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Photo-Reversibility of Cinnamylidene Acetic Acid Derived Crosslinks in Poly(ε-caprolactone) Networks

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ABSTRACT

Photoswitchable polymeric materials comprise moieties that undergo light-induced chemical reactions or conformational alteration. The reversibility of photo-responsive molecular switches has an influence on material functions observed on the macroscopic level such as reversibility of shape switching, especially with regard to the number of cycles. Cinnamylidene acetic acid (CAA) has received attention due to its reversible dimerization by [2+2] cycloaddition reactions. In the present study, possible side-reactions during photo-scission of the CAA dimers as netpoints in poly(ε-caprolactone) based materials were studied by fluorescence spectroscopy, HPLC and ^{1}H,^{1}H-COSY. Liberation of fluorescent fragments, which have their origin in the various dimer structures, could only be found in small amounts, while a non-identified species seems to be generated during dimerization and photo-scission. The results furthermore suggest that CAA-based switches in PCL-networks do not provide full reversibility of netpoint formation under the examined conditions, due to non-selective side-reactions, which could lead to an attenuation of the macroscopic effect in multiple photo-cycles. In perspective, the design of CAA derivatives with enhanced photo-reversibility should be targeted.

INTRODUCTION

Materials that could alter their physicochemical properties or structure in response to a light stimulus have found various applications, e.g. in the formation of photoresists [1] or, in case of reversible photo-reactions, as dynamic hydrogels [2, 3] and in the fabrication of polymeric shape-memory devices [4]. Versatile light responsive molecular switches for this purpose include cinnamylidene acetic acid (CAA) derived compounds, which can reversibly undergo intermolecular transitions by a [2+2] type cycloaddition. Nevertheless, the occurrences of various side-reactions during the cycloaddition (> 260 nm) were reported that diminish their photo-reversibility. It should be noted that such a limited photo-reversibility on the molecular level, particularly in case of multiple switching cycles, can translate in diminished macroscopic functions, even though not in all cases a 100% chemical reversibility will be needed to recall macroscopic effects. Reported side-reactions concluded a possibly photo-Fries rearrangement in case of aryl cinnamate modified polymers [5], as well as Cope-rearrangements in a topochemical reaction of the cinnamylidene acetic acid [6]. Also side-reactions during the photo-scission reaction that involve the styrylic double-bonds were suggested [7].

However, studies that focus on the causality between the formed dimerization products and their effect on the photo-scission products were not reported so far. Several regioisomers could theoretically be formed by the dimerization of CAA. Importantly, the structural requirement for a complete reversibility is only given for two out of six regioisomers. In case of bond cleavage

perpendicular to the formed sigma bonds, the liberation of molecule fragments should occur by four isomers, which do not correspond to the original educts.

In this study, possible photo-scission products should be studied that have their structural origin in the various possible regioisomers formed during CAA dimerization. For this purpose, CAA functionalized star-shaped oligo(ε-caprolactone) precursors (soCL-CAA) were selected due to the wide acceptance of PCL as biomaterial and used to prepare matrices in the shape of microparticles (high surface to volume ratio for better light penetration).

EXPERIMENT

Polyvinyl alcohol (Mowiol 4-88) was kindly provided by Kuraray (Frankfurt, Germany). Sodium hydroxide (5 N) was purchased from Carl Roth (Karlsruhe, Germany). Toluene, *trans*-stilbene, 1,4-diphenyl-1,3-butadiene (DPB) and 1,6-diphenyl-1,3,5-hexatriene (DPH) were obtained from Sigma Aldrich (Taufkirchen, Germany). Acetonitrile (HPLC gradient grade), chloroform, deuterated chloroform and hydrochloric acid (2 M) were purchased from Merck (Darmstadt, Germany). Tetravalent hydroxyl-terminated oligo(ε-caprolactone) with an number average molecular weight (M_n) of 8 kDa (CAPA 4801) was provided from Perstorp (Malmö, Sweden). Milli-Q water was used through all experiments.

