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THE GLUTATHIONE DIURNAL CYCLING IN *Dunaliella salina*

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ABSTRACT

Glutathione is intracellular low molecular weight thiols as detoxification agent in most marine microalgae. The short continuous culture was used to describe diurnal cycle of intracellular glutathione during light: dark period. The result showed that glutathione tend to follow light period and minimum levels at the end of dark period. Cysteine exhibited twofold diurnal variations, maximum levels at the early dark period. Those may prove that glutathione may have an important role as an antioxidant in marine phytoplankton.

Keyword : glutathione, diurnal cycling, *Dunaliella salina*

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INTRODUCTION

Glutathione was found as a wide range of low molecular weight in marine micro algae and event bacteria (Dupont *et al*., 2004). The glutathione concentration may found in from millimolar to > 90% of non protein thiols (Ahner *et al*., 2002). One major physiological function of glutathione is reactive oxygen species (ROS) detoxification in chloroplast. Glutathione acts as a removal agent of super-oxide radicals which can be generated during photosynthesis light saturation (Dupont *et al*., 2004). Therefore, intracellular concentration of glutathione may vary with light intensity and duration in marine phytoplankton.

Matrai and Vetter (1988) reported decreases glutathione concentration in natural assemblages of coastal phytoplankton in 24 h of dark incubation. More researchers showed the potential role of thiols as ligands in seawater by measuring glutathione and other thiols in field sampling (Tang *et al*., 2000; Al-Farawati and van den Berg, 2001). Marine phytoplankton is the one important source of thiols in surface seawater. Thus, it is important to understand possible controls on intracellular concentrations of those in marine phytoplankton.

The light is the one consider physical factor may effect production on the intracellular concentrations of the low molecular weight thiols (glutathione and cysteine) in marine phytoplankton. Thus, it is important to understand its possible controls on intracellular concentrations of those in marine phytoplankton.

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MATERIALS AND METHODS

The algae used were unicellular marine green algae *D. salina*. It was cultured in Aquaculture Laboratory University of Lampung pure isolate. The research was conducted on January-April 2008.

Samples for Chl-a and thiols analysis content were collected from a container receiving the outflow from the culture vessel. Samples were taken every 4 h for 60 h. light levels were reduced to 125 µmol photons m^{-2} s^{-1} with neutral density screening since cells did not grow well in full light. On the third day of sampling following 8 h of light, the culture vessel was darkened with black plastic. The light levels inside the un-shaded container were 380 µmol photons $m^{-2}.s^{-1}$.

At each sampling, three 50 ml and three 5 ml aliquots of culture were gently filtered onto a Whatman GF/F filters for particulate thiols and Chl-a measurements. Filters were immediately stored in liquid nitrogen until extraction and analysis. Chl-a was measured using fluorescence following GF/F filter extraction in the dark for 5-6 h room temperature in a solution of 45%: 45%: 10% dimetyl sulfoxide: acetone: water with 0,1% diethylamine (Dupont *et al*., 2004). For particulate thiols analysis, sample filters were heated to 70 ^{0}C in 10 mmol l^{-1} methanosulfonic acid (MSA) for 2 min and homogenized on ice (Dupont *et al*., 2004). The MSA extract was retained for derivatization with the fluorescence tag monobromobimane (Ahner *et al*., 2002).

Derivatized homogenate was taken then analyzed on a Beckman HPLC equipped with reversed-phased C-16 amide column and a 100 µl injection loop. Compounds were quantified post column using fluorescence detection (excitation 310-410 nm; emission 474-650 nm). The elution gradient and buffers used are described elsewhere (Method C; Dupont *et al.*, 2004). Stock solutions of cysteine and glutathione were used to develop standard curves for peak area calibration and to verify elution times. For both, the detection limit is about 200 femtomol per injection.

Field samples were collected from Lampung Bay at 1.2 m depth using 3 m pole sampler and an acid washed polycarbonate container. Duplicated water samples were immediately filtered onto Whatman GF/F filters and stored in liquid nitrogen until extraction of Chl-a and particulate thiols.

RESULTS AND DISCUSSION

The combined method was used to extend the time period during the exponential growth and to provide additional volume of culture to take the relative large samples needed for thiols analysis. The dilution rate used during both experiments was lower than the growth rate.

A gradual increase of cell density and extracted Chl a were observed over 32 h sampling period (**Fig. 1A**). The specific growth rate, µ, was 0,75 d^{-1} in the first stage batch culture. The dilution growth rate was 0,98 d^{-1} until 48 h. The Chl a cell level remain stagnant over the experiment. Minor variation showed but did not follow a diurnal pattern.

Light mediated changes in intracellular concentrations of glutathione and cysteine were clearly evident in bath culture whether the data normalized to Chl a or to cell number (**Fig. 1B, 1C**). Over 2 d culture, intracellular levels variated by a factor of approximately 2 with maximum of 500 µmol g^{-1} Chl a and a minimum of 250 µmol g^{-1} Chl a (**Fig. 1B**). The maximum stage did not occur at the same time both day, and the concentrations were generally higher during the light period. A sharp decline in glutathione followed light cessation consistently, and maximum levels always occurred at the end of the dark period. Particulate glutathione levels fell from approximately 415 µmol g^{-1} Chl a to 310 µmol g^{-1} Chl a when the culture was darkened after 6 h of light on the third day.

