Isolation and Purification of Bactericidal Species in Plasma-treated Water
プラズマ処理水に含まれる殺菌活性種の分離精製

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Plasma-treated water (PTW) prepared by irradiating atmospheric pressure plasma to distilled water showed strong bactericidal activity. This bactericidal effect was inactivated exponentially after the preparation and half-life of this activity was estimated to few minutes at room temperature. This fact suggests that the bactericidal species in PTW is short-lived substance not like hydrogen peroxide, nitric acid and nitrous acid. To identify the bactericidal species in PTW, respective components of PTW were isolated and purified by ion chromatography. In addition to peaks of hydrogen peroxide, nitrate ion and nitrite ion, a specific peak eluted after nitrite ion was detected in PTW. To evaluate the bactericidal activity of respective components, fractions from the ion chromatograph were collected to mix bacterial suspensions. As a result, strong bactericidal activity was observed only in fractions of the above-mentioned PTW-specific peak, suggesting that the bactericidal activity of PTW was due to a single substance.

1. Introduction

In order to apply a low-temperature atmospheric pressure plasma to medical disinfection, bactericidal technique in aqueous solutions is essential, because most bacteria and fungi grow in wet conditions.

In previous study, the successful method of bacterial inactivation in aqueous solutions was developed by the combination with the plasma irradiation and acidification of bacterial suspensions, which achieved strongly enhanced bactericidal activity (called "the reduced pH method") [1]. It is considered that strong bactericidal activity of the reduced pH method is brought by hydroperoxy radical (HOO•) generated from the association of hydrogen ion (H^+) and superoxide anion radical (O_2^-•). HOO•, which is without electric charge, can easily permeate into bacterial cytosol via hydrophobic cell membrane, and therefore has extremely stronger bactericidal activity than negative charged O_2^-• [2, 3].

By further investigation, it was found that plasma irradiated aqueous solutions had temporal bactericidal activity, called “plasma-treated water (PTW)”. Bactericidal activity of PTW was inactivated exponentially with incubation period and half-life of this activity was estimated to a few minutes at room temperature (Fig. 1). Although some similar experimental results with plasma-irradiated solutions showing bactericidal activity were reported, many of their activities were caused by more stable chemical species such as hydrogen peroxide or nitric and nitrous acids. PTW in this study is remarkably unique with relatively short half-life. Bactericidal activity of PTW was also enhanced in acidic condition, which is similar to plasma irradiating sterilization with the reduced pH method. From the analysis of pH-dependency, it

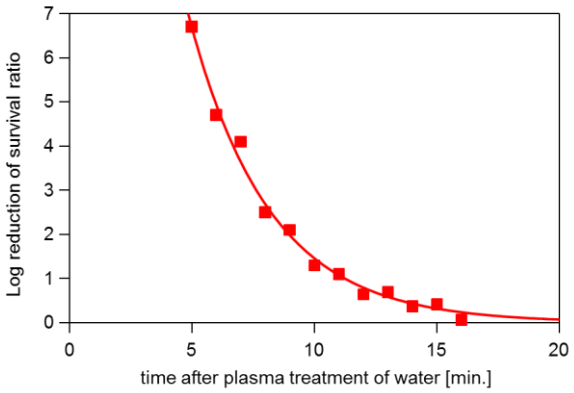

Fig. 1　Inactivation of bactericidal activity in PTW at room temperature.

was expected that O_2^-• was also involved in bactericidal activity of PTW by the permeation mechanism described above.

Because half-life of O_2^-• is known to a few seconds, it is unlikely that O_2^-• itself remains in PTW for a few minutes. It seems that the some compound of O_2^-• precursors exists in PTW. In this study, we tried to isolate and to purify the bactericidal species or its precursor from PTW and we examined its properties.

2. Results and discussion

After high concentration PTW was prepared by 1 m long special device which can continuously irradiate atmospheric-pressure cold plasma to distilled water flowing slowly with cooling, PTW was analyzed by the ion chromatograph. The analysis was carried out at low-temperature condition to maintain the bactericidal species in PTW. Result of the analysis (Fig. 2) revealed that PTW contained hydrogen peroxide, nitrate and nitrite. In addition to these components, a specific peak eluted after nitrite ion was detected around at 3 min retention time. This peak was not detected in heat-treated PTW showing no bactericidal activity, suggesting that a substance contained in this peak played an important role in the bactericidal activity of PTW.

To examine the bactericidal activity of respective peaks of PTW, eluate of the ion chromatograph were collected by 0.5 mL and bactericidal assay were performed with respective fraction. As a result, strong bactericidal activity was observed only with fractions around at a PTW-specific peak described above, and no bactericidal effects were observed in other peak fractions (Fig. 2). This result revealed that the bactericidal activity of PTW was due to single substance, not a combined effect of plural components.

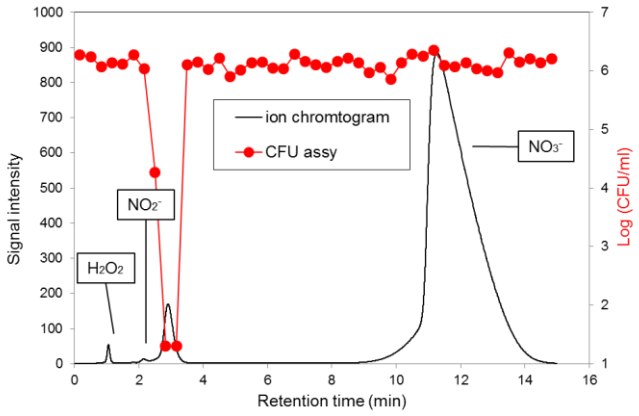

Fig. 2 Ion chromatogram of PTW and CFU assay of its fractions.

Furthermore, fractions containing bactericidal species (or its precursor) were inactivated by heating and applied to the ion chromatograph again. Consequently, only nitrate and nitrite were detected. This means that degradation products of the bactericidal species are nitrate and nitrite ions, strongly suggested the bactericidal species is a compound consisting of oxygen and nitrogen atoms.

We are conducting further investigations to identify this bactericidal species. It is expected to elucidate the molecular mechanism of bacterial inactivation with PTW by additional investigations.

3. Summary

Although PTW has many chemical components, bactericidal species with the reduced pH method was isolated and purified by the ion chromatograph with a fraction collector. Other components in PTW showed no bactericidal effects, meaning that concentrations of these disinfectant components were low enough. The details of the observed specific peak are now under investigation.

References

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