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Response of phytoplankton to nitrogen addition in the Taiwan strait upwelling region: Nitrate reductase and glutamine synthetase activities

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ABSTRACT

This study investigated the activities of two nitrogen assimilation enzymes, nitrate reductase (NR) and glutamine synthetase (GS) in phytoplankton in relation to sample, as well as the nutrient levels and phytoplankton biomass (Chl-*a* concentrations) during an upwelling event in the southern Taiwan Strait during an upwelling period from 6 to 12 July, 2005. The results showed that high NR activity (NRA) was always found with low nitrate and high Chl-*a* concentrations, while GS activity (GSA) exhibited positive correlations with ammonium and Chl-*a* concentration. Both NRA and GSA varied with the time and stage of upwelling: high NRA and GSA were observed initially at the subsurface layers in the early stage of upwelling, accompanied by the consumption of nutrients and the increase of Chl-*a* concentration; and then at the surface with high Chl-*a* concentrations in the middle and late stages of upwelling. Results from *in situ* enzyme bioassays on water samples along the tracing of upwelling track and on board mesocosm experiments on board the ship showed that there was a time-lag between nitrate addition and NRA and GSA, but. However, no time-lag was found between ammonium addition and GSA. The present results indicated that both NRA and GSA reflect the status of ambient nitrogen levels and the assimilation process of the phytoplankton, and could be used as effective parameters for the analysis of the physiological response of the phytoplankton to nitrogen variations during upwelling periods. Measurements of NRA and GSA in the phytoplankton in newly upwelled water appeared to provide ecophysiological indicators of phytoplankton, which will make it possible to trace during the sequence of upwelling events (such as nutrient supplementation) leading to enhanced productivity.

1. Introduction

A coastal upwelling system is described as a conveyer belt of nutrients and carbon (Wilkerson and Dugdale, 1987), which exhibits rapid nutrient turnover rates and high biological production (Botas et al., 1990; Fernández and Bode, 1991; Fernández et al., 1991). Early studies in upwelling regions have shown that a series of increased physiological rates occur along the conveyor (MacIsaac et al., 1985; Wilkerson and Dugdale, 1987; Dugdale and Wilkerson, 1990; Bode et al., 1997). Phytoplankton cells transported to the surface undergo an upward energy shift in the high irradiance and high nutrient surface environment, and rates of nutrient-uptake increase along the upwelling plume associated with an increase in phytoplankton biomass. An optimal environmental window for large cell phytoplankton (e. g. diatoms) has been reported in a previous study (Legendre and Le Fever, 1989). During this window, upwelled phytoplankton must be able to "shift-up" their rate of physiological processes in response to maximize nutrient uptake and growth processes (Hutchings et al., 1995; Kudela and Dugdale, 2000). A hypothesis that environmental shifts resulting from improved conditions induce a suite of molecular and physiological responses that lead to a higher nutrient uptake and growth rate of the phytoplankton has also been postulated (Schaefer et al., 1999). When N deficient cells are exposed to the improved growth conditions, the rate of induction of nitrate metabolism, in particular, may control the dynamics of the phytoplankton bloom (Smith et al., 1992).

Nitrate and ammonium are the two major inorganic nitrogen species that support new production (based on nitrate) and regenerated production (based on ammonium) in coastal upwelling systems (Dugdale and Goering, 1967). It is known that the utilization of nitrogen in phytoplankton cells involves complex processes including membrane transport, assimilation and incorporation of external dissolved nitrogen into biochemical compounds inside the cell via several enzymatic systems (Syreet, 1981; Falkowski, 1983; Dortch and Postel, 1989). Among these enzymes, nitrate reductase (NR; EC 1.6.6.1) and glutamine synthetase (GS; EC 6.3.1.2) are the two important enzymes involved in nitrogen assimilation.

