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 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 those organisms also makes them prone to large exchanges of material with the external medium.

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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.

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 cultures 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, 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

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

2.2 Nitrite

This compound is present in much lower concentrations than nitrate in the [illegible] undetectable to2 μmolN.l^{-1} and for this reason has not been studied as much as [illegible] but it can be used by a wide variety of species. Ks range from [illegible] to 25 μmolN.l^{-1} [illegible] 1000 μmolN.l^{-1}, nitrite can be toxic for green and blue-green algae, but some diatoms can tolerate up to 20000 μmolN.l^{-1}.

2.3 Ammonium

It 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 1 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 2[illegible] μmolN.l^{-1} for coastal diatoms.

2.5.2 Amino acids

The dissolved free forms (DFAA) are usually in very small concentrations [illegible]

$\mu molN.l^{-1}$) and represent a small part of DON (5-[illegible]). 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 $\mu mol\ N.l^{-1}$. 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 $0.01 \mu mol\ N.l^{-1}$), 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 $\mu molN.l^{-1}$ in oceanic waters and up to 1000 $\mu molN.l^{-1}$ 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 oxidised N compound is reduced to nitrite by the enzyme nitrate reductase (Ks between 22 and 31000 $\mu molN.l^{-1}$). Nitrite is then reduced to ammonium by the enzyme nitrite reductase (Ks between 24 and 4000 $\mu molN.l^{-1}$). 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 between 13-40000 $\mu molN.l^{-1}$). At low concentrations, glutamine synthetase (GS, Ks between 27-1000 $\mu molN.l^{-1}$) 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 3: Diagram of the major pathways of nitrogen assimilation in phytoplankton cells

Nitrate is reduced once inside the cell, the molecule is split, the N moiety (NH_3, NH_4^+) is 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 nitrogenous 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).

4. Accumulation and storage

4.1 Inorganic compounds

N can accumulate inside the cells at several stages of the assimilation process. In the case of nitrate, which is the most oxidized form of nitrogen, the first accumulation step is nitrate, with maximum values up to 30% of cell N. This can lead to 10000 fold concentration differences between the inside and the outside of the cell.

The second compound along the metabolic pathway is nitrite, and is generally not found to accumulate to any great extent (less than 1% of cell N), probably because it diffuses readily through the cell membrane to the external medium.

The extent of ammonium accumulation is similar to that of nitrate and amino acids in both N-sufficient and N-starved cells (Figure 2).

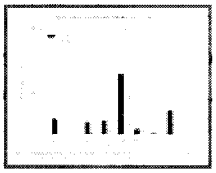

Figure 2: Percentage of N found in different forms within N-sufficient and N-starved phytoplankton cells. Bars represent mean values; error bars show the upper range of the values. The lower range (equivalent to the mean minus the error bar) is not shown.

The importance of these pools will depend on the species, its nutritional state and the time of day. They also represent transient accumulation pools mostly and indeed [illegible] between uptake and reduction processes, or between two steps of reduction.

The missing N in Figure 2 could be lipid N, proline or betaine [illegible] osmoregulation.

4.2 Organic compounds

Proteins: represented mainly by enzymes, which form the cellular machinery, among them those by Rubisco, the most abundant enzyme in the world which is responsible for carbon fixation in autotrophs. It can be stored in an inactive "crystalline" form in some species. Phycobiliproteins can be used as an N store in cyanobacteria.

Peptides: very diverse group with numerous functions. Among those, the following may be noted.

Glutathione: tri-peptide (gamma-glutamylcysteinglycine) responsible for intracellular detoxification of metals; precursor for phytochelatins.

Phytochelatins: small sulfhydril-containing peptides, with heavy metal-binding properties.

Amino acids: besides the usual 20 amino acids, there are more complex forms, which have specialized functions. For example, mycosporine like amino acids are UV absorbing compounds which are accumulated and also excreted in the medium. Others, such as B-dimethylsulphoniopropionate, glycine betaine and homarine, are used in osmoregulation.

Urea: transient accumulation ratios up to 3000 have been observed for cells with large vacuoles.

Vitamins: used as growth cofactors; some species require some vitamins and others produce them.

Vitamin B_{12} (14 N atoms): cofactor in reactions with intramolecular shifts of C or H and in group transfer reactions

Thiamin (3 N atoms): cofactor in decarboxylation of alpha keto acids.

