Reversible Association of PNA-Terminated Poly(2-hydroxyethyl acrylate) from ATRP

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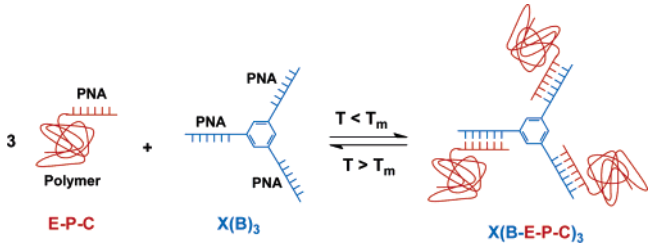
Introduction. In the interest of developing a system for the preparation of a thermally reversible aqueous gel, it would be desirable to prepare a well-defined water-soluble polymer (P) with end groups (E) designed to couple reversibly with complementary groups (B) on a small molecule core (X), with the design such that E will couple only with B, and vice versa. For example, a system comprising n E−P−E plus (n/m) $X(B)_m$ would lead in principle to a model gel if $m \geq 3$.[1−4] The first steps in that development are described here, involving the preparation and characterization of a polymer E−P−C and the cross-linker $X(B)_3$, where C is an end group that will couple with neither E nor B. A scheme to couple two E−P−C chains via the C end groups to produce the telechelic E−P_2−E is also described.

Atom transfer radical polymerization (ATRP)[5−9] will be used to prepare a poly(2-hydroxyethyl acrylate) (PHEA) with selected end groups. The E and B groups chosen comprise complementary peptide nucleic acids (PNA) designed to form a base-paired duplex with a melting temperature near room temperature. PNA is an achiral analogue of DNA consisting of a selection of the standard DNA bases attached to a synthetic pseudopeptide backbone, usually formed from *N*-(2-aminoethyl)glycine units, as is the case here.[10−13] These features make PNA capable of sequence-specific recognition of PNA, DNA, and RNA, obeying the Watson−Crick hydrogen-bonding rules, with hybrid complexes exhibiting extraordinary thermal stability, even at low ionic strengths. The literature on PNA duplex formation and the synthetic methods developed to prepare PNA are considerable assets.[14,15] As elaborated below, the PNA duplex formation may be followed spectroscopically, and in addition, the development of the star-shaped complex $X(B−E−P−C)_3$ formed by combination of 3 E−P−C with $X(B)_3$ may be followed by light scattering. The polymer and cross-linker used in this study comprise neutral structures soluble in an aqueous medium.

Prior work[16−18] demonstrated that a self-assembling hydrogel based on the protein avidin could be assembled in thermoreversible fashion using Watson−Crick base pairing between complementary DNA and biotinylated peptide nucleic acid (PNA) strands. We are interested in extending this self-assembly strategy to synthetic polymers, and this Communication describes the first step in this direction.

Design. The design of the self-assembling $X(B−E−P−C)_3$ complex is shown in Scheme 1. A synthetic polymer is covalently attached to a peptide nucleic acid (PNA) hexamer, while three copies of the complementary PNA hexamer are attached to a trifunctional benzene core. Mixing these components in a 3:1 ratio should allow thermoreversible hydrogen-bonded base pairing between the PNA strands to assemble the complex (Scheme 1). While a similar structure could be formed using DNA instead of PNA, the high thermodynamic stability of PNA−PNA duplexes allows the use of relatively short oligomers to form a stable complex. Moreover, the nonionic backbone of PNA allows it to be dissolved in various organic liquids, a feature important for the development of a PNA-based initiator for atom transfer radical polymerization (ATRP). In principle, the bromine end group on a PHEA prepared by ATRP could be replaced by a PNA-containing entity to give PNA−PHEA. However, ATRP leaves a small fraction of "dead" chains formed on termination by removal of the bromine end group, meaning that the PNA−PHEA prepared in such a way would be contaminated by PHEA and would be very difficult to purify. To avoid this effect, a new initiator containing the desired PNA was prepared for use in ATRP to directly obtain PNA−PHEA with a PNA end group on all chains.