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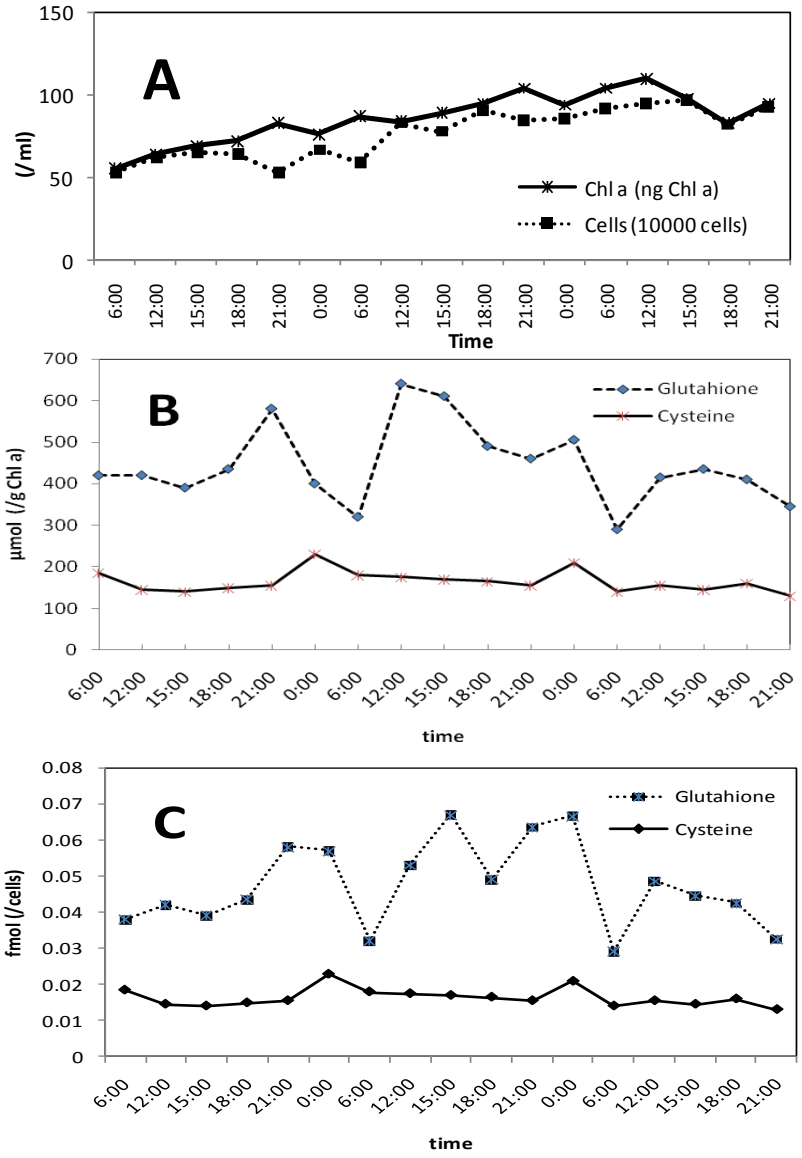

Fig1. Intracellular glutathione and cysteine concentrations with have 16:8 h light-dark cycle. Data are normalized to (A) Chl a, (B) cell number, and (C) Chl a concentration and cell number ml^{-1}.

Cysteine concentration exhibits in twofold diurnal variation. The maximum concentrations occurred early in the dark period with a gradual decline until the onset of the next dark period (**Fig. 1B, 1C**). An increase was not observed following the truncated light period at the end of the experiment.

The similar trend results were obtained from the field samples. Over the 24 h course, particulate glutathione in Lampung Bay describe a clear diurnal cycle similar to that

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observed in the culture experiments (**Fig. 2B**). Glutathione levels (normalized to Chl a) were within the range of those in Laboratory cultures. Glutathione concentrations gradually increase during the daylight hours and decrease during the night. The cysteine concentration in the darkened bottle was indistinguishable from that in the ambient water. The observed diurnal cycle was not driven by Chl a normalization, since the Chl a was generally higher during the daylight hours and lower at night (**Fig. 2A**). Normalization to Chl a actually serves to dampen the observed variation in total particulate glutathione concentration.

