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酸素ラジカル照射溶液による脂質分子拡散への影響の違い
Different influence on diffusion of lipid molecules due to oxygen-radical-exposed solutions

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1. Introduction

Non-equilibrium atmospheric-pressure plasmas (NEAPPs) are widely employed in recent biological researches. In the applications of NEAPPs, it is important that the understanding of reaction mechanism between plasma-generated oxygen and nitrogen reactive species (RONS) and cell membrane. Recently, our work revealed that the oxygen radicals formed nanopores on the artificial cell membrane, phospholipid bilayer, due to the lipid oxidation [1]. The size of nanopores in a range from 10 to 50 nm in diameter was observed by atomic force microscope (AFM). It is expected that the RONS can penetrate into the cell through the nanopores, resulting in the oxidization of intracellular organelles and the enhancement of pore formation on the cell membrane.

In this study, we measured the diffusion coefficient of the supported lipid bilayer (SLB), which is an artificial planar lipid bilayer system formed at a solid-liquid interface, as a function of immersion time in the oxygen-radical-exposed solutions using fluorescence recovery after photo-bleaching (FRAP) employing a confocal laser microscope.

2. Experimental procedure

In the experiment, we used 1,2-dioleoyl-sn-glycerol-3-phosphocholine (DOPC) of 0.26 mM and 1,2-dioleoyl-sn- glycerol-3-phosphoethanolamine-N-(lissamine rhodamine B sulfonyl) (Rb-DOPE) of 1.6 μM in the phospholipid solution. To develop SLB on the glass substrate, the phospholipid solution of 200 μl was dripped on the glass bottom dish and incubated for 2 hours at a constant temperature at 45 °C. In order to remove excess vesicles on the developed SLB, the sample was washed by PBS. We irradiated oygen radical to PBS using the radical source. Oxygen radicals were generated by a radical source (Fuji Machine MFG. Co., LTD. FPA-10). The radical source is based on an atmospheric-pressure high-density O_2/Ar plasma, which produces a high electron density of about 10^{16} cm^{-3}. The $O(^3P_j)$ density of about 10^{14} cm^{-3} was measured in our previous study [2]. Next, we replaced radical-exposed PBS and performed FRAP at 4 places every 5 minutes [3]. We also performed the same experiment on PB and DDW.

3. Results and discussion

From Fig.1, the diffusion coefficient decreased as immersion time increased in the case of PBS. On the other hand, the diffusion coefficient was almost constant in the case of PB.
Compering of PB and PBS, only PBS contains chloride ions. Thus, we estimated that HClO is the key species to decrease the diffusion coefficient of lipid bilayer.

In the case of DDW, the diffusion coefficient decreased by immersing SLB in DDW exposed to radicals. However, there is no dependence of immersion time on it. Because the pH decreased from 6.02 to 4.23 due to the irradiation, we estimated that the pH change due to NOx^- decreased the diffusion coefficient.

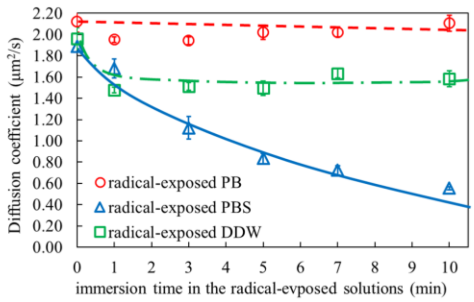

Fig.1 The diffusion coefficient of SLB as a function of immersion time in the radical-exposed solutions

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