Biochemical changes during life cycles of marine phytoflagellates

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SUMMARY: Changes in some chemical components of Chattonella antiqua (Hada) Ono (Raphidophyceae), Alexandrium catenella (Whedon et Kofoid) Balech and Scrippsiella trochoidea (Stein) Loeblich ‡V (Dinophyceae) during their asexual growth under laboratory conditions were examined. The energy charge (EC) value of these phytoflagellates was also calculated. The changes in cellular contents of ATP, carbon, nitrogen, phosphorus, and amino acids showed generally same pattern. They were markedly increased during late lag phase and early exponential phase of growth. After mid exponential phase, these biochemical components decreased and became approximately constant from late exponential phase to the end of experiment. The EC value of A. catenella and S. trochoidea rapidly increased after the inoculation, thereafter, it decreased and became approximately constant during stationary phase. These occurrences indicated that these phytoflagellates could increase their metabolic activities after the inoculation into the new culture medium within a few days.

KEY WORDS: Alexandrium catenella, amino acid, Chattonella antiqua, energy charge, Scrippsiella trochoidea, phytoflagellate

INTRODUCTION

More than two decades, physiological and biochemical studies on the growth of phytoplankton have been conducted to understand the mechanism of the outbreaks of their red tides. The studies of the chemical compositions of phytoplankton also have been motivated by the importance of such information in general physiology and ecology.1, 2) However, little is known concerning the changes in chemical compositions that seem to be associated with the outbreaks of red tides. Some of the published data are concerned with the life stages and focused on the biochemical changes during cyst formation.3,4,5) The authors 6) reported that the content of adenosine triphosphate (ATP) and amino acids in phytoflagellate changed remarkably through the life cycle. Such biochemical changes occurring inside the cell are needed to prepare the cell for division during the exponential phase. The biochemical changes may proceed in the phytoflagellates on the beginning of the outbreaks of red tides.

In the present study, to compare cell activities in various growth stages and organisms, the changes in the cellular ATP, carbon, nitrogen, phosphorus, and amino acids of marine phytoflagellates were monitored in batch cultures. The metabolic activities of these phytoflagellates were also examined by using the energy charge (EC) value. The results from this study should be the helpful information to understand a mechanism of the outbreak of red tides.

MATERIALS AND METHODS

Culture conditions and experimental design

The axenic culture strains of Chattonella antiqua (Hada) Ono and Alexandrium catenella (Whedon et Kofoid) Balech and the clonal axenic culture strain of Scrippsiella trochoidea were precultured in 300-ml Erlenmeyer flasks containing 100 ml of autoclaved (120 ºC, 20 min) ESM-enriched seawater from the Seto Inland Sea (salinity 31±1 psu) without soil extract.7) Illumination was provided by cool-white fluorescent lamps at an irradiance level of ca. 80 μE m⁻² s⁻¹ on a 14:10 hr LD cycle. Temperature was kept at 21±1 ºC. The precultured species in the stationary phase were inoculated into a 10-l glass carboys containing 9 liters of ESM-enriched seawater under conditions described above. Sub-sampling were taken 6 hours after switching on the fluorescent lamps. Each organism sample was taken at appropriate culture intervals for about one month. Cell density, cellular ATP, cellular organic carbon and nitrogen, cellular phosphorus, cellular amino acids of all organisms were estimated except cellular amino acid of C. antiqua.
The ATP content in cyst of *A. catenella* and *S. trochoidea* was also examined. Cysts were obtained in ESM-encystment medium under culture conditions as described by Lirdwitayaprasit et al. All samples were first filtered through glass fiber filters (Whatman GF/C) free of organic matter by ignition at 450 °C, 2 hours) and stored at -20 °C until analysis, except samples used for cellular ATP measurement which were extracted with Tris-HCl buffer (0.025 M, pH 7.7) at 100 °C for 20 min.

**ATP and related compound analyses**

Concentration of ATP in the extracts was determined by the bioluminescent reaction utilizing firefly luciferin-luciferase on a TD 4000 lumiphotometer (Labo Science). The ATP related compounds in the extracts was determined by HPLC.

