Thermoreversible Copolymers with Enzyme-Dependent Lower Critical Solution Temperatures

Derek J. Overstreet, Harshil D. Dhruv, Brent L. Vernon.
School of Biological and Health Systems Engineering, Center for Interventional Biomaterials,
Arizona State University, Tempe, Arizona 85287, USA

Statement of Purpose: Poly(*N*-isopropylacrylamide) (poly(NIPAAm)) and its copolymers are promising for use as *in situ* forming biomaterials due to a lower critical solution temperature (LCST) in aqueous solutions between room temperature and body temperature. The LCST of NIPAAm-based copolymers can be controlled by adjusting the composition and hydrophilicity of comonomers. Hydrophilic comonomers such as acrylic acid increase the LCST, while hydrophobic comonomers such as butyl methacrylate lower the LCST (Feil H. Macromolecules. 1993;26(10):2496-2500.). Several groups have demonstrated time-dependent increases in LCST using comonomers with hydrolyzable ester groups in order to create an injectable degradable material (Cui Z. Biomacromolecules. 2007;8(4):1280-1286.). Here, we report the synthesis and characterization of poly(NIPAAm)-based copolymers with enzyme-dependent LCSTs. In this proof-of-concept study, comonomers containing the collagenase-labile amino acid sequence Ala-Gly-Pro-Leu were incorporated in polymer side groups to elicit enzyme-specific LCST change. These materials can be prepared as an injectable liquid rather than a pre-formed cross-linked gel, providing increased flexibility in delivery properties, final gel properties, LCST, and degradation properties.

Methods: The solvent for enzyme degradation, differential scanning calorimetry (DSC), and cloud point determination was 145 mM HEPES buffer with 5 mM $CaCl_2$ (pH 7.4). Custom peptides Gly-Ala-Pro-Gly-Leu-NH_2 (GAPGL) and Gly-Ala-Pro-Gly-Leu-Phe-NH_2 (GAPGLF), >98% purity, were purchased from American Peptide Company (Sunnyvale, CA). Poly(*N*-isopropylacrylamide-*co*-*N*-acryloxysuccinimide) at a 90:10 molar feed ratio was synthesized in anhydrous THF with AIBN as the initiator. Custom peptides were conjugated to the polymers by nucleophilic substitution. Custom peptide and triethylamine (equimolar with the peptide) were added at 5 mol% monomer feed ratio to poly(NIPAAm-*co*-NASI). Remaining NASI side groups were back-reacted to NIPAAm with isopropylamine to obtain poly(NIPAAm-*co*-GAPGL) and poly(NIPAAm-*co*-GAPGLF). Control polymer was back-reacted to poly(NIPAAm) without peptide. Polymer solutions (5 wt%) were degraded at 25°C by collagenase (0.4 mg/ml added daily for 5 days). Materials were analyzed before and after degradation by ^{1}H NMR spectroscopy (Varian Gemini-300 MHz, Palo Alto, CA), DSC (Calorimetry Sciences Corp. 4100 Microcalorimeter, Spanish Fork, UT), and cloud point determination. Molecular weight and polydispersity was measured by gel permeation chromatography (Shimadzu Corp. LC-10AD, Columbia, MD) in conjunction with static light scattering (Wyatt MiniDawn, Santa Barbara, CA).

Results: Successful synthesis was confirmed by ^{1}H NMR. A new peak at 0.85 ppm in both peptide-conjugated samples is due to the 6 methyl protons of the leucine residue isopropyl group. In poly(NIPAAm-*co*-GAPGLF), a broad peak at 7.25 ppm is attributed to the 5 phenyl protons of the phenylalanine residue. Both peaks are reduced after enzyme treatment. The peptide content of poly(NIPAAm-*co*-GAPGL) and poly(NIPAAm-*co*-GAPGLF) was estimated to be 4.3 and 6.1 mol%, respectively. All weight average molecular weights were in the range 9000-11000 g/mol with polydispersities 1.55-1.76. DSC results show a significant change in LCST for both enzyme-sensitive materials and no significant change for poly(NIPAAm). The LCST of poly(NIPAAm-*co*-GAPGL) decreased by about 0.8°C, and the energy of the transition was reduced. This is consistent with published data for poly(NIPAAm-*co*-acrylic acid) (Vernon B. J. Biomed. Mater. Res. A. 2000;51(1):69-79.). The LCST of poly(NIPAAm-*co*-GAPGLF) increased from 34.23±0.25°C to 37.03±0.23°C after enzyme treatment, and the energy of the transition was also reduced. The cloud point data (shown in Figure 1) shows an increase in LCST (half-max absorbance temperature) after enzyme action of 1°C for poly(NIPAAm-*co*-GAPGL) and an increase of 9°C for poly(NIPAAm-*co*-GAPGLF).

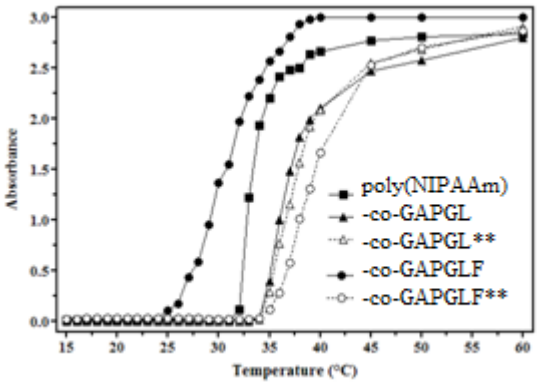

Figure 1. Cloud point data. **= after collagenase action

Conclusions: Thermoreversible copolymers poly(NIPAAm-*co*-GAPGL) and poly(NIPAAm-*co*-GAPGLF) were successfully synthesized. Results from NMR spectroscopy indicate that degradation of peptide side groups caused pendant Leu or Leu-Phe to be cleaved from the polymer in the presence of collagenase. The change in structure led to a change in LCST behavior that was confirmed by both DSC and cloud point determination. Further, the loss of the more hydrophobic group, Leu-Phe, caused a greater increase in copolymer solubility than loss of Leu alone. This result implies that the substrate peptide or pendant leaving group can be chosen to elicit a desired change in LCST for a given enzyme or application. Further studies on this class of materials will include characterization of rheological properties and enzyme-triggered drug release in concert with degradation. Additional substrate sequences will also be investigated.