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Photodimerized 7-hydroxycoumarin with improved solubility in PMMA: Single-photon and two-photon-induced photocleavage in solution and PMMA films

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Abstract

tert-Butyldimethylsilyl-chloride (TBS) revealed to be a photostable protecting group for the photodimerization of 7-hydroxycoumarin in a [2 + 2]-cycloaddition. TBS-functionalized coumarin dimers show an about 100-fold increased solubility in organic solvents enabling them to be easily incorporated into polymeric films, e.g., PMMA. In the described photochemical dimerization reaction almost pure anti-head-to-head isomer is obtained. The single- and two-photon absorption-induced cycloreversion reactions in acetonitrile as well as in PMMA matrix were investigated and the two-photon absorption cross sections and quantum yields were determined to be around 1 GM and about 0.36, respectively. The only product obtained upon photocleavage of the dimer is the TBS-protected 7-hydroxycoumarin monomer. The TBS-protecting group withstands the high light intensities required for two-photon absorption-induced photocleavage without any noticeable degradation. The mild deprotection conditions for *tert*-butyldimethylsilyl-ethers (TBS-ethers), the chemical stability of the compound as well as its significantly improved solubility in organic solvents and its miscibility with acrylic polymers, make this a very useful compound for potential applications in 3D volumetric optical data storage and photocontrolled drug delivery.
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Keywords: 7-Hydroxycoumarin; Coumarin dimer; Photodimerization; Two-photon absorption; Solubility; *tert*-Butyldimethylsilyl-ether

1. Introduction

Dimerization of coumarin and its derivates has been intensively investigated in view of fundamental as well as applied aspects. The basics of the dimer formation are reviewed, e.g., in [1–3]. Coumarin dimers recently were employed, e.g., as photocontrolled molecules opening and closing a silica pore, wherefrom guest molecules are released, or in photodegradable polymers [3,4]. In our research efforts coumarin dimers play an important role as photocleavable linker molecules for two-photon absorption controlled drug delivery [5]. The refractive index changes accompanying the photocleavage of the cyclobutane structure may be of use in optical and holographic recording [6]. The main advantage of two-photon absorption-induced photochemistry in both applications is the precise 3D spatial control of the photoreactions. In the case of our main application, the photocontrolled drug delivery from polymeric intraocular lenses, an additional advantage of the two-photon absorption photochemistry is, that a photochemical reaction can be triggered behind a barrier absorbing photons required for the single-photon excitation.

The coumarin dimer is a photocleavable linker between a polymer backbone and a drug molecule. In order to attach coumarin dimers to polymers, reactive side groups are required. A first choice would be 7-hydroxycoumarin, but due to the properties of the phenolic group it cannot be photochemically dimerized easily [3] because upon light irradiation, the phenolic group forms rather stable radicals which lead to undesired side products. As a protecting group for the phenolic group acetylation has been employed, but this group is only cleavable at rather harsh conditions [3], what is undesired for the further steps. A further requirement to the dimer is that it should have a good solubility in organic solvents and polymers. The coumarin dimer itself is very poorly soluble in common

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Table 1
Overview of determined photochemical characteristics of TBS-C dimer in acetonitrile solution and PMMA matrix

	Cycloreversion quantum yield (SPA = TPA)	SPA cross section (cm^2)		TPA cross section (cm^4 s $photon^{-1}$)
		266 nm	532 nm	532 nm
ACN solution	0.36	In both cases		1.1×10^{-50}
PMMA matrix	0.37	7.85×10^{-18}	1.70×10^{-20}	1.9×10^{-50}

organic solvents, what is a problem for the further coupling steps.

In summary, a photostable hydroxyl group protection group for the photodimerization, a significantly improved solubility of the dimer for the further processing, and comparably mild deprotection chemistry for the linkage step with other molecules, e.g., drugs is required.

