Available online at www.sciencedirect.com

ScienceDirect

Tetrahedron Letters 49 (2008) 3794–3796

Tetrahedron Letters

Synthesis of photoaffinity probe based on the leaf-opening factor from genus *Albizzia*

Masahiro Okada, Akira Matsubara, Minoru Ueda *

Tohoku University, Department of Chemistry, Aoba-ku, Sendai 980-8578, Japan

Received 29 January 2008; revised 31 March 2008; accepted 1 April 2008
Available online 8 April 2008

Abstract

The circadian rhythmic leaf-movements in legumes, called nyctinasty, are regulated by a pair of leaf-closing and -opening factors. Recent fluorescence studies revealed that *cis-p*-coumaroylagmatine (**1**), the leaf-opening factor from genus *Albizzia*, specifically bound to motor cells in a joint of leaf and stem. In order to identify the receptor protein, which is expected to be involved in the plasma membrane of the cell, we designed and synthesized a photoaffinity probe (**2**), containing a photoreactive azide group on the aromatic ring and FLAG segment as a peptide antigen for immunoprecipitation.
© 2008 Elsevier Ltd. All rights reserved.

Keywords: *Albizzia saman*; Nyctinasty; Leaf-opening factor; Photoaffinity probe

1. Introduction

Most leguminous plants close their leaves in the evening, as if sleeping, and open them in the morning. This circadian rhythm, which is characterized by leaf-closing and -opening movements and is known as nyctinasty, is regulated by a pair of leaf-closing and -opening factors (LCF and LOF, respectively).[1,2] *cis-p*-Coumaroylagmatine (**1**) was isolated as the LOF of the genus *Albizzia*.[3] Recent fluorescence studies revealed that **1** binds to motor cells in the joint of the leaf and stem[4] where they interacted with specific receptors on the cells.[5,6] These findings indicated that **1** played a crucial role as an endogenous signaling molecule in the control of nyctinastic leaf-movement through binding to the corresponding receptor. However, the receptor protein of **1** and the signaling pathway of nyctinasty have not yet been resolved. In order to elucidate the molecular mechanism responsible for nyctinastic leaf-movement, identification of the receptor protein for **1** is necessary. We considered the combination of photo-affinity-labeling and affinity-purification with immunoprecipitation to be a useful approach for purifying the receptor of a ligand. Specifically, immunoprecipitation using the FLAG peptide (Asp-Tyr-Lys-Asp-Asp-Asp-Asp-Lys)[7] is an effective method that is widely used for the purification of a protein. Here, we report the synthesis

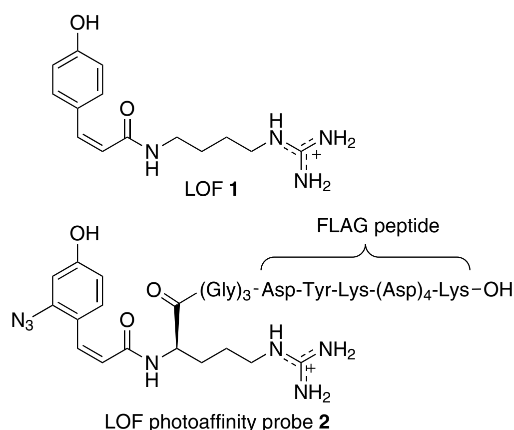

Fig. 1. The chemical structure of LOF **1** and the LOF photoaffinity probe **2**.

* Corresponding author. Tel./fax: +81 22 795 6553.
E-mail address: ueda@mail.tains.tohoku.ac.jp (M. Ueda).

0040-4039/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2008.04.002

of photoaffinity probe **2**, containing an azide on the aromatic ring as a photoreactive group and a FLAG segment for use as an antigen for immunoprecipitation (Fig. 1).

2. Results and discussion

We designed an efficient photoreactive probe in order to identify the receptor for **1** using a photoaffinity-labeling approach. In general, an aryl azide derivative such as azidobenzoic acid is usually employed as a photoreactive group.[8] However, the addition of a large photoreactive unit into a small molecule results in a marked increase in the molecular size of the probe and would result in decreased affinity between a ligand and the corresponding receptor. Thus, we attempted to synthesize a novel photoaffinity probe that contained a photoreactive group as a part of the LOF molecule to minimize the increase in probe size.

