Molecular design, chemical synthesis, and evaluation of novel anthracene derivatives as a new family of protein photocleavers

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Abstract

The newly designed and synthesized artificial anthracene derivatives possessing a deoxyamino sugar effectively and randomly cleaved proteins, such as bovine serum albumin (BSA) and hen egg lysozyme (Lyso), during photoirradiation using a long wavelength UV light (365 nm) without any further additives. Furthermore, it was found that the cleaving ability of anthracene could be improved by the attachment of a suitable substituent into anthracene core skeleton.

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1. Introduction

The development of photochemical protein cleaving agents, which cleave proteins by irradiation with a specific wavelength of light under mild conditions and without any additives, such as metals and reducing agents, is very interesting from chemical and biological standpoints. These agents could be useful for structure–activity studies of proteins, investigation of protein structural domains, design of novel therapeutic drugs, and conversion of large proteins into smaller fragments that are amenable for sequencing and preparing peptide-based functional materials [1]. However, only a few successful examples, such as N-(1-phenylalanine)-4-(1-pyrene)butyramide [2], 2-bromo-4-nitrocacetophenone [3], enediyynes [4,5] and cobalt(III) complexes [6] have been reported as chemical protein photocleavers. Among them, only 2-bromo-4-nitrocacetophenone [3] and enediyynes [4,5] are organic protein photocleavers without metals. In this context, we have very recently announced anthraquinones as new protein photocleavers [7]. Herein, we disclose the molecular design, chemical synthesis, and protein photocleaving properties of novel and artificial light activatable protein cleaving agents possessing anthracene structure as the core unit (Fig. 1).

2. Experimental procedure

2.1. Chemical synthesis

General procedures. Anthracene (1) and 2-hydroxymethylanthracene (2) were purchased from Sigma–Aldrich Co. Melting points were determined on a micro hot-stage Yanako MP-S3 and were uncorrected. Optical rotations were measured on a JASCO DIP-360 photoelectric polarimeter. 1H NMR spectra were recorded on a Lambda 300 (300 MHz) using TMS as internal standard unless otherwise noted. Silica gel TLC and column chromatography were performed on Merck TLC 60F-254 (0.25 mm) and Kanto Chemical Co. Inc. silica gel 60 N (spherical, neutral), respectively. Air- and/or moisture-sensitive reactions were carried out under an atmosphere of argon with oven-dried glassware. In general, organic solvents were purified and dried by the appropriate procedure, and evaporation and concentration were carried out under reduced pressure below 30 °C, unless otherwise noted.

2-Anthrylmethyl 3-amino-2,3,6-trideoxy-α-D-arabino-hexopyranoside (4): to a stirred mixture of 2 (65.4 mg, 0.314 mmol), 6 [8] (67.3 mg, 0.226 mmol) and MS 4 Å (47.1 mg) in dry THF (2.6 mL) was added TMSOTf (56.8 µL, 0.271 mmol) at 0 °C. The reaction mixture was stirred at 0 °C for 30 min and poured into ice-cold saturated NaHCO₃ aq. The resultant mixture was extracted with EtOAc and the extracts were washed with brine, dried over anhydrous Na₂SO₄ and concentrated in vacuo. Purification of the residue by column chromatography (10.6 g of silica gel, 3/1 n-hexane/
ETOAc) gave the \( \alpha \)-anomer 7 (55.8 mg, 53\%) and its \( \beta \)-anomer 8 (42.5 mg, 46\%) as pale yellow solids. To a stirred solution of 7 (55.8 mg, 0.138 mmol) in dry CHCl\(_3\) (1.1 mL) was added 5 N NaOMe (82.6 \( \mu \)L, 0.414 mmol) at 0 °C. After stirring at 25 °C for 3 h, the reaction mixture was neutralized with dry ice and NaOMe (82.6 mg, 0.339 mmol). The reaction mixture was stirred at 365 nm, 100 W) was used as a UV lamp for the photocleavage of proteins by anthracene derivatives.

**Fig. 1.** Photocleavage of proteins by anthracene derivatives.

SDS/polyacrylamide gel electrophoresis (SDS-PAGE) experiments were performed as reported \[9\]. After addition of a 4.8 \( \mu \)L solution containing SDS (5\%, wt/vol), glycerol (27\%, vol/vol), 2-mercaptoethanol (13\%, vol/vol) and bromophenol blue (0.007\%, wt/vol) to the photoirradiated samples, the resultant mixtures were heated for 10 min. Gels (8\% for BSA and 12\% for Lyso) were run by applying 110 V for 1.5 h for BSA or 2.5 h for Lyso. The gels were stained with Coomassie blue for 1 h, destained in acetic acid (10\%, vol/vol) and methanol (30\%, vol/vol) for 1 h, and dried at 65 °C for 1.5 h. The gels were scanned with a Canon scanner (CanoScan FB1210U) and images were processed by using Adobe Photoshop software. Molecular weight markers were used in each gel for calibration.

