Target-selective degradation of proteins by porphyrins upon visible photo-irradiation†

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A porphyrin derivative effectively and selectively degraded the target transcription factor, human estrogen receptor-α (hER-α), upon visible light irradiation, in the absence of additives and under neutral conditions.

The development of novel methods for selective control of specific protein functions is of considerable importance in the fields of chemistry, biology, and medicine. In this context, the possibility of developing an organic photochemical agent which can degrade proteins upon irradiation with a specific wavelength of light under mild conditions and without any additives (such as metals or reducing agents) has attracted much attention due to the potential for selectively controlling specific protein functions.[1] We recently reported that certain anthraquinones and 2-phenylquinolines can act as protein photocleavers under long-wavelength UV photo-irradiation.[2,3] However, there are no reports of methods in which such a light-activated agent has been used for the selective degradation of a target protein leading to fragmentation under visible light irradiation. Here, we report the target-selective degradation of a protein induced by a light-activated porphyrin derivative. In the present study, certain porphyrin derivatives were found to be capable of degrading proteins leading to fragmentation upon long-wavelength UV and visible photo-irradiation, without the need for additives and under neutral conditions. Furthermore, a porphyrin derivative, 5,10,15,20-tetrakis(4-hydroxyphenyl)-21*H*, 23*H*-porphine (**1**), effectively and selectively degraded the target protein, the transcription factor human estrogen receptor-α (hER-α). To the best of our knowledge, this is the first example of target-selective degradation of a protein using a porphyrin derivative by light switching under neutral conditions. We anticipate that the present method will be used as a "smart" technology for selective control of specific functions of target proteins; in addition, it should prove useful for structure–activity studies of proteins, investigation of structural domains, and design of novel therapeutic drugs targeting proteins.

Certain porphyrin derivatives are well known to be efficient agents for DNA photocleavage, and have been used in photodynamic therapy (PDT) against cancer.[4–9] However, there are no reports of methods using a light-activated porphyrin for degradation of proteins leading to fragmentation. If a porphyrin derivative could be made to produce a radical or a reactive oxygen species (ROS) by photo-excitation, we hypothesized that this could be used for degradation not only of DNA, but also of protein molecules. To investigate this hypothesis, we selected 10 commercially available porphyrin derivatives as candidates for protein photo-degrading agents (see: ESI Fig. S1†), and bovine serum albumin (BSA) as the protein for the preliminary experiments.

First, we examined the photo-induced protein-degrading activity of the selected porphyrin derivatives at concentrations of 15, 5.0, 1.5 and 0.5 μM against 1.5 μM BSA using a long-wavelength UV lamp (365 nm, 100 W) for photo-irradiation. The progress of the photo-degradation reaction was monitored by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE).[10] Four porphyrin derivatives **1–4** (Fig. 1) were found to degrade BSA in a dose-dependent manner, as shown in Fig. 2a–d. In each figure, comparison of lanes 3 and 4 with lane 2 shows that neither photo-irradiation of BSA in the absence of each porphyrin derivative (lane 3) nor treatment of BSA with each porphyrin derivative without photo-irradiation (lane 4) resulted in a change in the SDS-PAGE profile. In contrast, lanes 5 and 6 show fading of the BSA band after exposure to each porphyrin derivative following photo-irradiation, indicating degradation of BSA. These results show that some porphyrin derivatives are capable of degrading a protein, BSA, upon irradiation with long-wavelength UV light and without further additives. Because degradation of BSA by these porphyrin derivatives did not take place in the absence of light, UV light must trigger the initiation of protein degradation by the porphyrin derivatives.

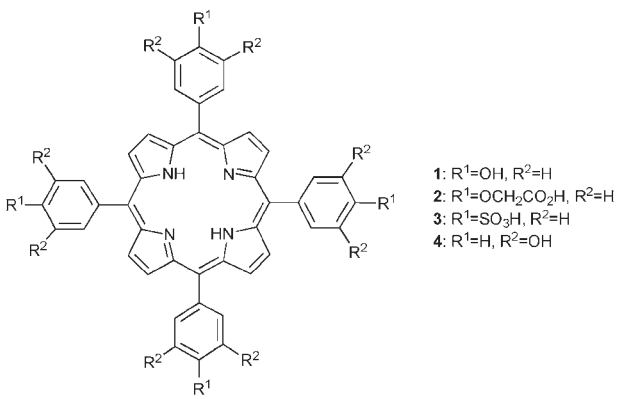

Fig. 1 Chemical structures of porphyrin derivatives **1–4** showing protein photo-degrading activity.