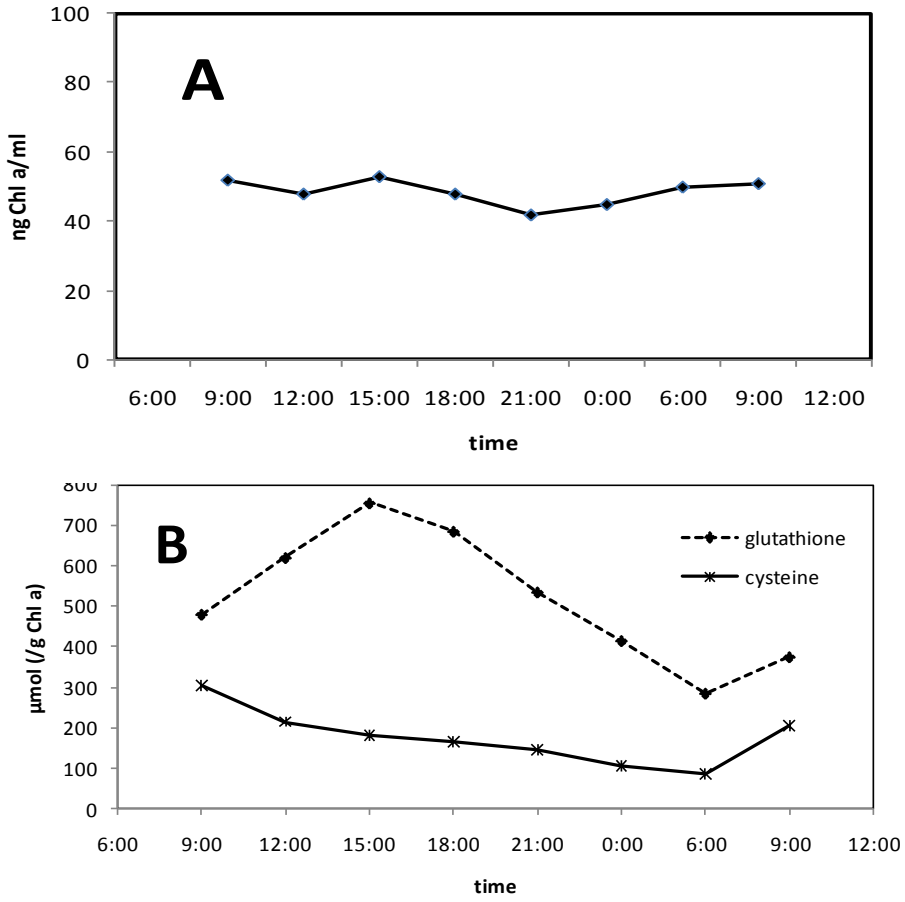

Fig. 2 (A) Chl a concentration. (B) Intracellular glutathione and cycteine concentrations (µmol g^{-1} Chl a) measured from Lampung Bay.

In field samples, cysteine did not appear to cycle with light, but particulate concentrations in Lampung Bay were very high (60-110 µmol g^{-1} Chl a). Fahey, *et al.* (1987) found that some cyanobacteria produce similar or even greater concentration of cysteine compared with glutathione. Wakeham, *et al.* (1987) found significant number of bacteria which has similar ratios of glutahione to cysteine compared to marine eukaryotes.

Other low molecular weight thiols are also important biochemically and are precursors in glutathione metabolism (Dupont

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et al., 2004). Cysteine is a substrate for γ-glutamyl-cycteine synthetase, which froms γ-glutamyl-cysteine (γ-Glu-Cys). γ-Glu-Cys is then adjoined to glycine by glutathione synthetase to produce glutathione. Cysteine-Glycine (Cys-Gly) and Cys are also generated during glutathione degradation by transpeptidase and peptidase (Meister and Anderson, 1983). By this experiment, γ-Glu-Cys concentration, the newly identified thiols Arg-Cys, and Gln-Cys varied by as much as twofold but did not follow diurnal trend consistently. Particulate Cys-Gly and phytochelatin were measured in the field samples and did not follow a diurnal cycle. Cystein did cycle in *D. salina*, although the maximum concentration level often occurred at night. The peak cysteine concentration that occurs early in the dark period may be the result of sulfur assimilation in preparation for the synthesis of sulfuring containing proteins.

The variation of glutathione observed in laboratory and field experiments are potentially driven by the need to scavenge H_2O_2 generated during photosynthesis saturation at high light levels. The ratio of oxidized to reduced glutathione might also be expected to vary as a function of light as well as glutathione reductase activity responsible for the cycling between these two pools. Another possibility to explain that was the cultures become synchronized with respect to cell cycle (Nelson and Brand, 1979). Glutathione concentration in phytoplankton might be linked to this cycle.

The results indicate that glutathione play multiple defenses against oxidative stress. The reduced sulfur compounds dimethyl sulfoxide (DMSO) and dimethyl sulfide (DMS) have recently been suggested to act as antioxidants in algae (Sunda *et al.*, 2002), in the case of light induced stress. The rate of which thiols may be released from the particulate to the dissolved phase through nonspecific means is certainly influenced by intracellular concentrations. It is also possible that intracellular pool influenced the biologically controlled exudation of specific thiols.

CONCLUSION

Glutathione and Cysteine have different trend according to the response of light intensity. Glutathione has follow the diurnal cycle of light. In the other hand, cysteine did not appear to cycle with light intensity. Intracellular concentration of glutathione fluctuation showed may be related to cell defense mechanism against oxidative stress, respectively.

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