Damage to Cultivated Japanese Pearl Oysters by Oxidative Stress That Was Related to “Mass Mortality”

Yuushi UCHIMURA,1 Hirofumi YAMASHITA,1 Makoto KURAMOTO,2 Kohji ISHIHARA,3,* Manabu SUGIMOTO,4 and Nobuyoshi NAKAJIMA5,

1Ehime Prefectural Fisheries Experimental Station, Uwajima, Ehime 798-0104, Japan
2Advanced Instrumentation Center for Chemical Analysis, Ehime University, Matsuyama, Ehime 790-8577, Japan
3Department of Chemistry, Kyoto University of Education, Fushimi-ku, Kyoto 612-8522, Japan
4Research Institute for Bioresources, Okayama University, Kurashiki, Okayama 710-0046, Japan
5Department of Nutritional Science, Okayama Prefectural University, Soja, Okayama 719-1197, Japan

Received May 20, 2003; Accepted August 11, 2003

Increased blood-DNA breakage was observed in diseased pearl oysters. They showed significant formation of 8-hydroxydeoxyguanosine (8-OHdG) and malondialdehyde (MDA), whereas the oysters that had a low mortality rate from the disease had high activity of superoxide dismutase (SOD) and low amounts of 8-OHdG and MDA. These results suggest that radical damage had occurred only in the diseased pearl oysters with the cytolyis of their haemocytes, which was related to the mass mortality of the Japanese pearl oysters. Therefore, the corpuscle collapse was considered to be an important factor for understanding the symptoms of this disease. The accumulation of carotenoid, abnormal metabolism of sterols, and formation of toxin were also demonstrated in the diseased oysters. Herein, we report the formation of radical-damaged products due to oxidative stress in the diseased oysters, which was related to the mass mortality of the Japanese pearl oysters. We would also propose a plan in order to produce “oyster’s seedlings” that are not so susceptible to the disease.

Key words: pearl oyster; 8-hydroxydeoxyguanosine (8-OHdG); malondialdehyde (MDA); superoxide dismutase (SOD); oxidative stress

The large-scale death of the cultivated Japanese pearl oysters, Pinctada fucata martensii, with their soft tissue being discolored to red, has occurred in several sea areas around Japan since 1996. The mass mortality of the raised pearl oysters caused damage amounting to “18.3 billion yen” also in Ehime, Japan. In 1999, the amount of mother-shellfish production and total pearl production decreased by 15.6% and 14.0%, respectively, compared to those of the past few years.1)

The cause of the mass mortality has been reported to be an infectious disease in the circulatory organs, based on infection experiments.2) When the haemolymphs of the diseased pearl oysters were inoculated into the ordinary pearl oysters, cytolyis of the haemocytes, collapse of the skin in the blood vessels, and damage of the organs occurred in ordinary oysters. Therefore, the corpuscle collapse was considered to be an important factor for understanding the symptoms of this disease.3) The accumulation of carotenoid, abnormal metabolism of sterols, and formation of toxin were also demonstrated in the diseased oysters.4) Herein, we report the formation of radical-damaged products due to oxidative stress in the diseased oysters, which was related to the mass mortality of the Japanese pearl oysters. We would also propose a plan in order to produce “oyster’s seedlings” that are not so susceptible to the disease.