Biotin (2 N atoms): cofactor in carboxylation and transcarboxylation reactions.

Toxic products

Saxitoxin (7 N atoms): one of the most toxic non-protein substance known; produced by dinoflagellates, it represents a N storage form when excess N is present in the growth medium. This compound is a sodium channel blocker and is responsible for Paralytic Shellfish Poisoning (PSP)

Domoic acid (1 N atom) is responsible for Amnesic Shellfish Poisoning (ASP)

Part of the molecule is similar to glutamate, which acts as a neurotransmitter.

5. Nutrient classification and preferences

New vs. regenerated production: this conceptual framework was proposed for a two layer ocean and distinguishes mainly two N sources: nitrate and ammonium. Nitrate, coming from deeper layers of the ocean and supplied to the euphotic zone by either advection or diffusion fuels new production (NP). In contrast, ammonium, produced in the euphotic zone by zooplankton excretion or mineralization of organic matter by bacteria fuels regenerated production (RP). To be complete, urea has to be added to RP and molecular N

to NP.

When nitrogen is available under several forms, phytoplankton will either use one, two or more depending on the experimental conditions. It may also use one in preference to another. For example, large phytoplankton such as diatoms generally show a preference for nitrate, while smaller forms such as flagellates use ammonium preferentially.

The most extensively studied interaction is that between nitrate and ammonium at the uptake level. Two distinct processes can be identified: preference and inhibition, whose relative importance depends on environmental conditions and species.

Preference can be assessed by comparing Vmax values for each N source separately. On short time scales (minutes to hours), Vmax for ammonium generally exceeds (up to ten times) Vmax for nitrate. On longer time scales, the difference is smaller, due to the inducible nature of the nitrate uptake system (see *section 2*). Ks for ammonium uptake are also generally lower than Ks for nitrate uptake.

From an energy point of view, ammonium uptake requires less reducing power than nitrate uptake (Table 2), and this can explain the increased preference for this N compound at low light levels. At high light, nitrate can be used as an electron sink to prevent photoinhibitory damage to photosystem II, so this could be a reason for nitrate preference under certain conditions.

Table 2: The energetics of CO_2 and N assimilation

Inhibition of nitrate uptake by ammonium is a complex phenomenon, depending on species and environmental conditions. This interaction ranges from no effect to complete inhibition of nitrate uptake by ammonium. The ammonium concentration threshold ranges from 0.1 to 90 $\mu molN.l^{-1}$. Increasing the concentration of nitrate sometimes mitigates such inhibition, indicating competitive inhibition. Low light or darkness may also favor this phenomenon. In some cases, nitrate can inhibit ammonium uptake, and small amounts of ammonium can stimulate nitrate uptake.

6. Plasticity in cell composition

Under the influence of changing environmental conditions, phytoplankton has a remarkable capacity to adapt its chemical composition. For example, under N starvation, they will continue to photosynthesize and divide, therefore reducing their N content. Depending on the number of cell divisions following N exhaustion from the medium, this N content can decrease by a factor of three to four. All compounds will decrease under such conditions, except DNA (Figure 2). The main N storage reserves are the chlorophyll-protein complex and the enzyme Rubisco, which can represent up to 50% of soluble proteins. A net decrease in cellular protein may also be induced by high irradiance levels.

Although accounting for less than 1% of cell N, chlorophyll a is [illegible] harvesting molecule. Its cellular content can decrease by a factor of [illegible] nitrate supply and by a factor of ten during N starvation.

7. Overflow mechanisms: excretion and release processes

The accumulation of internal pools of N compounds will lead to their release in the external medium along a concentration gradient. Of all the nitrogen compounds studied, nitrite appears to be the one most prone to being released during nitrate assimilation. Release rates in a wide variety of species ranged from zero to 100% of nitrate uptake. This is due to the nitrite reductase being a limiting step in the assimilation of nitrate. Factors influencing such a process include light intensity, temperature, growth rate, nitrate concentration, and the N deficiency of the cells. Following release, nitrite is generally taken back up by the cells once the nitrate is exhausted. This occurs under low PAR and is thought to be due to the light requirement of nitrite reduction.