We studied *tert*-butyldimethylsilyl-chloride (TBS^+Cl^-) [7] as a protecting group for the coumarin's hydroxyl group, because a wide choice for the deprotection conditions is reported in the literature [7–11]. In particular the mild deprotection with potassium hydrogensulfate in aqueous methanol or lithium hydroxide in dimethylformamide [8,12] is very attractive for our applications. We present an efficient route for the photochemical dimerization of 7-hydroxycoumarin after protection with TBS^+Cl^-, leading to 7,7′-(*tert*-butyldimethylsilyloxy)-dicoumarin (TBS-C dimer). In addition to the required photochemical stability, the employed protection group increases the solubility of TBS-C dimer compared to 7,7′-hydroxydicoumarin about 100-times. This enables the preparation of highly loaded polymer films.

We characterized the photochemical properties of the TBS-C dimer in acetonitrile solution as well as in PMMA films. Absorption cross section and the quantum yield for photocycloreversion were experimentally determined for both, single-photon absorption (SPA) as well as two-photon absorption (TPA) (Table 1). The TBS-protection group was stable in both photochemical processes investigated, as checked by HPLC analysis. Highly loaded polymer films containing TBS-C-dimer in combination with TPA-induced photocleavage are required for photocontrolled drug delivery from polymeric intraocular lenses [13].

2. Experimental

7-Hydroxycoumarin 99% (Acros Organics), *tert*-butyldimethylsilyl-chloride (Fluorochem), imidazole p.a. (Fluka), benzophenone p.a. (Fluka), acetonitrile HPLC grade (Fisher Scientific), polymethylmethacrylate (PMMA) pellets (Plexiglas®, Degussa), silica gel 60 (Merck) were used as received. Tetrahydrofuran (THF) was dried over sodium. THF, ethylacetate and *n*-pentane were distilled before use. Water was prepared using a Milli-Q gradient system (Millipore).

HPLC analysis was done using a Hewlett-Packard Model 1050. The isomers were separated on a 250 mm × 4 mm RP18 column (Nucleosil, 3 μm, Bischoff) using acetonitrile and water as an eluent.

UV–vis absorption spectra were recorded on an UVIKON 922 (Kontron) spectrophotometer.

Elemental analysis was done on a CHN-Rapid (Hereaus).

EI-MS measurements were done on a CH7A (Finnigan). ESI-MS measurements were done on a LCQ-duo (Thermoquest Finnigan) equipped with an ESI ionization (positive mode). A spray voltage of 4.50 kV, a capillary temperature of 200 °C and a capillary voltage of 10.00 V were used.

^{1}H-NMR (300 MHz) and ^{13}C-NMR (75 MHz) spectra were collected on a Bruker AC-300 spectrometer using $CDCl_3$ as solvent.

3. Synthesis

3.1. 7-(tert-butyldimethylsilyloxy)-coumarin

For the synthesis of 7-(*tert*-butyldimethylsilyloxy)-coumarin (TBS-C) (see Fig. 1) 3.36 g (21 mmol) 7-hydroxycoumarin (I) and 3.53 g (53 mmol) imidazole were dissolved in 75 mL dry THF in a Schlenk flask under argon atmosphere. After a clear solution was obtained, 3.75 g (25 mmol) *tert*-butyldimethylsilyl-chloride were added. The mixture was heated to 50 °C for 3.5 h and then cooled down to room temperature and stirred for another 22 h. The ammonium salts were filtered off and the solution was reduced under vacuum and then washed with 5% aqueous sodium hydrogen carbonate ($NaHCO_3$) solution. Then it was extracted three times with 50 mL chloroform ($CHCl_3$)

