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Triggered copolypeptide hydrogel degradation using photolabile lysine protecting groups

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ABSTRACT: We have prepared a new L-lysine based N-carboxyanhydride monomer containing a photolabile *o*-nitrobenzyloxycarbonyl protecting group. This monomer was used to prepare poly(L-lysine)-*block*-poly(*o*NB-L-lysine) block copolypeptides that formed hydrogels with tunable physical properties and the capability to be degraded by UV irradiation. In these materials, the *o*NB-lysine residues were found to be excellent surrogates for the hydrophobic residues typically used to form block copolypeptide hydrogels, thus adding functionality without adversely altering self-assembly characteristics. Upon irradiation, full cleavage of the *o*-nitrobenzyloxycarbonyl groups was observed, resulting in dissolution of the product, poly(L-lysine), and complete hydrogel disruption. When dye molecules were entrapped in the hydrogels, photolysis resulted in release and mixing of these molecules with surrounding media.

Hydrogels possess many useful properties and have considerable potential for use in biology and medicine, including for the delivery of molecules and cells and as structural scaffolds.¹⁻³ There has been much recent interest in tuning hydrogel physical properties using external stimuli, i.e. temperature, redox, and light,^{4,5} with particular attention to photoresponsive hydrogels since the input light can be highly selective, spatially localized, and minimally disruptive to cells and tissues.⁶ In this area, polypeptide based photoresponsive materials are attractive due to their relevance to biology and medicine, yet methods for preparation of physically-cross-linked photoresponsive polypeptide hydrogels are needed.^{4,5} In effort to address this challenge, we describe a new L-lysine based N-carboxyanhydride monomer containing a photolabile *o*-nitrobenzyloxycarbonyl protecting group (*o*NB-Lys NCA). This monomer was used to prepare diblock copolypeptides designed to form hydrogels with tunable physical properties (Scheme 1). Upon UV irradiation, cleavage of the *o*-nitrobenzyloxycarbonyl groups resulted in complete hydrogel disruption and release and mixing of entrapped molecules with surrounding media. This photoswitchable solubility makes *o*NB-Lys NCA useful for preparation of self-assembled, photoresponsive polypeptide materials.

Photodegradable hydrogels have been prepared using a variety of chemistries, and involve the light induced disruption of either chemical or physical crosslinks.⁶⁻¹⁰ There are also many examples where photoresponsive groups have been attached to polypeptides in order to alter material properties upon irradiation. Many early studies focused on altering polypeptide chain conformations by attaching groups whose polarity can be switched by light.¹¹ Recent work has focused on using similar conjugation of photoresponsive groups in polypeptides to obtain photoswitchable assemblies.¹² Post-polymerization conjugation of photoactive groups to polypeptide segments in block copolymers has been used by many groups to create micelles that can either photodegrade or pho-

tocrosslink.^{13,14} Preparation of NCA monomers containing photoresponsive side-chain modifications has also been explored.

Scheme 1. Synthesis of photodegradable, hydrogel forming $\text{K}_m \text{K}_n^{\text{oNB}}$ diblock copolypeptides.

While preparation of functional NCAs requires more synthetic effort up front, the ability to precisely incorporate these functional residues in a controlled polymerization provides advantages for preparation of well-defined materials.¹⁵ L-Glutamate NCAs containing either 4,5-dimethoxy-2-nitrobenzyl¹⁶ or cinnamyl¹⁷ photoresponsive groups, and *o*-nitrobenzyl-L-cysteine NCA¹⁸ have been prepared and were used to prepare block copolymer micelles or vesicles that could be either crosslinked or disrupted by light. To obtain photocrosslinked polypeptide hydrogels, Yamamoto prepared 7-coumaryloxyacetyl-L-lysine NCA and copolymerized it with benzyloxycarbonyl-L-lysine NCA (Z-Lys NCA) to give

statistical copolypeptides of different compositions.¹⁹ Selective deprotection of the Z-L-lysine residues gave water soluble (L-lysine)-(7-coumaryloxyacetyl-L-lysine) copolymers that were photocrosslinked to obtain covalent hydrogels.

Here, we sought to prepare physically cross-linked hydrogels that could undergo photodegradation as a means to potentially effect triggered release of entrapped molecules. Our design was based on our family of diblock copolypeptide hydrogels (DCH) composed of L-lysine and L-leucine segments, e.g. poly(L-lysine)₁₈₀-*block*-poly(L-leucine)₂₀, **K**₁₈₀**L**₂₀.²⁰ We have previously shown that these physical hydrogels are injectable for minimally invasive application, can entrap and passively release both protein and small molecule cargos, and can form deposits and are well tolerated in central nervous system tissues *in vivo* in mice.^{21–24} To create photodegradable DCH, we envisioned replacement of hydrophobic leucine residues with lysine residues containing photolabile protecting groups, where the protected residues would be hydrophobic to promote gelation, and once deprotected the lysine residues would become hydrophilic resulting in dissolution (Scheme 1).

