Controlling the Aggregation of Conjugates of Streptavidin with Smart Block Copolymers Prepared via the RAFT Copolymerization Technique

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Block copolymers containing stimuli-responsive segments provide important new opportunities for controlling the activity and aggregation properties of protein−polymer conjugates. We have prepared a RAFT block copolymer of a biotin-terminated poly(*N*-isopropylacrylamide) (PNIPAAm)-*b*-poly(acrylic acid) (PAA). The number-average molecular weight (M_n) of the (PNIPAAm)-*b*-(PAA) copolymer was determined to be 17.4 kDa ($M_w/M_n = 1.09$). The PNIPAAm block had an M_n of 9.5 kDa and the poly(acrylic acid) (PAA) block had an M_n of 7.9 kDa. We conjugated this block copolymer to streptavidin (SA) via the terminal biotin on the PNIPAAm block. We found that the usual aggregation and phase separation of PNIPAAm−SA conjugates that follow the thermally induced collapse and dehydration of PNIPAAm (the lower critical solution temperature (LCST) of PNIPAAm is 32 °C in water) is prevented through the shielding action of the PAA block. In addition, we show that the cloud point and aggregation properties (as measured by loss in light transmission) of the [(PNIPAAm)-*b*-(PAA)]−SA conjugate also depended on pH. At pH 7.0 and at temperatures above the LCST, the block copolymer alone was found to form particles of ca. 60 nm in diameter, while the bioconjugate exhibited very little aggregation. At pH 5.5 and 20 °C, the copolymer alone was found to form large aggregates (ca. 218 nm), presumably driven by hydrogen bonding between the −COOH groups of PAA with other −COOH groups and also with the −CONH− groups of PNIPAAm. In comparison, the conjugate formed much smaller particles (ca. 27 nm) at these conditions. At pH 4.0, however, large particles were formed from the conjugate both above and below the LCST (ca. 700 and 540 nm, respectively). These results demonstrate that the aggregation properties of the block copolymer−SA conjugate are very different from those of the free block copolymer, and that the outer-oriented hydrophilic block of PAA shields the intermolecular aggregation of the block copolymer−SA bioconjugate at pH values where the −COOH groups of PAA are significantly ionized.

Introduction

The ability to sequentially control biomolecular recognition and activity by two different stimuli, for example, to turn activities of different enzymes on or off in sequence, could open new opportunities or improve existing applications in the bioprocessing and diagnostic fields. Current methods for the control of protein and enzyme activities are still largely based on nonspecific and relatively large changes in solution conditions such as temperature and pH. We have developed a different approach to the control of biomolecular recognition that is based on coupling the stimulated collapse (dehydration) or expansion (rehydration) of "smart" polymer coils with recognition events such as protein−ligand or enzyme−substrate binding reactions. The smart polymers serve as both antennae and actuators, to sense signals and respond to them, leading to control of biorecognition events.[1] Their characteristic sharp responses in coil size and physical properties to small changes in pH, temperature, and/or UV−visible light permits rapid and precise control of molecular events. In addition to the control of bioactivities, stimuli-responsive conjugates with protein and DNA have also drawn considerable attention due to their reversible aggregation properties, which can be exploited for separations and processing applications.[2−4]

Two different mechanisms have been developed for switching biomolecular activities (Figure 1). The first relies on the direct stimulation of a smart polymer chain[5] that is conjugated to a protein at a specific position relative to the protein's active site (Figure 1A). The molecular collapse and expansion of the polymer chain as a function of temperature, pH, or specific light wavelength causes steric blocking and unblocking of the protein's active site. To prevent the intermolecular aggregation of the protein-smart polymer conjugates, the bioconjugates are immobilized onto a solid phase support such as chromatography beads.[6−8] While this is a convenient format for some diagnostic and separations applications, it is a limitation for others. A second mechanism has been more recently developed that exploits the aggregation behavior of stimuli-responsive polymer−protein bioconjugates that are free in solution before stimulation of the smart polymer to collapse (Figure 1B). If the polymer is conjugated near the active site, then in the aggregated state, the binding site is inaccessible and the protein is turned off.[9−11] However, if the polymer is attached far away from the active site, the protein's activity may be retained in both the free and aggregated states.

