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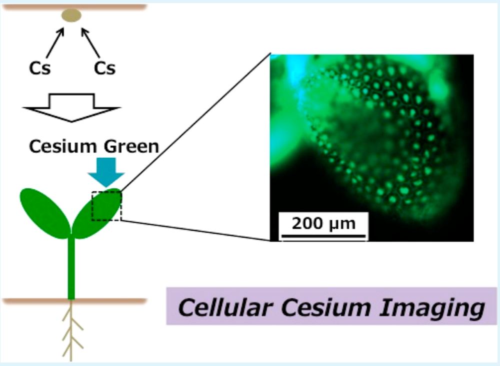
Intracellular Imaging of Cesium Distribution in *Arabidopsis* Using Cesium Green

Masaaki Akamatsu,[†,‡] Hirokazu Komatsu,*[,‡] Taizo Mori,[‡] Eri Adams,[§] Ryoung Shin,[§] Hideki Sakai,[†] Masahiko Abe,[†] Jonathan P. Hill,[‡,∥] and Katsuhiko Ariga*[,‡,∥]

[†]Department of Pure and Applied Chemistry, Tokyo University of Science, 2641 Yamazaki, Noda, Chiba 278-8510, Japan
[‡]WPI-MANA, National Institute for Materials Science (NIMS), 1-1 Namiki, Tsukuba, Ibaraki 305-0044, Japan
[§]RIKEN Center for Sustainable Resource Science, 1-7-22 Suehiro-cho, Tsurumi-ku, Yokohama, Kanagawa 230-0045, Japan
[∥]JST-CREST, 1-1 Namiki, Tsukuba, Ibaraki 305-0044, Japan

Supporting Information

ABSTRACT: The accident at the Fukushima Daiichi nuclear power plant, which was one of the most serious adverse effects of the Great East Japan Earthquake, was accompanied by the release of a large quantity of radioactive materials including ^{137}Cs to the environment. In a previous report, we developed and proposed a cesium (Cs) fluorescent probe, "Cesium Green", that enables the detection of cesium carbonate particles by spraying an alcoholic solution of the Cesium Green probe. In this paper, the sensing activity of this probe was investigated for its selectivity (by using an optode method) and for its application to detect micrometer-sizes Cs particles. Cesium Green was also assessed for its use in plant cellular imaging of Cs localization in *Arabidopsis*. Cesium Green enabled high-resolution Cs imaging of Cs-containing particles and of Cs contained in plants.



KEYWORDS: *cesium, fluorescent probe, imaging, plant, cell*

1. INTRODUCTION

The accident at the Fukushima Daiichi nuclear power plant, which was one of the most serious adverse effects of the Great East Japan Earthquake, was accompanied by the release of a large quantity of radioactive materials to the environment. Of the radioactive species released, ^{137}Cs with a half-life of 30.17 years has the potential to be a long-term radioactive hazard detrimental to human health and agricultural production for generations.[1] Furthermore, high concentrations of ^{137}Cs remain in the vicinity of the nuclear reactors' meltdown site, making a large area of land uninhabitable.

Currently available detection and quantification methods for the radioactive isotopes of cesium (Cs) entail the use of radiation meters and scintillation detectors tuned for that purpose.[2,3] However, the spatial resolution of these methods is poor (greater than centimeters) so that an imaging method with sufficiently high resolution to detect Cs localized at hot spots is required. There exist supramolecular approaches for environmental sensing of analytes[4] with fluorescence-based imaging techniques[5] offering high sensitivities and higher resolutions than the micrometer level. Thus, the development of a fluorescent probe for Cs involving such techniques would meet requirements of high sensitivity and resolution and, in addition, would enable cellular imaging.

In previous work, we reported the design and synthesis of a fluorescent probe for Cs-containing particles, applying the label "Cesium Green" to that fluorophoric compound.[6] Cesium Green contains a terphenyl fluorophore and an ethylene glycol binding moiety together with a nitrophenyl group as an electron-withdrawing group. This compound exhibits green fluorescence (505 nm) upon application of its methanolic solutions to solids containing, for example, cesium carbonate. It was demonstrated in the same report that Cesium Green exhibits green fluorescence when sprayed on (as a methanolic solution) the freeze-dried stems of plants that had been treated with cesium carbonate. However, there are no reports regarding the observation of cellular localization of Cs in plants despite its potential importance for determining the mechanism and location of Cs^+ uptake in plants and animals. This is of particular interest from the point-of-view of Cs^+ bioaccumulation and for potential application in its phytoremediation.

