Archives of Biochemistry and Biophysics 605 (2016) 26–33

Contents lists available at ScienceDirect

Archives of Biochemistry and Biophysics

journal homepage: www.elsevier.com/locate/yabbi

Nanopore formation process in artificial cell membrane induced by plasma-generated reactive oxygen species

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A R T I C L E I N F O

Article history:
Received 31 December 2015
Received in revised form
17 May 2016
Accepted 18 May 2016
Available online 20 May 2016

Keywords:
Atmospheric pressure plasma
Lipid bilayer
Lipid oxidation
Reactive oxygen species
Atomic force microscope

A B S T R A C T

We investigated morphological change of an artificial lipid bilayer membrane induced by oxygen radicals which were generated by non-equilibrium atmospheric pressure plasma. Neutral oxygen species, $O(^3P_j)$ and $O_2(^1\Delta_g)$, were irradiated of a supported lipid bilayer existing under a buffer solution at various conditions of dose time and distances, at which the dose amounts of the oxygen species were calculated quantitatively. Observation using an atomic force microscope and a fluorescence microscope revealed that dose of the neutral oxygen species generated nanopores with the diameter of 10–50 nm in a phospholipid bilayer, and finally destructed the bilayer structure. We found that protrusions appeared on the lipid bilayer surface prior to the formation of nanopores, and we attributed the protrusions to the precursor of the nanopores. We propose a mechanism of the pore formation induced by lipid oxidation on the basis of previous experimental and theoretical studies.

1. Introduction

Reactive oxygen species (ROSs) are reactive factors and/or mediators in biological and physiological reactions shown to be either beneficial or harmful [1–3]. Atmospheric pressure plasma is a novel and valuable tool to generate ROSs intentionally, as well as reactive nitrogen species (RNSs) and electric field, and is applied in the fields of medicine and biology in these decades [4–6]. Recent studies demonstrated the multifarious applications of atmospheric pressure plasma in these fields; sterilization [7–13], selective killing of tumor cells [14,15], cellar regulation [16,17], and gene transfection [18,19]. Various kinds of ROS and RNS are generated and included in these reactions induced by atmospheric pressure plasma [12], and play crucial roles in these phenomena. Some studies showed that radical species are requisite for the transportation of materials through a cell membrane [19,20]. Recently we developed a neutral oxygen radical source on the basis of non-equilibrium atmospheric pressure plasma [21], which effectively inactivates *Penicillium digitatum* spores [22–26] and proliferates/inactivates *Saccharomyces cerevisiae* [27]. This radical source excludes the effects of electric field and lights, and supply only ROSs. The ROSs provided by this radical source are characterized and their dose amounts were quantitatively evaluated [21,25–27]. The active species critical for these reactions are attributed to the atomic oxygen at the ground state $O(^3P_j)$, and its amount is on the order of 10^{19} cm^{-3}.

In spite of the various applications of the atmospheric pressure plasma, however, what ROSs causes on cell membranes and how ROSs pass through cell membranes are still unclear. Biological cell membrane is the outermost cell organ and acts as a barrier and gates controlling the transportation of materials and information. Everything needs to pass through a cell membrane when it enters into a cell and causes an effect inside the cell. The plasma-generated ROSs also access to the cell membranes first. The critical effect of ROSs on cells may be a direct effect on cell membranes, or indirect one through physiological cascades inside cells and/or genetic damages. Some previous studies showed that cell

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http://dx.doi.org/10.1016/j.abb.2016.05.014
0003-9861/