2.3. Electrophoresis

After addition of a 4.8 \( \mu \)L solution of dithiothreithol (2.3\%, wt/vol) to the photoirradiated samples, the resultant mixtures were kept at 25 °C for 10 min and dried under vacuum. The samples were dissolved in acetonitrile–0.1% trifluoroacetic acid (2/1) and analyzed for intact molecular weight by using matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) on Bruker Ultraflex (Bruker Daltonics, Billerica, MA) with sinapinic acid (Sigma–Aldrich) as the matrix. Positive ionization was used and the detection was in the linear mode.

**3. Results and discussion**

In our approach, to investigate such novel and organic protein cleaving molecules, we noted the anthracene structure, because we have previously found that certain anthracene derivatives were efficient agents for DNA photocleavage \[10\]. Based on these results, we expected that if a photo-excited anthracene derivative could produce some radical species, not only DNA, but also proteins would be damaged and cleaved by photoirradiation. All the protein cleavage experiments were performed with BSA or Lyso (15 \( \mu \)M) in a volume of 10 \( \mu \)L containing 20% acetonitrile in 50 mM Tris–HCl buffer (pH 7.0) at 25 °C for 2 h under irradiation of the UV lamp placed at 10 cm from the mixture. The protein-sample levels were varied as indicated in the figure captions.

2.4. Mass spectrometry

**3. Results and discussion**

In our approach, to investigate such novel and organic protein cleaving molecules, we noted the anthracene structure, because we have previously found that certain anthracene derivatives were efficient agents for DNA photocleavage \[10\]. Based on these results, we expected that if a photo-excited anthracene derivative could produce some radical species, not only DNA, but also proteins would be damaged and cleaved by an anthracene derivative during photoirradiation. To confirm our hypothesis, we first examined the photo-induced protein cleaving activity of commercially available anthracene (1) and 2-hydroxymethylanthracene (2) (Fig. 2) in concentrations of 5–150 \( \mu \)M using bovine serum albumin (BSA, 15 \( \mu \)M) in Tris–HCl buffer (50 mM, pH 7.0) by photoirradiation using a long wavelength UV light (365 nm) without any additives. The progress of the protein photocleavage was monitored by 8% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) \[9\]. As obvious from (a) and (b) in Fig. 3, anthracene (1) and...
2-hydroxymethylanthracene (2) exhibited very week BSA cleaving abilities under the photolrradiation. Thus, 1 and 2 cleaved BSA in concentrations over 150 μM. However, these results clearly demonstrate, for the first time, that an anthracene derivative is able to cleave a protein, BSA, upon irradiation with a long wavelength UV light without any additives. It was also confirmed that no BSA cleavage by 1 and 2 was observed in the absence of light. Thus, the UV light functioned as a trigger to initiate the anthracene derivative for the protein scission. In addition, it was found that since the band corresponding to BSA rarefied and smear bands were observed on the SDS-PAGE for the BSA cleaving pattern, 1 and 2 must have randomly cleaved BSA [11]. On the other hand, 2-hydroxymethylnapthalene (3) showed no protein cleaving activity under similar conditions ((c) in Fig. 3). This result demonstrated that the aromatic ring system of anthracene was essential for the protein photocleavage.

To further improve the protein cleaving ability of the anthracene derivative, we designed and synthesized hybrid compounds 4 and 5 consisting of anthracene and a deoxyamino sugar, which was a suitably hydrophilic molecule possessing hydroxyl and amino groups. This is because we expected that the hydrophilic moiety of the hybrid with hydroxyl and amino groups would enhance the interaction with proteins due to its amphipathic nature and by forming the hydrogen bond(s). The newly designed hybrids 4 and 5 are anomeric anomers of each other (Fig. 2).

The chemical synthesis of the hybrids 4 and 5 is outlined in Scheme 1. The glycosidation of 2-hydroxymethylanthracene (2) and the known glycosyl acetate 6 [8] using TMSOTf in the presence of MS 4 Å in THF at 0 °C for 30 min smoothly proceeded to afford the α-glycoside 7 and its β-anomer 8 in a 93% yield and a ratio of 1.3:1. After the separation of 7 and 8 by column chromatography, the deprotection of acetyl groups in 7 and 8 employing NaOMe in CHCl3 yielded 9 and 10, respectively, in good yields. Finally, the reduction of the azido groups in 9 and 10 using Ph3P in THF–H2O furnished the desired hybrids 4 and 5, respectively, in high yields.

With the designed hybrids 4 and 5 in hand, the photoinduced DNA cleaving activities of these hybrids were assayed under the same conditions. Based on the results summarized as (a) and (b) in Fig. 4, the anthracene derivatives 4 and 5 caused effective protein cleavage during irradiation with the long wavelength UV light. It was also confirmed that the cleaving ability was dependent on the concentrations of 4 and 5, and the cleaving activity increased as the concentrations of 4 and 5 increased. In addition, it was again confirmed that no protein cleavage by 4 and 5 was observed in the absence of light. The cleaving activity was independent of the configuration of the anomeric center of the hybrids. Thus, 4 and 5 cleaved protein, BSA, in concentrations over 50 μM, and caused near 100% protein degradation at concentrations over 150 μM against 15 μM of BSA. Furthermore, the sugar component of the hybrids 11 [8] lacking the anthracene moiety did not cleave BSA at all as shown as (c) in Fig. 4. These results clearly indicate that the introduction of a suitable substituent into anthracene can significantly enhance the protein cleaving activity.