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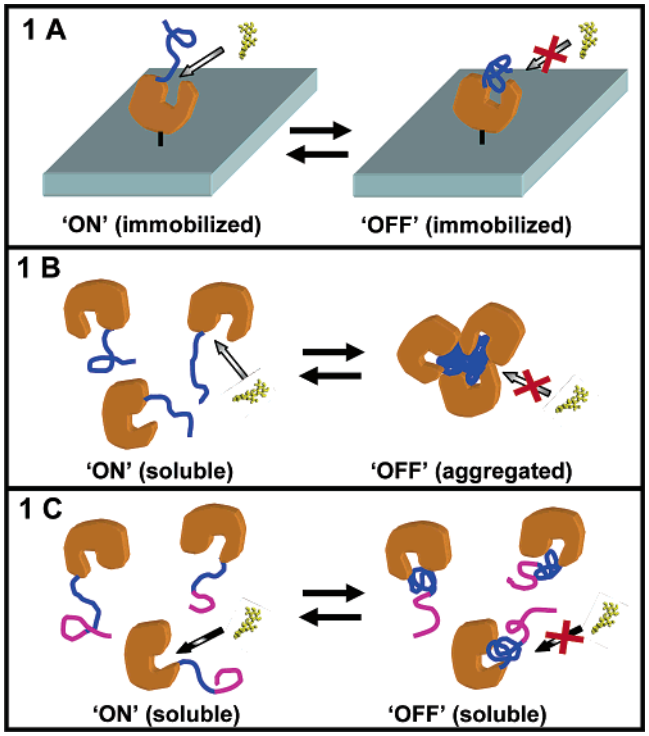

Figure 1. Schematic of the action of the smart block copolymer to switch enzymes "on" and "off" in solution without the associated aggregation or need to immobilize on a surface. (A) For an enzyme-smart homopolymer conjugate immobilized on a surface, when the polymer is expanded, the substrate has access to the enzyme binding pocket and the enzyme is "on". When the polymer is collapsed, it blocks the enzyme active site and the enzyme is switched "off". (B) For an enzyme-smart homopolymer conjugate free in solution when the polymer is hydrated, the substrate has access to the enzyme binding pocket. When the polymer collapses on application of a stimulus, the conjugate molecules aggregate into mesoscopic-size particles. This aggregate blocks access of the substrate to the binding pocket and enzyme is "off". (C) For an enzyme-smart block copolymer conjugate free in solution when the copolymer is expanded, the substrate has access to the enzyme binding pocket. When the PNIPAAm block collapses at higher temperature, aggregation is prevented by the hydrophilic PAA block. However, the collapsed PNIPAAm blocks substrate access to the binding pocket and enzyme is "off". In this mechanism, enzyme switching occurs in solution without the formation of aggregates.

These studies have motivated the design of a polymer for bioconjugation that provides the stimuli-responsive, reversible collapse and expansion and associated blocking and unblocking of the active site *without the associated aggregation behavior*. We hypothesized that the addition of a hydrophilic block to the end of the poly(*N*-isopropylacrylamide) (PNIPAAm) chain, which was directly conjugated to the protein, would prevent the usual intermolecular aggregation directed by the collapsed PNIPAAm segment, but still allow for the associated control of the protein's activity (Figure 1C). Previous studies of block copolymers of PNIPAAm with segments such as poly(ethylene glycol) (PEG) have shown that, above the lower critical solution temperature (LCST), the blocks formed polymeric micelles with a core of collapsed PNIPAAm stabilized by a corona of PEG.[12,13] If such a block copolymer were to be conjugated to a protein, we would expect the conjugate to be stabilized in solution by the PEG. In our study reported here, we concluded that it was desirable to have as the hydrophilic block a weak anionic polyelectrolyte instead of PEG because that could add pH (i.e., acid) sensitivity to the bioconjugate. Additionally, the pH sensitive block could be used to recover the enzyme or protein after switching applications by lowering the pH and phase separating the conjugate. In this study, dynamic light scattering has been used to investigate the phase separation and particle formation of a site-specific streptavidin (SA) bioconjugate that is conjugated with a poly(*N*-isopropylacrylamide)-*block*-poly(acrylic acid) (PNIPAAm-*b*-PAA) copolymer near the SA biotin binding site. The data indicate that, at pH 7.0, there is no thermally induced phase separation of the bioconjugate, in contrast with a PNIPAAm−SA conjugate which phase separates.