The adenylate energy charge (EC) was calculated after Atkinson (1968) as follows:

\[ EC = \frac{ATP + 1/2ADP}{ATP + ADP + AMP} \]

**Cellular carbon, nitrogen, and phosphorus analyses**

The filtered samples were freeze dried. Carbon and nitrogen were measured with a Yanaco MT-3 CHN analyzer. Total cellular phosphorus was determined using the method of Anderson and Lindquist.

**Amino acid analysis**

The samples were hydrolyzed with 6 N HCl at 105 °C for 22 hours. The hydrolysate was evaporated until dry under vacuum, redissolved in 0.01 N HCl and then analyzed using the ninhydrin method with a Hitachi L-8500 high speed amino acid analyzer.

**RESULTS**

**Changes in cellular content of ATP**

Growth curves and the changes in cellular content of ATP of *C. antiqua*, *A. catenella* and *S. trochoidea* during the growth processes are shown in Fig. 1. Growth phases were defined based on the sigmoid curve. *C. antiqua*, *A. catenella* and *S. trochoidea* grew at a rate of 0.23, 0.26 and 0.18 divisions/day, respectively. During the early exponential phase, they can grew at a rate of 0.34, 0.93 and 0.22, respectively. Changes in cellular content of ATP in all phytoflagellates were generally same pattern. The highest ATP content of these phytoflagellates was observed during the early exponential and mid exponential phases of growth. After the inoculation for 4 days, the ATP pool of *C. antiqua* and *A. catenella* cells increased remarkably to the value of about 2.5 times higher than the initial levels. The cellular content of ATP in *S. trochoidea* also increased remarkably to the value of about 2 times higher than the initial level within 3 days after the inoculation.

![Fig. 1](image-url)
throughout the growth cycles (Fig. 2). Their contents reached a maximum level only two or three days after the inoculation. The amount of nitrogen per cell in *C. antiqua* and *A. catenella* increased about 40% and 53% of their initial level after 3 days since the start of incubation. In *S. trochoidea*, the amount of nitrogen per cell increased from 217 pg/cell to 219 pg/cell after 1 day since the start of incubation, and then decreased rapidly. An increase was again observed when cell reached the mid exponential phase of growth.

Changes in EC value

The energy charge value of *C. antiqua* calculated from the HPLC analytical data during exponential phase was remarkably higher than those during stationary phase (Fig. 4A). In *A. catenella* and *S. trochoidea*, the EC values increased rapidly after the inoculation (Fig. 4 B, C). The EC values in the cysts of *A. catenella* and *S. trochoidea* are lower than those of their vegetative cells inoculation (Fig. 4 B, C).

DISCUSSION

All of the results in this study demonstrated that the biochemical components of phytoflagellates changed remarkably through their growth cycle. The changes in cellular ATP in each phytoflagellates followed generally the same pattern as changes in those of cellular carbon, cellular nitrogen and phosphorus. The maximum level of these biochemical components were observed during the early exponential phase of growth. These results demonstrate that during this period, the cells can increase production of ATP and other biochemical compounds by increasing the accumulation rate of nitrogen and phosphorus from acid per cell increased rapidly after 1 day since the start of incubation, and then decreased gradually (Fig. 3). An increase was again observed when cell reached the mid exponential phase of growth.

Changes in cellular content of total amino acid

Total amino acid of *A. catenella* gradually increased during the lag and the early exponential phases of growth (Fig. 3A). The change in cellular content of total amino acid followed generally the same pattern as changes in those of cellular ATP and cellular carbon. In *S. trochoidea*, the amount of total amino
the new culture medium. It was observed that cell numbers increased rapidly after the cellular contents of ATP, phosphorus and nitrogen markedly increased and reached a maximum level. These results confirm that cells can rapidly increase their photosynthetic rate and synthetic the biochemical compounds necessary for cell division in the first 3 or 4 days.

Overall, this study provided more detailed information, clarifying the cause of the outbreaks of red tide. In nature, when the surrounding environment is in optimal conditions for growth and nutrient concentrations in seawater are high enough for cell growth, phytoflagellates can rapidly replicate and occur as the red tides.

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REFERENCES