear hydrophobic unit in the centre of the oligopeptide shows a relatively weak hydrophobicity than that attached at the chain end, leading to a higher T_c [compare **P8-Dec** with **P8(Dec)**]; and (3) all phase transitions from **P8-Dec** and **P8(Dec)** are very sharp (<1 K), and the hystereses are small (<2 K), which should be resulted from the absence of strong hydrogen bonding from the secondary amide linkage within oligoprolines in contrast to convenient peptides. The effects of peptide concentration on the thermoresponsive behavior of **P8-Dec** and **P8(Dec)** were also investigated, and results are summarized in Fig. 2b (for turbidity curves, see Fig. S1 in ESI†). Overall, the T_c of **P8-Dec** shows much high concentration dependence than that for **P8(Dec)**. From concentration of 0.25 wt% to 1.0 wt%, the T_c for **P8-Dec** decreases 12 K, while the T_c for **P8(Dec)** decreases only 5 K. This result indicates that alkyl



Scheme 1 Synthesis of modified oligoprolines **P8-Ada**, **P8-Dec** and **P8(Dec)**. *Reagents and conditions*: (a) LiOH·H_2O, MeOH, H_2O, −5 °C–r.t., 4 h (92–98%); (b) pentafluorophenol, EDC·HCl, DCM, −15 °C–r.t., overnight (60–74%); (c) Ada-NH_2, DIEA, DCM, 0 °C–r.t., overnight (64%); (d) *n*-decylamine, DCM, 0 °C–r.t., overnight (60%); (e) 1-bromo-decane, KI, 15-crown-5, NaH, THF, r.t., 20 h (40%); (f) DiPEA, DCM, −15 °C–r.t., overnight (91%, 64% and 65%, respectively); and (g) TFA, DCM, 0 °C–r.t., 4 h (100%).

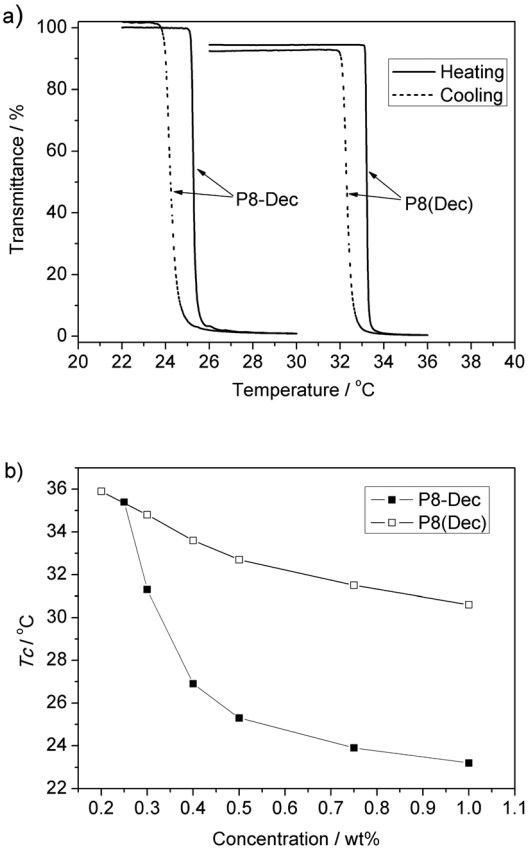


Fig. 2 a) Plots of transmittance *vs.* temperature for 0.5 wt% aqueous solutions of **P8-Dec** and **P8(Dec)** and (b) dependence of T_cs on concentrations of **P8-Dec** and **P8(Dec)**. Heating and cooling rate = 0.2 °C min^{-1}.

moieties at the end of peptide chains enhance the inter-molecular aggregation more pronouncedly. Besides the T_c, the broadness of the phase transitions for both peptides is also dependent on the concentration. With the decrease of solution concentrations, the transitions became significantly broad (Fig. S1 in ESI†). Above results demonstrate that the geometry and location of the hydrophobic units not only show significant influence on the phase transition temperature of the oligopeptides, but also afford them different aggregation behavior. The large concentration dependence of the thermoresponsive behavior of these oligopeptides is characteristic of thermoresponsive scaffolds with low molar masses.[14]

Thermoresponsive behavior of the oligoprolines was also investigated through host–guest interaction. α-Cyclodextrin (α-CD) can form complexes *via* host–guest interaction with alkyl chains with constants in the range of several hundreds,[15] thus, different amount of α-CD was introduced into the aqueous solutions of the oligoprolines to form supramolecular complexes. The influence of the supramolecular interaction on the thermoresponsiveness of both **P8-Dec** and **P8(Dec)** was examined, and the results are summarized in Fig. 3 (for the turbidity curves, see Fig. S2 in ESI†). Both oligopeptides show increased T_cs with increase of the α-CD/oligopeptide ratio. When equivalent α-CD was added, T_cs for **P8-Dec** and **P8(Dec)** increased to 68 and 55 °C, respectively. Even when excess amount of CD was added, T_cs increased further (data are not shown). The T_c change for **P8-Dec** is more pronounced than that for **P8(Dec)**. These results suggest: (1) complexation introduces strong hydrophilicity from