Experimental Procedures

Synthesis and Molecular Characterization of PNIPAAm-*b*-PAA. The synthesis of the block copolymer by RAFT polymerization has been described earlier.[14,15] First, acrylic acid was polymerized in methanol/water (4:1) with VAZO-88 as initiator and 1-cyanoethyl 2-pyrrolidone-1-carbodithioate as chain transfer agent at 90 °C. This polymer with $M_n = 7.9$ kDa (DP = 110) and $M_w/M_n = 1.19$ was purified and then used as a polymeric chain-transfer agent for the polymerization of *N*-isopropylacrylamide in methanol in the presence of AIBN at 60 °C. The block copolymer was found to have a total M_n = 17.4 kDa and $M_w/M_n = 1.09$; thus, the PNIPAAm block has $M_n = 9.5$ kDa and DP = 84. All molecular weights were measured by MALDI-TOF mass spectrometry and the polydispersity index by SEC in DMF.

The dithiocarbamate end groups of the synthesized polymers were hydrolyzed under basic conditions to yield the corresponding thiol-terminated polymers. The polymer was dissolved in a mixture of MeOH/aq 28% NaOH (7:3) for 3 days. Ethylenediaminetetra(acetic acid) (EDTA) was added to the solution to prevent oxidation of the resultant thiol groups. The reaction mixture was acidified with 88% formic acid, MeOH was evaporated, and the residue was dialyzed in phosphate buffer (PB), pH 7.2, overnight. The polymer was then lyophilized and stored as dry block copolymer.

Biotinylation of PNIPAAm-*b*-PAA. The block copolymer was biotinylated using a functionalized biotin, 1-biotinamido-4-[4′-(maleimidomethyl)cyclohexanecarboxamido]butane (BMCC) (Pierce). Briefly, BMCC was dissolved in DMSO at a concentration of 16 mg/mL. Four milligrams of the polymer was dissolved in 850 μL of PB, 50 mM, pH 6.6, just prior to the reaction. BMCC solution (75 μL) was added to the polymer solution, and the reaction was incubated at room temperature for 39 h. The excess BMCC was removed by desalting on a PD-10 column (Pierce) using deionized distilled water as an eluent after centrifuging residual undissolved BMCC. The efficiency of biotinylation of the copolymer was determined using the neutravidin−HABA assay as described by Lackey et al.[16]

Complexation of Biotin-PNIPAAm-*b*-PAA with SA. The biotinylated copolymer was mixed with SA in a 2:1 molar ratio in 50 mM PB, pH 7.2. The ratio was calculated by taking into account the biotinylation efficiency of the polymer. Briefly, 2 mg of the lyophilized biotinylated block copolymer was added to 1 mL of a 3 mg/mL solution of wild-type tetrameric SA to obtain a stock concentration of 58 μM for the bioconjugate. The conjugation of the polymer to the SA was confirmed by SDS−PAGE analysis. For the measurement of size, the conjugate was diluted to a final concentration of 0.5 μM in phosphate−citrate buffers at pH 4, 5.5, and 7.

Turbidimetric Measurements. The cloud point was determined as the temperature at 10% of the maximum absorbance

at 500 nm. PNIPAAm-*b*-PAA at a concentration of 0.2 wt % (equivalent to 116 μM) in phosphate−citrate buffered solvents at various pH values was used. The data were collected using a UV−vis spectrophotometer with a jacketed cuvette holder to control the temperature of the sample. A heating rate of 0.5 °C/min was used, and absorbance values were measured every 30 s. For the conjugate of PNIPAAm-*b*-PAA with SA, a concentration of 58 μM was used in pH 4.5 and pH 7 buffers.

