22E08

Analysis of the inactivation mechanism of bacteriophage MS2 and φX174 by atmospheric pressure non-equilibrium plasma

大気圧非平衡プラズマによる バクテリオファージ MS2 および φX174 の不活化メカニズムの解析

Yoshimasa Tanaka, Hachiro Yasuda, Hirofumi Kurita, Kazunori Takashima, Akira Mizuno
田中良昌, 安田八郎, 栗田弘史, 高島和則, 水野彰

Toyohashi University of Technology
1-1 Hibarigaoka, Tempaku-cho, Toyohashi, Aichi 441-8580, Japan
豊橋技術科学大学 〒441-8580 愛知県豊橋市天伯町雲雀ヶ丘 1-1

Atmospheric pressure non-thermal plasma has attracted attention as a method of virus inactivation because it can efficiently inactivate various viruses in a short time and at low temperature. However, the predominant factor in the inactivation is not clear yet. Bacteriophages, consisting of nucleic acid packed into proteins, are suitable for the study of the inactivation mechanism due to their simple structure. Analyzing the inactivation of bacteriophage MS2 and φX174 should be helpful in understanding the inactivation of influenza virus by plasma. When these bacteriophages are inactivated by dielectric barrier discharge (DBD), the damage should exist either on viral nucleic acids, coat proteins or both. It is possible to extract the nucleic acids from the bacteriophage so that the damaged coat proteins can be removed to avoid the effect of the proteins. And, the nucleic acids extracted from plasma-inactivated phage can be assayed its plaque forming activity by transfection of the nucleic acids. Using this assay, the damages of viral nucleic acids and coat proteins have been determined separately. As a result, the main cause of inactivation of bacteriophage MS2 and φX174 by DBD exposure should be due to the damage of MS2 RNA and φX174 coat proteins, respectively.

1. Introduction

Inactivation technology of viruses have been actively studied, because there is a serious problem which is communicable viral infection caused by influenza virus. In recent years, atmospheric pressure non- thermal plasma has attracted attention as a method of virus inactivation because it can strongly inactivate in a short time and at low temperature [1]. However, the predominant factor in the inactivation is not clear yet. Bacteriophages, consisting of a nucleic acid packed into proteins, are suitable for the study of the inactivation mechanism due to their simple structure [2]. In this study, bacteriophage φX174 which is consisted of only viral DNA and protein was investigated using atmospheric pressure dielectric barrier discharge (DBD) known as having highly microbicidal effect [3]. When bacteriophage is inactivated by DBD, the damage should exist either on viral nucleic acids, coat proteins or both. Bacteriophage φX174 and MS2 have some characteristics similar to influenza virus. Analyzing the inactivation of bacteriophage φX174 should be also helpful for understanding inactivation of influenza virus.

2. Experimental part

Figure 1 shows a DBD generator used in this study. A high voltage AC power supply was used to generate DBD in air gap (3 mm). In this report, discharge experiments were performed under fixed electrical conditions except the exposure time. The effective voltage, the frequency of the applied voltage, and the input power were 14 kV, 2.00 kHz, and 8.8 W, respectively.

A 50 μL aliquot of φX174 phages sample solution was spotted and widely spread to 3-4 cm^2 on a PET film and the DBD was immediately applied for the intended time. After that the sample solution was recovered by additional 50μL of the distilled water on the surface of the PET film. For the measurement of phage activity, the recovered phage solution was mixed with *Escherichia coli* ATCC13706 and poured on an agar media plate. Phage plaques on the plates were counted after incubation at 37℃ for 24 hrs. On the other hand the activity of DNA extracted from DBD-treated φX174 phages were measured by transfection, which is a method to form infectious φX174 phage particle by mixing purified DNA with competent

E.coli cells. Transfection allows measurement of DNA damage only without being affected by the damages of coat proteins. Damages of coat proteins were analyzed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE).

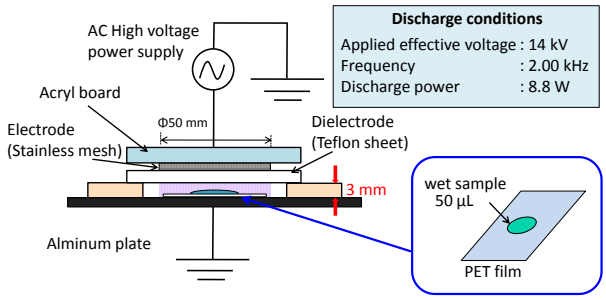

Fig. 1 Atmospheric pressure DBD generator

3. Results and discussion

Figure 2 shows the inactivation profile of the φX174 phage after DBD treatment, as well as the activity of DNA extracted from DBD-treated phages obtained from the transfection. These profiles were normalized with each control sample (0 sec samples) to give the PFU (plaque forming unit) value of 10^0. The profile exhibited a decrease in activity of φX174 phage. The activity of DNA also decreased in the first 10 sec. However, they were retained at long time from 20 sec to 40 sec. These results show that the predominant factor in the inactivation of φX174 phage is not DNA damage.

Figure 3 shows the analysis of coat proteins from DBD-treated φX174 phage by SDS-PAGE. These coat proteins degraded with the treatment time. In addition, band shifts with the treatment time showed that some modifications of proteins exist.

These results show that despite φX174 phage activity decrease with treatment time, the activity of DNA extracted from DBD-treated phage was retained. Moreover damages of coat proteins obtained from DBD-treated φX174 phage exist. Therefore the cause of inactivation of φX174 phage by DBD exposure should be mainly due to the damages of coat proteins.

In the case of MS2 phage, the activity of phage and viral RNA similarly decreased with DBD treatment time. However, coat proteins did not so degrade. Consequently, the main cause of inactivation of MS2 phage by DBD exposure should be due to the damages of RNA.

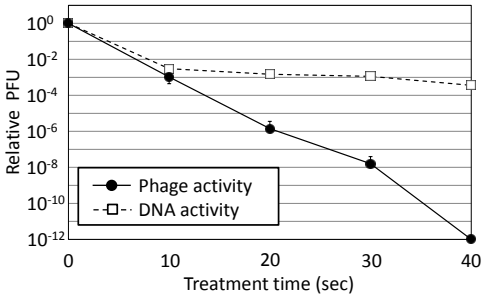

Fig. 2 Inactivation profiles of DBD-treated φX174 phage and φX174 DNA (PFU : plaque forming unit)

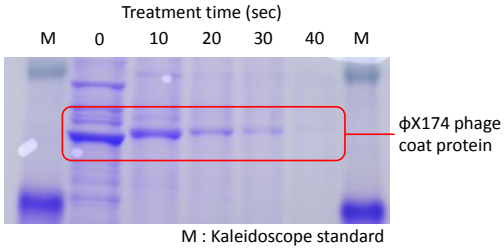

Fig. 3 Analysis of the DBD-treated φX174 phage coat protein by SDS-PAGE

4. Conclusions

Using bacteriophage φX174 and MS2, we have developed an assay method based on extraction of the viral nucleic acids from plasma-treated bacteriophage and transfection of the nucleic acids to the host bacteria. This assay was applied to the bacteriophage φX174 and MS2 which were inactivated by the treatment of atmospheric pressure non-thermal plasma. As a result, despite φX174 phage activity decrease with treatment time, the activity of φX174 DNA extracted from DBD-treated phages was retained. And, the activity of MS2 phage and MS2 RNA similarly decreased with DBD treatment time. MS2 coat proteins did not degrade so much. These results exhibited the main cause of inactivation of bacteriophage φX174 and MS2 by DBD exposure should be due to the damage of φX174 coat proteins and MS2 RNA, respectively.

References

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