Ammonium has been found to be released from cells upon increases in irradiance. This can represent up to 80% of nitrate uptake by diatoms.

DON: release as a percentage of gross N uptake ranges from 0 to 76 %, the higher values being exhibited by the cyanobacteria. Most of this material is not well characterized, but probably consists of amino acids, peptides and proteins. Apart from passive diffusion processes, there can be occasional considerable losses of cellular material due to protoplasm lysis during cell cycle events such as spermatogenesis induced by recovery from N starvation or by silicate limitation in diatoms.

8. Recycling of nitrogen within the cell

From Figure 1, it can be seen that ammonium is central to N assimilation. It is also central to N recycling in phytoplankton. While most of this aspect is dealt with in section 9, we will briefly mention the process of photorespiration here as it appears to be important in terms of N flux inside the cell. From the point of view of nitrogen, photorespiration is the production of ammonium from the transformation of glycine into serine, a pathway involved in protein turnover. This flux may be ten times greater than that through primary N assimilation (compare Table 1 and 3). This ammonium is refixed by glutamine synthetase into glutamate.

Table 3: Estimates of NH_3 / NH_4 reassimilation related to the photorespiratory carbon oxidation cycle and to phenylpropanoid synthesis

9. Degradation

9.1 Requirements for and roles of degradation

Nitrogen-containing compounds within phytoplankton cells must be degraded and turned over as part of normal cellular maintenance. The situation is best characterised for proteins, each of which have schedules of synthesis and degradation regulated by a range of environmental factors. For example, Rubisco is generally turned over in a period of days, while NR is turned over on a scale of hours. In contrast, the D1 protein of the Photosystem II reaction centre turns over on a scale of minutes. To some extent, the rates of turnover provide the means for cells to acclimate to changing condition.

In addition, however, degradation can be activated in response to environmental and life

history factors. Examples of such factors include:

a) Acclimation to changes in irradiance. Increased irradiance normally results in degradation of pigments (e.g. chl *a* or phycobiliproteins), and degradation of the protein complexes associated with pigments. Decreased irradiance may result in degradation of photosynthesis-related enzymes such as Rubisco. Under supra-optimal irradiance, degradation may also be used to shut down photosynthesis to avoid cellular damage: one important step in such an 'emergency' strategy is to degrade the D1 protein of Photosystem II.

b) Acclimation to changes in salinity. For some species, concentrations of N-containing compatible osmolites such as glycyl-betaine must be adjusted as conditions change. Degradation provides one means to remove them.

c) Acclimation to changes in nitrogen sources. As discussed above, cells have the ability to alter use of N-sources depending on availability. Many species of phytoplankton degrade the nitrate uptake system, and the enzymes NR and NiR, used for nitrate acquisition, when growing on ammonium. When alternative N-sources are available, many N_2-fixing species will degrade the nitrogen fixing enzymes and sometimes the special structures (e.g. heterocysts) required to fix nitrogen.

d) Degradation to release stored nitrogen during periods of extraordinary requirement (e.g. acclimations which require protein synthesis), or during periods of N-starvation. Clearly, cells could derive benefit from degrading compounds that are not currently being used in order to support synthesis of N-containing compounds that are immediate required.

e) Degradation of N-containing compounds for energy in periods of darkness. Proteins and other N-containing macromolecules could provide sources of energy via respiratory pathways. Degradation of amino acids that could enter such pathways is therefore necessary.

f) Acquisition of nitrogen in periods of heterotrophic growth or to supplement N-requirements during photosynthetic growth. As previously mentioned, a large portion of dissolved organic material in marine ecosystems is in the form of macromolecules such as dissolved proteins and peptides. Little is known about the precise pathways of acquisition, but given the cell's poor ability to transport such compounds, some degree of degradation is probably required (for example, extracellular proteases or amino acid oxidases).

g) Removing damaged or mis-synthesised N-containing compounds (e.g. proteins and nucleic acids). During periods of stress, such as nutrient limitation, exposure to elevated UV radiation or exposure to toxic compounds, macromolecules in cells may become damaged and the machinery involved in synthesis of proteins and nucleic acids becomes prone to errors. For example, cross-linkages can be formed in DNA strands, which can interfere with transcription and replication. In terms of proteins, mis-folding, loss of structure and oxidative damage are all found. Repair of such damage required degradation of the damaged or mis-synthesised compounds.