Dynamic Light Scattering. Sizing measurements were performed using a Brookhaven BI90Plus instrument, as described earlier.[11] A heater within the cuvette holder of the instrument was used to heat or cool the sample to various temperatures. The temperature of the sample was measured using a thin wire thermocouple (Cole Palmer). Measurements were performed at several temperatures between 15 and 45 °C (below and above the LCST of the PNIPAAm). Fresh samples were prepared on different days and measurements were performed in triplicate. A 656 nm laser source was used as the incident beam and the scattered light from the sample was detected at a 90° angle. Along with the particle size measurements, the average scattering intensity (number of counts per second, cps) was also recorded. The average count rate of the background was 700 cps and that of the measurements were in the range of 40−200 × 10^3 cps.

The intensity autocorrelation function (ACF), $G^{(2)}(\tau)$, was measured in the self-beating mode and can be expressed by the Siegert relationship:

$$G^{(2)}(\tau) = B(1 + f^2|g^{(1)}(\tau)|^2)$$

where τ is the decay time, B is a measured baseline, f is the coherence factor, and $g^{(1)}(\tau)$ is the normalized first-order electric field time autocorrelation function $E(\tau)$. $g^{(1)}(\tau)$ is related to the line-width distribution $G(\Gamma)$ by:

$$|g^{(1)}(\tau)| = \int_0^\infty G(\Gamma) \exp(-\Gamma\tau)\, d\Gamma$$

Using the cumulant analysis,

$$|g^{(1)}(\tau)| = \exp(-\Gamma\tau)[1 + (\mu_2/2!)\tau^2 - (\mu_3/3!)\tau^3 + ...]$$

the average line width $\langle\Gamma\rangle$ is obtained. This yields the apparent diffusion coefficient, $D_{app} = \langle\Gamma\rangle/q^2$. The hydrodynamic diameter, D_h, is then determined using the Stokes−Einstein relation:

$$D_h = k_B T/(6\pi\eta_o D)$$

where k_B, T, and η_o are the Boltzmann constant, the absolute temperature and solvent viscosity, respectively. For relatively narrow decay-rate distributions, the second moment, μ_2, designated as the polydispersity index, can be used to estimate the relative distribution width. Assuming a log-normal size distribution for the particles, the full width at half-maximum (fwhm) was calculated from D_h and μ_2 using:

$$\text{fwhm} = D_h\sqrt{\mu_2}$$

In addition to the method of cumulants, the intensity correlation function was also analyzed by the nonnegatively constrained least squares (NNLS) (multiple pass) to obtain a size distribution for the particles.[17,18] The method of cumulants yields a unimodal distribution for particle size, whereas both unimodal and multimodal distributions can be obtained from the NNLS method. After analyzing the correlation function by both methods, the size obtained by the method of cumulants is designated as D_h, and the peaks of the size distribution obtained from NNLS are designated as D_{NNLS}. The number-averaged D_{NNLS} was obtained from the *z*-averaged diameter by the method described, and the distribution was plotted as frequency against the D_{NNLS}. The relative proportion of particles under each peak in a multimodal distribution was obtained by calculating the area under each peak in this plot.

