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Development of 4-methoxy-7-nitroindolinyl (MNI)-caged auxins which are extremely stable in planta

Ken-ichiro Hayashi [a,*], Naoyuki Kusaka [a], Soma Yamasaki [a], Yunde Zhao [b], Hiroshi Nozaki [a]

[a] Department of Biochemistry, Okayama University of Science, 1-1 Ridai-cho, Okayama 700-0005, Japan
[b] Section of Cell and Developmental Biology, University of California at San Diego, La Jolla, CA 92093-0116, United States

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ABSTRACT

Phytohormone auxin is a master regulator in plant growth and development. Regulation of cellular auxin level plays a central role in plant development. Auxin polar transport system modulates an auxin gradient that determines plant developmental process in response to environmental conditions and developmental programs. Photolabile caged auxins allow optical control of artificial auxin gradients at cellular resolution. Especially, two-photon uncaging system achieves high spatiotemporal control of photolysis reaction at two-photon cross-section. However, the development of caged versions of auxin has been limited by the instability of the caged auxins to higher plant metabolic activities. Here, we describe the synthesis and application of highly stable caged auxins, 4-methoxy-7-nitroindolinyl (MNI)-caged auxins. Natural auxin, indole 3-acetic acid, and two synthetic auxins, 1-NAA and 2,4-D were caged by MNI caging group. MNI-caged auxins showed a high stability in planta and a rapid release the original auxin when photolyzed. We demonstrated that optical control of auxin-responsive gene expression and auxin-related physiological responses by using MNI-caged auxins. We anticipate that MNI-caged auxins will be an effective tool for high-resolution control of endogenous auxin level.

The plant hormone auxin plays a crucial role in almost every aspects of plant development including embryogenesis, cell elongation, vascular tissue differentiation, tropic responses to light and gravity, and lateral branching of shoots and roots.[1,2] The cellular auxin levels are modulated by auxin biosynthesis, auxin polar transport, and auxin catabolism. Indole-3-acetic acid (IAA), the major naturally occurring auxin, is biosynthesized from tryptophan in the indole 3-pyruvate pathway and is polar transported to generate auxin concentration gradients in plant tissues.[1–3] Auxin polar streams function in the establishment of the embryonic development, lateral development, and vascular patterning. Additionally, asymmetric redistribution of auxin in response to environmental and developmental cues drives the asymmetric tropic growth that allows plants to maintain positioning against the gravity vector and light. Therefore, spatiotemporal information of auxin levels would be crucial for dissecting the underlying mechanisms of the physiological roles of auxin gradients.[3,4]

Molecular biology and genetic studies have unraveled that the AUX1 family of auxin influx symporters, PIN family of auxin efflux carrier proteins, and ATP-binding cassette group B (ABCB) auxin transporters, coordinately modulate auxin transport.[4] The cellular level and localization of auxin transport proteins on plasma membrane was thought to determine the direction and rate of auxin movement to generate asymmetric auxin gradient in plant tissue. Furthermore recent studies demonstrated that locally synthesized auxin also participates in the formation of auxin gradients, and cellular auxin levels are preciously maintained by auxin inactivating enzymes, such as GH3, IAA-amino acid conjugating enzymes and auxin oxidases.[5–8] These studies indicated that auxin gradients would not only be formed by transport machinery, but also modulated by complicated processes involving local biosynthesis and inactivation of auxin. Visualization of cellular auxin gradients using auxin-responsive reporter lines has been widely utilized to dissect the role of auxin gradient formation in physiological response of plant.[9–11] As an alternative approach, using an artificial auxin gradient system would benefit studies of the positional impact of auxin levels on diverse type of cells and tissue in plants.

A caged compound is an inactivated bioactive molecule and can rapidly release the original active molecule upon photolysis of a photo-cleavable protecting group (caging group) with optical devices.[12,13] With caged compounds, the uncaging reaction can be spatiotemporally controlled at cellular level by precious control of light-irradiation. Additionally, the active molecule is

* Corresponding author. Tel.: +81 86 256 9661; fax: +81 86 256 9559.
E-mail address: hayashi@dbc.ous.ac.jp (K.-i. Hayashi).
URL: http://www.dbc.ous.ac.jp/labs/KHayashi/KhayashiEnglish.html (K.-i. Hayashi).

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intracellularly released, which can be repeated at any point during an experiment without physical perturbing. The intracellular levels of the released active molecule can be precisely controlled by the rate of uncaging.[12,14] We previously demonstrated that 2′,5′-dimethoxyphenyl-2-nitrobenzyl (DMPNB)-caged auxins were useful tools to establish an artificial auxin gradient.[15] DMPNB-caged auxin displayed useful features and advantages in comparison with classical methods of auxin application, such as a solution, a waxy paste, or an agar block containing auxin. Plant cells generally show higher metabolic capacity than mammal cells. Therefore, conventional caging groups used for mammalian biology are readily hydrolyzed by plant enzymes to release auxins without photolysis.[15] DMPNB-caged auxin was designed to be resistant against esterases and to have considerable stability both in vitro and in vivo. DMPNB-caged auxins enable the manipulation of cellular auxin levels within a single cell by fine control of optical devices, suggesting that an artificial auxin gradient can be achieved at a desired position by means of a caged auxin system.

In this study, we synthesized MNI (4-methoxy-7-nitroindolinyl)-caged IAA, NAA and 2,4-D in which the carboxylic acid was protected by a secondary amine of MNI caging group.

