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Anthraquinone derivatives as a new family of protein photocleavers

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Abstract—Certain anthraquinones, which are present in many biologically important natural products, effectively and randomly cleaved proteins (BSA or Lyso) during photoirradiation using a long wavelength UV light without any further additives. It was also found that this ability could be improved by the attachment of a suitable substituent into the anthraquinone core skeleton.

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-nitoroacetophenone,[3] enediynes,[4,5] and cobalt(III) complexes,[6] have been reported as chemical protein photocleavers. Among them, only 2-bromo-4-nitoroacetophenone[3] and enediynes[4,5] are organic protein photocleavers without metals. In this communication, we present the molecular design, chemical synthesis, and protein photocleaving properties of novel and artificial light activatable protein cleaving agents possessing an anthraquinone structure as the core unit (Fig. 1).

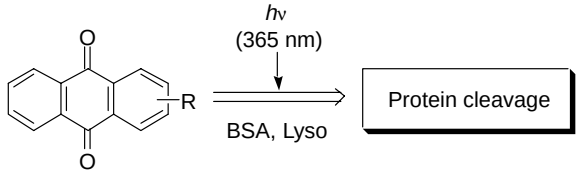

Figure 1. Photocleavage of proteins by anthraquinone derivatives.

In our first approach to investigate such novel protein cleaving molecules, we noted the anthraquinone structure, which appeared in several biologically important natural products, especially in antibiotics. Certain anthraquinone derivatives have been found to be efficient agents for DNA photocleavage by Schuster's group[7] and our group.[8] We expected that if a photo-excited anthraquinone derivative could produce some radical species, not only DNA, but also proteins would be damaged and cleaved by an anthraquinone derivative during photoirradiation.

To corroborate our hypothesis, we first examined the photo-induced protein cleaving activity of commercially available anthraquinone (**1**) and 2-hydroxymethylanthraquinone (**2**) in concentrations of 5–150 μM using bovine serum albumin (BSA, 15 μM) in Tris–HCl buffer (50 mM, pH 7.0) by photoirradiation using a long wavelength UV light (365 nm) without any additives (Fig. 2). The progress of protein photocleavage was monitored by 8% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE).[9] As obvious from (a) and (b) in

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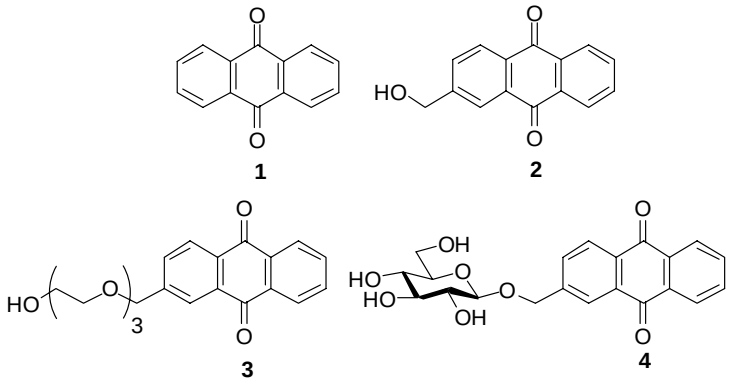


Figure 2. Anthraquinone derivatives.

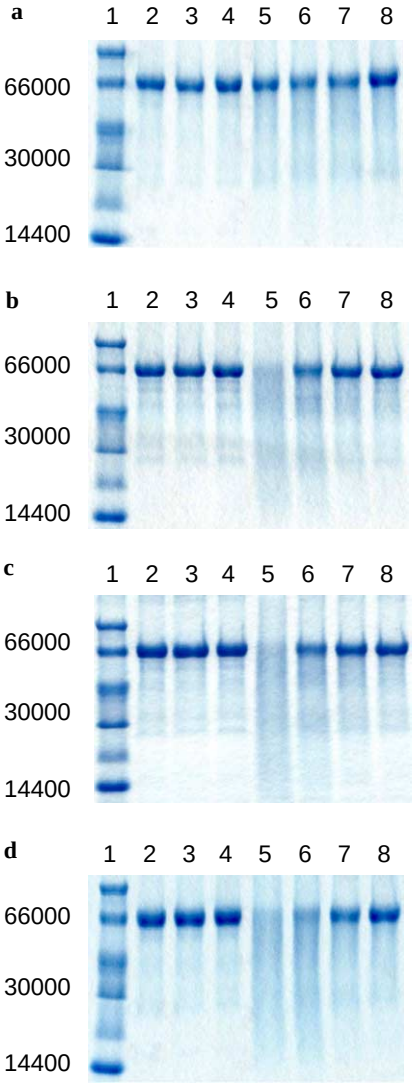


Figure 3. Photocleavage of bovine serum albumin (BSA). BSA (15 μM) was incubated with a compound in 20% acetonitrile Tris–HCl buffer (pH 7.0, 50 mM) at 25 °C for 2 h under the irradiation of a UV lamp (365 nm, 100 W) placed at 10 cm from the sample and analyzed by 8% Tricine–SDS–PAGE: (a), (b), (c), and (d) for the compounds **1**, **2**, **3**, and **4**, 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.