Ordinary pearl oysters (over 2 years old), P. fucata martensii, which were raised in Anamizu Bay, Ishikawa, Japan, were provided by the Pathology Division of the National Research Institute of Aquaculture, Fisheries Research Agency, Japan. Diseased pearl oysters (2 years old), raised in the southern part of the sea, Ehime, Japan, were used. The diseased stages of the haemocytes were individually classified into five groups by observation of the corpuscle-meter-number board using a phase-contrast microscope; as stage 1 (n = 9) and stage 2 (n = 11) in the ordinary pearl oysters, and as stage 3 (n = 1), stage 4 (n = 12), and stage 5 (n = 7) in the diseased pearl oysters. Haemolymphs were drawn with a syringe from the adductor muscles of both the ordinary and diseased pearl oysters (average 1 ml per oyster).5) The haemocyte DNAs were isolated using a “DNA Extraction Kit” (Wako Pure Chemical Industries, Ltd., Japan) from the haemolymphs (5 ml each) of the ordinary and diseased oysters. The isolated DNAs were analyzed by agarose gel electrophoresis.6) The amount of 8-hydroxydeoxyguanosine-
sine (8-OHdG) in the serum of the ordinary (stage 1) and diseased (stage 4) oysters was determined using a “New 8-OHdG Check Elisa Kit” (Japan Institute for the Control of Aging, Japan). The serum was prepared from the haemolymphs (4 ml) after centrifugation to remove the haemocytes and concentration (10- and 20-times) under vacuum conditions. For the measurement of malondialdehyde (MDA) in the adductor muscles of the ordinary and diseased oysters, the thiobarbiturate value (TBA value) was measured basically according to the method described previously; the muscles (five oysters each, 1 g wet) were suspended in 20 times (by volume) of 10% trichloroacetic acid and homogenized with a glass homogenizer in an ice bath to prepare the cell-free extracts. The relative value at 532 nm was measured after boiling for 10 min in the same volume of 0.67% thiobarbituric acid. The formation of the adduct of MDA with TBA was also confirmed by HPLC using a column of Sp-120-5 ODS-AP (4.6 mm i.d. × 150 mm, Daiso Co., Ltd., Japan, A: 0.1 M phosphate buffer, pH 4.0 and B: MeOH) with the authentic MDA-TBA adduct. Superoxide dismutase (SOD) activity was measured using a “SOD Assay Kit” (Bioxytech SOD-525, OXIS International, Inc., OR, USA). The cell-free extracts were prepared from the whole cells of the ordinary and diseased oysters (five oysters each, 15 g wet) by homogenization in 15 ml of phosphate buffered saline (pH 7.2) with a Teflon homogenizer in an ice bath. The supernatant solution (10 μl) was used as the enzyme solution for the SOD assay.

When the DNAs were electrophoresed on an agarose gel (Fig. 1), large amounts of high-molecular weight DNAs and small amounts of the fragmentated DNAs (below 23,130 bp) were found in both stage 1 and stage 2 of the ordinary oysters, whereas large amounts of the fragmentated DNAs and only small amounts of the high-molecular weight DNAs were observed in stages 4 and 5 of the diseased oysters. The fragmentation of the DNAs was remarkable in the diseased oysters. Similar results were observed in the cytolysis of the haemocytes; it was also the most severe in stage 4. Furthermore, the significant formation of 8-OHdG, which originated in the repaired DNA after the radical damage, was found in the serum of the diseased oysters (stage 4), as it was hardly detected in the ordinary oysters (stage 1) as shown in Table 1. MDA was also detected in the adductor muscles of the diseased oysters, although it was not detected in the ordinary oysters at all (Fig. 2). Thus, the breakdown of the haemocyte DNAs and the formation of 8-OHdG and MDA were found only in the diseased oysters.

The haemocytes of the pearl oysters were composed of leukocytes, which don’t play a role in oxygen transportation like red corpuscles or immunity like lymphocytes. The main function of the oyster’s haemocytes is recognized to be for cell defense. In the diseased oyster, the decrease in the haemocytes by cytolysis occurred after the increase in the haemocytes by the defense reaction, and the cytolysis of the haemocytes was found before the collapse of the endothelium of the blood vessel and the discoloration of the soft tissue, which led to the disruption of their circulatory organs. It was reported that MDA was formed by the peroxidation of unsaturated fatty acid and DNA by a hydroxy radical generated from hydrogen peroxide in the leukocytes. The haemocytes of the diseased oysters had significantly collapsed, and hydrogen peroxide in the haemocytes was found in the diseased oysters. The disruption of the circulatory organs of...
the diseased oysters, inducing the impediment of the nutrition supply, might be due to the radical damage as a result of the over-defense reaction of the hemocytes.

On the other hand, the SOD activity (97.7 units/ml of the cell-free extracts)\(^\text{14}\) of the “low-mortality group” (6.3% mortality through a year)\(^\text{1,3}\) of the pearl oysters was higher than that of the “high-mortality group” (28.8% mortality) whose SOD activity was 38.8 units/ml. The amounts of 8-OHdG and MDA in the “low-mortality group” were lower than those of the “high-mortality group” as described above. Thus, the pearl oysters showing the high-SOD activity were considered to be sufferable from the oxidative stress.

Therefore, the DNA breakage and the formation of 8-OHdG and MDA demonstrated in our studies suggest that the radical damage occurred only in the diseased oysters with the cytosis of their hemocytes and the disruption of their circulatory organs. These phenomena were found universally in all of the cultivated Japanese pearl oysters tested. This is a first report concerning to the formation of radical-damaged products due to oxidative stress in the diseased pearl oysters, which was related to the mass mortality of the Japanese pearl oyster, *Pinctada fucata martensii*, by tissue transplantation and cohabitation. *Fish Sci.* (in Japanese), 65, 241–251 (1999).


