Protist, Vol. 169, 603–614, xx 2018
http://www.elsevier.de/protis
Published online date 5 June 2018

Protist

ORIGINAL PAPER

The Uncoupled Assimilation of Carbon and Nitrogen from Urea and Glycine by the Bloom-forming Dinoflagellate *Prorocentrum minimum*

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Submitted January 30, 2018; Accepted May 29, 2018
Monitoring Editor: Ulrich Sommer

The spread of harmful dinoflagellate blooms has been linked to the increasing availability of nitrogen, including its dissolved organic forms. The relationships between organic and inorganic nutrient uptake by dinoflagellates are not completely understood; moreover, it is not clear whether organic substances are used exclusively as nitrogen or also as carbon sources. We used laboratory culture experiments to investigate the concurrent uptake of glycine and nitrate by *Prorocentrum minimum* and estimate a role of two widespread organic substrates, glycine and urea, as carbon sources. Glycine uptake exceeded the uptake of nitrate when both nutrients were present in equal nitrogen amounts. Carbon of urea and glycine constituted only 0.4% and 1.3% of the total carbon uptake by cells, respectively, and this amount of carbon was disproportionately small compared to nitrogen taken up from the same organic substrates indicating uncoupling of organic carbon and nitrogen assimilation. We suggest that the observed uncoupling of organic nitrogen and carbon assimilation is a result of urea and glycine metabolic processing by urease and the glycine decarboxylation complex. We argue that such uncoupling reduces the net dissolved inorganic carbon (DIC) removal by dinoflagellates since the acquisition of nitrogen from urea and glycine leads to DIC release.

Key words: *Prorocentrum minimum*; dinoflagellates; carbon uptake; nitrogen uptake; glycine; urea.

Introduction

Dissolved organic nitrogen (DON) represents a nitrogen source for many marine phototrophic protists, including such an ecologically important group as dinoflagellates (Antia et al. 1991; Bronk et al. 2007; Glibert 2017). Urea and dissolved free amino acids (DFAA) are the compounds most studied as potential substrates. Concentrations of these substances in seawater are typically higher than those of the other characterized DON species, especially in the case of urea representing the main nitrogenous fertilizer of our time. Agricultural use of urea can result in high loads of this compound in coastal zones of the ocean (Glibert et al. 2005, 2006; Switzer 2008). Both urea and DFAA are readily uti-

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https://doi.org/10.1016/j.protis.2018.05.006

lized by different dinoflagellates, often in preference to inorganic nitrate, and cover a noticeable fraction of their nitrogen demand in natural ecosystems (Anderson et al. 2002; Fan et al. 2003b; Jauzein et al. 2017; Li et al. 2011). Moreover, recently urea was shown to have a suppressive effect on the concurrent nitrate uptake by *Prorocentrum minimum* (Matantseva et al. 2016), which further increases the role of this compound in dinoflagellate N nutrition. At the same time, the effect of amino acids availability on the uptake of nitrate has not been reported.

It is less clear, whether dinoflagellates assimilate carbon from the same organic substrates they use as a nitrogen source. Only a few studies on the role of urea and DFAAs as carbon sources for phototrophic microeukaryotes have been published. On the one hand, the measured urea-C uptake was insignificant compared to the uptake of bicarbonate by the dinoflagellate *P. minimum* and the pelagophyte *Aureococcus anophagefferens* during their blooms (Fan and Glibert 2005; Mulholland et al. 2004). On the other hand, Andersson et al. (2006) showed that mixed natural microbial communities utilized both carbon and nitrogen of urea and DFAAs during the year, but in some months nitrogen was taken up preferentially to carbon. These studies revealed that uptake of organic carbon and nitrogen was uncoupled, i.e. nitrogen was assimilated by cells to a greater extent, but the magnitude of such uncoupling varied substantially. The observed effect was more carefully studied in the case of DFAAs. Carbon and nitrogen uptake uncoupling was explained by extracellular amino acid oxidation and putative respiratory losses (Mulholland et al. 1998, 2002, 2003).

Nitrogen and carbon uncoupling during utilization of organic substrates by microorganisms is likely to affect biogeochemical cycling of these elements significantly; moreover, such uncoupling can be a reason for underestimation of uptake rates when organic carbon is used in measurements as a tracer. Therefore, it is crucial to explore this phenomenon in ecologically relevant species. The dinoflagellate *P. minimum* is well known for its wide distribution, pronounced invasive capacity (Hajdu et al. 2005; Pertola et al. 2005; Telesh et al. 2016), versatile nutritional physiology and harmful environmental effect caused by its blooms (Brownlee et al. 2005; Glibert et al. 2008, 2012; Heil et al. 2005). *P. minimum* can assimilate various organic nitrogen sources, but the relationship between uptake of organic carbon and nitrogen was not sufficiently studied in this species.

We investigated the concurrent uptake of glycine-N and inorganic nitrogen (nitrate), as well as uptake of nitrogen and carbon of urea and glycine by *P. minimum*, aiming at an estimation of the ecological impact of these processes. Glycine is not only one of the most abundant DFAAs (Coffin 1989; Dittmar et al. 2001; Hubberten et al. 1994; Keil and Kirchman 1991; Mopper and Lindroth 1982; Zhang et al. 2015), but is also special because it cannot be efficiently utilized by extracellular amino acid oxidation (Palenik and Morel 1990a,b). Thus, urea and glycine are the compounds entering cells as whole molecules, and the fate of their carbon and nitrogen depends on their intracellular metabolism. The relevant genomic information is still scarce in the case of dinoflagellates, and the proteins responsible for most of the metabolic conversions performed in their cells have not been identified. The analysis of sequenced transcriptomes is a valuable approach to cope with this problem. It has already allowed identifying the sequences of putative urease, urea and nitrate transporters in *P. minimum* (Matantseva et al. 2016), as well as in other dinoflagellate species (Bellefeuille and Morse 2016). Therefore, following the determination of organic nitrogen and carbon uptake, we searched *P. minimum* transcriptomes for putative proteins involved in the uptake and metabolism of glycine to provide a more complete picture of carbon and nitrogen supply from organic substrates to these organisms.

Results

The Effect of Glycine Addition on Nitrate Uptake

Dinoflagellates growing on nitrate under luxury N conditions actively consumed glycine shortly after its addition to the culture medium. The rate of glycine-N uptake exceeded the rate of the concurrent nitrate uptake ($p = 0.046$) and was approximately two times higher than the latter. We revealed no statistically significant change in the nitrate uptake rate following the addition of glycine to the culture medium ($p = 0.272$) (Fig. 1A). After the input of glycine, glycine-N uptake constituted about 70% and nitrate-N uptake – about 30% of the total nitrogen uptake (Fig. 1A, B), although nitrate and glycine were provided at equal molar N concentrations.

Overall, the input of glycine led to an increase in the total nitrogen uptake by dinoflagellates ($p = 0.008$), but did not affect the total carbon uptake estimated as a sum of bicarbonate and glycine car-