非平衡大気圧プラズマ活性ラクテックにおける液相反応の解明

Mechanism of Aqueous Reactions in Non-equilibrium Atmospheric Pressure Plasma-activated Lactec Solutions

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1. Introduction

Non-equilibrium atmospheric pressure plasma (NEAPP) activated lactated Ringer's solution (PAL) induces the selective killing effects of cancer cells. [1] Reactive oxygen nitrogen species (RONS), such as H_2O_2 and nitrite [2], as well as reactive organic substances, such as pyruvic acid, glyoxylic acid and 2,3-dimethyl tartrate, were detected in PAL.[3] However, aqueous reactions of the reactive organics generation have not been clarified in details so far. In this study, we have analyzed the NEAPP-induced chemical reactions in regard with lactate by using various methods, such as electron spin resonance (ESR), colorimetric methods, nuclear magnetic resonance (NMR), and chromatography-mass gas spectrometry (GC-MS).

2. Experimental

Different concentrations from 0 to 20 mM of organics were prepared. NEAPP (Ar 2.0 slm; 9 kVp-p; 60 Hz; distance of 6 mm) irradiated 3 mL of solutions filled in a 35 mm dish for 5 min. Just after 1-min irradiation, using spin-trapping agent such as 5,5-Dimethyl-1-pyrroline N-Oxide (DMPO) or 3,5-dibromo-4-nitrosobenzene sulfonate (DBNBS) to detect radicals by ESR. For 5 min-irradiated samples, concentrations of H_2O_2 were colorimetrically determined by using amplex red hydrogen peroxide assay (λ = 560 nm). ¹H NMR spectra of the irradiated organics dissolved in heavy water were measured. Also, 1 µL of samples were analyzed by GC-MS.

3. Results and discussion

In 1-min NEAPP irradiated samples, radicals generation such as OH, H, CH₂COO⁻, CH₃, COO⁻ detected by ESR were depended on the initial lactate concentration. After 5 min irradiation, pH value decreased and all samples were acidic. The NEAPP generated H₂O₂ concentration was correlated with lactate, formate and acetate but not pyruvate and glyoxylate. From the results, acetate and formate are arisen from pyruvate and glyoxylate. Intermediates, including ethanol, acetaldehyde were formed and oligomerized to form higher mass components, which were detected in the GC-MS measurements. Processes of dehydrogenation and oxidation are the possible pathways of plasma-activated organics originated from lactate.

References

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- [2] N. Kurake *et al.*, Arch. Biochem. Biophys. 605, 102 (2016).
- [3] Y. Hosoi *et al.*, Fall Meeting JSAP (Nagoya, 2018) 21a-144-10.