Coumarin-Caged Polyphosphazenes with a Visible-Light Driven On-Demand Degradation

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Polymers that, upon photochemical activation with visible light, undergo rapid degradation to small molecules are described. Through functionalization of a polyphosphazene backbone with pendant coumarin groups sensitive to light, polymers which are stable in the dark could be prepared. Upon irradiation, cleavage of the coumarin moieties exposes carboxylic acid moieties along the polymer backbone. The subsequent macromolecular photoacid is found to catalyze the rapid hydrolytic degradation of the polyphosphazene backbone. Water-soluble and non-water-soluble polymers are reported, which due to their sensitivity toward light in the visible region could be significant as photocleavable materials in biological applications.

There is considerable demand for stimuli-responsive polymers that can undergo chemical or physical changes in response to triggers such as pH,[1] biomolecules,[2] temperature, oxidation,[3] and light.[4] Macromolecules which undergo triggered backbone disassembly or degradation upon response to stimuli could be of particular importance for a range of future applications including sensory materials, lithography, and triggered release systems.[5] One approach involves self-immolative polymers[6] which are designed to undergo end-to-end disassembly upon a triggering event.[7] More recently, a small number of chain-shattering polymers have been described, a term used to describe polymers with multiple responsive cleavage sites along the backbone.[10–8]

Among the investigated stimuli, light is notably attractive due to its ability to exert spatial and temporal control over the desired response. Consequently, several photo-responsive polymers have been prepared that have great promise in diverse applications such as drug delivery, porous membranes, and film patterning.[6b,9] Macromolecules that undergo photochemical backbone degradation are also of significant interest.[10] Most reported photo-cleavable systems require ultraviolet (UV) light to achieve cleavage, which can be a drawback especially in biomedical environments due to low penetration and biocompatibility, hence a shift to longer wavelengths is required.[11] Coumarin and its analogs are widely employed in various fields such as medicine, polymer science, cosmetics, and biology.[12] Coumarin derivatives can be readily synthesized and functionalized to red-shift the light absorption.[12] Cage the desired molecule as protecting groups,[13] and/or to be incorporated on the polymer main chain as photoresponsive units.[14] It is also reported that coumarin photocages can be cleaved via two-photon processes with near-infra-red irradiation.[15] In addition, functionalized aliphatic polycarbonates,[16] photodegradable hydrogels,[17] and drug delivery systems[18] based on coumarin derivatives have been developed. Furthermore, the ability of some coumarin polyesters to undergo chain scission or crosslinking upon certain wavelength irradiation has been demonstrated.[14,19]

Herein, we present a novel approach to photodegradable polymers based on coumarin-functionalized polyphosphazenes. Polyphosphazenes are unique due to their hydrolytically unstable backbone which can be tailored through the incorporation of different substituents.[20] Furthermore, the polyphosphazene degradation pathway is known to be acid-catalyzed,[21] a process which can be intramolecular when acidic substituents are present on the polymer backbone.[22] Hence we proposed that through functionalization of polyphosphazenes with a coumarin-caged amino acid as a pendant group along the backbone, the sensitivity of the polymers to hydrolysis would be accelerated upon irradiation by effectively producing a macromolecular photoacid which could subsequently catalyze its own degradation.

First 7-{(N,N-diethylamino)-4-(hydroxymethyl)coumarin 1 (Figure S1, Supporting Information) was prepared according to literature procedures.[15] After reaction with N-(tert-butoxycarbonyl)glycine (Boc-gly-OH) and deprotection, this gave the coumarin-caged glycine 3 (Figures S2 and S3, Supporting Information).[23] Coumarin 1 was chosen as caging group as it is expected to be photochemically active in the visible region
due to the electron-donating diethylamino group.\cite{24} The kinetics of the elementary photochemical reaction of 3 has been investigated recently by flash-photolysis\cite{23} but not under steady illumination. Hence, we first investigated the photocleavage reaction of compound 3 upon irradiation (100 W, ≥395 nm) in MeOH/H₂O (4:1) solution. UV–vis spectroscopy (Figure S4, Supporting Information) showed a decrease in absorption intensity and shifted to lower wavelength (from 382 to 377 nm) after irradiation, indicative of the decaging of the glycine moiety (Figure 1a). Interestingly, an increase in intensity and slight hypsochromic shift (from 478 to 473 nm) was observed in the emission spectra upon irradiation (Figure 1b). This ceased after approximately 25 min irradiation, indicating completion of the photosolvolysis reaction. ¹H NMR spectroscopy (Figure 1c) also confirmed the nature of the photocleavage reaction, albeit with a lower reaction rate due to the higher concentration required (10⁻⁷ mol L⁻¹ vs 0.082 mol L⁻¹).