Scheme 1. Schematic Illustration of Thermally Reversible Coupling via PNA Moieties on a Cross-Linker and the Complementary Groups on the Polymer End Group



PNA Synthesis and Characterization. The PNA hexamers used in this study have the general structure H−R−NH_2, where −R− is given by −(NH−$(CH_2)_2$−N(S_j)−CH_2−CO)$_6$−, with the sequence and structures S_j of the bases chosen to adjust the melting temperature of the duplex formed by the complementary pairs in the ATRP initiator and the cross-linker molecules. On the basis of prior knowledge,[19] the PNA sequences (−R−) selected for this purpose, $−R_E− = −$ATCTAC− and the complementary $−R_B− = −$GTAGAT− for use in E and B, respectively, were expected to form a duplex with a melting temperature slightly above room temperature. Thus, H−R_E−NH_2 and H−R_B−NH_2 were synthesized by standard solid-phase methods[20,21] for use in the polymer and the cross-linker, respectively. As elaborated in the Supporting Information, a solution containing equimolar amounts of the two hexamers in 10 mM sodium phosphate buffer (pH = 7) showed the anticipated absorbance behavior, with the absorbance at 260 nm decreasing sharply with decreasing temperature, or vice versa, with little hysteresis over the 5−85 °C range studied, to give a melting temperature $T_m \approx 30$ °C (Figure S1).

Core Molecule Synthesis and Characterization. A flexible linker designed to reduce steric hindrance during duplex formation was incorporated into the PNA component of the core molecule, giving $H_2N−(CH_2)_5−CO−R_B−NH_2$. The three-arm, star-shaped core molecule then was synthesized by coupling 3 equiv of $H_2N−(CH_2)_5−CO−R_B−NH_2$ with 1,3,5-benzenetricarboxylic

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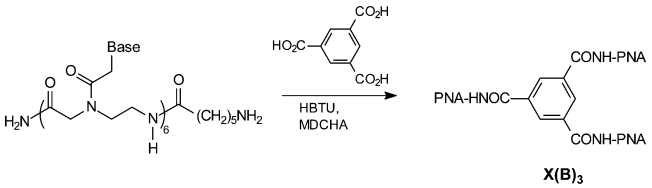
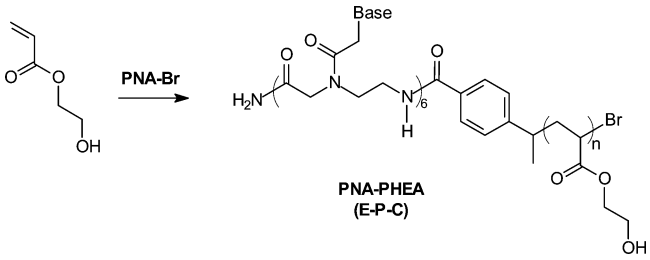
Scheme 2. Formation of the Trifunctional Core Molecule Containing PNA Functional Groups



Scheme 3. Formation of a Polymer with a PNA End Group by ATRP



acid (Scheme 2). The resulting trifunctional star-shaped core molecule $C_6H_3[-CO-NH-(CH_2)_5CO-R_B-NH_2]_3$ was purified by HPLC and confirmed by mass spectroscopy (Supporting Information).

Although the three PNA arms of the cross-linker have identical sequences, and should not form Watson−Crick double helices with one another, in either an intra- or intermolecular sense, solubility problems were encountered, especially at the lower temperatures used. As elaborated in the Supporting Information, the addition of 20% NMP tended to suppress this tendency, giving the normal melting and formation behavior when mixed with the complementary PNA hexamer (square symbols, Figure 1a).

Polymer Synthesis and Characterization. Polymers of similar molecular weight were prepared by ATRP, differing by the presence (PNA−PHEA) or absence (PHEA) of the PNA oligomer end group. Although the use of ATRP to prepare PHEA has precedence in the literature,[22,23] the conditions used here differ from those reported. Here, $H-R_E-NH_2$ was modified by reacting 4-(1-bromoethyl)benzoic acid with the PNA N-terminus, either before or after cleavage of the PNA from the solid support. The resulting $Br-(CH-CH_3)-C_6H_4-CO-R_E-NH_2$ initiator was purified by HPLC and gave satisfactory mass spectral data. The initiator was dissolved in the 2-hydroxyethyl acrylate monomer and readily initiated ATRP at room temperature with CuBr/tris[2-(dimethylamino)ethyl]amine (Me_6-Tren) catalyst[24] to produce PNA−PHEA (Scheme 3). An SEC analysis in DMF solution of the polymer revealed a narrow single peak, with $M_n = 18\,500$ and $M_w/M_n = 1.06$. This compares favorably with a prior report in which poly(2-hydroxyethyl methacrylate) was prepared by ATRP using a pentapeptide-linked alkyl bromide initiator.[25] In that case, the peptide initiator was immobilized on a solid support, while in our case the PNA initiator was dissolved in the monomer.

