NITROGEN METABOLISM IN PHYTOPLANKTON

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Summary

Phytoplankton use a large variety of nitrogen compounds and are extremely well adapted to fluctuating environmental conditions by a high capacity to change their chemical composition.Degradation and turnover of nitrogen within phytoplankton is essential for many processes including normal cell maintenance, acclimations to changes in light, salinity, and nutrients, and cell defence against pathogens. The

pathways by which N degradation is accomplished are very poorly understood, but based on work in higher plant species, protein degradation is likely to be of central importance.

1. Introduction

After carbon, nitrogen is the second most important nutrient in phytoplankton. Relative to higher plants, nitrogen is also more important in unicellular algae because they lack structural material (essentially carbon). For example, the C/N composition ratio of higher plants ranges from 18 to 120 (by atoms) while that of phytoplankton ranges from 5 to 20. In the marine environment and in contrast to freshwater, nitrogen is also generally considered to be the element limiting phytoplankton growth. The very high surface/volume ratio of these organisms also makes them prone to large exchanges of material with the external medium.

Phytoplankton is responsible for around 70% of global nitrogen assimilation on earth (Table 1) and is therefore of major importance in transforming incoming solar energy into biomass.

Primary N assimilation	**Global yearly N assimilation (Tmol/year)**		
	Total	**Oceans**	**Land and freshwater**
Total net	584	417	167
As N_2	<10.9	<1.1	<9.8
As NO_3 or NO_2	<160.9 to <286	82.3 to 208	<78.6
As NH_3 / NH_4(and other reduced sources)	<266.6-<412.2	208-333.6	>78.6

Source: Raven et al. (1993)

Table 1. Estimates of global primary assimilation of N by photolithotrophs and the N species involved

The information given below is based on laboratory studies of phytoplankton under controlled culture conditions, with the caveat that only about 10% of the species living in nature are able to grow under laboratory conditions.

2. Availability and use of different forms of nitrogen

A wide variety of N compounds of different oxidation states are available and used by phytoplankton: nitrate, nitrite, ammonium, molecular nitrogen, organic nitrogen such as urea, amino acids, peptides, and proteins. Because of the very dilute nature of the medium in which they live, phytoplankton have developed extremely efficient ways of acquiring nutrients from the surrounding environment. Such acquisition by microalgae can take place through diffusion, active transport or a combination of both processes. Although encompassing the activities of several enzymes such as permeases, reductases and dehydrogenases, uptake is generally modeled according to simple enzyme kinetics (Michaelis-Menten). The rectangular hyperbola model is characterized by two parameters: the maximum uptake velocity (Vmax) and the half-saturation constant (Ks). Vmax depends on the incubation duration, with generally high values for short incubation times and lower values with longer incubation times for compounds such as ammonium whose assimilation requires constitutive enzymes. One can distinguish between an “externally controlled” Vmax, depending on the substrate concentration in the medium, and an “internally controlled” Vmax, which is due to feedback by products of assimilation. For compounds such as nitrate whose assimilation involves inducible enzymes such as nitrate reductase, Vmax increases with incubation time following a period of deprivation for example.Ks, the so-called half-saturation “constant”, is not really a constant as it can be modified by the nutrient regime: for example, N starvation will decrease its value on time scales of hours to days.When subjected to alternating light-dark cycles, phytoplankton exhibits variations in uptake, with higher values during the first half of the light period, and lower values in the dark.

2.1 Nitrate

Nitrate is one of the main N sources for phytoplankton, and one of the most extensively studied. Its concentration ranges between undetectable and 50 µmolN.l^{-1} in oceanic waters, and up to 500 µmolN.l^{-1} in coastal waters. Saturation kinetics are observed in the range of 0 to about 10 µmolN.l^{-1}, with Ks values ranging from 0.1 to 10 µmolN.l^{-1}. At higher concentrations (up to 100 to 300 µmolN l^{-1}), biphasic or multiphasic uptake systems are then involved, with Ks values up to 80 µmolN.l^{-1}. This compound can be toxic at concentrations above 1000 µmolN.l^{-1}.