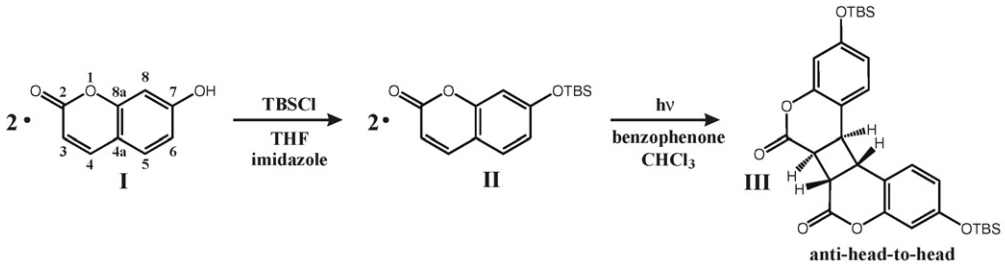

Fig. 1. Synthesis of 7,7′-(*tert*-butyldimethylsilyloxy)-dicoumarin (TBS-C dimer). Anti-head-to-head TBS-C dimer (III) is obtained by photodimerization of 7-(*tert*-butyldimethylsilyloxy)-coumarin (II, TBS-C) which is prepared from 7-hydroxycoumarin (I, 7HC).

each. The combined $CHCl_3$ solutions were dried over magnesium sulfate ($MgSO_4$). After a further reduction step in vacuum the product was purified by flash chromatography using 700 mL of silica gel and a 1:4 ethylacetate/*n*-pentane mixture as an eluent. The yield was 4.7 g (81%) of product.

^{1}H-NMR, δ_H [ppm]: 0.24 (s, 6H), 0.98 (s, 9H), 6.24 (d, 1H, $J = 9.4$ Hz), 6.75 (d, 1H, $J = 8.7$ Hz), 6.77 (s, 1H), 7.33 (d, 1H, $J = 9.0$ Hz), 7.63 (d, 1H, $J = 9.5$ Hz).

^{13}C-NMR, δ_C [ppm]: −4.3 (Si-CH_3), 18.4 (Si-C), 25.6 (C-CH_3), 107.8 (C_{ar}), 113.3 (C=C), 113.5 (C_{ar}), 117.5 (C_{ar}), 128.8 (C_{ar}), 143.4 (C=C), 155.7 (C_{ar}), 159.5 (C_{ar}), 161.2 (C=O).

Elemental analysis: (calculated) C 65.22 %, H 7.25 %; (found) C 64.65 %, H 7.55 %.

Mass (EI, m/e): 276 (M^+, 14.65%), 219 (100%), 163 (30.19%), 89 (11%), 73 (7.54%), 29 (9.27%).

3.2. 7,7′-(tert-Butyldimethylsilyloxy)-dicoumarin

7,7′-(*tert*-Butyldimethylsilyloxy)-dicoumarin (III) was obtained from TBS-C (II) by photodimerization (see Fig. 1). The reaction was performed in 5 pyrex tubes (diameter = 1 cm, height = 12 cm), each filled with 0.2 g (0.72 mmol) TBS-C (II), 25 mg (0.14 mmol) benzophenone and 5 mL $CHCl_3$. The solution was degassed and irradiated for 20 h in a Rayonet-type photoreactor equipped with 12 UV-lamps Eversun 40W/79 K (Osram). After 20 h the solutions were combined and reduced under vacuum. The product was dissolved in 5 mL THF and purified on a preparative HPLC (Pump 64 and UV-detector, Knauer, detection wavelength 280 nm, column EnCaPharm 100 RP18 250 mm × 32 mm, 10 μm) with a 80:20 mixture of acetonitrile:water as an eluent. Yield was 746 mg (79%).

During dimerization of TBS-C (II) four isomers may be obtained. At the conditions chosen we obtained just one of the isomers with 95% yield as indicated by HPLC analysis. Elemental analysis, mass spectroscopy and UV–vis spectra (Fig. 2A) were recorded to characterize the dimer. From the NMR spectra the obtained 7,7′-(*tert*-butyldimethylsilyloxy)-dicoumarin (III) was assigned to be the anti-head-to-head isomer according to the analysis published by Yu et al. [14]. The two signals at 3.74 and 3.88 ppm do have the same shifts and structure as described there [14].

^{1}H-NMR, δ_H [ppm]: 0.23 (s, 12H), 0.99 (s, 18H), 3.74 (d, 2H, $J = 7.9$ Hz), 3.88 (d, 2H, $J = 7.7$ Hz), 6.58 (d, 2H, $J = 2.5$ Hz), 6.62 (d, 1H, $J = 2.3$ Hz), 6.65 (d, 1H, $J = 2.4$ Hz), 6.95 (s, 1H), 6.98 (s, 1H).