In this work, we demonstrate application examples of Cs determination and imaging using Cesium Green. Initially, we developed an optode method for application of Cesium Green derivatives. Additionally, methods for micrometer resolution imaging of particles containing cesium carbonate and those for cellular imaging of the Cs-treated plant cells were developed.

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2. RESULTS AND DISCUSSION

Development of an Optode Using a Cesium Green Derivative. The Cs binding site of Cesium Green is considered to be the ethylene glycol chain. It is also known that deprotonation of phenol enhances the sensitivity of its fluorescence response. Although Cesium Green responds to solid Cs-containing particles, a quantitative estimation of the selectivity is not possible in the form of a solid. Thus, a lipophilic analogue of Cesium Green, Cesium Green-C12, was synthesized and applied as an ionophore in the optode method (Figure 1).[7−9] In optode systems, the ionophore and membrane extraction affect the protonation of a pH-responsive dye, inducing a color change of the dye. By using the optode method, aqueous-phase Cs detection and estimation of the selectivity by organic-phase extraction become possible. Also, there exist other optode methods for determination of Cs^+, which may be useful for comparative purposes.[10,11] In this work, the optode membrane was prepared from Cesium Green-C12, the pH-response dye KDM-13,[12] the cation exchanger NaTFPB, and the plasticizer dioctyl sebacate with poly(vinyl chloride) as a matrix.

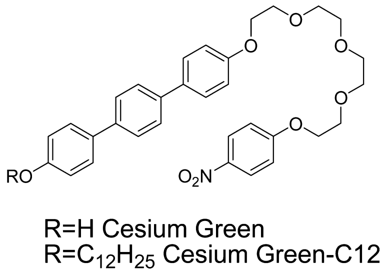

Figure 1. Cesium Green and its *n*-dodecyl ether derivative.

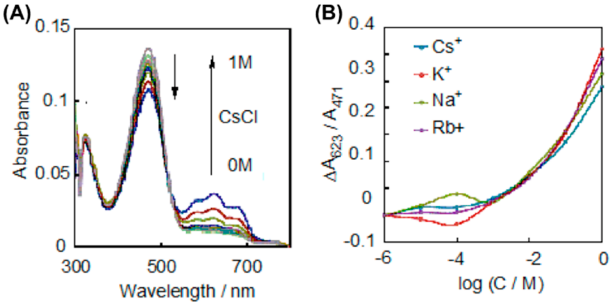

Figure 2. Cesium Green-C12-based optode. (A) Responses to Cs ([CsCl] = 1×10^{-6}, 5×10^{-6}, 1×10^{-5}, 5×10^{-5}, 1×10^{-4}, 1×10^{-3}, 1×10^{-2}, 1×10^{-1}, 1 M). (B) Selectivity for CsCl, NaCl, KCl, and RbCl.

The responses of the Cesium Green-C12-based optode membrane are shown in Figure 2 and the poor selectivities of the optode for alkali-metal cations were revealed. Using Cesium Green-C12, aqueous-phase Cs^+ detection could be established, although the selectivity was moderate.

From a comparison of the organic-phase extraction (membrane) selectivity with the solid-phase Cs selectivity, it seems that the sensitivity of Cesium Green is derived not only from the binding of Cs cations at the ethylene glycol unit but also from the optical selectivity for Cs cations in the solid state.

Micrometer-Scale Imaging of Cs Particles Using Fluorescence Microscopy. Detection of cesium carbonate particles using Cesium Green is based on fluorescence; thus, its resolution can be greater than that of radiation detectors. To illustrate the potential of Cesium Green for micrometer-scale imaging, cesium carbonate and potassium carbonate particles were mixed prior to imaging by fluorescence microscopy. Green and blue fluorescence emitted by Cs- and potassium-containing particles, respectively, were observed (as shown in Figure 3).

