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Effects of exogenous Ca^{2+} on the membrane permeability, MDA and SH group content of *Alexandrium* sp. LC3 under surfactant stress

LI Hao[1,2], MIAO Jin-lai[2], CUI Feng-xia[3], LIU Xiao-guang[2], LI Guang-you[2]
(1. Marine Life Sciences College, Ocean University of China, Qingdao 266003, China. E-mail: lihaoh@163.com; 2. Key Laboratory of Marine Biological Active Substances, SOA, Qingdao 266061, China; 3. Fishery College, Ocean University of China, Qingdao 266003, China)

Abstract: The effect of Ca^{2+} on the removal of *Alexandrium* sp. LC3 under HDTMAB stress was investigated. The results showed that the toxic effect of HDTMAB on *Alexandrium* sp. LC3 was significantly reduced in the presence of Ca^{2+}, especially under 4 mmol/L of Ca^{2+}. To understand the underlying mechanism, the SH group and MDA content of the cell membrane and membrane permeability were measured. It was found that the SH content of cell member increased, the MDA content and membrane permeability decreased when *Alexandrium* sp. was treated with Ca^{2+} and HDTMAB complex, compared with using HDTMAB only. The data suggested that Ca^{2+} might promote HDTMAB stress resistance of *Alexandrium* sp. LC3 by reducing the permeability and increasing the stability of cell membrane.

Keywords: Ca^{2+}; surfactant; *Alexandrium* sp. LC3; cell membrane permeability

Introduction

Recently, the red tides have occurred more frequently all over the world, which resulting in the economic loss and the environmental pollution(Zhou, 2001; Miao, 2002). Red tides are formed usually by the mass-gathering of microalgae, protozoan and bacteria which change the color of the sea water or do harm to the other marine organisms, then lead to the ecological abnormality (Zeng, 2004). Sometimes red tides are called harmful algae bloom (HABs) too. *Alexandrium* is one of the most harmful red tide alga distributing in the world (Ferrier, 2002), about thirty dinoflagellates in this species can release many types of natural toxin which caused massive kills of marine animals, and threaten human. Therefore, it is necessary to prevent of the occurrence of *Alexandrium*.

Currently, spread clay and chemicals are employed widely for eliminating harmful algal blooms (HABs) (Yu, 1993, 1999; Zhao, 2001; Miao, 2002; Ferrier, 2002). However, those methods are not feasible on account of the cost and cause recontamination. The surfactant is applied safely in the medicine and food industry as a kind of bactericides(Yang, 2000). It can influence the biological function and selective permeability of the cell membrane which mainly consists of lipoid through changing its structure and biological characterization.

It was previously reported that Ca^{2+} could safeguard the integrality of the cell membrane(Weimberg, 1983), and it was considered to be a kind of membrane protectors(Luan, 1987). In this study, hexadecyltrimethyleamiue bromide (HDTMAB), one of the cationic organic surfactants, associated with Ca^{2+} were used to eliminate the red tide. The influence of Ca^{2+} at various concentrations on the effect of HDTMAB eliminating the algae was investigated by detecting the lipid peroxidation level of cells, the plasma membrane permeability and the change of the content of sulfhydryl group. The purpose of this study was to explore the mechanism of Ca^{2+} preventing surfactant from eliminating the algae and the application potential of surfactant for red tide prevention.

1 Materials and methods

1.1 Strains and cultivation

Alexandrium sp.LC3 used in this study was provided by Fisheriy College of China Ocean University. This algae was incubated at 21℃ in f/2 medium under 35—45 μE/(m^2·s) light with a cycle of 12 h light and 12 h dark. The culture of algae was shaked three times everyday.

1.2 Reagent

Hexadecyltrimethyleamiue bromide (HDTMAB) was purchased from AiBi Chemical Industry, Co., Shanghai, China.

1.3 The detection of cell numbers

Alexandrium sp. LC3 cells were counted with a hemocytometer.

1.4 Measurement of the content of MDA

The content of MDA (Malonaldehyde) of the cell was measured by TBA method (Li, 1989). After addition of phosphate buffer(concentration 50 mmol/L, pH 7.0) and a little quartz sand, 0.5 g of the harvested cells of the culture of *Alexandrium* sp. LC3 was triturated in ice-bath and then centrifuged at 20000 × g for 20 min at 4℃. The final volume of the supernatant was quantified to 5 ml. 1 ml of the supernatant with 3 ml 27% tricarboxylic acid and 1 ml 2% thiobarbituric acid were incubated at 95℃ for 30 min and cooled in ice-bath immediately, then centrifuged at 1500 × g for 10 min. The absorbance of the supernatant was measured at 532 nm. After the deduction of the absorbance at 600 nm, the content of MDA was measured by using an extinction coefficient of 155 L/(mmol·cm).

