Production and Decomposition Mechanisms of Reactive Oxygen Species by Red-tide Causing Phytoplankton — Case Study for Hydrogen Peroxide.

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Key words: H_2O_2, *Chattonella antiqua*, *Heterocapsa circularisquama*, photochemical reaction, red-tide, H_2O_2 production and decomposition

In this doctor's thesis, the distribution and the behavior of Reactive Oxygen Species: ROS (mainly hydrogen peroxide (H_2O_2)) in the ocean was studied. I have studied the biological generation of H_2O_2 as well as the production by photochemical processes. Especially the phytoplankton that may cause harmful algal bloom with the mortality of cultured fish and bivalves, in late spring to early summer in the Seto Inland Sea and other coastal seas in Japan were investigated for their ability of ROS production and decomposition by analyzing of natural red tide seawater and cultured samples.

Firstly, previous studies of production, distribution and decomposition of H_2O_2 in the environment mainly in the atmosphere and the ocean was summarized and on the basis of previous studies, the aim and significance of this study were described.

Secondly, the concentration and the behavior of H_2O_2 in the Hiroshima Bay seawater was investigated during 8 cruises in 1996 to 2002 (except 2000). H_2O_2 was characterized as higher concentrations at the surface water with decreasing trend with depth. The H_2O_2 concentration showed higher during the daytime (140-450 nmol L^{-1} at 5:00-19:00) than during the nighttime (85-260 nmol L^{-1} at 20:00-4:00) and suggested that H_2O_2 at the surface seawater was generated by photochemical reaction and also partly by biological production on the process of photosynthesis by phytoplankton. The correlation of H_2O_2 with environmental factors such as salinity, water temperature, solar radiation, concentration of dissolved organic matter was examined by statistical analysis and H_2O_2 concentration was found to be controlled by mainly salinity and water temperature in Hiroshima Bay probably due to the influence of river waters running into the bay. H_2O_2 photo-production rate was estimated to be 8.0-16 nmol h^{-1} by solar irradiation experiment and indicated faster production rate than those in other sea areas reported previously. Estimated H_2O_2 half-life time under the dark condition was 12-14h and seemed to be faster decomposition rate compared with those in other sea areas. Decomposition of

広島大学総合科学部紀要Ⅳ理系編、第31巻（2005）
*広島大学審査学位論文
口頭発表日：2005年２月９日、学位取得日：2005年３月４日

H_2O_2 was prevented by filtration of seawater before the incubation, suggesting that the decomposition was taken place by microorganisms including phytoplankton in seawater.

Thirdly, biological production of H_2O_2 in Hiroshima prefecture coast seawater was observed. Concentration of H_2O_2 in natural red tide seawater was compared with that in natural seawater (containing no red tide causing species). Natural red tide seawater was taken from 3 harbors in Hiroshima prefecture. I have observed a phytoplankton *Chattonella ovata* (Raphidophyte) that had never occurred red tide until now in Japan has caused big blooms and generated high concentration of H_2O_2 (1,700-5,600 nmol L^{-1}). After the separation of *C. ovata* from the red tide seawater, the production ability of H_2O_2 under the artificial culturing condition (21℃, 12h light: 12h dark, 42-62 μ photons) was observed and confirmed that *C. ovata* produces H_2O_2, with the increase of cell number, as the same phenomenon has been observed in other Raphidophyte species such as *Chattonella antiqua* and *Chattonella marina* in previously reports.

Fourthly, production mechanism of H_2O_2 by *C. antiqua* was observed. I have measured the activity of Super Oxide Dismutase (SOD) to estimate an enzymatic formation of H_2O_2 from O_2^- in algal cells. High SOD activity was found in the cell of *C. antiqua*, indicating large production of H_2O_2 while low SOD activity was detected in the cell of *H. circularisquama*, indicating little or no production of H_2O_2. The result of Native-PAGE active staining analysis for cultured samples suggested that both *C. antiqua* and *H. circularisquama* contain Mn-SOD in the cell as a kind of antioxidant system.

Fifthly, the mechanism of H_2O_2 decomposition by *C. antiqua* and *H. circularisquama* was observed. Decomposition ability of H_2O_2 was completely diminished by filtration of the culture solution containing the phytoplankton studied, which suggested that cell itself (probably cell surface) is involved in the decomposition process of H_2O_2. *H. circularisquama* has shown strong decomposition ability compared with that of *C. antiqua*. I found that *C. antiqua* has a high catalase activity (5.2 units^{-1} 10^3 cells mL^{-1}) but *H. circularisquama* indicated the low activity (1.3 units^{-1} 10^3 cells mL^{-1}). The confirmatory test was done by using 3-amino-1,2,4-triazole (3AT) which inhibits the activity of catalase. Since higher concentration of 3AT added to the cell suspension resulted in higher H_2O_2 concentration, the involvement of catalase against the decomposition of H_2O_2 was highly likely. Ascorbate peroxidase (APX) activity was found to be strong with *H. circularisquama* (1.73 μ mol mg Chl^{-1} min^{-1}) and low with *C. antiqua* (0.55 μ mol mg Chl^{-1} min^{-1}). To decompose the harmful H_2O_2, *C. antiqua* will use mainly catalase to keep the balance of H_2O_2 concentration both inter-cellular and outer-cellular environments. In other hand, *H. circularisquama* will use both catalase and APX to rapidly decompose H_2O_2.

At the last, I have summarized and discussed on all the experiments mentioned above. From my results, H_2O_2 in Hiroshima Bay seawater is produced by both photochemical reaction and biological process. The photochemical reaction is probably the dominant pathway of generation of H_2O_2 during no blooms of phytoplankton in Hiroshima Bay while during some phytoplankton

bloom period biological process may be the dominant for H_2O_2 generation. Now it is clear that some Raphidophyte species that cause red tide in Japan and other countries have the specific mechanism of H_2O_2 production and decomposition. Considering significant fishery damage reported by this species, further clarification of production and decomposition processes of ROS is needed.