Our approach was to extend this photodecaging phenomenon to “cage” a hydrolytically unstable glycine-substituted phosphazene moiety through incorporation of the coumarin-caged glycine onto a polyphosphazene backbone. Polydichlorophosphazene was first prepared via phosphine-mediated polymerization of trichlorophosphoranimine\cite{25} (Scheme 1, see Supporting Information for detailed procedure). First, the coumarin-cage 3 was added in order to substitute the majority of chlorine atoms. For related amino acid esters, it is known that monosubstitution at the phosphorus atom is strongly favored due to the higher reactivity of -Cl₂PN- in comparison to -CIRPN-.\cite{26} therefore, we expect a distribution of the coumarin groups along the backbone. Thereafter, the remaining chlorine atoms were substituted with either Jeffamine M-1000, a polyether monoamine, or glycine ethyl ester to obtain polymer P1 and polymer P2, respectively.

Polymer P1 was synthesized using Jeffamine M-1000 as the second substituent to give a water-soluble polymer. M-1000 was also chosen to ensure that the hybrid polymer remained hydrolytically stable in the timeframe of the photochemical reactions\cite{21} and hence to be able to induce a photochemical degradation without significant interference from undesired hydrolytic degradation. The polymer was purified by dialysis in the dark and characterized by ¹H and ³¹P NMR spectroscopy (Figure S6, Supporting Information), size exclusion chromatography (SEC) in DMF containing 10 mM LiBr (Figure S7, Supporting Information, Mₙ/GPC = 149 000 g mol⁻¹, and Mₘ/Mₙ = 1.03, measured using multidetector calibration) and dynamic light scattering (DLS) (Figure S8, Supporting Information, d = 12.25 nm ± 0.38 nm in H₂O at 1 mg mL⁻¹). According to ¹H and ³¹P NMR spectroscopy complete backbone substitution in a ratio of nearly 34:66 (coumarin derivative:M-1000) could be observed with no additional peaks in the ³¹P NMR corresponding to partially substituted phosphorous atoms. UV–vis spectroscopy (Figure S9, Supporting Information) showed the loading to be approximately 14 wt%, which corresponds roughly to 35:65 ratio, coumarin derivative to M-1000 substituents.

The polymer was irradiated (100 W, ≥395 nm) in aqueous solution to investigate its photochemical properties. The photo-reaction was followed by UV–vis (Figure 2a) and fluorescence spectroscopy (Figure 2b). In the UV–vis spectra, a prominent long wavelength absorption band with a maximum at 386 nm.
could be observed, which corresponds to the absorption of coumarin. The maximum of the emission band was approximately 500 nm. Aqueous solutions of the polymer showed no changes during 24 h in the dark (Figure S10, Supporting Information). However, under irradiation with visible light, the sample changed color (from yellow to orange) and significant changes in the UV-vis and fluorescence spectra could be observed. The intensity of the absorption band at 386 nm gradually decreased and shifted to 381 nm. Four isosbestic points at 457, 359, 266, and 236 nm are visible indicative for a clean photo-reaction. The fluorescence spectra showed both an increase of the emission band and a hypsochromic shift from 508 to 499 nm. This behavior is comparable to the small-molecule studies on compound 3.

Since the photo-cleavage was deemed to be completed after 90 min irradiation in H₂O, a further sample was irradiated at the same concentration (0.16 mg mL⁻¹) and analyzed by SEC (Figure 2e). Meanwhile, an identical sample held for 24 h (Figure 2f) and even 7 days (Figure S11a, Supporting Information) without irradiation showed no signs of degradation. This confirmed the destabilizing effect of glycine after decaging rendering the polymer hydrolytically unstable.

As the degradation upon irradiation was significant but incomplete, the polymer was kept in the dark for a further period (Figure S11b, Supporting Information). However, total degradation of the polymer could not be achieved even keeping it for 7 days in aqueous solution after irradiation. Since Jeffamine M-1000 (34:66 ratio) was used in excess, parts of the polymer might consist of blocks constituted only by the M-1000 substituent which are relatively stable against hydrolytic degradation. It is known that some polymers containing coumarin derivatives can undergo +2 photodimerization of the 3,4-double bond forming a cyclobutane ring. For the small-molecule caged glycine 3, we could not identify any photodimerization product. Furthermore, the predominance of the photocleavage in the polymers was also clearly observed (Figure S12, Supporting Information).

