Target-selective photo-degradation of HIV-1 protease by a fullerene-sugar hybrid†

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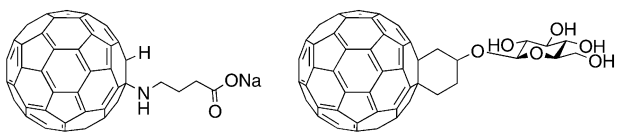
Received (in Cambridge, UK) 9th July 2008, Accepted 22nd August 2008
First published as an Advance Article on the web 2nd October 2008
DOI: 10.1039/b811726h

A designed fullerene-sugar hybrid effectively and selectively degraded the target protein, HIV-1 protease, which has high affinity for the fullerene moiety; degradation was achieved using long-wavelength UV or visible photo-irradiation, in the absence of any additives and under neutral conditions.

Proteins are key players in many biological events. The development of new methods for the 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 has attracted much attention.[1] Here, we report the target-selective degradation of a protein induced by a light-activated fullerene derivative. This fullerene derivative was found to be capable of degrading proteins upon long-wavelength UV or VIS photo-irradiation, without the addition of additives and under neutral conditions. Furthermore, the designed and synthesized fullerene-sugar hybrid effectively and selectively degraded the target protein, HIV-1 protease. To the best of our knowledge, this is the first example of target-selective degradation of a protein by a fullerene derivative using light switching under neutral conditions.

Certain fullerene derivatives have been found to be efficient agents for DNA photocleavage.[2] However, there are no reports of methods in which a light-activated fullerene is used for the degradation of proteins. In this context, we expected that if a fullerene derivative could be made to produce a radical or a reactive oxygen species (ROS) by photo-excitation, this derivative could be used for degradation not only of DNA, but also of protein molecules. To investigate this hypothesis, we selected a commercially available and water soluble fullerene derivative **1** as a protein photo-degrading agent, and human immunodeficiency virus-1 (HIV-1) protease as the target protein (Fig. 1). It has previously been reported that a fullerene derivative binds with high affinity to HIV-1 protease due to hydrophobicity and steric size compatibility with a pocket in HIV-1 protease.[3] Furthermore, modulation of HIV-1 protease function is a very important factor in HIV diseases such as AIDS.[4]

First, we examined the photo-induced protein-degrading activity of **1** at concentrations of 1.5, 0.5, 0.15 and 0.05 μM against 1.5 μM HIV-1 protease in PBS buffer (pH 7.0, 50 mM) 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);[5] the results are shown in Fig. 2. Comparison of lanes 3 and 4 with lane 2 shows that neither photo-irradiation of HIV-1 protease in the absence of **1** (lane 3), or treatment of HIV-1 protease with **1** without photo-irradiation (lane 4), resulted in a change in the SDS-PAGE profile. In contrast, lane 5 shows fading of the SDS-PAGE band corresponding to HIV-1 protease after exposure to **1** upon photo-irradiation, which indicates that degradation of HIV-1 protease took place. These results show that fullerene derivative **1** is capable of degrading a protein, HIV-1 protease, upon irradiation with long wavelength UV light in



1 2

Fig. 1 Chemical structure of fullerene derivative **1** and fullerene-sugar hybrid **2**.

Fig. 2 Photo-degradation of human immunodeficiency virus-1 (HIV-1) protease using **1**. HIV-1 protease (1.5 μM) was incubated with **1** in PBS buffer (pH 7.0, 50 mM) at 25 °C for 2 h under irradiation with a UV lamp (365 nm, 100 W) placed 10 cm from the sample, and analysed using tricine-SDS-PAGE. Lane 1, size marker; lane 2, HIV-1 protease alone; lane 3, HIV-1 protease upon UV irradiation; lane 4, HIV-1 protease + **1** (1.5 μM) without UV irradiation; lanes 5–8, HIV-1 protease + **1** (concentrations 1.5, 0.5, 0.15 and 0.05 μM, respectively) upon UV irradiation.

