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Trigger-Responsive Poly(β-amino ester) Hydrogels

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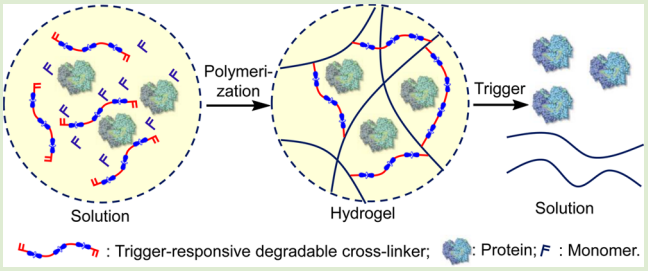
Supporting Information

ABSTRACT: Water-soluble, acrylate-terminated poly(β-amino esters) with built-in trigger-responsive domains were synthesized through Michael addition of trigger-responsive diacrylates and primary amines. They were used as macromolecular precursors for photoinitiated cross-linking reactions to prepare trigger-responsive hydrogels for protein encapsulation. The encapsulated proteins could be rapidly released upon external triggering.



Polymeric hydrogels are extensively used in various biomedical applications, including drug delivery and tissue engineering.[1−6] They are network materials with a hydrophilic polymer physically or chemically cross-linked. Covalent cross-linking is typically achieved through photoinduced radical reactions[7−10] in which terminal vinyl or pendant functional groups of the polymers are cross-linked, often with the addition of vinyl monomers. The photoinduced vinyl cross-linking reaction, because of its simplicity, has been routinely used for in situ preparation of polymeric hydrogels.

Dynamic, trigger-responsive, degradable hydrogels that can undergo rapid chemical, physical, mechanical, or morphological changes in response to external triggers have attracted much attention.[11−13] To develop trigger-responsive hydrogels for in situ encapsulation and triggered release applications, the trigger-responsive motifs should be either placed in polymer backbones that can lead to polymer degradation or in cross-linkers that can be cleaved upon trigger activation. It is essential that both polymeric building blocks and cross-linkers are water-soluble for in situ hydrogel formation. Given the limited number of water-soluble polymers with built-in trigger-responsive domains, prior studies have been primarily focused on the design of water-soluble, trigger-responsive cross-linkers. Water-soluble cross-linkers responsive to pH,[14−17] enzyme,[1] redox,[18−20] or light[21] have been developed and used for in situ hydrogel formation. Nevertheless, synthesis of these hydrophilic small molecular cross-linkers may require multiple steps and may become quite challenging.[1,14−27] Developing an alternative strategy that allows in situ formation of trigger-responsive hydrogels is of great interest.

Amine-containing polymers are able to achieve water solubility through ionization of the amine groups that absorb large amounts of water and, therefore, are excellent building blocks for making cross-linked polymer hydrogels. These polymers, however, can be fairly expensive and difficult to make (e.g., polylysine[28]); some may not have well controlled structures and may lack desirable properties (e.g., branched polyethylenimine that lacks structure control and degradation capability). For the intended biomedical and biological applications of hydrogels, it is ideal that the amine-containing polymers have built-in degradable functional groups, such as ester bonds. Linear polyesters bearing pendant amine functional groups are rare and may not be stable because of the amidation reaction of pendant primary or secondary amines with the main-chain ester bonds. In 2000, Lynn et al. reported the synthesis of poly(β-amine ester) (PBAE), a class of polyesters bearing a non-nucleophilic tertiary amine in their backbones via the Michael addition of a diacrylate (bearing ester bonds) with a primary amine or a bis-secondary amine.[29] The chemistry is easy to control and the resulting PBAEs have versatile structures and properties, given the wide availability of both diacrylate and amine monomers. While they have been primarily used for gene and siRNA delivery,[30,31] PBAE as building blocks for making hydrogels have also been reported.[32−34]

In this paper, we report the design of diacrylates containing acid-sensitive ketal bonds or reduction-responsive disulfide bonds, and use them to make trigger-responsive PBAE macromers for cross-linking reactions and in situ hydrogel formation. The degradation capability of hydrogels can be greatly enhanced by increasing the number of trigger-responsive, degradable domains in the backbone of PBAE.[35−39] Protein-encapsulated, trigger-responsive hydrogels were prepared by a photoinitiated cross-linking reaction of the PBAEs (terminated with acrylate) in the presence of added acrylamide. These hydrogels were able to provide excellent control over the release of encapsulated proteins in response to external triggers (Scheme 1). Trigger-responsive PBAEs can potentially be broadly used as water-soluble, degradable materials for the design of trigger-responsive structures that

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can control burst cargo release or in cell and tissue engineering area for the investigation of the biological response to the kinetics of scaffold degradation and rapid environmental changes.