^{13}C-NMR, δ_C [ppm]: −4.3 (Si-CH_3), 18.3 (Si-C), 25.7 (C-CH_3), 40.1 (C_{cyclo}), 43.7 (C_{cyclo}), 109.2 (C_{ar}), 113.2 (C_{ar}), 117.5 (C_{ar}), 128.5 (C_{ar}), 151.9 (C_{ar}), 156.8 (C_{ar}), 166.3 (C=O).

Elemental analysis: (calculated) C 65.22%, H 7.25%; (found) C 64.40%, H 7.63%.

Mass (ESI, m/e): 570.6 [$M^+ + H_2O$].

3.3. Solubility

One key aspect in the selection of the protection group for the phenolic group of 7-hydroxycoumarin was the solubility of the resulting dimer. Saturated solutions of both dimers, 7,7′-hydroxydicoumarin as well as 7,7′-(*tert*-butyldimethylsilyloxy)-dicoumarin, were prepared in chloroform. The solutions obtained were diluted and the concentrations of the dimers were photometrically determined, assuming that the specific absorption at 266 nm of both dimers is the same. A 100-fold increased solubility of TBS-C dimer (III) compared to 7,7′-hydroxydicoumarin in chloroform was found.

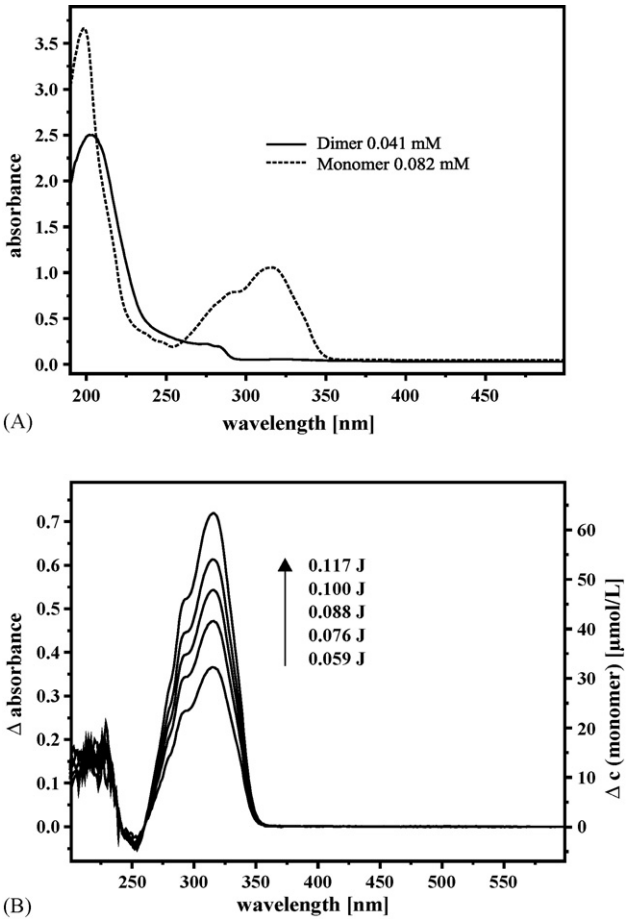

Fig. 2. Single-photon-absorption (SPA)-induced photocleavage of 7,7′-(*tert*-butyldimethylsilyloxy)-dicoumarin (TBS-C dimer). (A) The absorption spectra of 0.082 mM monomeric (dashed line) 7-(*tert*-butyldimethylsilyloxy)-coumarin (TBS-C) and 0.041 mM TBS-C dimer (solid line) solutions in acetonitrile (ACN) are shown. The maximal absorption of the monomer observed is at 316 nm. (B) Difference spectra taken from a TBS-C dimer (III) solution (0.36 mM in ACN) tracing the SPA-induced photocleavage of the dimer by 266 nm light. The absorption increase observed is due to the formation of monomer. The intensity of 266 nm light was 195 μW/cm^2. The total energies applied are given.