Since our aim was to prepare block copolypeptides, we chose to use a photoreactive NCA, as opposed to post-polymerization modification, to obtain discrete copolymer segments via living copolymerization. Lysine was chosen as the photoresponsive residue since, once deprotected, the resulting poly(L-lysine) would be expected to have good water solubility. Glutamate and cysteine based monomers, as described above, were not utilized since anionic poly(L-glutamate) produced after photolysis could complex with the cationic poly(L-lysine) chains of DCH, and poly(L-cysteine) chains are known to exhibit poor water solubility.²⁵ Since Yamamoto's coumarin functionalized lysine NCA undergoes crosslinking reactions and not deprotection upon irradiation, it was also not suitable for our purpose. Hence, we prepared a new L-lysine based NCA monomer containing the photolabile *o*-nitrobenzyloxycarbonyl protecting group (oNB-Lys NCA) (see SI, Scheme S1).

o-Nitrobenzyloxycarbonyl-L-lysine amino acid was prepared according to known procedures^{26,27} and then converted to the target NCA using phosgene. The oNB-Lys NCA was purified and obtained in satisfactory yields of greater than 80%. Since initiation of oNB-Lys NCA polymerizations using Co(PMe₃)₄ in THF led to polymer precipitation and side-reactions with the oNB groups, we tested the ability of this monomer to prepare well-defined polypeptides in a chain-extension experiment. An active segment (degree of polymerization = 60) of poly(Z-L-lysine) was prepared from Z-Lys NCA and Co(PMe₃)₄ in THF (see Table S1).²⁸ To aliquots of these active chains were added different quantities of oNB-Lys NCA to prepare poly(Z-L-lysine)-*block*-poly(oNB-L-lysine) copolypeptides. All of these copolymerizations went to completion of monomer consumption and gave copolypeptides in greater than 95 % isolated yield. Lengths of poly(oNB-L-lysine) segments were obtained by comparison of ¹H NMR integrations for oNB-L-lysine and Z-L-lysine residues, and showed excellent agreement with monomer to initiator feed stoichiometry (see Table S1). GPC analysis of the initial poly(Z-L-lysine) segment and block copolypeptides also showed the copolymers were well-defined with narrow chain length distributions (see Table S1, Figure S1).

Having verified that oNB-Lys NCA was of sufficient purity and suitable for preparation of well-defined block copolypep-

tides, we prepared a series of poly(Z-L-lysine)₁₈₀-*block*-poly(oNB-L-lysine)_x, **K**₁₈₀**K**^{oNB}_x, samples, where *x* = 20, 30 and 40, designed to mimic known DCH compositions (Scheme 1).²⁰ To obtain samples with compositions intermediate between **K**₁₈₀**K**^{oNB}_x and DCH, we also prepared another series of copolypeptides with equimolar L-leucine and oNB-L-lysine statistically copolymerized as the hydrophobic segment, i.e. poly(Z-L-lysine)₁₈₀-*block*-poly(oNB-L-lysine_{0.5}-stat-L-leucine_{0.5})_x, **K**₁₈₀(**K**^{oNB}/**L**)_x, *x* = 20 and 30 (see Scheme S2). Selective deprotection of the Z-L-lysine residues in all samples was then accomplished with 33% HBr/HOAc in TFA using optimized reaction conditions (see Table S2). Characterization of the copolypeptides showed that all samples were obtained in high yields, had compositions within 1.5 mol% of target values, and narrow chain length distributions (see Table S3).

Figure 1. Rheology of copolypeptide hydrogels. (A) Storage modulus, G' (Pa), and (B) loss modulus, G'' (Pa), for hydrogels of different compositions and concentrations (m/v). All G' and G'' values measured at 1 Hz, 22 °C.

All deprotected **K**₁₈₀**K**^{oNB}_x and **K**₁₈₀(**K**^{oNB}/**L**)_x copolypeptides were found to form transparent hydrogels in Milli-Q water over a range of concentrations (Figure 1, see Tables S4, S5). **K**₁₈₀**K**^{oNB}₄₀ was found to be the poorest gel former, as it formed very weak gels that precipitated at higher concentrations, likely due to the large hydrophobic content in this sample. All other samples formed elastic gels over a broad frequency range above a minimum concentration and increased in stiffness (G' , storage modulus) at higher concentrations, following trends similar to previously studied DCH (see Figure S2).²⁰ The use of oNB-L-lysine in the hydrophobic segments was found to increase stiffness of the gels at a specific concentration relative to samples incorporating L-leucine, likely due to the rigidity and large dipoles of the oNB groups. This trend can be seen in Figure 1 by comparison of G' values for **K**₁₈₀**K**^{oNB}_x and **K**₁₈₀(**K**^{oNB}/**L**)_x samples of identical *x* and

concentration. Overall, these data show that poly(oNB-L-lysine) is capable of replacing poly(L-leucine) as the hydrophobic component in preparation of DCH, and provides hydrogels with increased stiffness compared to leucine containing analogs.

