Damages of biological components in bacteria and bacteriophages exposed to atmospheric non-thermal plasma

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Résumé

Mechanism of inactivation of bio-particles exposed to non-thermal plasma, NTP, has been studied using *B. subtilis, E. coli* and bacteriophages. States of different biological components were monitored during the course of inactivation. Analysis of green fluorescent protein, GFP, introduced into *E.coli* cells proved that NTP causes a prominent protein damages without cutting peptide bonds. We have invented a biological assay which evaluates in vivo DNA damage of the bacteriophages. Different doses of the plasma were applied to wet state of λ phages. From the discharged λ phages, DNA was purified and subjected to in vitro DNA packaging reactions. The re-packaged phages consist of the DNA from discharged phages and brand-new coat proteins. Survival curves of the re-packaged phages showed extremely large *D* value (*D*=25 s) compared to the previous *D* value (*D*=3 s) from the discharged phages. The results indicate that DNA damage hardly contributed to the inactivation, and the damage in coat proteins is responsible for inactivation of the phages.

Introduction

There are strong concerns about the emergence of the next pandemic influenza, considering the recent worldwide outbreaks of highly pathogenic avian flu (H5N1) or swine flu (H1N1) and sporadic occurrence of human infections. Non-thermal atmospheric pressure plasma is hopeful about dealing with such menaces because, in principle, it can decontaminate both the surface of materials [1,2] and room air [3,4] at low gas temperature without using bactericides. Though the study of plasma decontamination is expanding [5-9], the intricacy and the high reactivity to any organic materials of the plasma sources have so far made it difficult to prove the inactivation mechanism of microorganisms.

In this study, we have invented a biological assay which evaluates DNA-specific damage in λ phages treated with cold plasma [10]. It was found that λ phage DNA was hardly damaged in the course of plasma application in comparison with easily affected coat proteins. We also evaluated the damages to *B. subtilis* and *E. coli* [11].

Strategy for assay of DNA damage

Generally, phage DNA by itself obtains total functions of the phage if it comes into the host cell. Therefore, in vivo function of the damaged phage DNA can be investigated by its transfection to the host cells. Figure 1 illustrates a concept for how to estimate the DNA-specific damage in plasma treated λ phages. Putative damage is introduced in both protein and DNA of the plasma treated phages (Phage A). DNA is extracted from Phage A and packaged in vitro to form newly packaged phages (Phage B). Phage B does not have any protein damage, but carries DNA damage originated from Phage A. Therefore, all of the damage for inactivation of Phage B belongs to DNA damage originally introduced to Phage A. Comparison of the *D* value (Decimal value: time for a one log10 reduction) of survival curves from Phage A and Phage B enables to evaluate the DNA damage, and as a consequence, to evaluate the protein damage.

Damage of the plasma treated λ phage having double-stranded DNA

λ phage suspended in 0.1M Tris-HCl (pH=8.0), 1×10^{-3}M of $MgSO_4$ was treated with the atmospheric pressure DBD. Inactivation profile (survival curves of Phage A) is shown as dotted line in Figure 2. The number of infectious phages decreased quickly and 6-orders of magnitude inactivation was achieved at 20 s discharge treatment. The profile exhibited the characteristic of a single slope curve and the *D* value was about 3 s.

The solid line in Figure 2 represents the survival curves obtained from the re-packaged λ phages (Phage B). Until 20 s discharge, the profile exhibited the characteristic of a single slope curve and the *D*

value was about 25 s. The large *D* value of re-packaged phages means very slow decrease of their viability. The shift of *D* value from 3 s to 25 s by re-packaging indicates that DNA damage introduced by the plasma hardly affected to the inactivation.

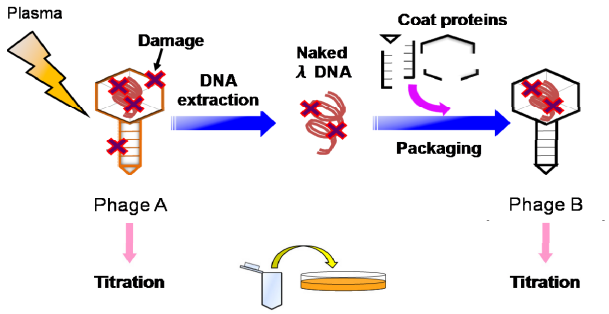

Fig. 1: Schematic procedure to estimate the DNA damage in plasma treated λ phages.

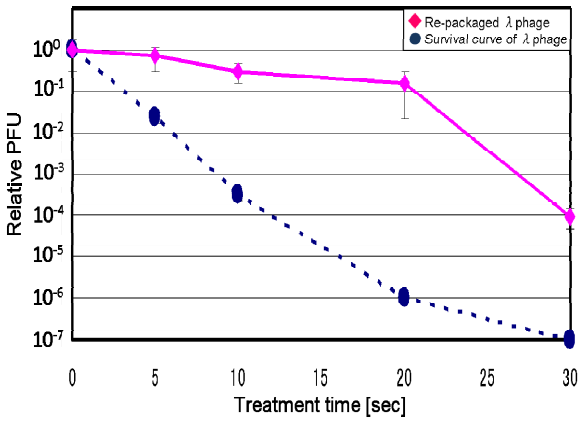

Fig. 2: Survival curves of λ phages subjected to the atmospheric pressure DBD. Dotted line represents the results from plasma-treated phage (Phage A). Solid line represents the results from re-packaged phage (PhageB). PFU: plaque forming units

Damage to the M13 phages having single-stranded DNA

For comparison, damages to M13 phages were evaluated. After the exposure to DBD, the DNAs were extracted, and were transfected to E.coli to measure the infection rate. The result indicated that the infection ability did not recover. The comparison shows that double-stranded DNA is stronger against the exposure to DBD, as the DNA repairment system of the host cell works if the damage remains on one strand.

Conclusion

λ and M13 phages, *E.coli* and *B. Subtilis spore* were inactivated effectively by the DBD exposure. Degradation of proteins seems major cause of inactivation for the microbes and λ phage. This damage could be denaturation or chemical modification such as oxidation or reduction but not degradation. The result showed that the double-stranded DNA of λ phage can be repaired. For M13 phage having single-stranded DNA, the repairmen system did not work, and degradation of both protein and DNA were observed.

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