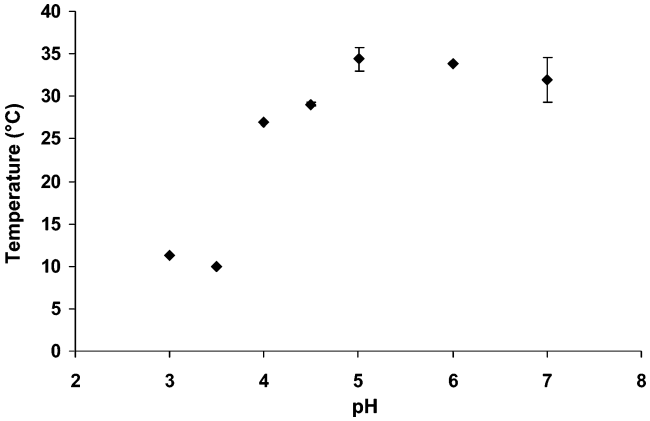

Figure 2. Dependence of the cloud point of PNIPAAm-*b*-PAA on pH is shown. The transmittance of the copolymer sample at a concentration of 0.2 wt % in various pH conditions was measured as the temperature of the sample was raised at a rate of 0.5 °C/min. The cloud point is defined as the temperature at which there is 10% change in transmittance. Error bars represent ± one standard deviation from mean.

Results & Discussion

Synthesis and Biotinylation of PNIPAAm-*b*-PAA. Using the RAFT technique, PNIPAAm-*b*-PAA with a narrow molecular weight distribution was synthesized. From MALDI-TOF studies, the number-average molecular weight (M_n) of the PNIPAAm-*b*-PAA was determined to be 17.4 kDa, and the weight-average molecular weight (M_w) was 18.75 kDa (M_w/M_n = 1.09). The PNIPAAm block had an M_n of 9.5 kDa, and the poly(acrylic acid) (PAA) block had an M_n of 7.9 kDa. The dithiocarbamate end group on the polymer from the chain-transfer agent was cleaved to generate a functional thiol end group that was used for end-specific biotin conjugation. Biotinylation yields were determined by the HABA assay and showed complete reaction within experimental error. The biotinylation efficiency was used to calculate the amount of polymer to be added to SA for complexation. In these studies, a molar ratio of two biotinylated PNIPAAm polymer chains to one SA tetramer was used. The conjugation of SA to the block copolymer was confirmed by SDS−PAGE analysis. Recently, a novel and facile method to conjugate biotinylated polymers to SA has been demonstrated by Maynard and co-workers.[19] By using biotinylated ATRP initiators, a greater number of polymers could be attached to SA. In our study, however, we have used a traditional method of conjugating biotinylated polymer to SA.

Cloud Point of PNIPAAm-*b*-PAA and Dependence on pH. As reported previously, the block copolymer displays temperature sensitivity in a pH-dependent fashion.[14] At pH values close to the pK_a of the PAA (ca. 4.5−5.0), the solubility of the PAA block decreases. Under these low pH conditions, the highly protonated PAA block lowers the cloud point of the copolymer by adding both H-bonding capability as well as hydrophobic character. The dependence of the cloud point on the pH of the solution is shown in Figure 2. A similar dependence of the cloud

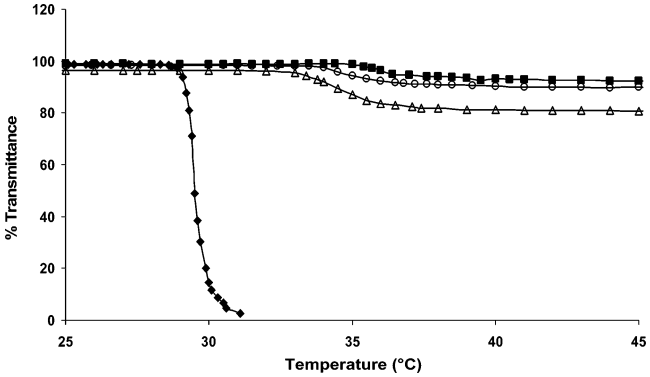

Figure 3. Turbidimetric properties of a 0.2 wt % solution of PNIPAAm-*b*-PAA as a function of pH is shown. The transmittance of the copolymer sample in various pH conditions was measured as the temperature of the sample was raised at a rate of 0.5 °C/min in pH 4.5 (−◆−), pH 5 (−■−), pH 6 (−○−) and pH 7 (−△−). Data points are means of three experiments.