1.5 Measurement of the content of SH group

The content of SH group of the cell was measured by DTNB method (Ellman, 1959). After addition of 75 μl diluted buffer, 25 μl DTNB reagent and 400 μl methanol, 25 μl extracted protein of the algae was centrifuged at 3000 × g for 5 min. The absorbance of the supernatant was measured at 412 nm.

Diluted buffer(pH 8.2) containing Tris-HCl(30 mmol/L) and EDTA(3 mmol/L) was stored at 4℃.

1.6 Measurement of the permeability of the membrane

The permeability of the cell membrane was measured by ultraviolet absorption method(Liu, 1985). The conductivity was measured using a conductometer (DDS-Ⅱ A). The relative permeability of the cell membrane was evaluated by the ratio of relative conductivity to the total conductivity.

2 Results

2.1 Effect of various concentrations of Ca^{2+} on the HDTMAB mitigation rate of *Alexandrium* sp. LC3

The critical micelle concentration of hexadecyltrimethyleamiue bromide (HDTMAB) was 0.92 mmol/L. It

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was associated with Ca^{2+} to eliminate *Alexandrium* sp. LC3. After incubation of 50 ml of exp. growth-phase culture of this algae(10^5 cells/L) was inoculated into f/2 fresh medium with HDTMAB(final 0.5 cmc). And various Ca^{2+} level with 0, 2, 4, 6, 8 mmol/L were added in the medium, respectively. After six days incubation, cells were counted with a hemocytometer. As the culture without HDTMAB or Ca^{2+} was controlled, the survival rate of *Alexandrium* sp. LC3 was calculated. The extinguishment rate of the algae cells with only HDTMAB treated was regarded as 100%. And the extinguishing rates of algae with various concentrations of Ca^{2+} were evaluated (Fig. 1). The extinguishment rates of *Alexandrium* sp. LC3 by HDTMAB were lowered by Ca^{2+} obviously. The relative extinguishing rate of algae with 2 mmol/L Ca^{2+} was less than 70% of that without Ca^{2+}. The inhibiting effect by Ca^{2+} was most remarkable at the 4 mmol/L.

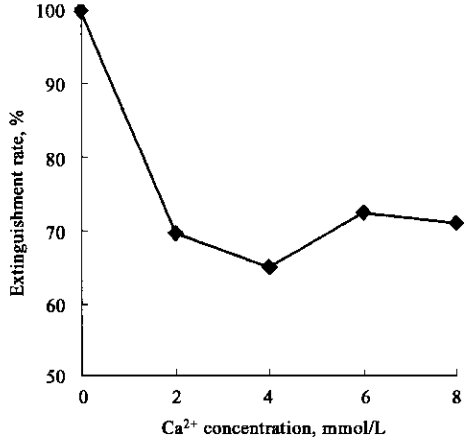

Fig. 1 Effect on the extinguishment rate of *Alexandrium* sp. LC3 by HDTMAB and different concentrations of Ca^{2+}

2.2 Effect of various concentrations of Ca^{2+} on the membrane peroxidatic level of *Alexandrium* sp. LC3 under HDTMAB stress

Malonaldehyde, which can oxidate SH group and cross-link lipids, nucleic acid, saccharide and protein, is one of the main products of the peroxidation of the cell membrane (Zeng, 1991). LC3. The cell membrane peroxidatic level can be evaluated by the content of MDA. Ca^{2+} was associated with HDTMAB (final 0.5 cmc) to extinguish *Alexandrium* sp. LC3. The final concentration of Ca^{2+} was 0, 2, 4, 6, 8 mmol/L, respectively. As the culture without HDTMAB or Ca^{2+} was as control, the content of MDA was measured after six days incubation (Fig. 2). The content of MDA of algae with HDTMAB was remarkably higher than that of controlled group, which showed that HDTMAB promoted the cell membrane peroxidatic level notably. The MDA content of each group under complex operation was always lower than that of algae culture with only HDTMAB operation. The algae culture with 4 mmol/L Ca^{2+} under complex operation reached the lowest MDA content which was still higher than that of controlled group. The cell membrane peroxidatic level could be alleviated by addition of Ca^{2+}.