2.2. Nitrite

This compound is present in much lower concentrations than nitrate in the oceans (undetectable to 2 µmolN.l^{-1}) and for this reason has not been studied as much as nitrate, but it can be used by a wide variety of species. Ks range from 0.1 to 25 µmolN.l^{-1}. Above 1000 µmolN.l^{-1} , nitrite can be toxic for green and blue-green algae, but some coastal diatoms can tolerate up to 20 000 µmolN.l^{-1}.

2.3. Ammonium

Ammonium is generally present in small quantities (undetectable to 2 µmolN.l^{-1}), except in polluted areas (up to 600 µmolN.l^{-1}). It is preferred to nitrate because its more reduced state makes it less energetically expensive to assimilate. Inside the cell, ammonium can be produced by several processes such as photorespiration, protein degradation and amino acid deamination. Ammonium saturation kinetics are observed in the range of 0 to about 10 µmolN.l^{-1}, with Ks values ranging from 0.1 to 9.3 µmolN.l^{-1}. At higher concentrations, up to 100 µmolN.l^{-1}, Ks ranges from 8 to 30 µmolN.l^{-1}, depending on the growth rate. This compound can be toxic at concentrations as low as 25 µmolN.l^{-1} for some dinoflagellates. In contrast, some green algae can tolerate levels up to 1000 µmolN.l^{-1}.

2.4. Molecular N_2

This compound is the most abundant form of N but it is used only by a particular class of phytoplankton called cyanobacteria. Among these, only cells with heterocysts can fix N_2 in presence of oxygen. Otherwise, N fixation is a strictly anaerobic process. N_2 reduction to ammonium is carried out by the enzyme nitrogenase. This process requires a large amount of energy (4 times more ATP than C fixation for example). Dinitrogen gas enters the cells by passive diffusion. Because of its high concentration in seawater (700-1100 µmolN.l^{-1}), there is apparently no active uptake system for this compound.

2.5. Dissolved organic N (DON)

Under this loosely defined term are grouped a huge number of different molecules (urea, free and combined amino acids, peptides, and proteins).

2.5.1. Urea

This compound contains 2 N atoms and is a good nitrogen source for many species of phytoplankton. Its concentration ranges from undetectable to 1 µmolN.l^{-1} in oceanic waters and up to 25 µmolN.l^{-1} in coastal waters. Saturation kinetics are observed in the range 0-50 µmol N.l^{-1}. At higher concentrations, no saturation occurs up to 1000 µmolN.l^{-1}. Toxic level can be as low as 100 µmolN.l^{-1} for dinoflagellates and as high as 2000 µmolN.l^{-1} for coastal diatoms.

2.5.2. Amino acids

The dissolved free forms (DFAA) are usually in very small concentrations (less than 0.5 µmolN.l^{-1}) and represent a small part of DON (5-10%). The combined forms (DCAA: peptides and proteins) represent a somewhat larger percentage (10-20%). Ks for dissolved free forms are in the range 0.4-150 µmol N.l^{-1}. In some species, there are two transport systems, differing in Ks by factors of between 4 and 20.

2.5.3. Humic substances

These are large organic compounds with complex structures adsorbing DFAA, DCAA and other N-containing macromolecules. They can contribute up to 30% of DON concentration and represent up to 40% of N used by some species of phytoplankton, probably by direct uptake or by pinocytosis.

2.5.4. Purines

Adenine and guanine can be used as sole N sources by several species of phytoplankton; their degradation products (xanthine, hypoxanthine, and uric acid) can be used as well. Microalgae sometimes require a long adaptation period (days to weeks) before being able to use those compounds.

2.5.5. Vitamins

Although present in very low concentrations in natural waters (less than 0.01µmol N.l^{-1}), one or more of those compounds (vitamin B_{12} , thiamin, biotin) are essential for phytoplankton growth depending on the species considered.