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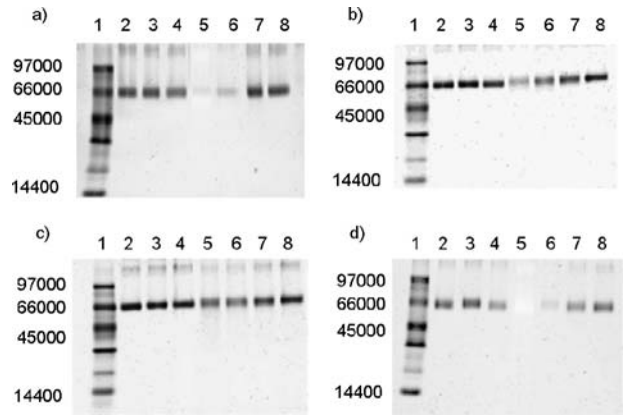

Fig. 2 Photo-degradation of BSA using **1–4** upon UV photo-irradiation. BSA (1.5 μM) was incubated with **1**, **2**, **3** or **4** in 20% acetonitrile–Tris-HCl buffer (pH 7.0, 50 mM) at 25 °C for 2 h while being irradiated by a UV lamp (365 nm, 100 W) placed 10 cm from the sample. The products were analyzed by tricine-SDS-PAGE. Gels (a), (b), (c) and (d) represent **1**, **2**, **3** and **4**, respectively: lane 1, size marker; lane 2, BSA alone; lane 3, BSA with UV; lane 4, BSA + each compound (15 μM) without UV; lanes 5–8, BSA + each compound (concentrations 15, 5, 1.5 and 0.5 μM, respectively) with UV.

In addition, since the SDS gels contained both faded and smeared bands, degradation of BSA by the porphyrin derivatives apparently occurs in a random fashion.[11]

In order to examine whether visible (Vis) light can initiate porphyrin-mediated photo-degradation of proteins, we first measured the UV–Vis spectra of porphyrin derivatives **1–4**. As shown in Fig. 3 (only the spectrum of **1** is shown), each porphyrin derivative shows absorption in both the UV and Vis regions. These results prompted us to examine the photo-degradation activity of the porphyrin derivatives under visible light irradiation. Whether visible light (diffuse sunlight, 75 W xenon lamp) was used (Fig. 4, only the case of **1** is shown) or UV, the photo-degrading abilities of porphyrin derivatives **1–4** were quite similar.

Porphyrin derivative **1** has some similarity to estrogens such as an estradiol (**5**), which has high affinity for hER due to its intraphenolic structure and hydrophobicity.[12] We therefore expected that porphyrin derivative **1** might selectively degrade the transcription factor, human estrogen receptor-α (hER-α). Indeed, the distance between the hydroxyl group of the A-ring and the 17β-hydroxyl group of the D-ring in **5** is similar to that between the two phenolic hydroxyl groups in **1** (Fig. 5). Modulation of hER-α function is an important factor in a variety of diseases, including breast cancer and osteoporosis.[13]

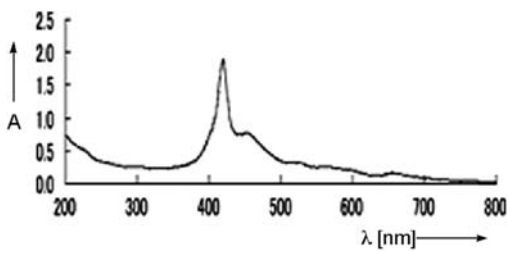

Fig. 3 UV–Vis spectrum (20% acetonitrile–Tris-HCl buffer (pH 7.0, 50 mM)) of **1**.

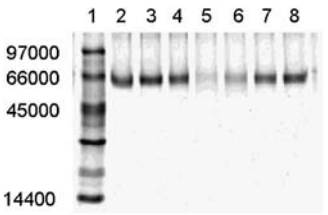

Fig. 4 Photo-degradation of BSA using **1** under Vis photo-irradiation. BSA (1.5 μM) was incubated with **1** in 20% acetonitrile–Tris-HCl buffer (pH 7.0, 50 mM) at 25 °C for 2 h under irradiation from a Vis lamp (diffuse sunlight, 75 W) placed 10 cm from the sample, and the products were analyzed by tricine-SDS-PAGE. Lane 1, size marker; lane 2, BSA alone; lane 3, BSA with visible irradiation; lane 4, BSA + **1** (15 μM) without irradiation; lanes 5–8, BSA + **1** (concentrations 15, 5, 1.5 and 0.5 μM, respectively) with visible irradiation.