Acid-responsive PBAE-KT, a PBAE containing acid-sensitive ketal bond in each repeating unit, was prepared via the addition reaction of ((propane-2,2-diylbis(oxy)) bis(ethane-2,1-diyl) diacrylate (KTDA) and 2-(2-(2-methoxyethoxy)ethoxy)ethan-1-amine (mOEG-NH_2; Scheme 2). The KTDA/mOEG-NH_2 ratio was controlled at 5:4 to ensure the resulting PBAE-KT macromer (M_n = 2.2 kDa and M_w/M_n = 1.70, entry 1, Table 1) to have acrylate terminal group on both ends. Using disulfanediylbis(ethane-2,1-diyl) diacrylate (SSDA) by following the same strategy, we also prepared PBAE-SS, a PBAE macromer containing a disulfide bond. The ratio of SSDA/mOEG-NH_2 was also controlled at 5:4 to ensure PBAE-SS macromer to have acrylate terminal groups for cross-linking reaction. PBAE-CT, a control PBAE that does not have trigger-responsive domain, was similarly prepared by reacting hexanediol-diacrylate (HDA) with mOEG-NH_2 at a molar ratio of 5:4. Both PBAE-SS and PBAE-CT have molecular weights (MWs) and molecular weight distributions (MWDs, MWD = M_w/M_n) similar to PBAE-KT (entries 2–3, Table 1). All three PBAEs were found to have excellent water solubility (>100 mg/mL). We also observed thermal responsiveness with these materials in phosphate buffered saline (1× PBS, pH 7.4), a property observed in mOEG-containing polypeptide, as reported by Li and co-workers,[40,41] with lower critical solution temperature (LCST) around 40–43 °C (Figure S4).

Scheme 1. Schematic Illustration of In Situ Preparation of Protein-Encapsulated, Trigger-Responsive Hydrogels and Protein Release via Triggered Disassembly of Hydrogels

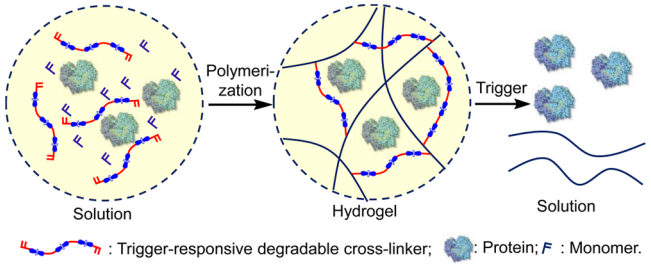


Table 1. Synthesis of PBAE Macromers

entry	polymer[a]	diacylate	amine	M_n[b] (×10^{-3})	MWD
1	PBAE-KT	KTDA	mOEG-NH_2	2.2	1.70
2	PBAE-SS	SSDA	mOEG-NH_2	2.5	1.64
3	PBAE-CT	HDA	mOEG-NH_2	2.3	1.56

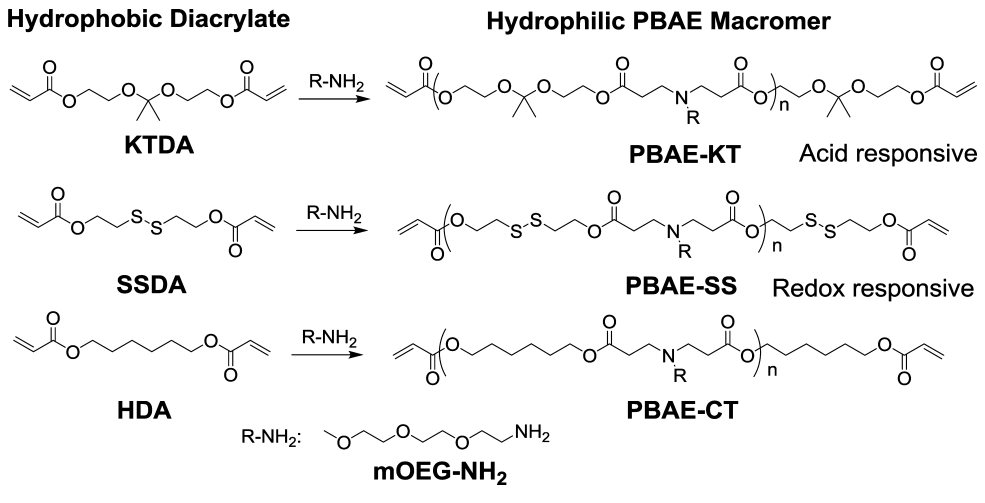
[a]The polymers were prepared by solvent-free Michael addition from diacrylates and mOEG-NH_2 with a 5/4 ratio of diacrylate/mOEG-NH_2 at 60 °C for 24 h. [b]The MW obtained by GPC.