The 4-arm star-shaped oligo(ε-caprolactone) precursors were functionalized by esterification with an excess of cinnamylidene acetyl chloride in dry tetrahydrofuran and triethylamine as proton receptor, with slight variations (in melt at 65 °C) to the procedure described in [1] to give CAA modified oligo(ε-caprolactone) precursors (soCL-CAA) with a degree of functionality of $d_f \approx 4$ and a M_n of 11 kDa.

As matrices, microparticles with a diameter of approximately 60 µm were prepared by droplet-based microfluidics. A 10 wt% solution of soCL-CAA in chloroform was hydrodynamically focused by a 5 wt% solution of PVA in a glass capillary device [8]. The particles were collected in an aqueous bath under permanent stirring for 24 hours to remove the organic solvent. The particle suspension was then heated to 70 °C and irradiated by UV-light (320-480 nm, Omnicure S2000, Lumen Dynamics, Mississauga, Canada) for 20 minutes. The conversion of CAA endgroups was determined by FTIR spectroscopy (Nicolet 6700, Thermo Scientific, Waltham, USA).

For isolation of the formed dimers, the microparticles were rinsed with water, dried and then resuspended in a 5 N solution of sodium hydroxide for 4 days at 40 °C. The pH of the obtained clear orange colored solution was then adjusted to pH 4.8 to protonate the expected CAA-dimers, which were then extracted three times with toluene. The aqueous solution was neutralized and analyzed by ^{1}H-NMR (500 MHz, Bruker Avance, Billerica, USA). The toluene fraction was dried under high vacuum conditions until yellow oil was obtained and analyzed by ^{1}H,^{1}H-COSY.

For photo-scission, the dimer solution was irradiated with UV-light of 254 nm (Herolab S2000, Wiesloch, Germany). Fluorescence spectroscopy of the isolated dimers, photo-scission products and reference molecules were performed in chloroform on a Carry eclipse fluorospectrometer (Varian, Palo Alto, USA).

HPLC analysis of the isolated dimer, photo-scission products and reference molecules were performed on an Agilent 1200 (Agilent, Santa Clara, USA), equipped with a diode-array UV/Vis detector and fluorescence detector. 5 µL of sample dissolved in chloroform were injected and separated at 20 °C with a gradient method [9] at a flow of 1.2 mL·min^{-1} of an acetonitrile/water

eluent system. A RP-18 column with 5 µm pore-size (LiCrospher 100, Merck, Darmstadt, Germany) was used.

RESULTS

Poly(ε-caprolactone) based networks were prepared by UV-irradiation (> 260 nm) of soCL-CAA precursors in a microparticle template. CAA dimerization was confirmed by the complete disappearance of the vibrational signal of the carbon double-bond at 1625 cm^{-1} via FTIR. The dimerization of CAA could theoretically lead to six different regioisomers by [2+2] cycloaddition (Fig. 1) acting as covalent netpoints in the PCL-based material. Similarly to reported findings for cinnamic acid [10], the photo-scission of these CAA dimers could either lead to a reformation of the CAA or to the liberation of molecule fragments by bond cleavage perpendicular to the formed sigma bonds during dimerization (Fig. 1). Only two regioisomers fulfill the structural requirements for complete chemical reversibility regardless energetical favors. The other four isomers can release molecule fragments, while the polymer-junction is unaffected in 3 of 4 isomers (Fig. 1, shown in bold), i.e. no cleavage of the netpoints in the PCL networks occurs. The identification of the formed isomer is therefore useful for the evaluation of possible side-reaction during the photo-scission.