The mechanism of selective detection of cesium carbonate by Cesium Green is considered to be due to the combined effects of deprotonation of the phenol group together with the binding of the dye to Cs cations at the particles' surface. Deprotonation

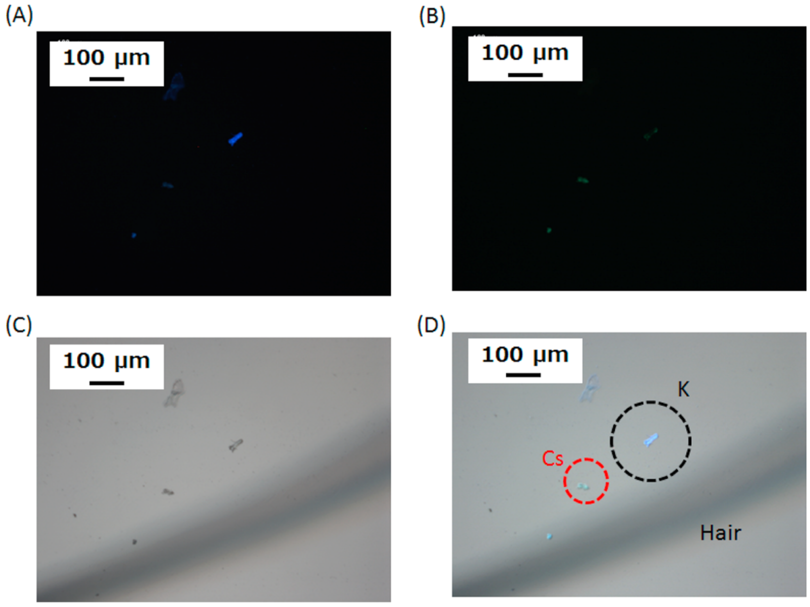

Figure 3. Imaging of cesium carbonate and potassium carbonate using Cesium Green: (A) blue fluorescence (Ex. 360 nm, Em. 425 nm); (B) green fluorescence (Ex. 480 nm, Em. 527 nm). (C) Optical microscopy image (transmission mode). (D) Overlaid image. Dimensions of the particles can be estimated by reference to the human hair visible in the image (average diameter of the human hair: ~100 μm).