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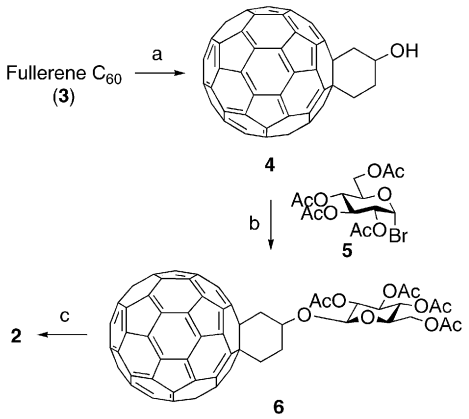
† Electronic supplementary information (ESI) available: Synthesis of fullerene derivatives and degradation reactions of proteins. See DOI: 10.1039/b811726h



Scheme 1 Synthesis of fullerene-sugar hybrid **2**. (a) Ref. 8; (b) $AgClO_4$, $CaCO_3$, $CaSO_4$, PhMe, rt, 4 h, 44%; (c) NaOMe, MeOH–PhMe (2/1), rt, 0.5 h, 73%.

the absence of further additives, although degradation by **1** is not particularly high. Since the pattern obtained for HIV-1 protease degradation by **1** contained faded and smeared bands, it was concluded that degradation of HIV-1 protease by **1** took place in a random fashion.[6]

In order to improve the protein degrading ability and selectivity of the fullerene, we designed and synthesized hybrid molecule **2**, which consists of a fullerene and a sugar (Fig. 1).[7] The design was based on the expectation that the hydrophilic hydroxyl group(s) of the hybrid would enhance the interaction with HIV-1 protease, due to its amphipathic nature and to the formation of one or several hydrogen bonds. The synthesis of hybrid **2** is outlined in Scheme 1. Known fullerene derivative **4**,[8] which was prepared from the commercially available fullerene C_{60} (**3**), was glycosylated with glycosyl bromide **5** in the presence of $AgClO_4$, $CaCO_3$ and $CaSO_4$ in PhMe[9] to give protected fullerene glycoside **6**. Subsequent deprotection of the acetyl (Ac) groups in **6** using NaOMe in MeOH–PhMe gave the desired hybrid **2**.

The target-selective photo-degradation of proteins by fullerene-sugar hybrid **2** was examined. Photo-induced degradation of three proteins—HIV-1 protease, bovine serum albumin (BSA) and hen egg lysozyme (Lyso)—was carried out using **2**, and the reaction progress was monitored by SDS-PAGE. The results are summarized in Fig. 3a–c. When hybrid **2** was exposed to HIV-1 protease under photo-irradiation conditions, significant degradation took place (Fig. 3a). The degradation ability of **2** was found to be much greater than that of **1**. This result was in sharp contrast to that obtained with the other proteins (BSA and Lyso), which showed no degradation upon photo-irradiation in the presence of **2** (Fig. 3b and c). Furthermore, it is noteworthy that when HIV-1 protease and BSA were both present in the reaction mixture, only HIV-1 protease was degraded by **2**, as shown in Fig. 3d. In addition, the target-selectivity of **2** against HIV-1 protease was higher than that of **1**. These results clearly indicate that the fullerene-sugar hybrid **2** causes selective degradation only of the target protein, HIV-1 protease upon photo-irradiation, in the absence of any additives and under neutral conditions.

In order to examine the possibility of photo-degradation of proteins by fullerene-sugar hybrid **2** upon irradiation by visible

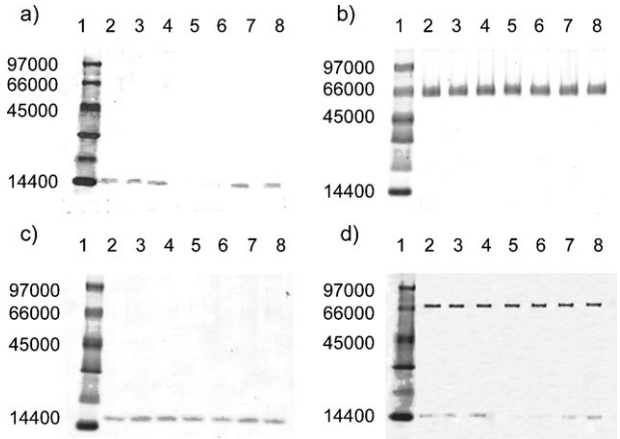


Fig. 3 Photo-degradation of proteins using **2** upon UV irradiation. Each protein (1.5 μM) was incubated with **2** in 10% dimethyl formamide/PBS buffer (pH 7.0, 50 mM) at 25 °C for 2 h under irradiation with a UV lamp (365 nm, 100 W) placed 10 cm from the sample, and the products were analysed by tricine-SDS-PAGE. Gels (a)–(c) and (d) represent HIV-1 protease, BSA, Lyso and HIV-1 protease + BSA, respectively. Lane 1, size marker; lane 2, protein alone; lane 3, protein upon UV irradiation; lane 4, protein + **2** (1.5 μM) without UV irradiation; lanes 5–8, protein + **2** (concentrations 1.5, 0.5, 0.15 and 0.05 μM, respectively) upon UV irradiation.

