論 文 内 容 の 要 旨

The number and use of resin nanoparticles have been rapidly increased, which has led to growing awareness of their adverse effect on living organisms, particularly on aquatic organisms. It has been reported that resin nanoparticles made of isobutyl cyanoacrylate polymers (iBCA-NPs) acutely induce cell death in a wide range of microgreen algae; this was accompanied by excess accumulation of reactive oxygen species (ROS). In this study, analysis of the fundamental mechanism of how resin NPs induce cell death in algae, determination of how widely distributed algal species differ in their sensitivity, and investigation of transcriptome changes induced by iBCA-NPs were performed. First, to examine the potency of iBCA-NPs to induce cell death in diverse non-green algal species (e.g. species in the SAR and Hacrobia clades) and the difference in sensitivity to iBCA-NPs among the species, 18 non-green algal species were co-incubated with laboratory-prepared iBCA-NPs (mean diameter: 180 nm). After exposure to 100 mg L^{-1} iBCA-NPs for 24 h, cell death was induced in two of three *Bacillariophyceae* species, all three *Cryptophyceae* species, four of six Dinophyta species, three of four Haptophyta strains, and all three *Raphidophyceae* species. However, exposure to a concentration of 1 g L^{-1} iBCA-NPs induced cell death in all the examined species. ROS scavenger *N*-acetyl-L-cysteine (NAC) substantially delayed cell death induction, suggesting that ROS generation is a direct cause of induced cell death. The cells of the *Raphidophyceae* species that lack covering structures and a Haptophyta strain bearing no coccoliths were more sensitive than those of species bearing covering structures. This finding suggests that cell covering structures act as barriers against the invasion of NPs.

Second, to better understand the molecular mechanism of cell death induced in algae by iBCA-NPs, altered gene expression changes along with the increase of the cell death ratio were investigated in *Chlamydomonas reinhardtii* (Chlorophyceae) through chronological transcriptome analyses using next generation sequencing. Transcriptome data indicated upregulation of genes coding for the antioxidant

enzymes included in the oxidative stress response, such as glutathione peroxidase (GPX5), Fe-superoxide dismutase (Fe-SOD), and glutathione S-transferase (GSTS1) when the cell death ratio reached ~3%. Besides these genes, 9 out of 20 heat shock protein (HSP)-coding genes and Cre13.g605200, a gene identified to code for cell wall hydrolytic enzymes, were prominently upregulated (log_2 fold-change, up to 11.73). The tag-insertion mutant for the Cre13.g605200 gene showed considerable resistance to nanoparticle-induced cell death, suggesting that its overexpression is essential for the induction of acute cell death by iBCA-NPs.

Nucleosomal units of laddering DNA were barely detected in the smeary digested DNA of *C. reinhardtii* cells exposed to iBCA-NPs, This indicates that induced cell death is primarily a necrosis-like type, while it is also partly accompanied by the programmed cell death (PCD)-like type. Direct chemical reactions between the largely accumulated ROS and intracellular substances such as proteins, lipids, and DNA can be the cause of necrosis.