2.3 Effect of various concentrations of Ca^{2+} on the SH group content in *Alexandrium* sp. LC3 under HDTMAB stress

After six days incubation, the SH group content in

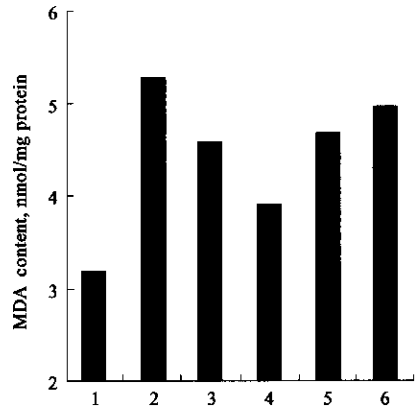

Fig.2 Effect on the MDA content in *Alexandrium* sp. LC3 by different concentration of Ca^{2+} under complex operation
1: Controlled group; 2: 0 mmol/L; 3: 2 mmol/L; 4: 4 mmol/L; 5: 6 mmol/L; 6: 8 mmol/L

Alexandrium sp. LC3 with various concentrations of Ca^{2+} under HDTMAB stress was measured (Fig. 3). Compared with that of controlled group, SH group content was lower obviously. The SH group content of each group under complex operation was always higher than that of algae culture with only HDTMAB operation. The algae culture with 4 mmol/L Ca^{2+} under complex operation reached the highest SH group content which was still lower than that of controlled group. It was important for SH group to maintain the normal conformation of the protein and the membrane, and usually oxidated by MDA. As MDA accumulated in the cell, SH group content decreased as being oxidated. Therefore, the content of SH group was negatively correlated with that of MDA.

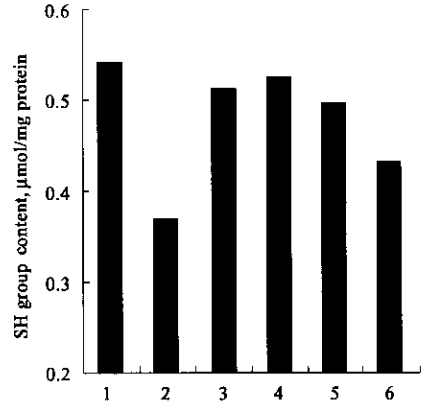

Fig.3 Effect on the SH group content in *Alexandrium* sp. LC3 by different concentrations of Ca^{2+} under complex operation
1: Controlled group; 2: 0 mmol/L; 3: 2 mmol/L; 4: 4 mmol/L; 5: 6 mmol/L; 6: 8 mmol/L

2.4 Effect of various concentrations of Ca^{2+} on the cell membrane permeability of *Alexandrium* sp. LC3 under HDTMAB stress

The permeability of the cell membrane could be used as a kind of physiological criterions to evaluate the response of the algae cell under stress. As the selective permeability of plasma membrane altered under stress, the salt or organic compound leaked from cell to peripheral space and led to the increase of peripheral space conductivity. Therefore, the degree of the damage of the plasma membrane was attained through determining the change of conductivity of the

peripheral space (Fig. 4). The results showed that the membrane permeability with HDTMAB was remarkably higher than that of controlled group. The decrease of the membrane permeability of these groups with Ca^{2+} indicated the cell damage alleviated. Under complex operation, the algae culture with 4 mmol/L Ca^{2+} reached the highest SH group content and the lowest MDA content and membrane permeability. The membrane permeability was positively correlated with the content of MDA while negatively correlated with that of SH group.

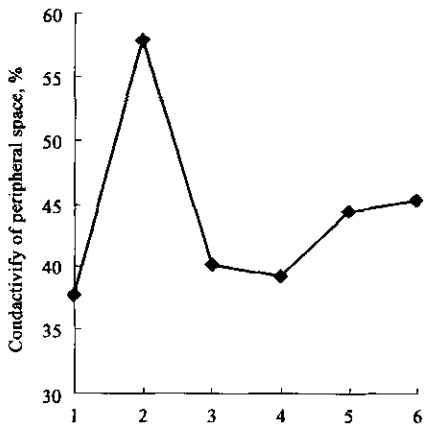

Fig.4 Effect on membrane permeability of *Alexandrium* sp. LC3 by different concentrations of Ca^{2+} under complex operation
1: Controlled group; 2: 0 mmol/L; 3: 2 mmol/L; 4: 4 mmol/L; 5: 6 mmol/L; 6: 8 mmol/L

3 Discussion

Cupric sulfate used to control the red tide algae was ever a widely accepted method worldwide. Although cupric sulfate can effectively eliminate the red tide algae, it will do harm to other organisms and marine ecological environment due to the extremely high cupric concentration while it is used directly.