light, we measured the UV/Vis spectrum of **2**. The results shown in Fig. 4 indicate that **2** absorbs in both the UV and visible regions. These results prompted us to examine the photo-degradation activity of **2** upon visible light irradiation. Similar results were obtained when visible light (diffuse sunlight, 75 W xenon lamp) was used instead of the UV lamp (Fig. 5a and b): **2** still effectively and selectively degraded the target protein, HIV-1 protease, rather than the other proteins, BSA and Lyso.

In order to investigate the mechanism behind this photo-degradation of proteins, a scavenger assay was conducted. It was found that the photo-degrading activity of **2** significantly decreased in the presence of the $HO^{\bullet}$, H_2O_2 and $^1O_2$ scavengers, DMSO, KI, and histidine. Therefore, HIV-1 protease degradation is likely to be due to ROS produced by photo-excitation of fullerene and O_2.[10]

In conclusion, it was found that certain fullerene derivatives degraded not only DNA but also proteins upon long-wavelength UV or visible light irradiation, in the absence of

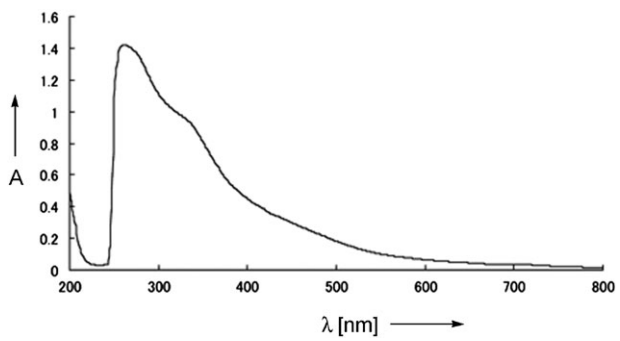


Fig. 4 UV/Vis spectrum (10% dimethylformamide/PBS buffer (pH 7.0, 50 mM)) of **2**.

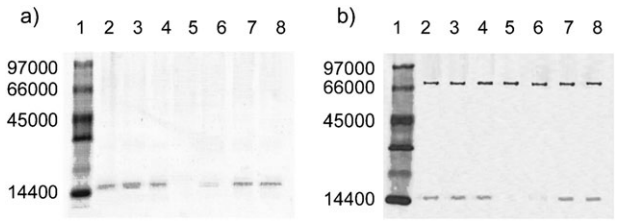


Fig. 5 Photo-degradation of proteins using **2** upon Vis irradiation. Each protein (1.5 μM) was incubated with **2** in 10% dimethyl formamide/PBS buffer (pH 7.0, 50 mM) at 25 °C for 2 h under irradiation with a visible light (diffuse sunlight, 75 W xenon lamp)-placed 10 cm from the sample, and the products were analysed by tricine-SDS-PAGE. Gels (a) and (b) represent HIV-1 protease, and HIV-1 protease + BSA, respectively. Lane 1, size marker; lane 2, protein alone; lane 3, protein upon VIS irradiation; lane 4, protein + **2** (1.5 μM) without Vis irradiation; lanes 5–8, protein + **2** (concentrations 1.5, 0.5, 0.15 and 0.05 μM, respectively) upon Vis irradiation.

any additives and under neutral conditions. Furthermore, we have developed a new method for selective degradation of the target protein, HIV-1 protease, by photo-irradiation using a fullerene-sugar hybrid. The results presented here will contribute to the molecular design of novel artificial protein photo-degradation agents.

This research was supported in part by the 21st Century COE Program 'Keio Life-Conjugated Chemistry', High-Tech Research Center Project for Private Universities: Matching Fund Subsidy, 2006-2011, and Scientific Research (B) (No. 20310140) from the Ministry of Education, Culture, Sports, Science and Technology of Japan (MEXT).

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