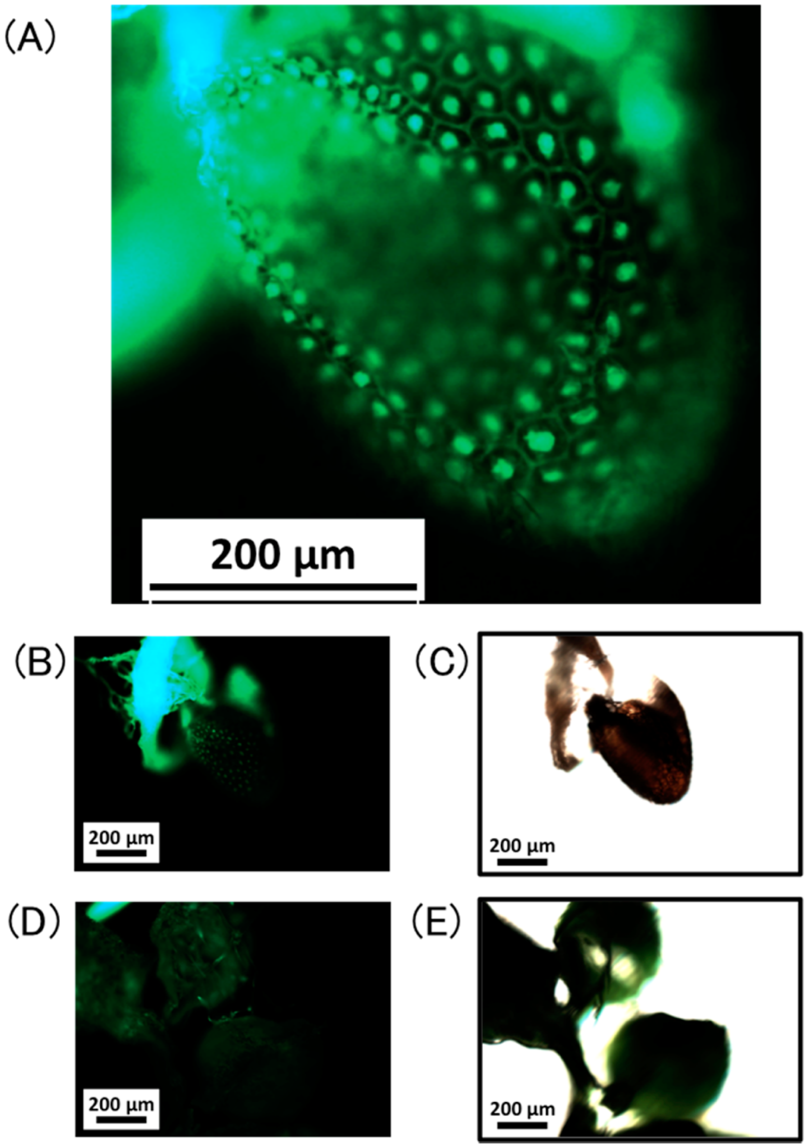

Figure 4. Cs imaging of the cotyledon of *Arabidopsis* treated with a methanolic solution of Cesium Green (0.02 wt %). (A and B) Fluorescence image (Ex 480 nm, Em 527 nm) of cotyledon of *Arabidopsis* treated with 0.5 mM Cs_2CO_3. (C) Optical micrograph in the presence of 0.5 mM Cs_2CO_3. (D) Fluorescence image of the plant grown in the absence of Cs_2CO_3. (E) Optical micrograph image in the absence of Cs.

causes a red shift in the electronic spectra based on an intramolecular-charge-transfer-type mechanism.[13] Additionally, ethylene glycol binds to the surface of Cs cations, causing a red shift. Consequently, Cs-bound Cesium Green specifically emits observable green fluorescence. The fluorescent probe operating in the solid state suggests a novel mechanism and a novel application for environmental Cs detection.

Plant (*Arabidopsis*) Cell Imaging of Cs. We considered that micrometer-scale imaging of Cs particles could be extended to cellular-level imaging. Cs uptake by plants occurs via the potassium transport system, and resulting intracellular Cs distributions in plants are of interest, although an imaging method has not been established to date. Determination of the intracellular localization of Cs in plants is particularly important in the scope of phytoremediation or for detection of plants contaminated with Cs. Thus, we applied Cesium Green to plant cell imaging using *Arabidopsis* cotyledon. Here, Cs imaging was performed using plants treated with high concentrations of Cs ions.[14,15]

Arabidopsis seeds were germinated and grown for 9 days on a medium containing 0.5 mM cesium carbonate. Elemental analyses revealed a Cs^+ content in the resulting plants of 67 ± 11 nmol of Cs/mg of dry weight (Figure S2 in the Supporting Information) Subsequently, the resulting seedlings were freeze-dried and soaked in a 0.02 wt % methanolic Cesium Green solution prior to imaging by fluorescence microscopy.

Upon a comparison between the fluorescence images of Cs-treated and nontreated plants (Figure 4), bright fluorescent spots are visible in the cotyledons of the Cs-treated plants but not of the untreated plants. It is likely that these fluorescent spots are vacuoles, which are known to play a role in detoxification of potentially toxic substances (such as sodium under salt stress[16]) by sequestration. It is possible that plants sequestrate Cs in vacuoles to avoid its negative effects in the cytoplasm. From this result, it is apparent that Cesium Green is suitable for detection of Cs in plants at the organelle level. This might contribute to a better understanding of the mechanisms by which plants accumulate Cs, indicating its potential in phytoremediation applications.

3. CONCLUSION

In this work, the Cesium Green derivative "Cesium Green-C12" was synthesized and applied for optode-type sensing, which enabled aqueous-phase Cs detection, although the selectivity of this protocol was only moderate. High-resolution (microns) cesium carbonate imaging by observing its green fluorescence was also demonstrated using Cesium Green. Application of Cesium Green for plant cell imaging suggested that Cs absorbed from growth media might be sequestrated in the vacuole in *Arabidopsis*. This result highlights the capability of Cesium Green for visualization of Cs accumulated in plants at the organelle level. Thus, the use of Cesium Green enables micrometer-level analysis of Cs cations, and we anticipate that this capability will soon be extended to the nanoscale.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental sections and figures of Cs particle imaging. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Authors

*E-mail: KOMATSU.Hirokazu@nims.go.jp.

