博　士（環境科学）　林　　　春

学位論文題名

Studies on Toxicity of Multi-Wall Carbon Nanotubes on Plants

（植物におけるカーボンナノチューブの毒性影響評価）

学位論文内容の要旨

Because plants play key roles in our ecosystem and in the food chain, the possible toxicities of nanomaterials towards plants must be clarified off the nanomaterials being industrially popularized. To date, however, information on the possible toxicity of the nanomaterials on plant is seriously insufficient. In this study, I have evaluated the possible toxicities of multi-walled carbon nanotubes (MWNTs), one of the typically engineered nanomaterials which has already been produced in industrial quantities, on plant by using *Arabidopsis thaliana* (*Columbia* ecotype) suspension cell lines, and cucumber (*Cucumis sativus*) seeds as the representative targets through *in vitro* and *in vivo* experimental studies. It was demonstrated experimentally that MWNTs are toxic to plant at both the cell and the tissue levels, warning a carful risk management including the hazard control is need. This dissertation consisted of 5 chapters.

In ***chapter 1***, engineered nanomaterials, especially the so-called carbon nanotubes have been highlighted. Carbon nanotubes are the key nanomaterials epitomizing the field of nanotechnologies. Carbon nanotubes are classified as single-walled nanotubes (SWNTs, a single SWNT consisted of only one graphene cylinder) and multi-walled nanotubes (MWNTs, a single MWNT having 2 – 50 graphene cylinders with a common long axis). The possible risk and toxicity of carbon nanotubes towards the ecosystem are summarized; also, the purpose of this study is addressed.

In ***chapter 2***, the possible toxicities of MWNTs toward *Arabidopsis* T87 suspension cells were evaluated by culturing the suspension cells in media containing MWNTs. Decreases in values of cell dry weights, cell viabilities, cell chlorophyll contents and superoxide dismutase (SOD) activities were observed, indicating a fact that MWNTs are toxic to the *Arabidopsis* T87 suspension cells. The certain toxicities were found to be dependent strongly on the size of the agglomerates of MWNTs. Agglomerates of smaller size induce stronger toxicity

than the agglomerates of larger size. The agglomerates of smaller sizes (including the individual tubes) of MWNTs can mimic either as a viral, or bacterial, or fungal elicitor, which induces hypersensitive signals. The defense responses cascaded, and as a result, cells died through either the apoptotic or the necrotic manner.

In *chapter 3*, residuals of the heavy metals involved in MWNTs were eliminated by purifying the as-received MWNTs with hydrochloric acid. The purified MWNTs showed toxicities which were almost identical in magnitudes to that observed for the as-received MWNTs. The experiments were conducted by cultivation of *Arabidopsis* T87 suspension cells in media containing the MWNTs. Contents for ROS, catalase, a typical antioxidative enzyme, and glutathione, a key anti-oxidant, were quantitatively analyzed. Increase in level for ROS and decrease in contents of catalase and glutathione were observed for cells after the exposure to MWNTs. The MWNT exposure also caused cell membrane disintegration and cellular organs structure change, such as bulb-like vacuole. These experimental data gave evidences to support the conclusion that the intrinsic morphologies and/or the unique physiochemical properties of the MWNTs are the key parameters responsible for causing the toxicities to the *Arabidopsis* T87 suspension cells.

In *chapter 4*, a hydroponic culturing system was established for studying the possible toxicity of MWNTs through the *in vivo* experimental approaches with cucumber (*Cucumis sativus*) being used as the targeted species. Root fresh weight, root hair length/density have been significantly affected after the exposure of the roots to MWNTs. ROS contents rose sharply, malondialdehyde, a final product of lipid peroxidation, was largely produced, indicating the oxidative damage to membranes by ROS overproduction occurred. Lignifications and Ca^{2+} ion transportations have been drastically enhanced; this signs the defense lines of the roots functioned. A MWNT induced, postulated pathogen-like hypersensitive model is suggested to explain the possible toxic mechanism for causing the root tissue damaging.

In *chapter 5*, general summary and further prospect were drafted. It was experimentally demonstrated that MCNTs are toxic to plant cells and plant in both *in vitro* and *in vivo* culturing systems. All the experimental data obtained in this study indicated that the ROS overproduction induced oxidative stress is the key mechanism responsible for causing cell death after the MWNT exposure. Whether MWCNTs can penetrate into plant cells through the wall need further studies; long-term experiment can offer a right solution to this question. Nevertheless the findings obtained in this study together with the others reports on the toxicology of the engineered nanomaterials will be extraordinarily useful for establishing a methodological field for assessment of the possible risks of this new class of materials to our ecosystem.