MNI-caged auxins showed higher in vivo stability than DMPNB-caged auxins. Additionally, MNI-caged auxins can be used for two-photon uncaging system, as the MNI-caging group was originally developed to be a photolabile group for the two-photon excitation system,[16] and the MNI-caged L-glutamate and MNI-caged-D-aspartate are widely used as commercial reagent for two-photon uncaging in neuroscience.[13] Two-photon uncaging takes advantage of the high spatial resolution in comparison with one-photon uncaging, because photolysis was occurred at the cross-section of the two-photon excitation (Fig. 1A).[17] Additionally, near infrared light (760 nm) used in two-photon excitation can reach into deeper tissue compared with UV light in one-photon excitation (360 nm). However, higher concentrations of caged molecules are required due to the low uncaging efficiency by two-photon excitation. We herein demonstrate the high stability of MNI-caged auxins in *Arabidopsis* plants and the photo-triggered spatiotemporal controls of auxin response in roots by one-photon excitation system.

Synthesis of MNI-caged auxins: Napthalene-1-acetic acid (NAA) and 2,4-dichlorophenoxy acetic acid (2,4-D), two well characterized synthetic auxins, and the natural auxin IAA are (Scheme 1) widely used for physiological experiments. NAA and 2,4-D exhibited a different transport profile from IAA. The transport profiles of the two typical synthetic auxins have been extensively characterized.[3,4] PIN carrier proteins export NAA to outside of the cell, but NAA is not actively imported into the cell. In contrast, 2,4-D is imported by AUX1, but is not actively exported.[4] Therefore, these two synthetic auxins have been used as diagnostic tools for influx or efflux auxin transport machinery.

As the initial step of auxin response, auxin is perceived by TIR1-Aux/IAA receptor complex in *Arabidopsis*. The crystal structures of the auxin-bound TIR1-Aux/IAA receptor complex revealed that aromatic ring and carboxylic acid in auxin molecule are essential for the affinity to the binding site of the receptor complex.[18] Based on the structural information, caging of the carboxylic acid in auxin would diminish the binding activity of auxin molecules to yield physiologically inert caged auxin molecules.

Plant cells seem to show higher esterase activities and the sterically accessible ester linkage in caged compound, such as NPE-IAA (**4**) are readily hydrolyzed in planta. In our previous studies, DMPNB-caged auxin was designed to be esterase-resistant caged

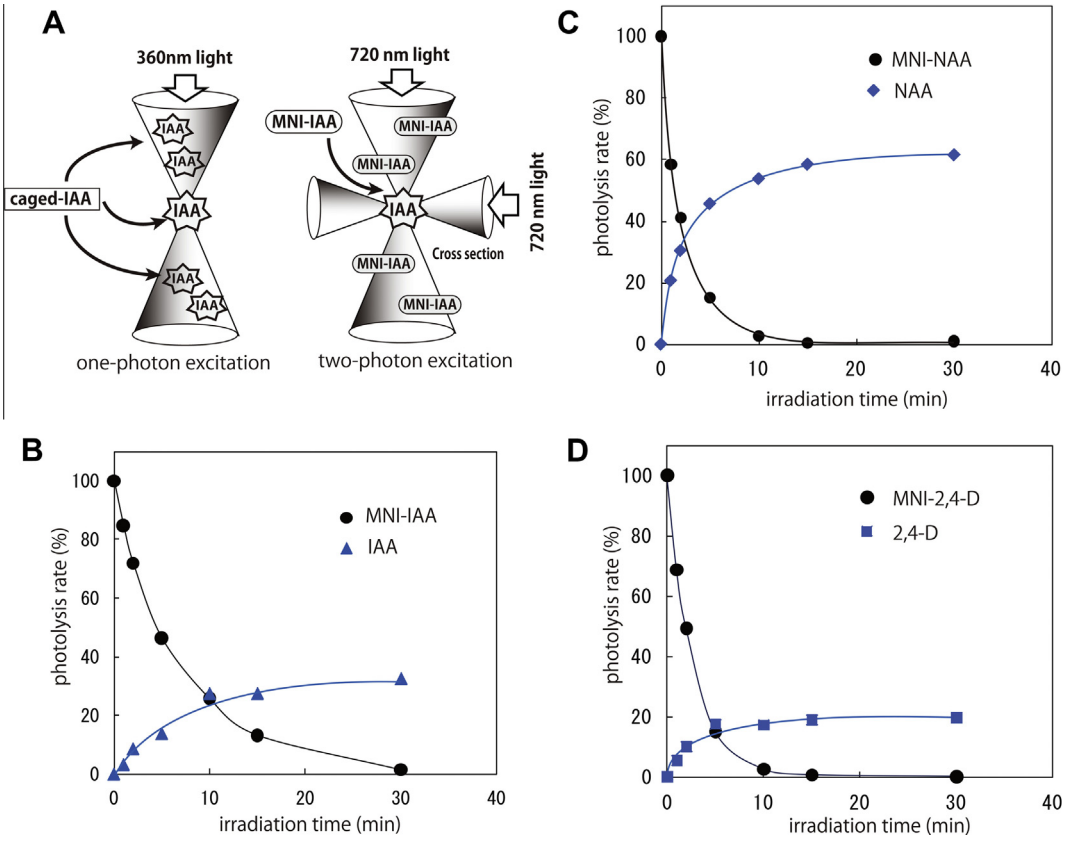

Figure 1. In vitro uncaging of MNI-auxins. (A) A schematic diagram for one-photon and two-photon excitation. (B–D) In vitro uncaging rates of MNI-caged auxins. (B) MNI-IAA, (C) MNI-NAA (D) MNI-NAA, 100 μM MNI-caged auxin solution (85% aqueous EtOH) was irradiated by a fluorophotometer (365 nm), and photolyzed solution were analyzed by HPLC at regular intervals. The amount of released auxins was plotted as the relative uncaged yield (%) from the initial amount of caged auxins.