

DOCTORAL THESIS

**Measurement, Dynamics and Roles of Lipid
Hydroperoxide, Singlet Oxygen and OH Radical in
Natural Waters**

(Summary)

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General summary (English)

学 位 論 文 の 要 旨

論文題目 Measurement, Dynamics and Roles of Lipid Hydroperoxide, Singlet Oxygen and OH Radical in Natural Waters.

(天然水中の脂質過酸化物, 一重項酸素、OH ラジカルの測定、動態、役割に関する研究)

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In chapter one, a brief introduction to lipids and their roles in natural waters was provided. Also, OH radical ($\cdot\text{OH}$) and singlet oxygen ($^1\text{O}_2$) were introduced. Despite the presence of lipids in natural water and the availability of conditions necessary to oxidized them, quantification of lipid hydroperoxides (LHPs) have not yet been reported perhaps due to lack of a selective analytical method to determine them. Hence, the need to selectively quantify LHPs was presented. Also, the need to determine $\cdot\text{OH}$ and $^1\text{O}_2$ in various natural waters in Japan was discussed. Therefore, this present study involved development of analytical method to selectively determine LHPs in natural waters. Also, the lack of information on the $^1\text{O}_2$ generation in natural waters in Japan and expansion of available data on $\cdot\text{OH}$ formation rate were addressed.

Chapter two reports the development of a selective analytical method to determine LHPs in natural waters. A method was developed to selectively determine LHPs in river water using the

fluorescent probe Liperfluo (2-(4-diphenylphosphanyl-phenyl)-9-(3,6,9,12-tetraoxatridecyl)-anthra[2,1,9-def:6,5,10-d'e'f']diisoquinoline-1,3,8,10-tetraone). The optimum conditions for the application of the probe were established. The conditions and the probe used in this study showed high selectivity for LHPs over other natural water hydroperoxides, including hydrogen peroxide, methyl hydroperoxide and ethyl hydroperoxide. The method was applied to the quantification and fate determination of LHPs in water from the Kurose River (Japan). The concentrations ranged from below detection limit to 98 nM (ave. 37.2 nM; $n = 12$). The LHPs in river water samples were found to quickly photo-decomposed within an hour. The irradiation of LHP-spiked river water using a solar simulator resulted in an increased H_2O_2 concentration, suggesting that H_2O_2 formation may be a possible sink for LHPs in river water.

The spatial distribution of photogenerated $^1\text{O}_2$ in Seto Inland Sea, which was hitherto lacking, was reported in chapter 3. Furfuryl alcohol(FFA) was used as a probe to quantify $^1\text{O}_2$ generation. It reacts with $^1\text{O}_2$ to form 6-Hydroxy-2*H*-pyran-3(6*H*)-one (6-HP-one). The steady state concentration $[\text{}^1\text{O}_2]_{\text{ss}}$ varied by a factor of about six with 1.16×10^{-14} M in Kii Channel to 7.3×10^{-14} M in Osaka bay. In addition, the chromophoric dissolved organic matter (CDOM) absorption characteristic such as absorption co-efficient (a_{300}), spectra slope and carbon-specific absorption were determined. A strong positive correlation between $[\text{}^1\text{O}_2]_{\text{ss}}$ and a_{300} was obtained ($r=0.93$, $P<0.001$). This correlation was even stronger ($r=0.998$, $P<0.001$) within Osaka bay samples. A linear equation between $[\text{}^1\text{O}_2]_{\text{ss}}$ and CDOM concentration in Osaka bay was derived. Predictions made using this equation were in good agreement with experimentally

obtained values suggesting that such equation may be a useful model for the estimation of $[^1\text{O}_2]_{\text{ss}}$ in Seto Inland Sea based on CDOM data.