First, we designed and synthesized the LOF analog (**3**), which has an azide group on the aromatic ring of **1**. The molecular size of **3** is approximately the same as that of **1**. The synthesis of **3** is summarized in Figure 2. The iodide **5** prepared from commercially available **4** in two steps was coupled with propargyl alcohol by the Sonogashira reaction[9] to give **6** in an excellent yield. Reductions of **6** with $Na_2S_2O_4$ and then with Lindlar's catalyst gave *cis*-olefin (**7**). Successive treatment of **7** with triflyl azide gave **8**. The resulting **8** was oxidized to give **9** in 67% yield in two steps.[10] Finally, **9** was coupled with agmatine to give **3**.[11] We tested the biological activity of **3** using the leaves of *Albizzia saman* according to a previous method.[3] The reason for using *A. saman* in bioassay is that *A. saman* will be suitable for the future study on the identification of receptor of LOF. *A. saman* would be most useful for the collection of a large amount of motor cells among genus *Albizzia*. Analog **3** was as effective as **1** in the bioassay and showed leaf-opening activity at 300 μM.

In order to confirm the photoreactivity of an azide in **3**, an aqueous solution containing **3** was irradiated at 5.0 cm distance with UV light (365 nm and 1820 μW/cm^2). After UV irradiation for 5 min, the products were analyzed by LC–MS (Fig. 3). Analog **3** was consumed completely, and concurrently observed new signals at 254 nm were weakened. This result indicated that the azide group in **3** was degradated by UV irradiation. Additionally, a new signal corresponding to a denitrogenated degradate of **3** was mainly observed at m/z 290.0, and a minor signal, a water adduct of the denitrogenated degradate of **3**, was also observed. Thus, **3** was confirmed to be degraded by UV

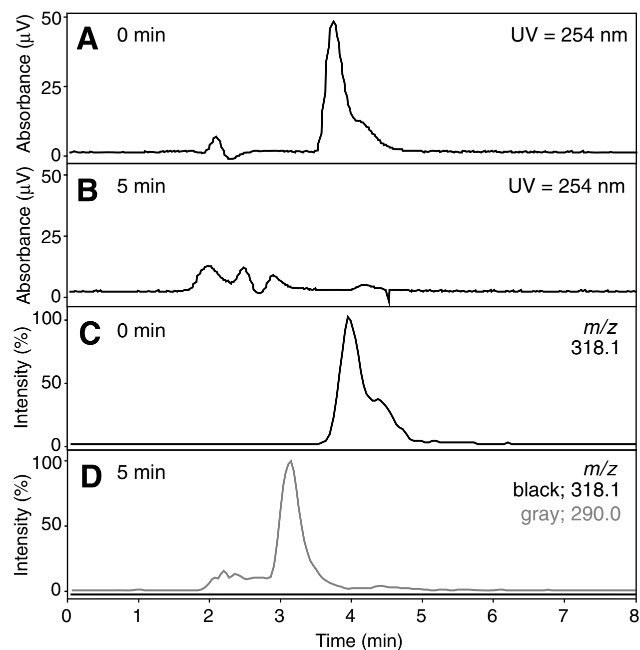

Fig. 3. Photodegradation analysis of the LOF analog **3** with UV irradiation at 365 nm. (A) LC/ESI-MS analysis of the initial aqueous solution containing the LOF analog **3** by UV at 254 nm and (C) the extracted ion chromatogram m/z 318.1. (B) LC/ESI-MS analysis of an aqueous solution containing LOF analog **3**, which was irradiated for 5 min at 5.0 cm distance with UV light (365 nm and 1820 μW/cm^2), by UV at 254 nm and (D) the extracted ion chromatograms m/z 318.1 (represented by the black line) and m/z 290.0 (represented by the gray line).