2.6. Particulate nitrogen (PN)

This class of compounds ranges from 0.1 to 0.4 µmolN.l^{-1} in oceanic waters and up to 1000 µmolN.l^{-1} in coastal waters, and is mainly made up of proteins (more than 70%). PN can be ingested by some members of phytoplankton and thus serve as a N source for growth. Phagotrophy is induced in some dinoflagellates by nutrient limitation. This change in nutrition mode can result in cell size increases up to 60%.

3. Assimilation pathways

Nitrate, the most oxidized N compound, is reduced to nitrite by the enzyme nitrate reductase (Ks between 22 and 31000 µmolN.l^{-1}). Nitrite is then reduced to ammonium by the enzyme nitrite reductase (Ks between 24 and 4000 µmolN.l^{-1}). For ammonium, several pathways exist. At high substrate concentrations, ammonium assimilation occurs by reductive amination of alpha-ketoglutarate by the enzyme glutamate dehydrogenase (GDH, Ks 13-40 000 µmolN.l^{-1}). At low concentrations, glutamine synthetase (GS, Ks between 27-1000 µmolN.l^{-1}) and glutamate synthase (GOGAT) are involved. GS catalyzes the reaction of glutamate with ammonium, forming glutamine. GOGAT then catalyses the transfer of the amido group of glutamine to alpha-ketoglutarate, forming 2 molecules of glutamate. While one of these molecules is recycled back by GS, the other is at the base of the formation of amino acids by transamination of the amino nitrogen of glutamate to various alpha-keto acids (see Figure 1)

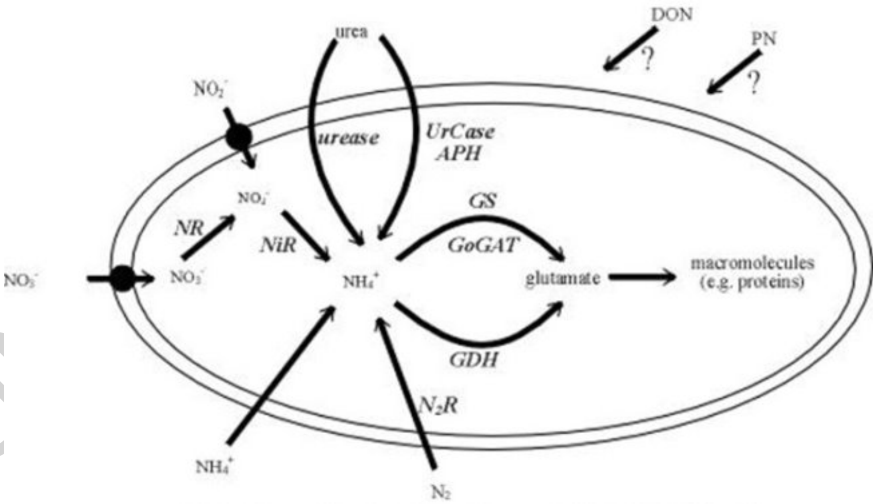

Figure 1. Diagram of the major pathways of nitrogen assimilation in phytoplankton cells.

Concerning urea, once inside the cell, the molecule is split, the N moiety (NH_3/NH_4+) being released and then assimilated and the C moiety (CO_2) sometimes being released to the external medium, and sometimes being used as a C source. Amino acids are either taken up directly, or processed by cell surface enzymes (amino acid oxidases) and the ammonium part being assimilated. The carbon may be expelled, respired or incorporated. Several essential amino acids, pyrimidines and purines are produced from anaplerotic carboxylations. Amino acids can also be produced by protein degradation by extracellular proteases (section 9) and N recycling within the cells (section 8).

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Biographical Sketches

Yves Collos was born in June1949, in Paris, France. He is a French citizen. He obtained his BSc in Biology at Old Dominion University (Norfolk, Virginia, USA)1969, an MSc in Biological Oceanography from University of Washington (Seattle, Washington, USA) in 1973, and a DSc from Université Aix-Marseille (France) in 1982.

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