4. Results and discussion

4.1. Photo-induced cleavage of the cyclobutane ring in solution

A 0.36 mM solution of TBS-C dimer (III) in acetonitrile was used for the measurement of the quantum efficiency of the photocycloreversion in solution. Single- and two-photon absorption-induced photocleavage was analyzed in quartz

cuvettes with 10 mm path length. The cuvettes were filled with 2.5 mL of the 0.36 mM solution of TBS-C dimer.

As a light source for single-photon absorption (SPA)-induced dimer cleavage the excitation beam of a fluorescence spectrophotometer was used (RF-1502, Shimadzu). The intensity of the excitation beam was measured by a calibrated photo diode (S1337-1010BQ, Hamamatsu). For dimer cleavage by two-photon absorption a Q-switched Nd:YAG laser (Infinity 40–100, Coherent) operating at 532 nm was employed. The pulse length was 3 ns, the repetition rate 20 Hz and the beam diameter was 5.5 mm.

The photocycloreversion was monitored by UV–vis spectroscopy. The photocleavage of TBS-C dimer (III) is measured by determining the amount of TBS-C (II) monomer formed. For this analysis the molar extinction coefficient of TBS-C of $\varepsilon_{316} = 11347.6\,\mathrm{L\,mol^{-1}\,cm^{-1}}$ was employed. The absorption band at 316 nm is characteristic for the TBS-C monomer, as it reflects the increase in conjugation which occurs during the cycloreversion step [13].

For the SPA photocleavage of TBS-C dimer ($\varepsilon_{266} = 4729.1\,\mathrm{L\,mol^{-1}\,cm^{-1}}$) excitation at 266 nm was chosen. This wavelength corresponds to the double frequency of 532 nm which was used for two-photon excitation. In Fig. 2A the absorption spectra of a 0.041 mM solution of dimer and a 0.082 mM solution of monomer in acetonitrile are compared. The difference spectra in Fig. 2B show the increase in absorbance at 316 nm and the formation of monomer, respectively. The wavelength 316 nm is free from any interference by the dimer absorption. The quantum yield for SPA photocleavage was determined by measuring the initial reaction rate calculated from the changes in absorbance at 316 nm. Because the absorption at 266 nm of the initial 0.36 mM solution used for these experiments is about OD (266 nm) = 2, it is assumed in good approximation that 100% of the photons exposed to the solution are absorbed at the beginning of the reaction. Interfering absorption from the formed coumarin monomer is negligible in the initial phase of the cycloreversion reaction. A quantum yield of $\Phi \approx 0.36$ is found for TBS-C dimer, a value about 50% higher than the one found for coumarin dimer [12].

The absorption changes induced by two-photon absorption (TPA) in dependence on the total energy exposed are shown in Fig. 3A. For the calculation of the TPA cross section and in order to prove that the cycloreversion at 532 nm is induced by a TPA mechanism, we tested different pulse energies (40.5 mJ, 50.5 mJ, 58.5 mJ, 68.0 mJ, 78.0 mJ) to cleave the cyclobutane ring. Fig. 3B shows the increase in absorption at 316 nm for the different energies. The TPA cross section was calculated assuming that the cycloreversion quantum yields for SPA and TPA are identical [13]. The TPA cross section found is 1.1 GM ($1.1 \times 10^{-50}\,\mathrm{cm^4\,s\,photon^{-1}}$). HPLC analysis of the irradiated solutions proved that monomeric TBS-C (II) is the only product.

Knowing that a TPA-induced photoprocess depends on the square of light intensity, a double logarithmic plot of the incident power versus the initial rate of cycloreversion is used to prove the TPA-nature of this process [15]. The experimentally obtained slope of 1.81 (Fig. 3C) confirms TPA nature of this process.