Figure 2. Effects of irradiation ($I_0 = 125 \text{ mW/cm}^2$) on a 2 wt% hydrogel of $\mathbf{K}_{180}\mathbf{K}^{\text{oNB}}_{30}$ in Milli-Q water. (A) Compliance, J (Pa⁻¹), vs. time (s) as determined by measurement of creep compliance after different durations of hydrogel irradiation time t (min). (B) Percent deprotection of oNB-L-lysine residues as determined by ¹H NMR integrations (blue); and change in viscosity η (Pa·s, red, secondary axis) as determined from data of compliance vs. irradiation time (min).

With hydrogel forming samples in hand, we next studied the photoresponsive properties of these materials. We used $\mathbf{K}_{180}\mathbf{K}^{\text{oNB}}_{30}$ for these experiments as it forms relatively stiff, transparent hydrogels at low concentrations. Samples of 2 wt% hydrogel of $\mathbf{K}_{180}\mathbf{K}^{\text{oNB}}_{30}$ in 5 to 20 mM semicarbazide hydrochloride (1 eq per oNB-lysine, *o*-nitrosobenzaldehyde scavenger) in Milli-Q H₂O were irradiated for different times ranging from 0 to 30 minutes. The samples were then analyzed by ¹H NMR to determine extent of oNB-L-lysine deprotection, as well as creep compliance to evaluate hydrogel degradation (Figure 2). Compliance measurements showed significant loss of elasticity and decrease in viscosity even at very short irradiation times, while NMR analysis showed that deprotection of oNB-L-lysine groups also occurred quickly and were complete at *ca.* 15 min. The rapid photodegradation of these samples is encouraging for applications and is due to the high irradiation intensity ($I_0 = 125 \text{ mW/cm}^2$, 395 nm) used in this study. A series of photodeprotection experiments showed that deprotection, and hence hydrogel degradation, could be controlled and

slowed significantly by variation of irradiation intensity (see Figure S3).

Figure 3. Coalescence of different colored hydrogel samples with or without irradiation. (A) Blue and red dye containing 2 wt% $\mathbf{K}_{180}\mathbf{K}^{\text{oNB}}_{30}$ hydrogel samples (thickness = *ca.* 2 mm) placed in contact without irradiation and allowed to stand for 48 h. (B) Same mixed hydrogel sample as in A, but irradiated ($I_0 = 125 \text{ mW/cm}^2$) for 5 minutes, and then let stand for 48 h.

To demonstrate the potential utility of these photodegradable hydrogels, we separately entrapped water soluble, anionic blue and red dyes into 2 wt% $\mathbf{K}_{180}\mathbf{K}^{\text{oNB}}_{30}$ hydrogels. Since the dyes were anionic, they were expected to complex the cationic poly(L-lysine) segments of the gels and only slowly diffuse from the gels.²² Red and blue dyed gels were placed in contact, and then allowed to stand for 48 hours, either with or without irradiation (Figure 3). In the control sample with no irradiation, only minimal dye diffusion across the red/blue interface was observed, which showed good dye entrapment within the hydrogels. In the sample irradiated for 5 min, complete mixing of dye was observed after incubation due to the resulting low viscosity liquid environment. These results highlight the potential to entrap molecules within these hydrogels, and then trigger release of the molecules into a liquid environment by application of light.

In summary, we have reported the synthesis of a new photoresponsive lysine NCA monomer, oNB-Lys NCA, which was used to prepare photodegradable block copolypeptide hydrogels. In these materials, the oNB-lysine residues were found to be excellent surrogates for the hydrophobic residues typically used to drive hydrogel assembly, thus adding functionality without adversely altering self-assembly characteristics. UV irradiation of these new hydrogels can give complete removal of oNB groups leading to conversion to water soluble poly(L-lysine) products and hydrogel degradation. We plan to explore applications of these materials for phototriggered release of active molecules in biological settings.

ASSOCIATED CONTENT

Supporting Information. Experimental and synthetic procedures, additional Schemes S1-S2, Tables S1-S5, Figures S1-S3, and ¹H NMR data of new molecules and polymers.

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