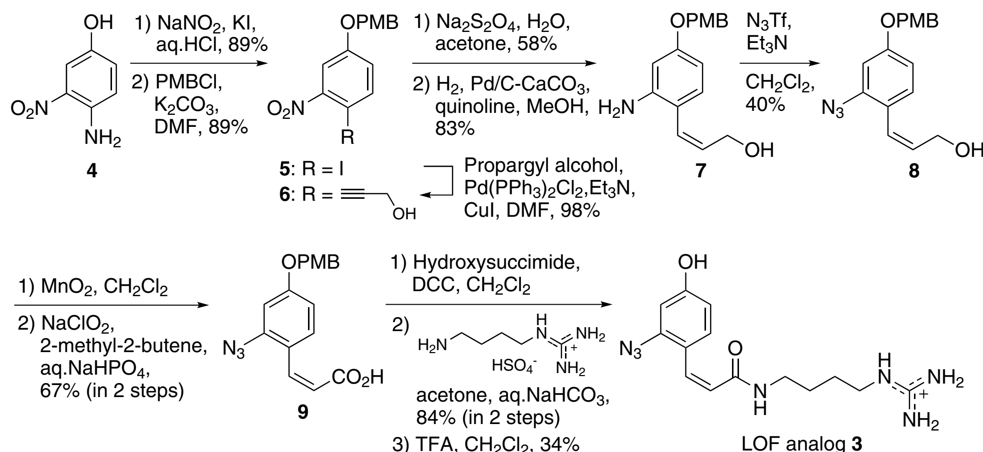

Fig. 2. Synthesis of the LOF analog **3**.

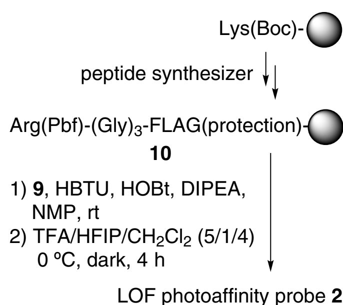

Fig. 4. Synthesis of the LOF photoaffinity probe **2**.

light immediately and was demonstrated to be a good photoreactive analog of **1**.

Based on the successful degradation of **3**, we designed an efficient probe for photoaffinity-labeling and immunoprecipitation of the receptor for **1**. Although biotinylated photoaffinity probes are usually used to identify binding proteins of small bioactive molecules,[8,12] it has been reported that purification based on biotin–avidin chemistry is often associated with high non-specific binding due the prevalence of numerous endogenous biotinylated proteins in the cell.[12,13] We designed photoaffinity probe **2** using a FLAG segment as an antigen because the FLAG peptide is widely used as a peptide tag for immunoprecipitation purification using anti-FLAG monoclonal antibody.[7] Since it is already known that the substitution of the agmatine moiety in **1** with arginine does not affect biological activity,[5,6] the FLAG peptide was introduced through peptide linkage. The synthesis of **2** was easily achieved using solid-phase peptide synthesis (Fig. 4). The protected dodecapeptide (**10**) on the polymer support was coupled with intermediate **9**. After acidic cleavage from the resin, the mixture was purified by HPLC to give the desired photoaffinity probe (**2**).[14] In a bioassay, probe **2** was approximately half as effective as **1**, and showed potent leaf-opening activity in *A. saman* at 500 μM. Despite the addition of the large FLAG segment, probe **2** retained its leaf-opening activity, which is three-fifth strong as that of natural LOF. This relative biological activity would be enough for future study on the purification of receptor of LOF.[6] Thus, we successfully synthesized biologically active photoaffinity probe **2** containing an aryl azide unit and a FLAG segment.

Given that a previous study revealed that the *cis*-*p*-coumaroyl moiety of **1** was essential for leaf-opening activity,[5] the photoreactive aryl azide unit in **2** is expected to interact with the binding site of the receptor. Thus, a higher yield can be expected in cross-link formation by photo irradiation. In addition, since several leaf movement factors possess a *cis*-*p*-coumaroyl moiety as well as **1**,[1,2] a similar approach would be applicable to the detection of specific receptors for other leaf-movement factors.