h) Morphological reorganisations such as creating resting stages, specialised structures like heterocysts, and reproductive phases. Many phytoplankton have different life history stages that are morphologically distinct. Diatoms, for

special cysts that act as resting stages, while many other species form specialised reproductive cells, for example. Degradation of N-containing compounds in existing cell structures is required every bit as much as synthesis of new ones.

f) Cell defence. Degradation of N-compounds may play key roles in defence against parasites and pathogens. This is discussed in more detail below (see *9.4*).

g) Creation and removal of intra- and inter-cellular signals. Cells produce a range of intra- and extracellular compounds (e.g. signaling peptides, and toxic compounds), the functions of which are still poorly understood. Some of these compounds are the result of degradation of larger N-containing compounds within the cells, thus requiring degradation. Alternatively, removal of such signals requires their degradation.

h) Degradation as a part of cell death phenomena. Processes of cell death within phytoplankton are only beginning to be examined. This is perhaps the ultimate degradation process for a phytoplankton cell and is discussed below (see *9.4*).

9.2 How is degradation accomplished?

Virtually nothing is known about the degradation of most nitrogen-containing compounds in phytoplankton. Even in the case of those that are better understood, the understanding is phenomenological rather than mechanistic. Much of the information in this section is drawn from what is known for higher plant systems. How applicable this may be is not yet clear.

Degradation is accomplished by enzymes within the cell. Regulation of such enzymes is necessarily strict; cells could not last long if this were not the case. There are three main methods of accomplishing this regulation:

a) Compartmentalise the enzymes within the cell and base control on the selective transport of N-containing compounds into the compartment. From what we understand at present, the vacuoles of phytoplankton cells may be the important compartment for degradation; in this respect, they are comparable to the lysosomes of animal cells. Vacuoles tend to have a lower pH that the cytoplasm, and have a wide range of proteases that exhibit optimal activity at lower pH.

b) Target compounds for degradation by selectively labelling them. This would allow the enzymes to selectively recognise and degrade only the desired compounds. An example of such a mechanism is the ubiquitin-labelling of proteins.

c) Regulate the enzymes themselves through activation/inactivation mechanisms or energy dependent pathways. This ensures that the enzymes remain inactive until required (i.e. in the form of an inactive zymogen, or inactivated through a mechanism such as phosphorylation), or simply cannot act until a source of energy is supplied (e.g. activation of some enzymes using associated ATP hydrolysing activity). Examples include the digestive enzymes of some facultative heterotrophic species, and the ATP-depended proteases found in chloroplasts.

In terms of the enzymes themselves, almost everything that is known about degradation involves proteins. There is virtually nothing known about, for example, the nucleases that could degrade RNA and DNA, or about the degradation of the complex molecules like

toxins and phytochelatins. Some details are available for chlorophyllases (enzymes involved in the degradation of chlorophyll *a*) from marine diatoms. The activity of these enzymes is potentially quite high (indeed, they can pose a threat to quantitative extraction of chlorophyll, a common procedure in oceanographic research) and they may be compartmentally regulated, because degradation proceeds very rapidly when cells are homogenised.

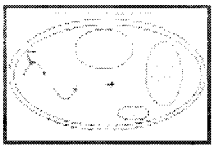

Figure 3: Major proteases and protein degradation pathways within phytoplankton cells.

Proteases activities have been examined in relatively few phytoplankton species, however bacterial, animal and higher plant proteases all share fundamental characteristics, so extrapolation from these systems is probably warranted. Proteases are traditionally divided into four classes, based on the key amino acid found in the active site of the enzyme: serine proteases, cysteine proteases, aspartic proteases and metalloproteases. These divisions are based on inhibition by specific inhibitors (e.g. phenylmethanesulfonyl fluoride specifically inhibits serine proteases), activation of the enzyme by thiol groups (e.g. the cysteine proteases), different pH optima (aspartic proteases are active in acid pH ranges), or the requirement for metal ions (metalloproteases often require zinc). More recently a number of enzymes have been characterised that defy such a classification systems because they exhibit mixed characteristics and even multiple activities. Protease activities are also classified by the nature of the cleavage that is catalysed. Proteases that recognise particular amino acids sequences within a protein and cleave it internally are termed endoproteases, or proteinases. In contrast, other proteases cleave from the end of proteins, either the end with a free amino group (termed aminopeptidases) or the end with a free carboxylic acid (termed carboxypeptidases).