Figure 3, anthraquinone (**1**) itself exhibited very little BSA cleaving ability under photoirradiation. In stark contrast, the anthraquinone derivative **2** possessing a hydroxymethyl group caused significant degradation of BSA in concentrations over 50 μM, and the process had a concentration-dependent component. These results clearly demonstrate, for the first time, that an anthraquinone derivative is able to cleave a protein, BSA, upon irradiation with UV light having a long wavelength without any additives. It was also confirmed that no BSA cleavage by **2** was observed in the absence of light. Thus, the UV light functioned as a trigger to initiate an anthraquinone derivative toward protein scission. In addition, it was found that since the band corresponding to BSA disappeared and smear bands were observed on the SDS–PAGE for the BSA cleaving pattern of **2**, **2** must have randomly cleaved BSA.[10] These results were also supported by the analysis using MALDI-TOF MS, as shown in Figure 4. Thus, only after the incubation of BSA with **2** under photoirradiation, the MS peak corresponding to BSA disappeared, and no MS peaks corresponding to the cleaving fragments could be detected due to the random cleavage, leading to many fragments of very small amounts.

To improve further the protein cleaving ability of the anthraquinone derivative, we designed and synthesized hybrid compounds consisting of anthraquinone and a hydrophilic molecule possessing hydroxyl group(s), such as a polyethylene glycol or a carbohydrate. This is because we expected the hydrophilic moiety with hydroxyl group(s) of the hybrid to enhance the interaction with proteins due to its amphipathic nature and by forming the hydrogen bond(s). The designed hybrid **3** is constructed from anthraquinone and triethylene glycol. The hybrid **4** is an anthraquinone–carbohydrate hybrid containing glucose as the hydrophilic moiety (Fig. 2).

The chemical synthesis of hybrids **3** and **4** is shown in Scheme 1. Thus, **2** was treated with CBr_4 and PhP_3 to give the bromide **5**. The obtained compound **5** was then subjected to a reaction using triethylene glycol (**6**), activated by NaH to furnish the desired hybrid **3**. On the other hand, glycosidation of **2** with the glucopyranosyl bromide **7** using $AgClO_4$ in the presence of $CaSO_4$ and $CaCO_3$ in $MeNO_2$[11] afforded the glycoside **8** with

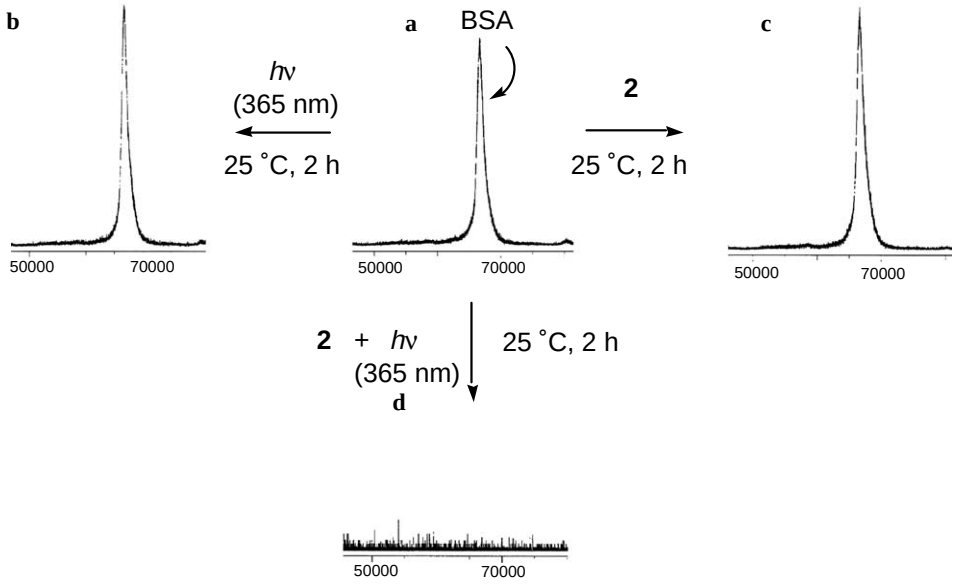
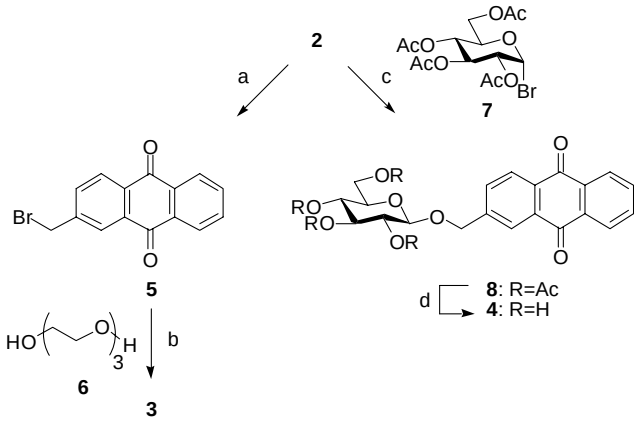