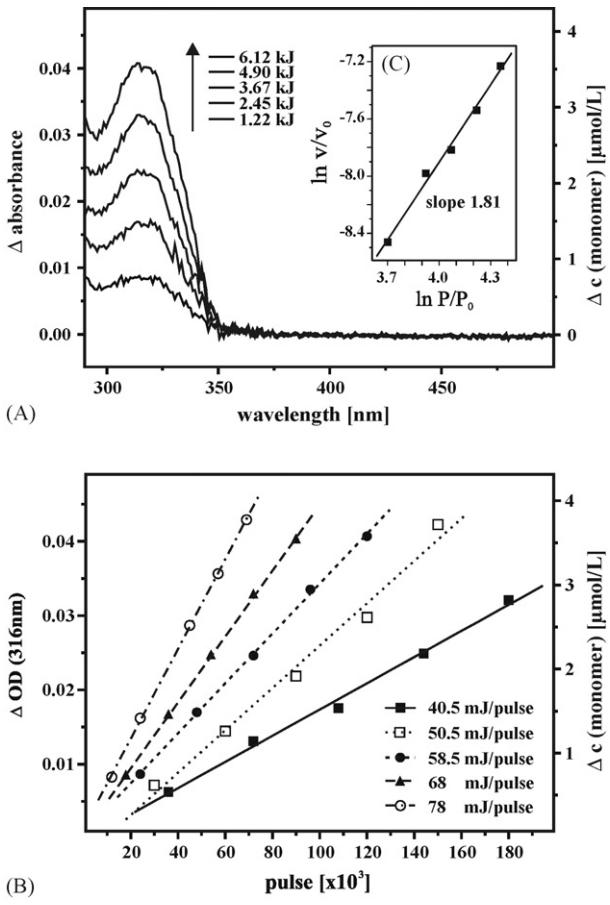

Fig. 3. Two-photon-absorption (TPA)-induced photocleavage of 7,7′-(*tert*-butyldimethylsilyloxy)-dicoumarin (TBS-C dimer). (A) The TPA-induced cycloreversion of TBS-C dimer was induced using 532 nm laser pulses of 3 ns length at a repetition rate of 20 Hz. The energy per pulse employed was 68 mJ/pulse. The absorption changes observed in a 0.36 mM solution in acetonitrile (ACN) after exposure to the indicated energies are shown as difference spectra vs. the unexposed sample. (B) Variation of the energy per pulse returns linear dependencies of the TPA-induced photocleavage from the total exposure in all cases. (C) A slope of 1.81 is derived from the double-logarithmic plot of the initial reaction rates vs. incident power. This indicates that the photocycloreversion of TBS-C dimer with 532 nm light is a TPA process.

4.2. SPA and TPA-induced cycloreversion of TBS-C dimer in PMMA matrix

As the solubility of the TBS-C dimer in organic solvents is about 100-fold higher than that of 7,7′-hydroxydicoumarin, PMMA films with a high load of TBS-C dimer were prepared, i.e., 5% (w/w). The question arises whether the photochemical properties of the TBS-C dimer are affected by matrix entrapment.

The PMMA films were prepared from a solution of 40 mg of TBS-C dimer and 800 mg of PMMA dissolved in 2 mL of chloroform. By knife coating 0.8 mm thick wet films were prepared on glass plates. After evaporation of the solvent PMMA films with a thickness of about 200 μm were obtained.

SPA and TPA properties were analyzed using conditions comparable to the ones used for the acetonitrile solutions. SPA was measured with 266 nm light of 44 $\mu W/cm^2$. TPA data were derived from experiments with 3 ns pulses at 532 nm which were