Now, spread clay used to control the red tide algae is a widely accepted method worldwide (Yu, 1999; Anderson, 1997; Sengco, 2001). Over the past 25 years, clays have been investigated in several countries as a mean of removing harmful algae from the water column. Red tide is controlled through the flocculation of clay particle to the red tide organism. Although clay was considered as a promising and attractive direct control option, clay is not available for some locations with HAB problems, and high transportation cost would quickly render this method uneconomical(Sun, 2004). Furthermore, this is limited in the long run. It is highly desirable to find alternative options.

The surfactant has been applied widely in the medicine and food industry as a kind of bactericides. It was previously reported(Cao, 2003) that HDTMAB could be regarded as an algaecide to eliminate the red tide algae effectively. Furthermore, compared with other surfactants with phenyl cycle, alkanes and many side chains, HDTMAB could be decomposed more than 90% of the total in one week due to its long linear chain structure. It would not bring secondary pollution to the marine environment. In this study, compared with the controlled group, the MDA content of the algae cell operated with HDTMAB increased obviously, the SH group content decreased and the membrane permeability got enhanced. All of the above results showed that the algae cell membrane was the target site of the surfactant. By the conjugation of hydrophobic headecanoic group with membrane lipid and hydrophilic cation with membrane protein, HDTMAB conjugated with and inserted or penetrated into the membrane(Luo, 1998). The separation of membrane protein and membrane lipid that was caused by micelle concentration of HDTMAB made the membrane permeability enhanced and membrane function damaged. Meanwhile, demagnesium chlorophyll produced by algae cell under HDTMAB stress affected the photosynthesis of the cell. HDTMAB used in this study assaulted the algae cell membrane and affected the integrality and function of the membrane, then eliminated the algae cell.

Ca^{2+} is an important regulon in cell metabolism and plays an important role in the extracellular signal coupling with intracellular physicochemical reaction as a secondary messenger. Recent researches showed that Ca^{2+} promoted the resistance of plant cell to stress and alleviates the cell damage (Zhao, 1993; Li, 1996; Lu, 1999; Zhou, 1999; Zhang, 2001). It has not been reported about the Ca^{2+} function in the *Alexandrium* sp. stress resistance process till now. In this study, the algae cell extinguishments by HDTMAB were obviously inhibited by Ca^{2+} that was added into the medium. Compared to the HDTMAB only operation group, the SH group content of the Ca^{2+} and HDTMAB complex operation group increased, the MDA content and the membrane permeability decreased. Ca^{2+} prevented the cell from HDTMAB stress and the algae cell got the smallest damage while cultured in 4 mmol/L Ca^{2+} medium. While being treated with 4 mmol/L Ca^{2+}, the cell death rate was only 65% of that of cell treated with HDTMAB only, SH group content increased 42%, MDA content and membrane permeability decreased 26.3% and 32%, respectively. The Ca^{2+} promotion of HDTMAB stress resistance of *Alexandrium* sp. LC3 was due to the reduced membrane permeability by Ca^{2+} and stimulation of Ca^{2+} to the stability of cell membrane which was fulfilled by the conjugation of Ca^{2+} with phosphate, phospholipid and carbonyle group of protein on the membrane surface (Zhang, 2001; Li, 2003). Ca^{2+} stimulated the stability of the algae cell wall structure too. Calcium pectic acid could be used as conjugon among those pectin protein complex compounds in the colloid layer of cell wall(Zhang, 2001). Furthermore, Ca^{2+} prevented HDTMAB from eliminating the algae cell by competing with HDTMAB for membrane conjugation site (Luan, 1987).

4 Conclusions

HDTMAB used in this study assaulted the *Alexandrium* sp. LC3 cell membrane and affected the integrality and function of the membrane, then eliminated the algae cell effectively. However, the extinguishment could be inhibited by addition of Ca^{2+}. The Ca^{2+} promotion of HDTMAB stress resistance of *Alexandrium* sp. LC3 was due to the reduced cell membrane permeability by Ca^{2+} and competing with HDTMAB for membrane conjugation site.

In order to enhance the effective utilization of HDTMAB, EDTA or EGTA could be added into the action system to chelate with Ca^{2+} in the process of eliminating red tide algae cell by HDTMAB. The effect of addition of EDTA or EGTA needed to be studied in the future.

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