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Effect of Opsonization of Zymosan on the Chemiluminescent Response of Haemocytes in the Pacific oyster *Crassostrea gigas*

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The effect of opsonization of zymosan with serum on the respiratory burst of haemocytes isolated from the Pacific oyster *Crassostrea gigas* was investigated. The reactive oxygen species (ROS) produced by the respiratory burst of haemocytes in response to the opsonized or unopsonized zymosan were measured using chemiluminescence (CL). The CL values were increased according to the increment of haemocyte number. The degree of CL amplification by increase of haemocytes from 0.1 x 10^6 to 0.5 x 10^6 cells/ml was 3-5 times, but comparatively low amplification was elicited by increase of haemocytes from 0.5 x 10^6 to 1 x 10^6 cells/ml. Chemiluminescences produced by the haemocytes in response to the zymosan opsonized with oyster serum were considerably higher than the CL produced by the haemocytes in response to the unopsonized zymosan.

Key words: Pacific oyster, Hemocyte, Respiratory burst, Opsonization, Chemiluminescence

As in vertebrates, the immune system of bivalves comprises cellular and humoral reactions. The cellular defence mechanisms involve phagocytosis of pathogens and their degradation by lytic enzymes and/or the production of reactive oxygen metabolites (Pipe, 1992; Winston et al., 1996; Carballal et al. 1997). Although there appear to be interspecies differences with respect to particular reactive oxygen species (ROS) generated by respiratory burst (Anderson, 1994; Greger et al., 1995), production of ROS by stimulated haemocytes of a variety of molluscan species has been documented (Nakamura et al., 1985; LeGall et al., 1991; Bachere et al., 1991; Bramble and Anderson, 1997). By analogy with vertebrate phenomena, ROS are postulated to participate in bivalve internal defence (Adema et al., 1991). The generation of ROS is currently being investigated by lumiol-enhanced chemiluminescence (CL), a method that amplifies the endogenous light emitted by excitation of these molecules, and this methodology has been developed for use in several mollusks (Bachere et al., 1991). Bivalves possess various types of non-specific humoral defense molecules including agglutinins, lectins, bactericidins, lysozymes and serine proteases (Roch, 1999). Among them, agglutinins and lectins are considered to function as opsonins (Vasta, 1992).

In the present study, we investigated the effect of opsonization of zymosan, a particulate extract from the yeast *Saccharomyces cerevisiae* cell wall, with serum on the respiratory burst of haemocytes isolated from the Pacific oyster *Crassostrea gigas*, and measured ROS production by CL assay.

The Pacific oysters measuring 7-10 cm in shell length, were purchased from a commercial source. The oysters were held at the laboratory in aerated aquaria and allowed to acclimate for two weeks prior to the start of the experiments. Haemolymph was intracardially collected from 10 oysters with a 23-gauge needle attached to a 1 ml syringe. The extracted hemolymph was pooled and immediately centrifuged at 900 g for 5 min. The supernatants were decanted and the cell pellets were resuspended in filtered sea water (FSW). Aliquots of the cell suspension were counted in duplicate using a haemocytometer. The

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isolated haemocytes were approximately 90% viable, which was confirmed by trypan blue exclusion test. Zymosan (Sigma) was mixed with the serum of oyster and incubated at 30°C for 30 min. The opsonized zymosan was separated by centrifugation, washed three times and suspended in FSW. The isolated haemocytes were adjusted to 1 x 10^6, 0.5 x 10^6 and 0.1 x 10^6 cells/ml with FSW. The reactive oxygen species (ROS) produced by stimulated haemocytes was quantified using an automatic photoluminometer (Bio-Orbit 1251, Finland). Each test cuvette contained 0.5 ml of luminol made according to the method of Scott and Klesius (1981), 0.5 ml of cell suspension, and 0.5 ml of opsonized or unopsonized zymosan, which was added just prior to measurement. In a control cuvette, 0.5 ml of FSW was added instead of zymosan. The measurements were made for 100 min, and the light emission was recorded as mV.

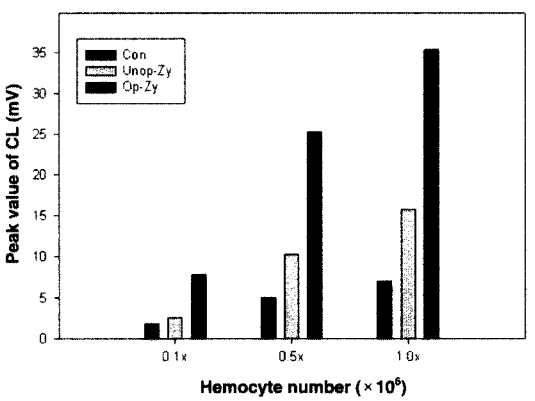

Fig. 1. Chemiluminescence (CL) of *Crassostrea gigas* hemocyte in response to opsonized zymosan (Op-Zy) and unopsonized zymosan (Unop-Zy) according to hemocyte number.

The results are shown in Fig. 1. The CL values were increased according to the increment of haemocyte number. The degree of CL amplification by increase of haemocytes from 0.1 x 10^6 to 0.5 x 10^6 cells/ml was 3-5 times, but comparatively low amplification was elicited by increase of haemocytes from 0.5 x 10^6 to 1 x 10^6 cells/ml. Therefore, the optimum hemocyte number for CL assay in Pacific oysters would be between 0.5 x 10^6 and 1 x 10^6 cells/ml.

In the present study, chemiluminescences produced by the haemocytes in response to the zymosan opsonized with oyster serum were considerably higher than the CL produced by the haemocytes in response to the unopsonized zymosan. Opsonization provides an important link between cellular and humoral defense mechanisms. Tripp (1966) demonstrated that eastern oyster haemolymph, which agglutinated rabbit erythrocytes, also enhanced the *in vitro* uptake of these erythrocytes by oyster haemocytes. However, little informations are available on the interaction between opsonization of foreign particles with oyster serum and respiratory burst activity of oyster haemocytes. The present results demonstrate that humoral factors in haemolymph of Pacific oysters serve as opsonins to link between nonself particles and haemocytes, and consequently increase the respiratory burst activity of haemocytes.

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