Figure 3a shows the proposed degradation mechanism induced by the photoreaction, in which cleavage of the coumarin cage exposes the free acid groups of the glycine moieties along the polyphosphazene backbone. Thus, once the protecting group was cleaved, the polymer undergoes a chain-shattering type of degradation. This process was further investigated by 31P NMR spectroscopy in which significant depletion of the polyphosphazene backbone peak was observed upon polymer irradiation (90 min irradiation at 0.164 mg mL⁻¹ concentration in H₂O and stored for 3 days in the dark, Figure S13a, Supporting Information). After a longer period, further degradation accompanied by the appearance of the sharp peak associated with phosphate formation could also be observed. The polymer kept in the dark even for 7 days did not show any signs of degradation. 1H NMR spectroscopy revealed the successful cleavage of the caging group upon irradiation with the appearance of sharp peaks corresponding to the coumarin group while no changes were observed in the dark sample (Figure S14, Supporting Information).

Amino acid ester-substituted polyphosphazenes are known as biodegradable polymers. Therefore, polymer P2 was synthesized using glycine ethyl ester as second substituent. After purification by dialysis in the dark, polymer P2 was successfully obtained as it could be confirmed by 1H and 31P NMR spectroscopy (Figure S15, Supporting Information), and SEC (M₉,GPC = 249 000 g mol⁻¹, M₀/M₉ = 1.8, measured using multidetector calibration in DMAc containing 57.6 mM LiBr and 0.1 mM acetic acid). A ratio of approximately 33:66 coumarin derivative to glycine ethyl ester could be confirmed by 1H NMR with complete backbone substitution observed in the 31P NMR spectroscopy.
Irradiation was performed in acetonitrile:H$_2$O solution (4:1) (Figure S16, Supporting Information) upon which the emission spectra showed a predominant increase especially in the first irradiation minutes with an approximate end after 45 min (Figure 3b). As the photocleavage seemed to have ceased after 45 min, a second sample was irradiated in the same solvent mixture (0.16 mg mL$^{-1}$) for 45 min and analyzed by SEC (Figure 3c). Significant degradation could be observed after irradiation. Compared to polymer P1, the degradation was more pronounced, presumably due to the better leaving-group ability of the chosen cosubstituent. Meanwhile, in the absence of light, only slight hydrolytic degradation over 3 days could be observed (Figure S17, Supporting Information).

Figure 2. Changes of a) UV–vis absorption spectra and b) emission spectra of polymer P1 in H$_2$O (0.167 mg mL$^{-1}$) upon irradiation with a HBO lamp (cut-off filter at 395 nm). Progress of c) absorbance intensity and d) emission intensity—each measured at the band maxima from spectra in (a) and (b)—versus time. SEC analysis of polymer P1 e) after irradiation in H$_2$O (0.163 mg mL$^{-1}$) and f) in the dark in H$_2$O (0.173 mg mL$^{-1}$) for 24 h (— before irradiation; –– 90 min irradiation; °24 h dark).
The design and synthesis of polyphosphazenes with a light-driven, on-demand degradation mechanism have been described. A coumarin-based photocage, which is sensitive to visible light, was used to protect the carboxylic acid group of a glycine moiety on a polyphosphazene backbone. Upon irradiation, the carboxylic acid was exposed which in turn catalyzed the polymer degradation. In the dark, the polymers were hydrolytically stable in the investigated timeframe, that is, the degradation could be initiated upon exposure to visible light. These results prove the potential to use visible light to switch the hydrolytic stability of polyphosphazenes and hence trigger backbone degradation. While complete degradation was observed for P2, an incomplete degradation was observed for P1, indicating the importance of the nature of the secondary substituents on the overall polymer stability. Hence, future work will aim to optimize the balance between the nature and loading of the photocage and further substituents with the aim of maintaining stability in the dark, while maintaining a complete on-demand degradation. The general proof-of-principle could also be extended to other photocages to prepare polymers which respond to irradiation with yet longer wavelengths in the red region which would have a considerable impact in biological applications.

Supporting Information
Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest
The authors declare no conflict of interest.

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[1] S. Binauld, M. H. Stenzel, Chem. Commun. 2013, 49, 2082.
[2] R. J. Amir, S. Zhong, D. J. Pochan, C. J. Hawker, J. Am. Chem. Soc. 2009, 131, 13949.