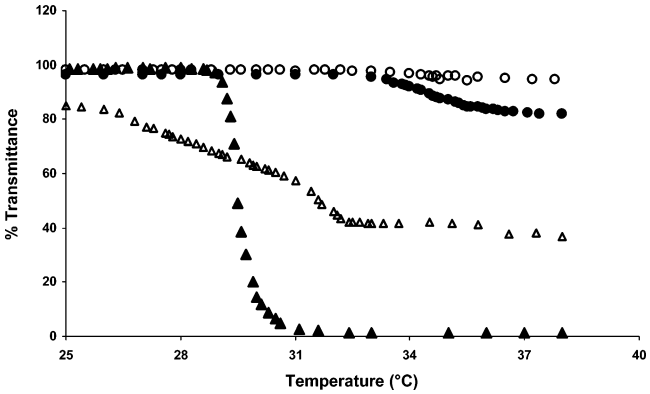

Figure 4. Turbidimetric curves of PNIPAAm-*b*-PAA and its streptavidin conjugate are shown. A concentration of 0.2 wt % (116 μM) was used for the block copolymer in pH 4.5 (▲) and pH 7 (●), and a concentration of 58 μM was used for the bioconjugate (the bioconjugate has two polymers per streptavidin, one on each binding face) in pH 4.5 (△) and pH 7 (○). Data points are means of three experiments.

point on pH for random and graft copolymers of NIPAAm and AA has been shown by Chen et al.[20] The underlying association mechanism and resulting particle properties for PNIPAAm-*b*-PAA were investigated by dynamic light scattering studies. In pH 7 buffer, ca. 60 nm particles (probably polymeric micelles) were formed above the LCST value as determined by Schilli et al.[14] These values were confirmed by our study, and seen optically as a small change in the transmittance of a copolymer solution (Figure 3). However, when the pH of the solution is reduced to near the pK_a of the PAA block, the polymer precipitates out of solution as mesoscopic (690 ± 170 nM) particles. The turbidimetric plots of the block copolymer at various pH values are shown in Figure 3.

Cloud Point of PNIPAAm-*b*-PAA−SA Conjugate and Dependence on pH. Figure 4 shows the effect of conjugation on the cloud point behavior of the copolymer−SA conjugate. At pH 7, no change in turbidity is detected in the conjugate solution on heating through the LCST range of the free block copolymer. The collapsed PNIPAAm block is sandwiched between the charged PAA block and the SA at this pH, with the hydrophilic SA and PAA moieties shielding the PNIPAAm and preventing micelle formation. At pH 4.5, however, the conjugate displays the typical LCST behavior of the free block copolymer. At this pH, the overall transmittance decreased to 40% on heating the conjugate, whereas for the copolymer by itself at equivalent concentrations, the transmittance decreased to almost 1%. This sharp phase separation is probably due to H-bonding between −COOH groups and other −COOH groups on the PAA block, as well as between the −COOH groups and the −CONH− groups of the PNIPAAm blocks. At this pH, the more gradual (less sharp) temperature response of the conjugate vs the sharp phase separation of the pure copolymer is probably caused by the stabilizing effect of the SA on smaller phase-separated particles, reducing the tendency of the particles to continue aggregating. A 90% change in transmittance occurs over a 2 °C range for the copolymer, whereas it occurs over a range of 6 °C for the bioconjugate.

Properties of Particles Formed by PNIPAAm-*b*-PAA and its Conjugate with SA. At pH 7 and 20 °C, no particle formation was observed for the copolymer alone at a concentration of 1 μM or its conjugate with SA at a concentration of 0.5 μM (the concentration describes molarity of one unit of the bioconjugate, consisting of one SA molecule and an average of two block copolymers). At this temperature and pH, all components of the conjugate, i.e., PNIPAAm, PAA, and SA, are hydrophilic, and there is no driving force for the aggregation. For the copolymer alone, a size (i.e., *z*-average hydrodynamic diameter) corresponding to unimers of the copolymer (8 ± 5 nm) was obtained (Table 1). The particle size of the conjugate (D_h = 16 ± 7 nm) indicates that the bioconjugate also remains in solution as unimers.