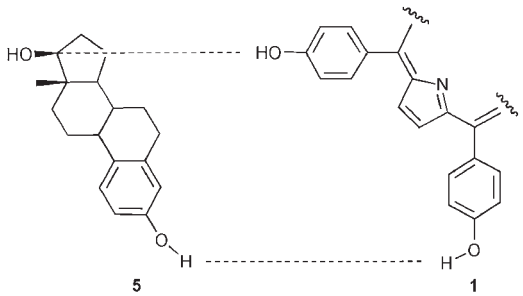

Fig. 5 Structural similarity of porphyrin derivative **1** and estradiol (**5**).

We then examined the application of porphyrin derivative **1** for the target-selective photo-degradation of proteins. Photo-induced degradation of three proteins—hER-α, BSA and hen egg lysozyme (Lyso)—was carried out using **1** at concentrations

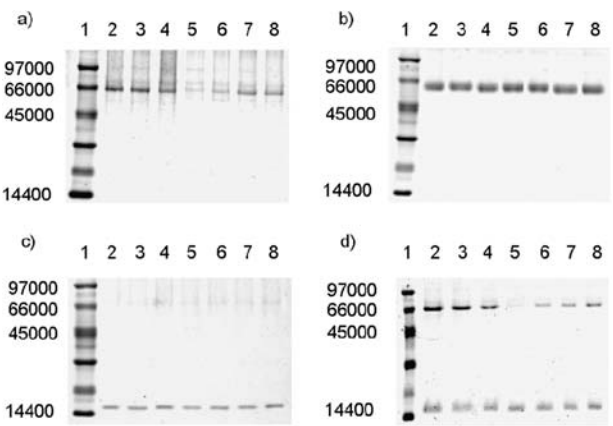

Fig. 6 Photo-degradation of proteins using **1** and visible wavelength irradiation. Each protein (1.5 μM) was incubated with **1** in 20% acetonitrile–Tris-HCl buffer (pH 7.0, 50 mM) at 25 °C for 2 h while irradiating with a visible wavelength lamp (diffuse sunlight, 75 W) placed 10 cm from the sample. The products were analyzed by tricine-SDS-PAGE. Gels (a), (b), (c) and (d) represent hER-α, BSA, Lyso and hER-α + Lyso, respectively: lane 1, size markers; lane 2, protein alone; lane 3, protein with visible wavelength irradiation; lane 4, protein + **1** (15 nM) without irradiation; lanes 5–8, protein + **1** (concentrations 15, 5.0, 1.5 and 0.5 nM, respectively) with visible wavelength irradiation.

of 15, 5.0, 1.5 and 0.5 nM (1000 times less than in the previous experiment) against 1.5 μM of each protein under visible light irradiation, and the reaction was monitored by SDS-PAGE. The results are summarized in Fig. 6a–c. When porphyrin derivative **1** was exposed to hER-α under photo-irradiation, significant degradation took place (Fig. 6a).[11] Degradation of hER-α by **1** was much greater (about 1000 times) than the degradation of BSA. The use of catalytic amounts of **1** was sufficient to effectively degrade hER-α. This result is in sharp contrast to the other proteins (BSA and Lyso), which showed no degradation under the same conditions (Fig. 6b and c). Furthermore, it is noteworthy that when hER-α and Lyso were both present in the reaction mixture, only hER-α was degraded by **1**, as shown in Fig. 6d. These results clearly indicate that porphyrin derivative **1** causes selective degradation of only the target protein, hER-α, upon visible photo-irradiation, in the absence of any additives and under neutral conditions.

hER-α degradation by porphyrin derivative **1** was found to decrease significantly in the presence of 17α-ethynylestradiol,[14] which also shows strong affinity for hER-α (see: ESI Fig. S2†). Furthermore, porphyrin derivatives **2** and **4**, which have a bulky substituent or two hydroxyl groups at the phenolic moiety, respectively, did not photo-degrade hER-α with high efficiency (see: ESI Fig. S3†). These results indicate that the strong and selective photo-degradation ability of **1** towards hER-α depends on the high degree of recognition of **1** for hER-α.

A scavenger assay was conducted in order to investigate the mechanism underlying this photo-degradation reaction. The photo-degrading activity of **1** significantly decreased in the presence of the $HO^{\bullet}$, H_2O_2 and $^1O_2$ scavengers EtOH, KI, and histidine (see: ESI Fig. S4†). Therefore, the degradation of hER-α must be due to reactive oxygen species (ROS) produced by photo-excitation of porphyrin and O_2.[15]

In conclusion, we have developed a new method for selectively degrading a target protein by visible wavelength photo-irradiation using a porphyrin derivative. The results presented here will contribute to the molecular design of novel artificial protein photo-degradation agents. We anticipate that this method will provide a means of controlling specific functions of certain proteins.

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