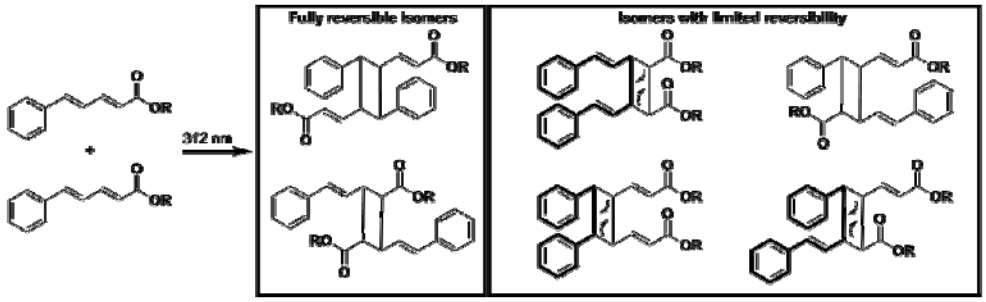

Figure 1. Possible regioisomers in the dimerization of cinnamylidene acetic acid derivatives. Possible cleavage products that were analyzed in this study are emphasized in bold.

The networks were hydrolyzed in 5 N NaOH and the dimer was successfully separated from the generated 6-hydroxycaproic acid, which was confirmed by ^{1}H-NMR. The dimer fraction was analyzed by ^{1}H,^{1}H-COSY (Fig. 2) and compared to dimerized pure CAA. The obtained characteristic peak pattern did not differ from non-hydrolyzed soCL-CAA solutions, i.e. PCL hydrolysis did not affected the CAA dimer structure. Surprisingly, no ^{3}J-couplings between cyclobutane protons (2.30-4.00 ppm) and carbon double-bonds could be observed in the dimer fraction. Regardless to that fact, a number of signals and ^{3}J-couplings appeared at 5.7-8.1 ppm and in the aliphatic region. Contrary to this result, the photo-dimerized pure CAA offered the expected scalar ^{3}J-couplings between the cyclobutane protons and the adjacent carbon double bonds in the region of 2.7-4.6/5.5-6.7 ppm.

The dimer fraction was then analyzed by fluorescence spectroscopy before and after photo-scission as triggered by UV-light of 254 nm. The dimer fraction did not indicate strong fluorescence emission in the measured range from 250 to 450 nm with one exception at λ_{ex} = 360 nm, where a low emission signal at λ_{em} = 425 nm could be observed (Fig. 3B, 0 min).

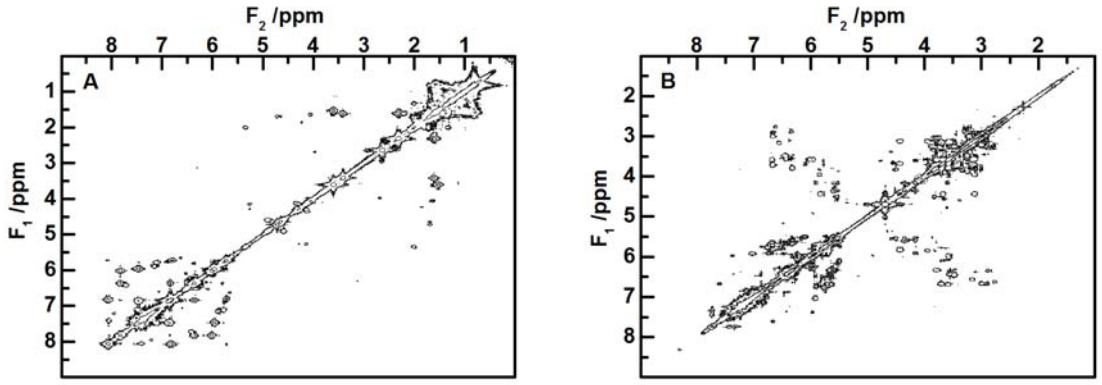

Figure 2. A) $^{1}H,^{1}H$-COSY spectrum of the dimer fraction isolated by PCL-network hydrolysis and B) $^{1}H,^{1}H$-COSY spectrum of dimerized pure CAA.