Figure 4. MALDI-TOF MS profiles of bovine serum albumin (BSA) photocleavage by **2**. (a) BSA alone; (b) BSA (15 μM) was incubated in 20% acetonitrile Tris–HCl buffer (pH 7.0, 50 μM) in the absence of **2** at 25 °C for 2 h under the irradiation of a UV lamp (365 nm, 100 W) placed at 10 cm from the sample; (c) BSA (15 μM) was incubated with **2** (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 **2** (150 μM) in 20% acetonitrile Tris–HCl buffer (pH 7.0, 50 mM) at 25 °C for 2 h under the photoirradiation.



Scheme 1. Synthesis of **3** and **4**. Reagents and conditions: (a) CBr_4, Ph_3P, CH_2Cl_2, rt, 15 h, 84%; (b) **6**, NaH, DMF, 0 °C, 0.5 h and then **5**, −60 °C, 5 h, 15%; (c) **7**, $AgClO_4$, $CaSO_4$, $CaCO_3$, $MeNO_2$, −15 °C, 1.5 h and then rt, 2 h, 55%; (d) NaOMe, MeOH, rt, 4 h, 61%.

β-stereoselectivity, whose protecting groups were removed using NaOMe to yield the targeted hybrid **4**.

With the designed hybrids **3** and **4** in hand, the photo-induced DNA cleaving activities of these hybrids were assayed under the same conditions. Based on the results given as (c) and (d) in Figure 3, the anthraquinone derivatives **3** and **4** caused effective protein cleavage during irradiation with the long wavelength UV light. It again confirmed that no protein cleavage by **3** and **4** was observed in the absence of light. Furthermore, the protein cleaving ability of the anthraquinone–carbohydrate hybrid **4** was found to be the strongest among the examined anthraquinone derivatives. Thus, the strongest **4** cleaved protein, BSA, at concentrations of over 15 μM, caused near 100% protein degradation at concentrations over 50 μM against 15 μM BSA. These results clearly indicate that the attachment of a suitable substituent into anthraquinone can significantly enhance the protein cleaving ability of anthraquinone.

To examine the generality of protein cleavage by anthraquinone derivatives, we next carried out the photo-induced protein cleavage using the strongest **4** 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 protein photocleavage was monitored by 12% SDS–PAGE (Fig. 5).[9] It was found that **4** caused a significant degradation of Lyso by photoirradiation using long wavelength UV light without any further additives. In addition, it was also confirmed that the cleaving ability is dependent on the concentration of **4**, and the cleaving

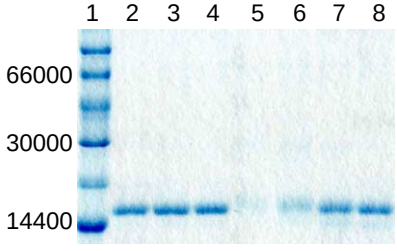


Figure 5. Photocleavage of hen egg lysozyme (Lyso). Lyso (15 μM) was incubated with a compound in 20% acetonitrile Tris–HCl buffer (pH 7.0, 50 mM) at 25 °C for 2 h under the irradiation of a UV lamp (365 nm, 100 W) placed at 10 cm from the sample and analyzed by 12% Tricine–SDS–PAGE: lane 1, sizemarker;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

activity increased as the concentration of **4** increased. These results clearly demonstrate that the anthraquinone derivatives cleave not only BSA, but also Lyso, thus showing the general ability for protein cleavage.

Since the protein cleaving activity of these anthraquinone derivatives significantly decreased in the presence of both hydroxyl radical and hydrogen peroxide scavengers, EtOH and KI, respectively, the protein cleavage must arise from the hydroxyl radical through hydrogen peroxide generated by the photoexcited anthraquinone and O_2.[12,13] Furthermore, it was confirmed that although the photocleaving reaction was not catalytic, the activity was time-dependent. Details of the reaction mechanism including the reaction pathway for protein cleavage are now under investigation.

In summary, we demonstrated here, for the first time, that the anthraquinone derivatives effectively caused protein cleavage under photoirradiation. This ability could be improved by the attachment of a suitable substituent into the anthraquinone core skeleton. The described chemistry and evaluation provide significant information about the molecular design of novel and artificial protein photocleaving agents. The targeted protein selective cleavage by attachment of a protein recognition moiety into anthraquinone is now in progress in our laboratories.

Acknowledgments

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