As the copolymer solution at pH 7 was heated to 45 °C, particles, micellar structures with mean hydrodynamic diameter (D_h) of 62 ± 1 nm were observed (observed by dynamic light scattering using the cumulants analysis method). The scattering intensity increased from 4 to 40 kcps due to the particle formation. The size of the micelles thus formed did not change even at higher concentrations, as shown in Figure 5. In contrast, heating the SA conjugate from 20 to 45 °C at pH 7 did not result in a significant increase in scattering intensity (5 vs 4 kcps). The *z*-average particle size increased to D_h = 35 ± 1 nm over triplicate measurements. Further analysis using the NNLS method showed the presence of two peaks at 16 and 100 nm in the number-weighted hydrodynamic diameter distribution function. The 16 nm peak is believed to correspond to unimers and the ∼100 nm peak to aggregates of the conjugates. Most of the particles observed at this temperature (99%) were in a nonaggregated form, while only a small fraction (1%) of the particles were larger aggregates (Figure 6). These results suggest that both SA and the ionized PAA block shield the PNIPAAm block in the conjugate against intermolecular association at pH 7. In comparison, we have previously reported that PNIPAAm−SA conjugates form mesoscale particles (defined as particles in the size range 100−1000 nm; ∼300−800 nm, depending on concentration, polymer molecular weight, and heating rate) when heated to 45 °C.[8] These composite results show that the addition of the PAA block to the PNIPAAm has a significant effect on modulating the particle size of the phase-separated protein conjugates.

The aggregation behavior of the pure block copolymer at pH 5.6 has been investigated by Schilli et al.[14] They found that, at 20 °C, H-bonded complexes in the size range of 40−200 nm were formed. In our study, at pH 5.5 and 20 °C, the copolymer formed similar large aggregates (D_h ∼ 220 ± 80 nm), whereas smaller aggregates were obtained with the SA bioconjugate (D_h ∼ 27 ± 10 nm). It can be inferred from this that the conjugation of the smart block copolymer to SA influences the nature of these H-bonded complexes. On heating the conjugate solution

Table 1. Size of Particles Formed by PNIPAAm-*b*-PAA and Its Conjugate with SA as Measured by Dynamic Light Scattering[a]

		pH 7			pH 5.5			pH 4		
	temp (°C)	D_h	σ	D_{NNLS}	D_h	σ	D_{NNLS}	D_h	σ	D_{NNLS}
PNIPAAm-*b*-PAA	20	8	±5	4	218	±84	43; 450	216	±11	24; 450
	45	62	±1	58	61	±1	42; 420	188	±48	60; 177; 655
PNIPAAm-*b*-PAA−SA	20	16	±7	21	27	±10	16; 582	541	±90	420; 750
	45	35	±1	16; 99	57	±5	36; 415	688	±170	25; 530; 1210
PNIPAAm−SA[b]	20									
	45	381	±7	316						

[a] A concentration of 1 μM is used for the copolymer and 0.5 μM for the bioconjugates (because there are two polymers per SA). D_h refers to the hydrodynamic diameter measured assuming unimodal distribution, and D_{NNLS} refers to hydrodynamic diameter measured assuming multimodal size distribution. σ represents the standard deviation over triplicate measurements. [b] Particle sizes for PNIPAAm−SA obtained from Kulkarni et al.[8] All sizes are in nm.

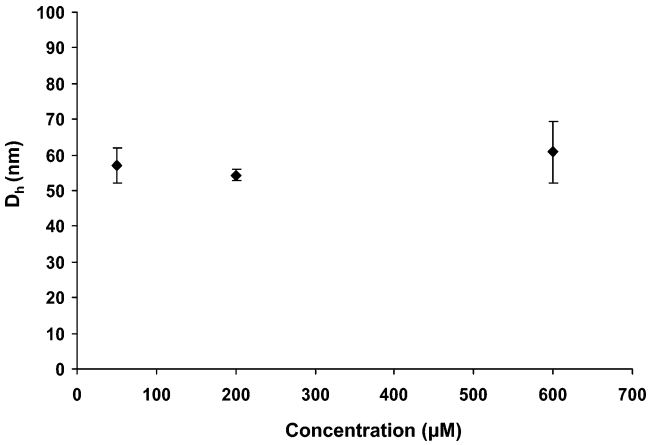

Figure 5. Concentration dependence of the size of micelle-like particles formed by the block copolymer at pH 7 and 45 °C is shown. D_h represents the hydrodynamic diameter and is the average of triplicate measurements at each concentration with freshly prepared samples. Error bars represent ± on standard deviation from mean.