PBAE-KT is expected to be stable at neutral or basic conditions yet should become unstable at low pH and subsequently degrade into low molecular weight fragments because of its built-in ketal bonds (Figure 1a). As expected, PBAE-KT at pH 8.0 was very stable. No degradation was observed by ^{1}H NMR after 4 h (Figure 1b). When the same PBAE-KT sample was dissolved in D_2O/DCl (pH = 2) for 4 h, it was completely degraded, as shown by the disappearance of the ketal methyl group at δ 1.35 ppm in Figure 1b.

We next studied the trigger responsiveness of the PBAE-KT hydrogel. Bovine serum albumin-fluorescein (BSA-Fluo) was prepared via the reaction of fluorescein isothiocyanate with BSA. BSA-Fluo encapsulated polyacrylamide (PAA) hydrogel was prepared via UV-induced radical reaction of PBAE-KT macromer and acrylamide in 1× PBS (pH 7.4, Figure 2a). Specifically, a mixture of PBAE-KT (1 wt %), acrylamide (AA, 5 wt %), 2-hydroxy-4′-(2-hydroxyethoxy)-2-methylpropiophenone (I-2959, UV initiator, 0.05 wt %), and BSA-Fluo (2.5 wt %) in PBS solution was irradiated by UV (365 nm, 10 mW/cm^2) for 10 min, resulting in BSA-Fluo encapsulated hydrogels. To test the pH-responsiveness of the PBAE-KT/PAA hydrogel, a pH 6 buffer solution was added to the vial containing the hydrogel. The hydrogel framework was completely disrupted and the materials were dissolved in the aqueous solution completely after 8 h (Figure 2a-iii and -iv). In comparison, BSA-Fluo encapsulated in PAA or PBAE-CT hydrogel treated with pH 6 buffer showed no sign of hydrogel structure disruption (Figure 2a-i and -ii).

As expected, the release kinetics of BSA-Fluo from the PBAE-KT/PAA hydrogels showed pH dependence. At pH 7.4, the hydrogel was perfectly stable and no obvious BSA-Fluo

Scheme 2. Synthesis of PBAE-KT, PBAE-SS, and PBAE-CT



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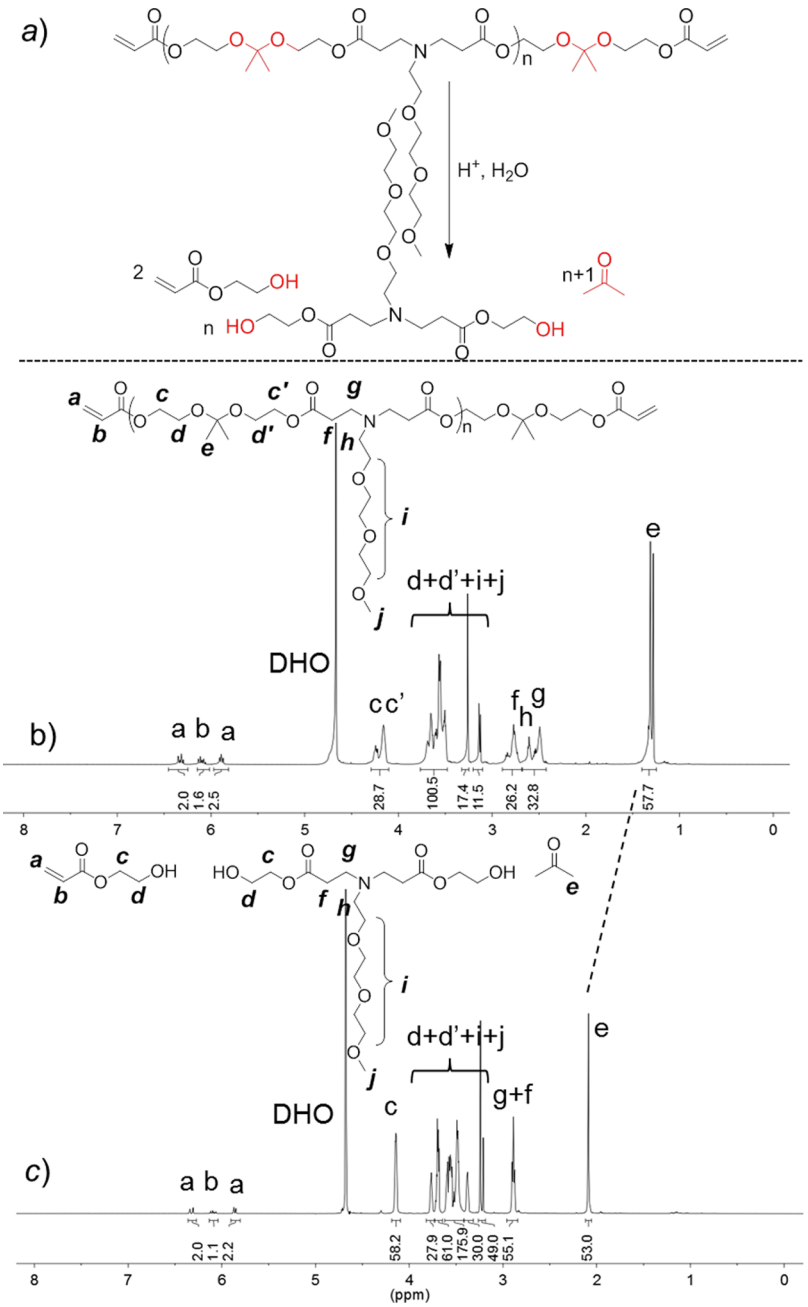