References and notes

1. Ueda, M.; Yamamura, S. *Angew. Chem., Int. Ed.* **2000**, *39*, 1400.
2. Ueda, M.; Nakamura, Y.; Okada, M. *Pure Appl. Chem.* **2007**, *79*, 519.
3. Ueda, M.; Tashiro, C.; Yamamura, S. *Tetrahedron Lett.* **1997**, *38*, 3253.
4. Satter, R. L.; Gorton, H. L.; Vogelmann, T. C. *The Pulvinus: Motor Organ for Leaf Movement*; American Society of Plant Physiologists, 1990.
5. Nagano, H.; Kato, E.; Yamamura, S.; Ueda, M. *Org. Biomol. Chem.* **2003**, *1*, 3186.
6. Nakamura, Y.; Matsubara, A.; Okada, M.; Kumagai, T.; Ueda, M. *Chem. Lett.* **2006**, *35*, 744.
7. Hopp, T. P.; Prickett, K. S.; Price, V. L.; Libby, R. T.; March, C. J.; Ceretti, D. P.; Urdal, D. L.; Conlon, P. J. *Biotechnology* **1988**, *6*, 1204.
8. Hatanaka, Y.; Sadakane, Y. *Curr. Top. Med. Chem.* **2002**, *2*, 271.
9. Sonogashira, K. In *Metal-Catalyzed Cross-coupling Cross-CouplingReactions*; Diederich, F., Stang, P. J., Eds.; Wiley-VCH: Weiheim, Germany, 1998; pp 203–229.
10. The intermediate **9**: ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.66 (d, 1H, $J = 8.3$ Hz), 7.30 (d, 2H, $J = 8.6$ Hz), 7.10 (d, 1H, $J = 12.5$ Hz), 6.92 (d, 2H, $J = 8.6$ Hz), 6.74–6.70 (m, 2H), 5.89 (d, 1H, $J = 12.5$ Hz), 5.01 (s, 2H), 3.81 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 171.3, 160.8, 159.6, 140.7, 140.1, 132.7, 129.3, 128.1, 119.0, 118.1, 114.1, 110.6, 104.6, 70.1, 55.3; ESI-HRMS calcd for $C_{17}H_{15}N_3O_4Na$ $[M+Na]^+$ 348.0955, found 348.0954.
11. The LOF analog **3**: ^{1}H NMR (500 MHz, CD_3OD): δ 7.39 (d, 1H, $J = 8.5$ Hz), 6.77 (d, 1H, $J = 12.4$ Hz), 6.61 (d, 1H, $J = 2.3$ Hz), 6.53 (dd, 1H, $J = 8.5$, 2.3 Hz), 5.88 (d, 1H, $J = 12.4$ Hz), 3.22–3.19 (m, 2H), 3.18–3.16 (m, 2H), 1.55–1.52 (m, 4H); ^{13}C NMR (125 MHz, CD_3OD): δ 170.0, 160.6, 158.6, 140.9, 133.6, 132.8, 123.3, 119.8, 113.0, 105.6, 42.1, 39.6, 27.5, 27.2; ESI-HRMS calcd for $C_{14}H_{19}N_7O_2$ $[M+H]^+$ 318.1673, found 318.1672.
12. Sugimoto, T.; Fujii, T.; Idutu, Y.; Yamamura, S.; Ueda, M. *Tetrahedron Lett.* **2004**, *45*, 335.
13. Masuoka, J.; Guthrie, L. N.; Hazen, K. C. *Microbiology* **2002**, *148*, 1073.
14. The LOF photoaffinity probe **2**: ^{1}H NMR (500 MHz, D_2O): δ 7.16 (d, 1H, $J = 8.1$ Hz), 7.07 (d, 2H, $J = 8.2$ Hz), 6.90 (d, 1H, $J = 12.5$ Hz), 6.81 (d, 2H, $J = 8.2$ Hz), 6.73 (s, 1H), 6.62 (d, 1H, $J = 8.1$ Hz), 6.07 (d, 1H, $J = 12.5$ Hz), 4.62–4.54 (m, 5H), 4.49 (t, 1H, $J = 6.8$ Hz), 4.30 (dd, 1H, $J = 7.4$, 6.6 Hz), 4.20–4.15 (m, 2H), 4.03–3.92 (m, 6H), 3.05–2.94 (m, 10H), 2.76–2.67 (m, 3H), 2.66–2.59 (m, 4H), 2.50 (dd, 1H, $J = 8.7$, 15.2 Hz), 1.84–1.63 (m, 8H), 1.39–1.27 (m, 8H); ESI-HRMS calcd for $C_{62}H_{88}N_{20}O_{26}$ $[M+2H]^{2+}$ 764.3084, found 764.3086.