![Fig. 2. Anthracene derivatives and their components.](image-url)

![Fig. 3. Photocleavage of bovine serum albumin (BSA). BSA (15 μM) was incubated with a compound in 20% acetonitrile Tris–HCl buffer (pH 7.0, 50 mM) at 25 °C for 2 h under the irradiation of a UV lamp (365 nm, 100 W) placed at 10 cm from the sample, and analyzed by 8% tricine–SDS-PAGE: (a), (b), and (c) for the compounds 1, 2, and 3, respectively: lane 1, sizemarker; lane 2, BSA alone; lane 3, BSA with UV; lane 4, BSA + compound (150 μM) without UV; lanes 5–8, the concentrations of the compound were 150, 50, 15, and 5 μM, respectively, with UV.]
ability of anthracene due to the increasing affinity of the anthracene derivatives with protein, BSA.

These protein photocleavage phenomena by anthracene derivatives were also supported by the analysis using MALDI-TOF MS as shown in Fig. 5. Thus, only after the incubation of BSA with 4 under photoirradiation, the MS peak corresponding to BSA disappeared, and no MS peaks corresponding to the cleaving fragments were detected due to the random cleavage leading to many fragments of very small amounts.

To examine the generality of the protein cleavage by the anthracene derivatives, we next carried out the photoinduced protein cleavage using 4, 5, and hen egg lysozyme (Lyso, 15 μM) in concentrations of 5–150 μM in Tris–HCl buffer (50 mM, pH 7.0). The progress of the protein photocleavage was monitored by 12% SDS-PAGE [9]. It was found, as indicated in Fig. 6, that 4 and 5 caused a significant degradation of Lyso by the photoirradiation using the long wavelength UV light without any further additives. These results clearly demonstrate that the anthracene derivatives cleave not only BSA but also Lyso, thus showing the general ability for protein cleavage.

It was found that the protein cleaving activity of these anthracene derivatives significantly decreased in the presence of both hydroxyl radical and hydrogen peroxide scavengers, DMSO and KI, respectively. Therefore, the protein cleavage must arise from the hydroxyl radical.
through hydrogen peroxide generated by the photo-excited anthracene and O₂ [12–14]. Although the precise mechanism of the protein photocleavage is still not clear at the present, one of the presumed reaction mechanisms based on the scavenger assay is illustrated in Fig. 7. Thus, hydrogen abstraction by the hydroxyl radical from the peptide backbone, followed by reaction with oxygen, led to the fragmentation of the proteins.

Fig. 5. MALDI-TOF MS profiles of bovine serum albumin (BSA) photocleavage by 4. (a) BSA alone; (b) BSA (15 μM) was incubated in 20% acetonitrile Tris–HCl buffer (pH 7.0, 50 mM) in the absence of 4 at 25 °C for 2 h under the irradiation of a UV lamp (365 nm, 100 W) placed at 10 cm from the sample; (c) BSA (15 μM) was incubated with 4 (150 μM) in 20% acetonitrile Tris–HCl buffer (pH 7.0, 50 mM) at 25 °C for 2 h without the photoirradiation; (d) BSA (15 μM) was incubated with 4 (150 μM) in 20% acetonitrile Tris–HCl buffer (pH 7.0, 50 mM) at 25 °C for 2 h under the photoirradiation.

Fig. 6. Photocleavage of hen egg lysozyme (Lyso). Lyso (15 μM) was incubated with a compound in 20% acetonitrile Tris–HCl buffer (pH 7.0, 50 mM) at 25 °C for 2 h under the irradiation of a UV lamp (365 nm, 100 W) placed at 10 cm from the sample, and analyzed by 12% tricine–SDS-PAGE: (a) and (b) for the compounds 4 and 5, respectively: lane 1, sizemarker; lane 2, Lyso alone; lane 3, Lyso with UV; lane 4, Lyso + compound (150 μM) without UV; lanes 5–8, the concentrations of the compound were 150, 50, 15, and 5 μM, respectively, with UV.

Fig. 7. Presumed mechanism of protein photocleavage by anthracene derivatives.

4. Conclusion

We demonstrated here, for the first time, that certain anthracene derivatives effectively caused protein cleavage under photoirradiation without any additives. This ability could be improved by the attachment of a suitable substituent into the anthracene core skeleton. The described chemistry and evaluation provide significant information about the molecular design of novel and artificial protein photocleaving agents. The selective cleavage of targeted protein by attachment of a protein recognition moiety into anthracene is now in progress in our laboratories.

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