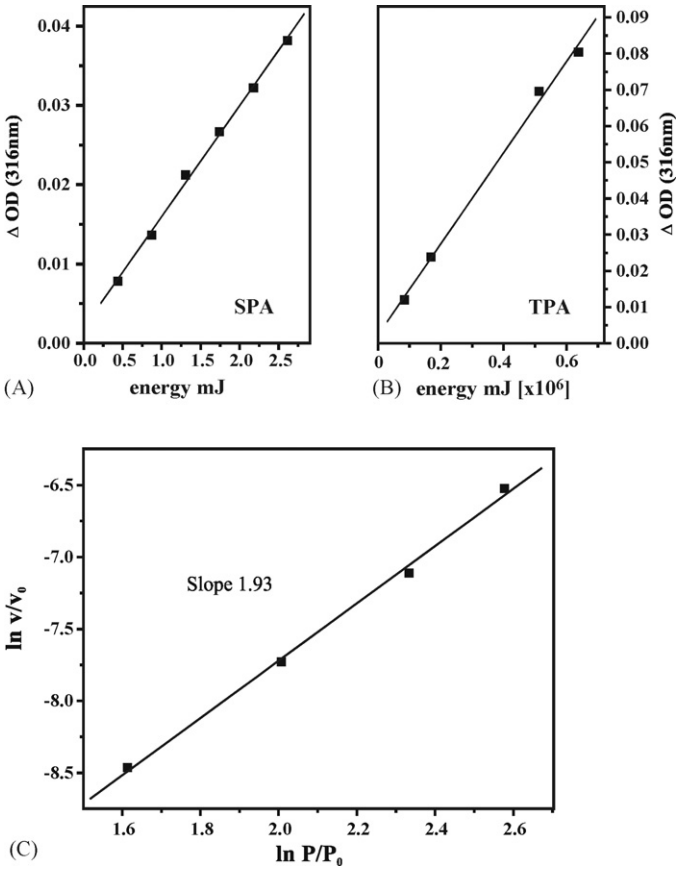

Fig. 4. Photo-cleavage of TBS-C dimer in PMMA films. (A) SPA-induced cycloreversion using 266 nm light in PMMA matrix (B). TPA-induced cycloreversion induced by 3 ns laser pulses of 532 nm at a repetition rate of 20 Hz and a pulse energy of 10.5 mJ/pulse in PMMA matrix. Linearity of the absorbance increase at 316 nm is observed in both cases. (C) A slope of 1.93 derived from the double-logarithmic plot of the incident power vs. the initial rate of cycloreversion indicates that the photocleavage of TBS-C dimer (III) in PMMA film with 532 nm light is a TPA process.

applied as pulse energies ranging from 5.0 to 13.5 mJ/pulse (beam diameter 5.5 mm). In Fig. 4A the linear increase of absorbance at 316 nm, which corresponds to the formation of monomer, is plotted versus the total energy exposed for SPA wavelengths. The corresponding experiment for the TPA-induced photoreversion is shown in Fig. 4B. The quantum yield for SPA and TPA triggered photocleavage in PMMA films was determined to be $\Phi \approx 0.37$, respectively. From the double logarithmic plot of the incident power versus the initial rate of cycloreversion (Fig. 4C) a slope of 1.93 is derived. Taking the experimental uncertainties into account, in particular slight variations in the film thickness, the TPA process seems to be not affected due to the matrix entrapment of the dimer molecules, but the TPA cross section in the PMMA film was found to be 1.9 GM, about 2-fold higher than the one found in solution.

5. Summary and conclusions

Photodimerization of 7-(*tert*-butyldimethylsilyloxy)-coumarin selectively leads to the formation of the anti-head-to-head isomer of the TBS-C dimer in high yield (95%). As the only product of the photocleavage process, induced either by single-photon absorption (SPA) or by two-photon absorption (TPA), the TBS-C monomer is observed in solution as well as in PMMA films. The SPA quantum yield was determined to be 0.36 in solution and 0.37 in PMMA matrix, respectively. The TPA cross section in solution was determined to be 1.1 GM and 1.9 GM in PMMA films. The TPA efficiency is not affected by matrix entrapment of the dimer. The solubility of TBS-C dimer in chloroform is >0.4 mol/L, about 100-times higher compared to 7,7′-hydroxydicoumarin dimer. The photostability of the TBS protecting group for the photodimerization as well as the improved solubility of the obtained dimer in organic solvents are both important for the further processing of this photocontrolled linker building block into polymer matrices and its final application in TPA triggered drug delivery.

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