Figure 1. (a) Proposed mechanism of disassembly of ketal bond containing pH-responsive PBAE cross-linker under acidic condition; (b) ^{1}H NMR spectra of PBAE-KT in D_2O (pH = 8.0, 4 h); (c) ^{1}H NMR spectra of PBAE-KT D_2O/DCl (pH = 2.0, 4 h).

release was observed for at least 5 h. At pH 6, substantial BSA-Fluo release was observed, substantiating that the triggered rupture of the hydrogel network can be controlled within a very small pH window. It is known that responsive pH of ketal bonds is around 5. However, even in a slightly acidic solution (pH 6), the hydrogel can be significantly responsive (Figure 2c). Such excellent responsiveness may be due to the design of the triggered degradable hydrogel with an average of five ketal bonds per PBAE macromer and cleavage of any one of the five ketal bonds would result in cleavage of the polymer and rupture of hydrogel structure (Figure 2b). In a control study, BSA-Fluo encapsulated in PAA or PBAE-CT hydrogels at pH 6 buffer showed negligible release of BSA-Fluo for at least 6 h. (Figure S5).

Using PBAE-SS and following the same strategy as with the PBAE-KT/PAA hydrogel, we prepared BSA-Fluo encapsulated PBAE-SS/PAA hydrogel and quantitatively assessed the release of BSA-Fluo at pH 7.4 in the presence or absence of DTT. Figure 3a illustrates the proposed mechanism of the disassembly of disulfide-containing redox-responsive hydrogel in response to DTT treatment. At pH 7.4, the release of BSA-Fluo from PBAE-SS/PAA hydrogel without DTT treatment was very slow with cumulative release of ~10% BSA-Fluo after 1.3 h (Figure 3b). When treated with DTT at a concentration of 20 mM, the release kinetics of BSA substantially increased, with a half-life of 0.59 h. After 1.3 h, the cumulative release ratios for all DTT-treated groups were close to be 100% (Figure 3b).

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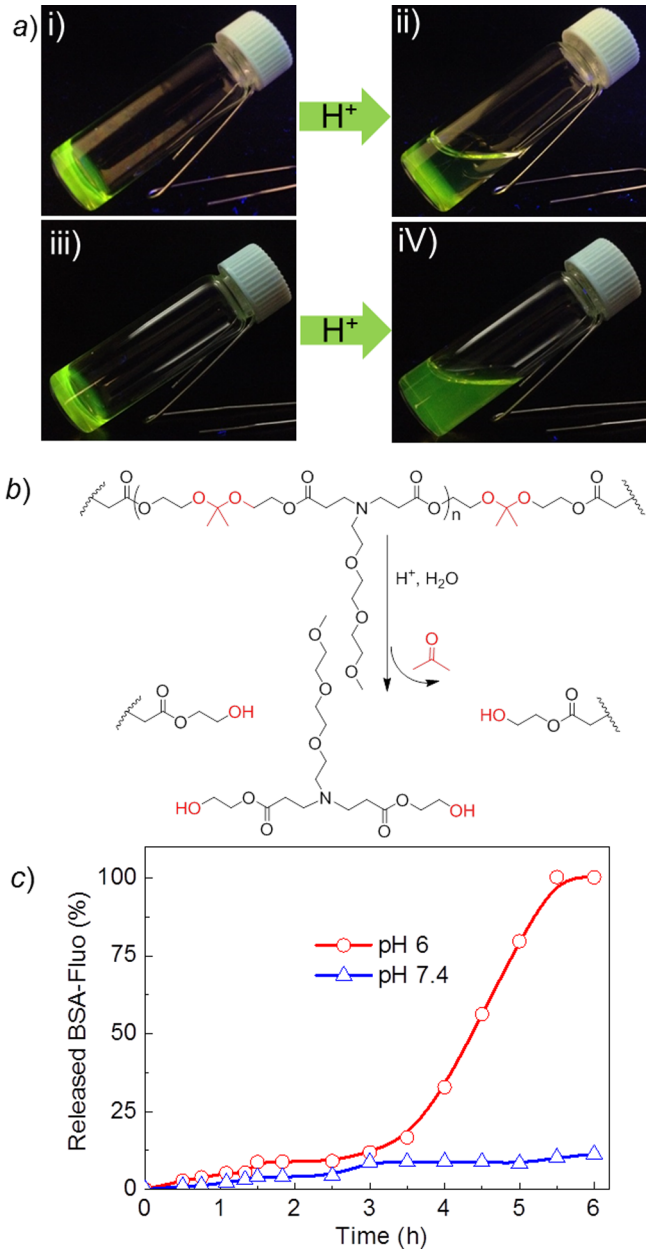