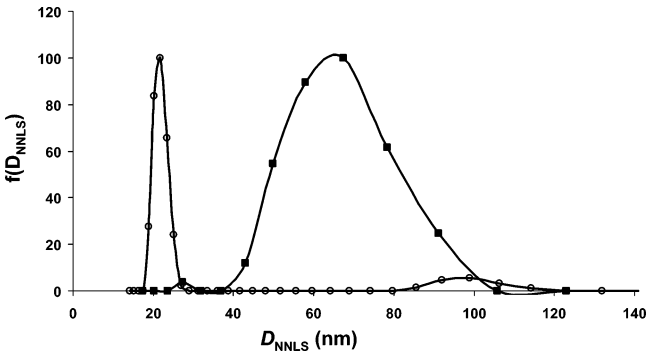

Figure 6. Number-weight average size distribution functions of the particles formed by the block copolymer and the bioconjugate at pH 7 and 45 °C. The distribution functions, $f(D_{NNLS})$, of the hydrodynamic diameter of the particles formed by the block copolymer and the bioconjugate in pH 7 phosphate−citrate buffer at 45 °C are shown. The size distributions are obtained by using the NNLS method of analysis assuming a multimodal size distribution of the intensity correlation function obtained by light scattering. The copolymer alone has a unimodal distribution, whereas the bioconjugate is seen to have a bimodal distribution. Data points are means of three experiments.

to 45 °C at pH 5.6, particles with D_h of 57 ± 10 nm were obtained. Similar particles with D_h of 61 ± 1 nm were formed by the copolymer alone.

At low pH conditions (pH 4 or 4.5), the PAA is sufficiently protonated to drive the aggregation of the conjugate. For the copolymer alone, particles in the size range 200−500 nm were formed at 20 °C.[14] For the conjugate, particles in the size range of 500−600 nm were observed. As the conjugate was heated, a 10-fold increase in scattering intensity was observed. Large particles in the size range of 600−800 nm were formed. The size of the particles formed at this pH was not stable over time, and the particles continuously aggregated to form larger particles (>1 μm). Complex H-bond interactions may be occurring to make the block copolymer precipitate more hydrophobic, which drives this aggregation process, as discussed above.

Conclusions

We have shown that a conjugate of streptavidin (SA) and a PNIPAAm-*b*-PAA block copolymer has significantly different particle-forming properties than either the corresponding PNIPAAm−SA conjugate or the isolated block copolymer. Most notably, the addition of the PAA block prevents the formation of large, mesoscale conjugate particles above the LCST at pH 7. This has important implications in protein switching applications, where thermally stimulated switching effects can be isolated from the aggregation behavior of enzyme−polymer conjugates, eliminating the need for immobilization of the conjugates. In addition, we have shown that the SA block significantly alters the properties of the H-bonded complexes formed by the block copolymer alone at lower pHs.

The properties of this nanoscale particle system provide interesting opportunities in the bioanalytical field, particularly for bioprocessing applications relying on sequential processes, such as enzymatic activity followed by separation. Using the block copolymer, it should be possible to control the enzyme activity first, in solution, by using the actuation switch at pH 7 and then recovering the enzyme by lowering the pH and phase-separating the enzyme−block copolymer complex.

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Abbreviations

PNIPAAm: poly(N-isopropylacrylamide)

PAA: poly(acrylic acid)

PNIPAAM-b-PAA: poly(N-isopropylacrylamide)-block-poly-(acrylic acid)

SA: streptavidin

NNLS: non-negatively constrained least squares

LCST: lower critical solution temperature

PB: phosphate buffer

DP: degree of polymerization

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