On mechanism of inactivation of bio-particles by the plasma exposure and evaluation of the toxicity using single DNA molecules

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Effect of Non-Thermal Plasma on bio-particles has been studied on *B. subtilis*, *E. coli* and bacteriophages. Plasma-jet with Ar, or dielectric-barrier-discharge in room air has been used. The bio-particles suspended in liquid or dried state were exposed to NTP, and states of different biological components were monitored during the course of the exposure. For spore of *B. subtilis* in liquid, turbidity of the suspension changed quickly and became transparent after the exposure to DBD. This finding suggests the cell surface has been modified to be more hydrophilic with the plasma exposure. Analysis of green fluorescent protein, GFP, introduced into *E.coli* cells proved that NTP causes a prominent protein damages without cutting peptide bonds.

NTP can also inactivate viruses. In the previous research, critical damages for the inactivation of λ phage were done on coat proteins and M13 phage on DNA. These results were obtained with wet phage sample [1]. We also tried to use dry sample. We used bacteriophage φX174 which is resistant to drying. When the bacteriophage is inactivated by NTP, the damage should exist either on viral nucleic acids or coat proteins or both. It is possible to extract the nucleic acids from the bacteriophage so that the damage to the DNA can be separately analyzed to exclude the effect of the proteins. The DNA extracted from the plasma-exposed phage can be assayed its plaque forming activity by transfection. The coat proteins were analysed by SDS-PAGE. Using these assays, the damages of viral DNA and coat proteins have been determined separately. The results for the dry φX174 phage inactivated by the exposure to DBD showed both DNA and coat proteins were damaged and the critical damage were done to the coat proteins. This is similar to the result of the wet sample.

We also report a single-molecule-based analysis of strand breakages on large DNA molecules induced by the plasma exposure. Single-molecule observation of DNA that involved molecular combing was used to measure the length of individual DNA molecules. The measured DNA length showed that plasma exposure caused a marked change in length of DNA molecules. The rate of plasma-induced strand breakage on large random-coiled DNA molecules was determined using a simple mathematical model. The measured rate shows good relation with the plasma exposure time, and could be used for safety evaluation of the plasma treated water.

References

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