Chapter 4 reports the photogeneration of $^1\text{O}_2$ and $\cdot\text{OH}$ and the optical characteristics of selected rivers across Japan was investigated. The sampled rivers include Kurose river and Ohta river in Hiroshima, Yamato river in Osaka and Kokubu river in Chiba prefecture. $^1\text{O}_2$ was determined using FFA as a probe while $\cdot\text{OH}$ was determined using benzene as a probe. The CDOM absorption coefficient (a_{300}) and spectra slope were also determined. The a_{300} was lowest in Ohta river ranging from 2.03 -3.13 m^{-1} . Yamato river ranged between 8.9 -13 m^{-1} while Kokubu river ranged between 9.6 – 11.4 m^{-1} . The highest CDOM was observed in Kurose river with values of 3.27-20.96 m^{-1} , 4.7 -22.43 m^{-1} and 3.36 -18.52 m^{-1} for samples obtained in August, October and December respectively. The spectra slope calculated for the river water samples were 16 – 18.2 μm^{-1} (Ohta river); 15.3 – 16.6 μm^{-1} (Yamato river); 13.1 - 15.9 μm^{-1} (Kokubu river). In Kurose river, the values were 14.4 – 17.1 μm^{-1} (August), 13.8 - 16.4 μm^{-1} (October), 11.6 - 16.2 μm^{-1} (December). For $^1\text{O}_2$ determination, the average values obtained for steady-state concentration $[^1\text{O}_2]_{\text{ss}}$ and photoformation rate ($R^1\text{O}_2$) were: Kurose ($9.64\text{-}20.5 \times 10^{-14} \text{ M}$, $24.1\text{-}51.2 \times 10^{-9} \text{ M s}^{-1}$); Kokubu ($12.4 \times 10^{-14} \text{ M}$, $31.2 \times 10^{-9} \text{ M s}^{-1}$); Yamato ($12.5 \times 10^{-14} \text{ M}$, $30.8 \times 10^{-9} \text{ M s}^{-1}$); Ohta ($4.24 \times 10^{-14} \text{ M}$, $10.58 \times 10^{-9} \text{ M s}^{-1}$). The average rate of $\cdot\text{OH}$ photoformation ($R^{\cdot\text{OH}}$) and steady-state concentration $[\cdot\text{OH}]_{\text{ss}}$ observed in the samples were $65.3 - 179 \times 10^{-12} \text{ M s}^{-1}$ and $338 - 734 \times 10^{-18} \text{ M}$ in Kurose river; $26.82 \times 10^{-12} \text{ M s}^{-1}$ and $192 \times 10^{-18} \text{ M}$ in Ohta river, $232 \times 10^{-12} \text{ M s}^{-1}$ and $1219 \times 10^{-18} \text{ M}$ in Yamato river; $528 \times 10^{-12} \text{ M s}^{-1}$ and $1260 \times 10^{-18} \text{ M}$ in Kokubu river which was the highest. This study demonstrated that the $^1\text{O}_2$ generation and $\cdot\text{OH}$

varied by one order among the river samples. Also, the $^1\text{O}_2$ and $\cdot\text{OH}$ generation in river water samples were generally one order higher than in seawater.

In chapter 5, the influence of $^1\text{O}_2$ and $\cdot\text{OH}$ on lipid peroxidation and fate of pollutants in natural waters were investigated. Both ROS are capable of reacting with lipids to initiate lipid peroxidation. The LHPs concentrations followed a trend similar to that observed for the generation of ROS across the sampled stations. This suggests that both ROS may influence peroxidation of lipids in natural waters but the more important ROS in this regard is not yet clear. The influence of $^1\text{O}_2$ and $\cdot\text{OH}$ on pollutants such as diazinon and methyl mercury in natural waters were investigated. The reaction rate constants of diazinon with $^1\text{O}_2$ and $\cdot\text{OH}$ were determined to be $7.29 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$ and $1.01 \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}$ respectively. Calculated degradation rate constants showed that degradation of diazinon in both seawater and river water will be mediated by $\cdot\text{OH}$. For methyl mercury, $^1\text{O}_2$ is more important for its degradation in seawater while $\cdot\text{OH}$ will mediate its degradation in river water.

In chapter 6, a simple, inexpensive set-up was assembled and applied to generate $^1\text{O}_2$ in the gas phase. A filter material typically employed in the air conditioner units was impregnated with Rose Bengal (a $^1\text{O}_2$ photosensitizer) and irradiated in the presence of oxygen. FFA was used as a substrate and its degradation was monitored. The degradation of FFA provided qualitative and quantitative information about $^1\text{O}_2$ from the treated filters. A minimum estimate of $1.18 \text{ nmole s}^{-1}$ of $^1\text{O}_2$ per cm^2 of filter was observed. The successful generation of $^1\text{O}_2$ by this assembly demonstrates that $^1\text{O}_2$ can be generated heterogeneously in the gas phase using simple, inexpensive laboratory set-up without the need for sophisticated materials.

Finally, in Chapter 7, a general discussion and conclusions on the main findings was provided.

Recommendations for future works were also provided.