When the dimer fraction was subsequently irradiated by UV-light of 254 nm in order to cleave the cyclobutane bond, the appearance of strong fluorescence signals could be noticed. Synchronous scans disclosed two emission maxima at λ_{em} = 370 nm and λ_{em} = 425 nm with Stokes shifts of 50 nm and 70 nm, respectively. With increasing irradiation time, these both emission maxima increased in intensity. In case of the λ_{em} = 370 nm, an excitation maximum was reached after 50 min of irradiation (Fig. 3A) and decreased subsequently. This was not the case in the fluorescence emission at 425 nm for at least 80 minutes of irradiation (Fig 3B). Beside the major excitation maxima at 317 nm (λ_{em} = 370 nm) and 352 nm (λ_{em} = 425 nm), a local maximum at 265 nm could be observed in both cases (Fig. 3).

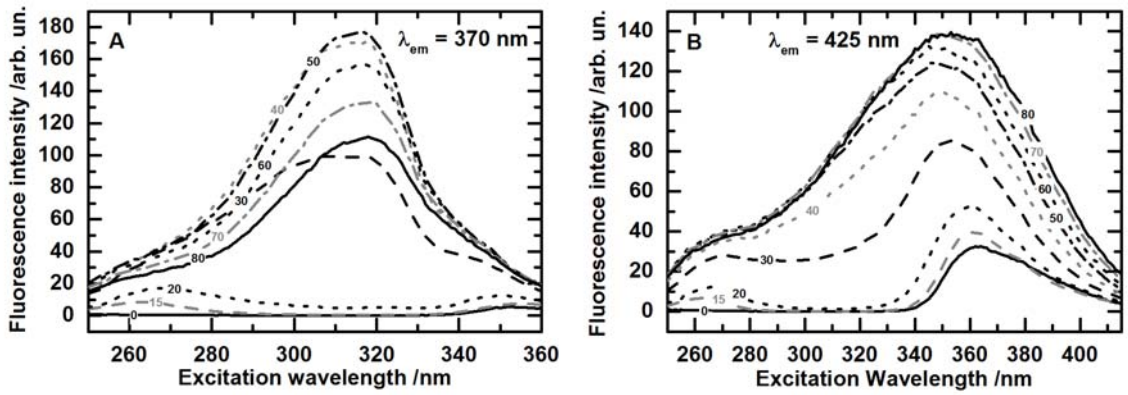

Figure 3. Excitation spectra of the dimer fraction and their subsequent photo-scission products in chloroform. A) λ_{em} = 370 nm. B) λ_{em} = 425 nm. Irradiation time (254 nm) in minutes as indicated.

The irradiation of pure CAA as negative control by the same procedure did not lead to an increase in fluorescence intensity at any excitation wavelength. The excitation spectra of two reference substances, namely stilbene and DPH that may theoretically be released by photo-scission (Fig. 1), showed a similar fluorescence behavior as the photo-scission products with excitation maxima at 302, 312 nm and 352 nm, respectively, and corresponding Stokes shifts of 50, 40 nm and 70 nm. The fluorescence pattern of DPB showed an excitation maximum at 333 nm and a corresponding emission maximum at 380 nm.

In order to verify if similar fluorescence pattern are related to structural similarities, the photo-cleaved fraction was then analyzed by HPLC after a total irradiation time of 80 minutes and compared to the reference molecules (Stilbene, DPB and DPH), the non-cleaved dimer, and monomeric CAA.

