学　位　論　文　内　容　の　要　旨

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Structure and stability studies on the lipid monolayers exposed to low-level ozone
（低濃度オゾンに曝露した脂質単分子膜の構造と安定性に関する研究）

Cell membranes play crucial roles in the bio-functionality of the cells such as mass transport, metabolism, information exchange, and energy conversion. The phospholipid bilayer, containing both saturated and unsaturated phospholipids, constructs the main frame structure of the cell membrane. The functionality of the cell membranes could be significantly affected by the stability of lipids, especially in an environment contaminated by oxidants such as ozone, which is a universal air pollutant. The ozone at the earth surface, with a typical concentration of a few tens of ppb, is mainly formed through photochemical reaction of volatile organic compounds (VOC) and nitrogen oxide (NOx) under ultraviolet (UV) radiation. The influence of ozone on the lipid molecules have been mainly evaluated in high concentration range (0.3 ~ 10 ppm) in the previous studies, much higher than the level of ozone in the ambient environment. The purpose of the present study is to investigate the molecular structure and stability of various phospholipid monolayer systems in a very low-level of ozone environment (20±10 ppb), close to our ambient environment, by sum frequency generation (SFG) vibrational spectroscopy in combination with π-A isotherm and atomic force microscopy (AFM) at a molecular level.

The present thesis consists of six chapters. In Chapter 1, after a brief introduction of lipid membrane, the previous studies on the lipid oxidation and the reaction mechanism are briefly reviewed. Finally, the purpose and outline of the present thesis are given.

In Chapter 2, a basic theory of SFG vibrational spectroscopy is provided, including a general theory of nonlinear optics, the principle of SFG vibrational spectroscopy and the spectral analysis. Furthermore, the procedures of molecular orientation analysis based on SFG results are also given.

In Chapter 3, the details about experiments in this thesis, including the chemicals, the sample preparation, the control of ozone concentration, π-A isotherm, and the optical system description about SFG vibrational spectroscopy and AFM measurement are described in detail.

In Chapter 4, an synthesized unsaturated lipid, 1,2-dioleoyl-sn-glycero-3-phosphocholine

(DOPC), and a natural saturated lipid, 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), were used to prepare single and mixed monolayers to mimic the cell membrane. The stability and structure of these monolayers exposed to nitrogen, and an extremely low-level of ozone (20±10 ppb) on the water surface had been explored by π-A isotherm, AFM, and SFG vibrational spectroscopy. It was found that the saturated DPPC molecules construct a well-defined monolayer on the water surface and are very stable in the low-level ozone environment. On the other hand, the unsaturated DOPC monolayer on the water surface is more disordered and can be oxidized by the low-level ozone easily. The *cis*-C=C bonds in the DOPC molecule are attacked by ozone and decomposed to oxidized phospholipids (oxPLs), nonanal and nonanoic acid. All of these products are unstable on the water surface and dissolve in the aqueous phase or go into gas phase soon after the reaction. After mixing with saturated DPPC, the conformational ordering of DOPC molecules in the monolayer is largely improved. After exposure to low-level ozone, the DPPC molecules in the mixed monolayer are still stable while the DOPC molecules are selectively oxidized. It is a noteworthy issue that a small amount of DOPC molecules in the mixed monolayers still remain on the water surface after the ozone oxidation, indicating that the presence of saturated DPPC molecules in the mixed monolayer can partially inhibit the oxidation process of unsaturated DOPC molecules under the low-level ozone.

In Chapter 5, another natural unsaturated lipid, 1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-choline (POPC), which is occupied a significant percentage of many membranes, has been studied in detail. It was found that the POPC monolayer is also largely affected by the low-level ozone but in a totally different way with DOPC monolayer. As confirmed by the *in-situ* SFG measurement, only the unsaturated chain of POPC is selectively oxidized by the low-level ozone, while its saturated chain remains. After exposure to ozone, oxPLs, nonanal and nonanoic acid are generated as the oxidation products. Due to the presence of a hydrophobic saturated alkyl chain, the solubility of oxPLs in aqueous phase is very low and thus oxPLs are much more stable on the water surface than that from DOPC. Furthermore, it was found the oxidation degree of the POPC molecules in the POPC-DPPC mixed monolayer become lower than that in the POPC monolayer, indicating that the DPPC molecules also have some shelter effect for POPC molecules from the oxidation damage of low-level ozone.

In Chapter 6, general conclusions for the thesis are summarized. (1) The saturated lipids show high stability in both nitrogen and the low-level ozone, while the unsaturated lipids are only stable in nitrogen but unstable in the low-level ozone environment. (2) The unsaturated lipids are selectively oxidized by the low-level ozone in the mixed monolayer with saturated lipids, while the presence of saturated lipids in the mixed monolayer can partially inhibit the oxidation process. (3) The structure of the unsaturated lipids significantly affects their response to the oxidation damage in the low-level ozone environment.