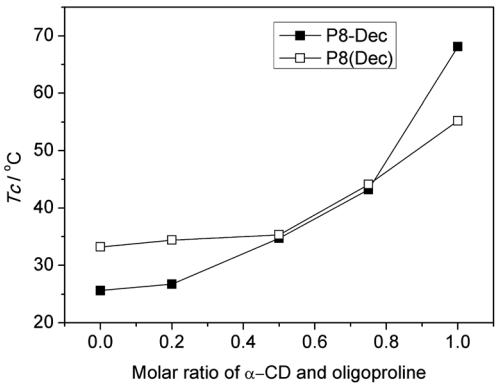


Fig. 3 Plots of T_c with the different ratio α-CD/oligopeptides for both **P8-Dec** and **P8(Dec)**. Heating and cooling rate = 0.2 °C min^{-1}. Concentration of the peptides = 0.5 wt%.

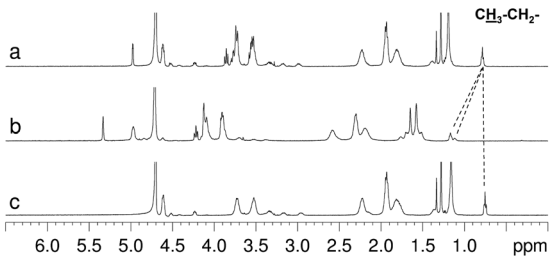


Fig. 4 ^{1}H NMR spectra of complex from **P8-Dec** and α-CD with the ratio of 1 : 0.5 below (23 °C), (a) and above (50 °C), (b) the T_c. The spectrum of pure **P8-Dec** in D_2O at 23 °C (c) is also included for comparison. The dotted lines are a guide for the eyes.

native α-CD into the inclusion complexes, (2) the inclusion complexes are dynamic with fast exchange between CD host and peptide guests, and (3) alkyl moieties at the end of peptide chains facilitate the complexation with α-CD because of less steric hindrance. In addition, temperature-varied ^{1}H NMR spectroscopy was utilized to follow the thermally induced dehydration of the supramolecular complexes (Fig. 4), and found: (1) most proton signals from oligoprolines became broad at elevated temperatures due to the dehydration and (2) proton signals from the terminal methyl group split into two groups at elevated temperature, indicating partial decomposition of the supramolecular complex due to the steric hindrance formed from the chain collapse.[16]

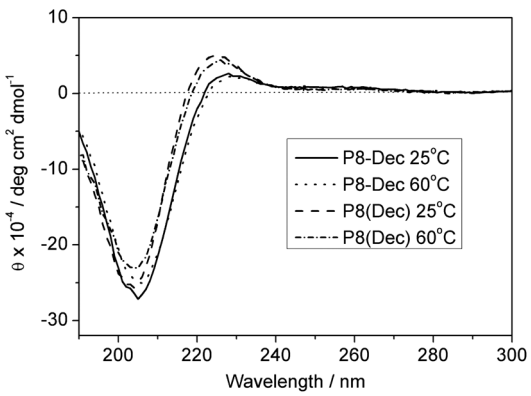


Fig. 5 Circular dichroism spectra of **P8-Dec** and **P8(Dec)** in aqueous solutions at 25 °C and 60 °C.

The helical conformations of the thermoresponsive oligoprolines were investigated with circular dichroism spectroscopy, and the results are plotted in Fig. 5. Typical circular dichroism spectra for PPII helices (a weak positive band around 228 nm and a strong negative band around 204 nm) were obtained for both **P8-Dec** and **P8(Dec)**. This helical conformation was retained without significant change at different temperatures, which suggests that the hydrophobic environment formed from the collapsed oligoprolines above their T_cs cannot lead to the conformation change from the more hydrophilic PPII to the more hydrophobic PPI. This PPII conformation remained even after keeping at elevated temperatures for several days. The reason for this high conformation stability may be due to the insufficient driving force from the hydrophobic aggregates or the cap effect from the terminal group.[17]

Conclusion

This work has demonstrated a powerful novel route to synthesize thermoresponsive peptides, which can be extended to other functional (poly)peptides. Both the shape and location of the hydrophobic units have been proved to have significant effects on the phase transition temperatures of the modified peptides. Due to the absence of strong hydrogen bonding of the secondary amide linkages, these peptides show unique thermoresponsive behavior with sharp transitions and small hysteresis. Additionally, the thermoresponsiveness of the oligoprolines can also be tuned easily through the host–guest interaction between the alkyl chain and α-CD. This work provides an alternative strategy for the design of promising thermoresponsive chiral scaffolds from polymers for bio-applications.

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