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The former, catalyzing the reduction of nitrate to nitrite, is the first enzyme involved in nitrogen assimilation and controls the process of nitrate metabolism in cells (Touchette and Burkholder, 2001), while the latter, catalyzing the formation of glutamine from ammonium and glutamate in the presence of ATP, plays an important role in regulating nitrogen metabolism, not only as the center for both nitrate and ammonium assimilation, but also as a key enzyme linking carbon to nitrogen metabolism by incorporating inorganic nitrogen into organic nitrogen via GS/glutamate synthase (GOGAT). NR activity (NRA) and GS activity (GSA) have been used as qualitative indicators and/or as quantitative measures of nitrate/ammonium assimilation in phytoplankton (Eppley et al., 1969; Collos and Slawyk, 1977; Berges and Harrison, 1995; El Alaoui et al., 2001), and show high environmental regulation in phytoplankton cells (Smith et al., 1992; Maurin and Gal, 1997). It is reported that nitrate uptake rate and NRA are enhanced under simulated upwelling conditions, and that modulation of NR gene expression and enzyme activity by environment factors affect the time scales of nitrate utilization and bloom formation in the sea (Smith et al., 1992).

The Taiwan Strait, a shallow shelf-channel linking the South China Sea (SCS) with the East China Sea (ECS), is characterized by highly dynamic seasonal and year-round upwelling events (Chen et al., 1982; Xiao, 1988; Hong et al., 1991; Tang et al., 2002; Shang et al., 2005). In the southern Taiwan Strait, phytoplankton blooms and the highest Chl-*a* concentration are generally observed in summer due to coastal upwelling induced by the southwest monsoon (Chen et al., 1992; Zhang et al., 1997; Tang et al., 2002; Shang et al., 2004). Many studies have shown that primary production, phytoplankton biomass, composition and size-fraction structure in the upwelling area vary significantly and rapidly in response to environmental shifts (Hong et al., 1991; Li and Wang., 1991; Yang et al., 1991; Wang et al., 2002), but the response mechanism of phytoplankton to the upwelling events, such as upwelled nutrients, is poorly understood. Nitrogen is regarded as one of the limiting factors to phytoplankton growth in the southern Taiwan Strait during the non-upwelling period (Wang et al., 1997; Zhang et al., 1997). In light of this, nitrogen supplementation and utilization dynamics in phytoplankton cells might control the successional process and phytoplankton bloom formation during the upwelling period.

In the present study, both the activities of two nitrogen assimilation enzymes, NR and GS transferase in phytoplankton samples, and the nutrient levels and phytoplankton biomass (Chl-*a* concentration) were investigated in the southern Taiwan Strait during an upwelling period from 6 to 12 July, 2005. The purpose of this study was to examine the nitrogen assimilation process of the phytoplankton *in situ* so as to obtain a better understanding of the cellular regulatory response to elevated nutrients, and hence provide insight both into the response of phytoplankton to ambient nutrient variation and the bloom formation mechanism.

2. Materials and methods

First, the temperature and salinity were investigated around the southern Taiwan Strait and a low temperature and high salinity center was found around a nearshore station, Stn. B0. Then Stn. B0 was selected for tracing the upwelling event during 6 to 12 July, 2005. Samples from the 5 depths (0, 5, 10, 15 and 25 m) at Stn. B0 were collected using a CTD-rosette system (SeaBird), equipped with 12 Go-Flo bottles (8 l each). NRA and GSA of the water samples as well as concentrations of nitrate (NO_3^-), nitrite (NO_2^-), ammonium (NH_4^+) and Chl-*a* were measured. To avoid the influence of irradiance, all samples were collected at the same time (around 10:00 am) each day at Stn. B0. The surface water samples were also collected at 16 stations in the southern Taiwan Strait (Fig. 1) and the same parameters were measured as Stn. B0 for mapping distribution.