Figure 2. (a) Photography for the BSA-Fluo encapsulated PBAE-CT/PAA and PBAE-KT/PAA hydrogel before and after reduction treatment under UV irradiation (365 nm): (i) PBAE-CT/PAA at pH 7.4 PBS buffer; (ii) PBAE-CT/PAA in a pH 6.0 buffer for 8 h; (iii) PBAE-KT/PAA in pH 7.4 PBS buffer; (iv) PBAE-KT/PAA in a pH 6.0 buffer for 8 h. (b) Proposed mechanism of disassembly of ketal-containing pH-responsive hydrogel under acidic condition. (c) Release profiles of BSA-Fluo from PBAE-KT/PAA hydrogels at pH 6 and 7.4.

In summary, water-soluble trigger-responsive degradable PBAE macromers were prepared via the Michael addition reaction between stimuli-responsive small molecular monomers and primary amines. These polymeric cross-linkers were used in the preparation of the protein encapsulated hydrogel. The resulting hydrogel demonstrated excellent controlled release of the protein in response to external conditions. We believe that the trigger-responsive cross-linked polymers may find applications in triggered release, drug delivery, and tissue engineering.

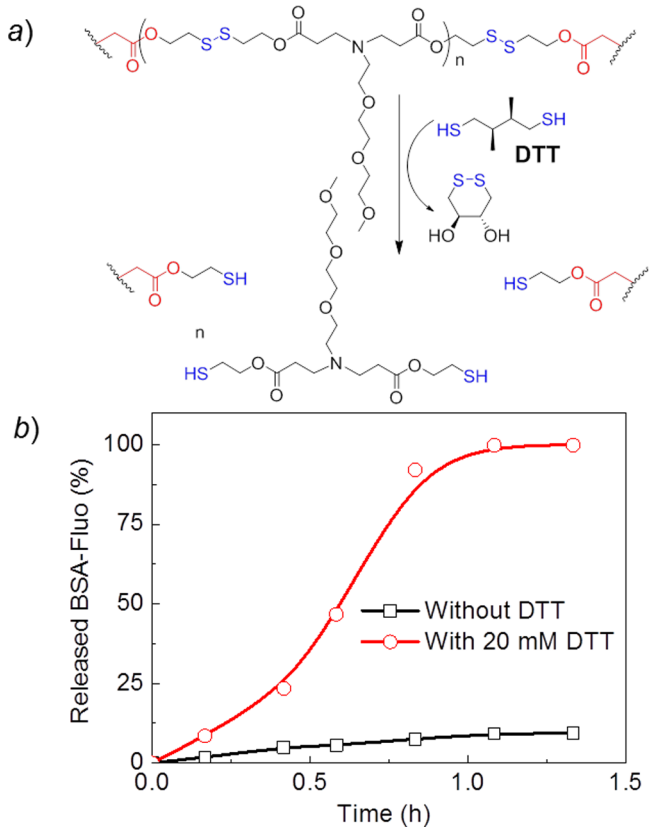

Figure 3. (a) Proposed mechanism of disassembly of disulfide-containing redox-responsive hydrogel under DTT conditions; (b) Release profiles of BSA-Fluo from PBAE-SS/PAA hydrogels at pH 7.4 with 20 mM DTT concentrations.

■ ASSOCIATED CONTENT

Supporting Information

Experimental details, including monomer synthesis and characterizations, polymerization, and polymer characterizations by NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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