Based on data from higher plants, vacuoles are likely to contain a wide variety of proteases active at low pH. In phytoplankton cells that have been examined to date, however, the majority of activity seems to be concentrated in the higher ranges of pH, from 7 to 9. In contrast, there are relatively few proteases characterised from the cytosol of cells. In eukaryotic cells a large (600-900 kDa), multi-subunit protease with multiple activities, the proteasome, is found. Many details of proteasomes remain unclear, but this protease complex appears to be involved in what is termed the ubiquitin-dependent proteolytic pathway. Ubiquitin is a highly-conserved 76-amino-acid peptide that can be covalently coupled to target proteins. Such labelling can be used to target a protein for degradation, thought the mechanism of selection is still not well understood. Proteins labelled with multiple ubiquitin molecules (poly-ubiquitinated proteins) are rapidly degraded by the proteasome. In contrast with many other proteases, the products of the reaction catalysed by the proteases tend to be free amino acids rather than smaller peptides. There is evidence that the majority of protein turnover within higher plant cells may occur via the ubiquitin/proteasome pathway.

Within prokaroytes and organelles of eukaryotic cells that have evolutionary roots in the prokaryotic domain, ubiquitin and proteasomes are not found. Indeed, relatively little is known about how protein turnover is accomplished in these species or in organelles such as the chloroplast and the mitochondrion. One system found in bacterial cells is termed the Clp protease. This protease is ATP-dependent, and evidence has been found for a related

system in the chloroplasts of higher plants.

Extracellular of cell-surface-related proteases have also been identified. These have tended to be in the aminopeptidase and carboxypeptidase classes. It is hypothesised that these enzymes may have roles in cell defence or in nutrient acquisition, but this has not been established. It would seem rather inefficient in a dilute system such as seawater to produce and release proteases into solution.

9.3 Variation in degradation

Little has been established regarding N-turnover and rates of degradation in marine phytoplankton. As discussed in section 9.1, these rates are critical for many processes within cells, and the lack on information on degradation rates and their variations is thus a serious gap. In addition to the implication for regulation of individual protein and requirements for nitrogen, it is worth noting that rates of growth depend not only on acquisition of N, but also on rates of N recycling and loss. Traditionally, experiments have focussed only on rates of nutrient uptake; much remains to be done.

9.4 Pathogenesis and Cell Death

Two exceptional cases of N-degradation in phytoplankton deserve special attention because of their implications. There is growing appreciation that mortality in phytoplankton species can be cause by a variety of organisms, principally bacteria and viruses. Moreover, there is speculation that some forms of mortality may involve the active participation of the phytoplankton's own degradatory capabilities. These phenomena have been variously classified as «lysis», and in the latter case, terms like «autocatalysed mortality» or «natural mortality» have been used. By analogy with processes described in multicellular organisms, terms like «cell suicide», «programmed cell death» and «apoptosis» have been used.

In the case of pathogenesis, there are many potential roles for N-degradation. Many toxic N-containing compounds may exist and the ability to detoxify them by degradation is a possibility that has received little attention. Bacterial attachment to phytoplankton cells is a potentially important step in mortality, and the adhesive mechanisms seem to involve N-containing compounds as well. Cell surface-active proteases may therefore play a role here. In the case of viral infection, protein and nucleic acid degradation may be important defence mechanisms. Viruses essentially hijack the phytoplankton cell's protein machinery and turn it over to production of viral nucleic acids and proteins. Infected cells might therefore have significant advantages if they possessed mechanisms to degrade foreign proteins and nucleic acids. In more extreme cases, inducing their own death might be a useful strategy. Consider the case of a phytoplankton cell in a clonal bloom. If infection with a virus occurred, the cell is at risk of producing more viruses which would infect the population. If the cell activated a 'suicide' mechanism, the virus would be stopped. Such a mechanism is not necessarily altruistic because the organisms saved would be genetically identical to the dying cell.