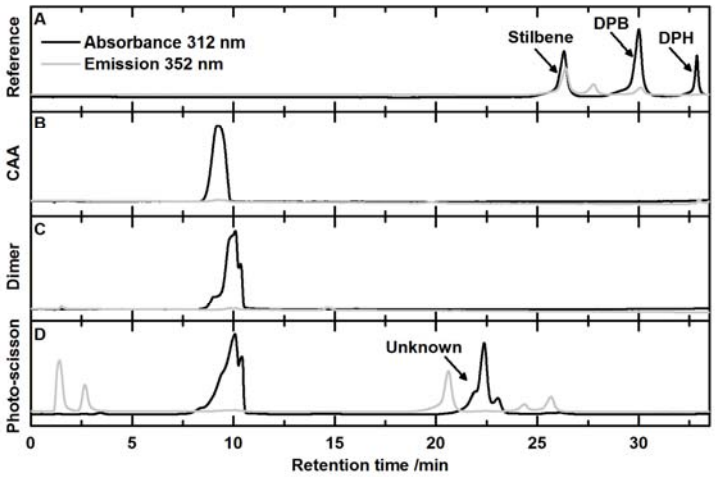

Figure 4. HPLC-chromatograms of the reference substances (stilbene, DPB and DPH), monomeric CAA, the dimer fraction and the photo-scission products (from top to bottom). Fluorescence signals of B,C,D are amplified by factor 10.

The reference molecules stilbene, DPB and DPH as potential products from photo-scission of CAA dimers were eluted after 26.3, 30.0 and 32.9 min (Fig. 4A). The non-dimerized CAA appeared at 9.2 min (Fig. 4B). The dimer appeared as a broad peak at a retention time (R_t) of around 10 min with a shoulder at R_t = 9.2 min (Fig. 4C). When the dimer was irradiated by UV-light of 254 nm, two dominant peaks were observed (Fig. 4D). The signal at 10 min remained, while the shoulder broadened to lower retention-times and with an intensity increase at 9.2 min. The second dominant peak appeared at $R_t \approx$ 20.6-23.5 min with a peak maximum at 22.3 min. The generated major peaks in the photo-scission fraction did not show the former observed fluorescence at 352 and 425 nm. However, several small peaks at $R_t \approx$ 1.4, 2.7, 20.6, 24.4 and 25.7 min emitted light of 352 nm, while no fluorescence could be verified for an emission of 425 nm.

DISCUSSION

CAA based crosslinking of PCL precursors successfully led to polymer network materials. The dimerization of pure CAA showed scalar ^3J-couplings between the cyclobutane and carbon double-bond protons. In contrast to that, an identification of the formed dimers by ^{1}H,^{1}H-COSY was not possible due to the absence of ^3J-couplings between the expected cyclobutane protons at 2.30-4.00 ppm and the residual protons from the carbon double-bonds (5.7-7.5 ppm). Interestingly, there were signals appearing at 7.83 and 8.07 ppm, which are usually found in electron-deficient aromatic compounds like polycyclic or electron-withdrawing group substituted aromates, while such a formation could not be explained by the so far known pericyclic reactions. A possible side-reaction could be a Norrish type reaction with subsequent substantial bond rearrangements, which was described for aliphatic α-di and α-tri substituted esters [11].

Another approach for the isomer identification was the analysis of photo-scission products, which according to Fig. 1 are well known fluorescent molecules, namely stilbene, DPB and DPH. It was shown by fluorescence spectroscopy that there is minor fluorescence before the photo-scission step, while fluorescence is generated after dimer irradiation with UV-light of 254 nm. Excitation and Emission spectra of the dimer after photo-cleavage corresponded to the spectra of the fluorophores stilbene and DPH, but not of DPB. The decrease of the emitted light of 352 nm after extensive irradiation was suggested to be reasoned by photo-bleaching. Nevertheless, the chromatograms from HPLC did not attest this indication, but a partial reconstruction of CAA monomer and the strong generation of other species were noticed.

CONCLUSIONS

Fluorescence spectroscopy, HPLC and $^{1}H,^{1}H$-COSY experiments confirmed the presence of side reactions during photo-dimerization and photo-cleavage of CAA, where the side-reactions do not follow the proposed perpendicular cleaving mechanism. While clearly CAA was reversibly formed by photo-scission with UV-light of 254 nm, substantial amounts of other species were formed. This may be responsible for a limited reversibility of CAA dimerization in a polymeric matrix, which might become a limitation in the number of reversibility cycles as the CAA moiety is consumed. A closer investigation of this species could give more insights into the ongoing photo-reactions and may help to design molecular functional groups with higher photo-reversibility.

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