学位論文審査の要旨

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副 査　教 授　田 中 俊 逸
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Studies on Toxicity of Multi-Wall Carbon Nanotubes on Plants

（植物におけるカーボンナノチューブの毒性影響評価）

The candidate, in this study, had evaluated the possible toxicity of multi-wall carbon nanotubes (MWNTs) on plant, by using *Arabidopsis thaliana* (*Columbia* ecotype) suspension cell lines and cucumber (*Cucumis sativus*) as the representative targets through *in vitro* and *in vivo* experimental studies.The achievements have been described in the thesis paper, which consisted of 5 chapters.

In chapter 1, engineered nanomaterials, especially the so-called carbon nanotubes (CNTs) have been highlighted by the candidate. CNTs are the key nanomaterials epitomizing the field of nanotechnologies. CNTs are classified as single-walled nanotubes (SWNTs) and multi-walled nanotubes (MWNTs). The possible risk and toxicity of CNTs towards the ecosystem are summarized; also, the purpose of this study is addressed. The candidate, in this chapter, had critically understood the relevant literatures and the importance of the studies.

In chapter 2, the possible toxicities of MWNTs toward *Arabidopsis* T87 suspension cells have been studied by the candidate, by culturing the suspension cells in media containing MWNTs. Decreases in values of cell dry weights, cell viabilities, cell chlorophyll contents and superoxide dismutase (SOD) were observed. The candidate observed also that the certain toxicities were dependent strongly on the size of the agglomerates of MWNTs. Agglomerates of smaller size induced stronger toxicity than the agglomerates of larger size. The candidate made a conclusion that the agglomerates of smaller size (including the individual tubes) of MWNTs can mimic either as a viral, or bacterial, or fungal elicitor, which induces hypersensitive signals. The defense responses cascaded, and as a result, cells died through either the apoptotic or the necrotic manner.

In chapter 3, the candidate had purified the as-received MWNTs with hydrochloric acid for removal of the residual heavy metals involved in MWNTs. The purified MWNTs showed toxicities which were almost identical in magnitudes to that observed for the as-received MWNTs. Contents for ROS, catalase, and glutathione were quantitatively analyzed by the candidate. Increase in level for ROS and decrease in contents of catalase and glutathione were observed for cells after the exposure to MWNTs. The MWNT exposure also caused cell membrane disintegration and cellular organs structure change, such as bulb-like vacuole. Based on these experimental data, the candidate made a conclusion that the intrinsic morphologies and/or the unique physiochemical properties of the MWNTs are the key parameters responsible for causing the toxicities to the *Arabidopsis* T87 suspension cells.

In chapter 4, the candidate had established a hydroponic culture system for studying the possible toxicity of MWNTs through *in vivo* experiments with cucumber (*Cucumis sativus*) as the targeted species. The candidate observed for the first time that the root fresh weight and the root hair length/density were significantly affected after the exposure of the roots to MWNTs. The candidate observed also that ROS contents rose sharply, malondialdehyde, a final product of lipid peroxidation, was largely produced. The candidate observed also that lignifications and Ca^{2+} ion transportations were drastically enhanced; those are the key signals showing the defense lines of the roots functioned. A MWNT induced, postulated pathogen-like hypersensitive model is suggested by the candidate to explain the possible toxic mechanism for damaging the root tissue.

In chapter 5, a general conclusion and further prospect were drafted by the candidate. The candidate had experimentally demonstrated that MWNTs are toxic to plant through both the *in vitro* and *in vivo* culturing investigations. All the biochemical data obtained by the candidate indicated that the ROS overproduction induced oxidative stress, which is one the key mechanism involving in the cell death after the MWNT exposure. Although whether MWNTs can penetrate into plant cells through the cell wall need further studies; nevertheless the candidate, in this study, had made an original contribution to the knowledge of this subject.

This thesis, as a whole, made an original contribution to the knowledge of this subject. The candidate had critically understood the relevant literatures. The methods adopted were appropriate to the subject. The experimental findings were suitably set out, also discussions were made logically. Qualities of English were satisfactory. The recommendations of all the examiners are that "the degree be awarded".