Unexpectedly, light scattering results on 10 mM phosphate buffer aqueous solutions of PHEA and PNA−PHEA samples of similar M_n (calculated either from the ATRP conditions or from SEC analysis on solutions in

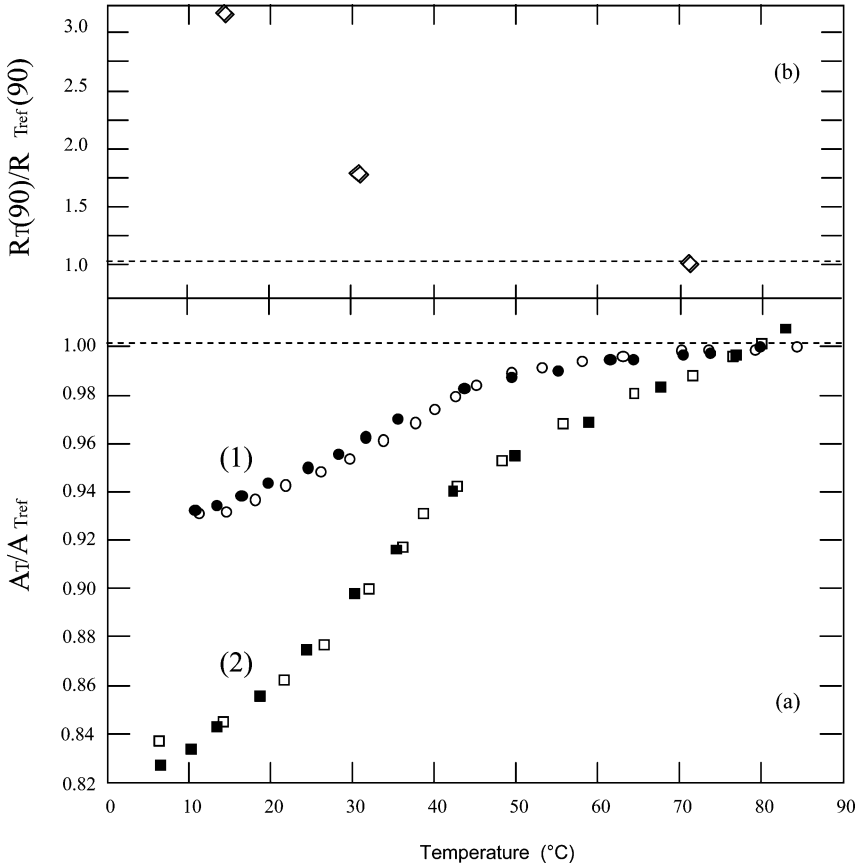


Figure 1. Properties of solutions at temperature T divided by that at $T_{ref} \approx 70$ °C vs temperature; the unfilled and filled symbols are for decreasing T, followed by increasing T, respectively. (a) Absorption solutions containing a stoichiometric ratio of either the cross-linker and the polymer (1) or the PNA hexamer (2). (b) Light scattering Rayleigh ratio at 90° scattering angle for solutions containing a stoichiometric ratio of the polymer.

DMF) gave very different results, with the apparent M_w for the PNA−PHEA sample much larger than that for the PHEA specimen. This indicates intermolecular association for the PNA−PHEA sample, even in the absence of the cross-linker, but similar to the behavior reported above for the cross-linker alone, the association was suppressed by the addition of 20% NMP.

Reversible Coupling of PNA−PHEA (E−P−C) and Star−PNA Core ($X(B)_3$). The absorbance and light scattering results obtained for a mixture of PNA−PHEA and the cross-linker in a 10 mM phosphate buffer aqueous solution with 20% NMP are shown in Figure 1. The thermoreversible duplex formation is shown by the transition in the absorption at 260 nm, similar to that seen in the same figure for the cross-linker and the oligomer containing the complementary PNA with the same sequence of nucleobases as that on the polymer. The light scattering intensity determined at 90° scattering angle shown at three temperatures is consistent with the formation of a star-shaped polymer at low temperature, with 3 times the molecular weight of the isolated PNA−PHEA existing at high temperature. An intermediate molecular weight is observed near the inflection of the absorption vs temperature response, as would be expected for a structure with an equilibrium extent of duplex formation at that temperature.

Almost all PNA−PHEA chains will also have a bromine end group, i.e., an E−P−C structure, with C and E the Br- and PNA-containing moieties, respectively. The bromine end groups may then be used in a subsequent reaction to couple two chains to produce a telechelic sample with PNA end groups, with this sample contaminated by any "dead" chains that lost Br during the ATRP process, which could be removed. The resulting telechelic PNA−PHEA−PNA could then be used to prepare a thermally reversible gel using methods similar to those described above. Finally, while the aggregation behavior described above for both the PNA−PHEA and PNA cross-linker components was problematic, simple addition of hydrophilic amino acids to the PNA components during solid phase synthesis will likely alleviate this problem, allowing assembly and study of gels in aqueous solutions without requiring an organic cosolvent.

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Supporting Information Available: Absorption spectra and MS spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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