General summary (Japanese)

論文題目 Measurement, Dynamics and Roles of Lipid Hydroperoxide, Singlet Oxygen and OH Radical in Natural Waters.

(天然水中の脂質過酸化化物, 一重項酸素、OH ラジカル の測定、動態、役割に関する研究)

本論文は、海水や河川水などの天然水中の脂質過酸化化物の測定および動態解明、そしてその生成機構に係る活性酸素種、特に一重項酸素 ($^1\text{O}_2$) およびヒドロキシルラジカル (OH) の測定および役割解明に関する研究である。天然水中には微量の脂質化合物が溶存し、その酸化分解過程で脂質過酸化化物が中間物として生成すると考えられるが詳細は不明である。また、OH や $^1\text{O}_2$ などはさまざまな有機物の酸化分解反応に関与することが知られているが、十分に解明されていない。本論文では、天然水中に存在するこれらの化学種に関して、測定法の開発も含めて動態や役割について研究した。

第1章は序論であり、研究の背景、意義、目的について述べている。

第2章は、天然水中の脂質過酸化化物の測定法の開発に関する研究である。蛍光試薬である Liperfluo を脂質過酸化化物と反応させ、生成物を高速液体クロマトグラフィー蛍光検出法で測定する方法を開発した。この方法を用い、黒瀬川河川水中の濃度を測定した結果、数十 nM レベルで脂質過酸化化物が存在することを明らかにした。さらに、脂質過酸化化物が光化学的に容易に分解することや、その分解過程で過酸化水素が生成することを明らかにした。

第3章は、瀬戸内海海水中の $^1\text{O}_2$ の測定、動態解明に関する研究である。光化学的に生成した $^1\text{O}_2$ をFurfuryl alcoholと反応させ、その反応物をHPLC-紫外検出法で測定することにより、 $^1\text{O}_2$ の生成速度や定常状態濃度を求めた。瀬戸内海における $^1\text{O}_2$ の光化学的生成速度および定常状態濃度はそれぞれ $10^{-9}\sim 10^{-8}\text{ Ms}^{-1}$ および 10^{-14} M レベルであり、大阪湾で最も高く、紀伊水道で最も低かったことを示した。海水中の有色溶存有機物(CDOM)濃度を併せて測定した結果、 $^1\text{O}_2$ との相関が高く、CDOMが $^1\text{O}_2$ の主な生成源であることが推定された。

第4章は、日本のいくつかの河川水中の $^1\text{O}_2$ およびOHの光化学的生成速度および定常状態濃度を測定した結果を示す。測定対象の河川は、国分川(千葉県)、大和川(奈良県・大阪府)、太田川(広島県)、黒瀬川(広島県)であった。河川水中の $^1\text{O}_2$ およびOHの光学的生成速度はそれぞれ $10^{-9}\sim 10^{-8}\text{ Ms}^{-1}$ および $10^{-11}\sim 10^{-10}\text{ Ms}^{-1}$ であり、また定常状態濃度はそれぞれ $10^{-14}\sim 10^{-13}\text{ M}$ および $10^{-17}\sim 10^{-15}\text{ M}$ であった。瀬戸内海と同様に、CDOMと $^1\text{O}_2$ との相関が高かった。

第5章は、天然水中において脂質の分解、あるいは農薬などの汚染物質の分解への $^1\text{O}_2$ およびOHの関与について反応速度論解析を述べている。これらの化学物質の分解反応においては、 $^1\text{O}_2$ もしくはOHのいずれかが主要な酸化剤として作用することが考えられた。脂質の場合はその疎水性の程度により $^1\text{O}_2$ およびOHのいずれかが主の酸化剤として働く。瀬戸内海海水中には、たとえば有機リン系殺虫剤であるDiazinonの分解にはOHが主な酸化剤として作用し、一方メチル水銀の分解には $^1\text{O}_2$ が主な酸化剤として作用すると予測した。

第 6 章は、 $^1\text{O}_2$ が有する有機物への強い酸化作用を利用して、空気浄化を行う方法の開発に関する研究であり、本研究の目的から二次的に派生したものである。ローズベンガルを塗布したフィルターに可視光 LED を照射することにより、フィルター 1 cm^2 当たり約 1 nmole s^{-1} の $^1\text{O}_2$ を効率的、簡便、安価で発生することに成功した。この方法は、空気中の悪臭物質の除去への適用が期待できる。

第 7 章は、総合討論であり、研究の総括および今後の課題を述べている。