*E-mail: ARIGA.Katsuhiko@nims.go.jp.

Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Tsuiki, M.; Maeda, T. Spatial Distribution of Radioactive Cesium Fallout on Grasslands from the Fukushima Daiichi Nuclear Power Plant in 2011. *Grassl. Sci.* **2012**, *58*, 153−160.

(2) Wakabayashi, G.; Uozumi, Y. Recent Activities in the Field of Radiation Measurement and Nuclear Data. *J. Nucl. Sci. Technol.* **2013**, *50*, 1127−1128.

(3) Heller, S.; Zanzonico, P. Nuclear Probes and Intraoperative Gamma Cameras. *Semin. Nucl. Med.* **2011**, *41*, 166−181.

(4) Albelda, M. T.; Frias, J. C.; Garcia-Espana, E.; Schneider, H. Supramolecular Complexation for Environmental Control. *Chem. Soc. Rev.* **2012**, *41*, 3859−3877.

(5) Terai, T.; Nagano, T. Small-molecule Fluorophores and Fluorescent Probes for Bioimaging. *Pfluegers Arch.* **2013**, *465*, 347−359.

(6) Mori, T.; Akamatsu, M.; Okamoto, K.; Sumita, M.; Tateyama, Y.; Sakai, H.; Hill, J. P.; Abe, M.; Ariga, K. Micrometer-level Naked-eye Detection of Caesium Particulates in the Solid State. *Sci. Technol. Adv. Mater.* **2013**, *14*, 015002.

(7) Bakker, E.; Simon, W. Selectivity of Ion-sensitive Bulk Optodes. *Anal. Chem.* **1992**, *64*, 1805−1812.

(8) Bakker, E.; Buhlmann, P.; Pretsch, E. Carrier-Based Ion-Selective Electrodes and Bulk Optodes. 1. General Characteristics. *Chem. Rev.* **1997**, *97*, 3083−3132.

(9) Buhlmann, P.; Pretsch, E.; Bakker, E. Carrier-Based Ion-Selective Electrodes and Bulk Optodes. 2. Ionophores for Potentiometric and Optical Sensors. *Chem. Rev.* **1998**, *98*, 1593−1687.

(10) Seo, H. S.; Lee, S. H. A Novel Fluorescent Cesium Ion-Selective Optode Membrane based on 15-Crown-5-Anthracene. *J. Fluoresc.* **2011**, *21*, 747−751.

(11) Zeng, H. H.; Dureault, B. Cesium-selective Optode Membrane Based on the Lipophilic Calix[4]biscrown in the 1,3-Alternate Conformation. *Talanta* **1998**, *46*, 1485−1491.

(12) Hisamoto, H.; Tohma, H.; Yamada, T.; Yamauchi, K.; Siswanta, D.; Yoshioka, N.; Suzuki, K. Molecular Design, Characterization, and Application of Multi-information Dyes for Multi-dimensional Optical Chemical Sensing. Molecular Design Concepts of the Dyes and Their Fundamental Spectral Characteristics. *Anal. Chim. Acta* **1998**, *373*, 271−289.

(13) de Silva, A. P.; Gunaratne, H. Q. N.; Gunnlaugsson, T.; Huxley, A. J. M.; McCoy, C. P.; Rademacher, J. T.; Rice, T. E. Signaling Recognition Events with Fluorescent Sensors and Switches. *Chem. Rev.* **1997**, *97*, 1515−1566.

(14) Hampton, C. R.; Bowen, H. C.; Broadley, M. R.; Hammond, J. P.; Mead, A.; Payne, K. A.; Prichard, J.; White, P. J. Cesium Toxicity in Arabidopsis. *Plant Physiol.* **2004**, *136*, 3824−3837.

(15) Adams, E.; Abdollahi, P.; Shin, R. Cesium Inhibits Plant Growth through Jasmonate Signaling in *Arabidopsis thaliana*. *Int. J. Mol. Sci.* **2013**, *14*, 4545−4559.

(16) Apse, M. P.; Blumwald, E. Na^+ Transport in Plants. *FEBS Lett.* **2007**, *581*, 2247−2254.