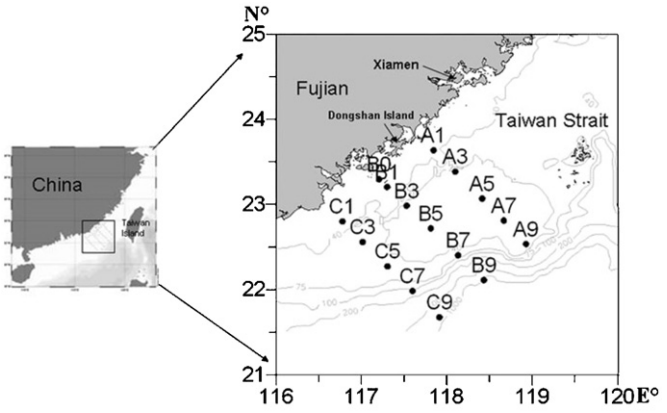

Fig. 1. Location of the study area in the Taiwan Strait.

Besides, a mesocosm culture experiment simulating the upwelling conditions at Stn. B0 was conducted on board in three column bags, the total volume of each bag (with a diameter of 1.0 m and depth of 1.5 m) was 700 l. The seawater was pre-filtrated using a 200 μm filter to remove the large grazers before the experiments were started. Seawater conditions at Stn. B0 were as follows: temperature 22.1 °C, salinity 34.0, Chl-*a* concentration 1.27 μg l^{-1}, and the concentrations of nitrate, phosphate and silicon were 0.70, 0.03 and 6.0 μmol l^{-1}, respectively. Nitrate, phosphate and silicon were added to the experimental bags on 6 July. Three mesocosm cultures were designed: M.1 was the control with no nutrient addition; M. 2 was the low nutrient treatment with the addition of 12 μmol l^{-1} SiO_3–Si (Na_2SiO_3), 0.5 μmol l^{-1} PO_4–P ($NaH_2PO_4 \cdot H_2O$) and 12 μmol l^{-1} NO_3–N ($NaNO_3$); and M. 3 was the high nutrient treatment with the addition of 48 μmol l^{-1} SiO_3–Si, 2 μmol l^{-1} PO_4–P and 48 μmol l^{-1} NO_3–N. The N/P ratio was designed according to the N/P ratio of the surface water of Stn. B0 on 6 July. Study on the seawater used for mesocosm showed that nitrogen and phosphate were both limiting factors for phytoplankton growth and silicon was not a limiting factor (Wang et al., 2008). Samples were collected at 8:00 am each day from 6 to 12 July, and NRA, GSA, NO_3^- and Chl-*a* data were analyzed. Before sampling, the seawater was mixed evenly using an oar and surface seawater was collected from each experimental bag. The mesocosm culture was under nature solar radiation and temperature was about 21–26 °C.

Concentrations of nitrate, nitrite and ammonium were measured immediately according to the procedures of Parsons et al. (1984) after being filtered using cellulose acetate membranes (Whatman, 0.45 μm). Chl-*a* concentration was determined using a fluorescence spectrophotometric method after filtration on GF/F membranes (Whatman) (Yentsch and Menzel, 1963). The fluorimeter was calibrated against a standard made from pure Chl-*a* (Sigma Chemical. Co.). The phytoplankton samples were filtered using cellulose acetate membranes (Whatman, 0.45 μm) for NRA and GSA detection.

NRA was determined according to the methods of Berges and Harrison (1995) and Joseph et al. (1997) with a small modification. Duplicate samples were collected on the cellulose acetate membranes (Whatman, 0.45 μm) and broken up in 2.0 ml extraction buffer with 200 mmol l^{-1} phosphate buffer, pH 7.9, 0.03% (wt/vol) dithiothreitol (DTT), 0.3% (wt/vol) polyvinyl pyrrolidone (PVP), 0.1% Triton X-100 (vol/vol), 5 mmol l^{-1} ethylenediaminetetraacetic acid (EDTA), and 3% (wt/vol) BSA using a sonicator. Homogenates