論 文 内 容 の 要 旨

It has been reported that *n*-butyl cyanoacrylate nanoparticles (NBCA NPs) can induce cell lysis in various Gram-positive bacteria, while no such effect exists for Gram-negative bacteria. Unexpectedly, we found that the similar solid resin nanoparticles (hereafter acrNPs), made from isobutyl cyanoacrylate, can acutely induce mortality in exponentially growing cells of the species *Chlamydomonas reinhardtii*. Co-incubations with three sizes of acrNPs (25 nm, 180 nm and 350 nm in diameter), immediately caused *Chlamydomonas* to display abnormal swimming with sudden and frequent orbital changes, and in approximately 60 minutes, swimming stopped completely. After four hr of co-incubation, most of the cells were stained with trypan blue, suggesting that these cells were bearing severely impaired plasma membranes. Observations of the cyto-ultrastructure demonstrated that the cell walls had severe damage, and acrNPs were located in the space between the cell wall and the plasma membrane (periplasmic space), as well as inside the cytoplasm. To analyze the mechanisms of such phenomena, we employed three types of *Chlamydomonas reinhardtii* strains, CC-124 wild-type, CC-503 (a mutant bearing a very thin cell wall), and CC-400 (a mutant lacking a cell wall), with varying degrees of sensitivity to acrNPs. Among these three strains, CC-400 is the most sensitive to the acrNPs, CC-503 is moderately sensitive, and CC-124 is the least sensitive to acrNPs under the same concentrations (weight/volume) in a wide range (10 mg/L to 1g/L). *Chlamydomonas* cell mortality ratios were the same in coincubation, with no regard to the different acrNP sizes, as long as the total NPs surface area in the medium is the same. This suggests that the surface of acrNPs plays an essential role in inducing cell mortality. Cell mortality was accompanied by the creation of reactive oxygen species, which were detected more readily in cells grown under constant light than those in the dark. It is worth noting that overgrown

cells were more resistant to the induction of mortality in the presence of acrNPs. Moreover, by co-incubating with acrNPs at a very high concentration (1g/L), around 60 % of the *Chlorella vulgaris* cells were changed into protoplasts or spheroplasts (protoplasts/spheroplasts), without the induction of cell mortality. At the same concentrations (w/v) of acrNPs, smaller particles worked much more efficiently than larger ones to induce mortality, or to generate protoplasts/spheroplasts. Filtrate prepared from the medium of *Chlorella vulgaris* co-incubated with acrNPs contained evidence of cell wall lytic activity. Random collisions of acrNPs with cells, which we observed directly with a dark-field observation system, seemed to stimulate the secretion of cell-wall hydrolytic enzyme(s).

Keywords: isobutyl cyanoacrylate nanoparticles (acrNPs), polymer nanoparticles, *Chlamydomonas*, *Chlorella*, cell mortality, protoplast