Based on our understanding of cell death in multicellular organisms, a division can be made between necrosis and apoptosis. Necrosis is characterised by swelling of cells, and a general breakdown of cellular structures in a disorganised manner. It is generally though to result from external injury to the cell. Apoptosis, in contrast, is much more ordered. Nucleic acid and proteins are systematically degraded as specific proteases and nucleases are synthesised and activated. Some organelles like the nucleus are targeted, while others

like the chloroplast are initially spared. Cells condense, shrink and lyse with little remaining after the process is complete. Apoptotic cell death often results from execution of a cell death programme, often in response to a relatively minor external stimulus.

In at least one species of marine phytoplankton, there is evidence for apoptotic cell death, based on morphology and biochemical evidence. In addition to gross morphological changes to cells, systematic degradation of nucleic acids and activation of a class of proteases called caspases have been observed. Caspases (Cysteinly ASPartate-specific proteinASES) are at the heart of a cell death pathway that is highly conserved evolutionarily. The precise triggers, characteristics and incidence of cell death in marine phytoplankton is currently unknown.

10. From uptake to growth: time-lag phenomena

The accumulation of nitrogen in intracellular pools at several stages of the assimilation process introduces time lags between nutrient uptake and cell division, which represents the integration of several processes. These lags will depend in part on the species involved. Two main strategies emerge at the genus level. One is the "growth" response, exhibited by genera which do not accumulate internal pools of inorganic nitrogen, whose uptake and growth are closely coupled, and which therefore process nutrients very rapidly into cells. The other is the "storage" response, found in genera which accumulate large pools of inorganic nitrogen, present extensive uncoupling between uptake and growth, and exhibits lags in cell division of up to 24 hours following nutrient resupply. The latter response type presents an ecological advantage when the nutrient pulsing frequency is lower than the cell division rate; the first response type providing a competitive advantage at high frequency pulses.

11. Relationships with carbon metabolism

From the chemical composition of phytoplankton (Figure 2) it can be expected that a lot of the nitrogen will be dedicated to the formation of proteins, and more specially ribulose biphosphate carboxylase. Carbon and nitrogen assimilation processes both compete for energy which is necessary to form biomass.

At the amino acid step (Table 2), N assimilation requires C skeletons in the form of ketoacids, which are intermediates of respiratory metabolism.

For N-limited cells in the light, an addition of inorganic N will lead to a reduction in net carbon fixation on time scales of minutes to hours depending on the magnitude of the nutrient pulse. This initial reduction can be due to several reasons: reduction in gross carbon fixation, increases in respiration, or increase in organic carbon release. This reduction will then be followed by a stimulation of C, which is due to a general improvement in the nutritional state of the cells.

For N-limited cells in the dark, an inorganic N addition will lead to a stimulation of C fixation. This effect is related to the oxidation state of the N compound added, ammonium leading to the strongest stimulation. This is called anaplerotic C fixation that is linked to amino acid synthesis.

Future directions:

A knowledge of time and space scales of variability in nutrient concentrations in the oceans and in Ks should point out directions for laboratory studies of uptake processes. Given the bewildering diversity of this class of organisms (in terms of size only, cell diameters range from 1 to 2000 μm), estimates of nitrogen utilization by phytoplankton should be improved by taking into account interspecific variability. Non invasive techniques for measuring release of compounds by delicate organisms would be most welcome.

We need to characterise the major protease systems within phytoplankton and discover how they are related to the systems we understand in bacteria, animals and higher plants. Because phytoplankton are so taxonomically diverse, there is likely to be substantial variation. Pathways of lysis and cell death in phytoplankton are very poorly characterised; we need to examine the phenomena in a range of different species and determine what importance it has in cell physiology and ecology of phytoplankton species.

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Related Chapters

Related links will be activated early 2003.

Glossary, Abbreviation and Symbols

Advection: movement of water masses
Anaerobic: living in absence of free oxygen
Anaplerotic: replenishing reactions
Apoptosis: a process of cell death involving ordered degradation and often associated with cell death programmes
ATP: adenosine triphosphate, energy rich molecule
Euphotic zone: upper layer of the oceans where light penetrates
C: carbon
Caspases: Cysteinly ASPartate-specific proteinASES that are involved in apoptotis
Chl a: chlorophyll *a*, the key photosynthetic pigment in phytoplankton
Chlorophyllases: chlorophyll-degrading enzymes
Clp protease: an ATP-dependent protease found in prokaryotes that serves a similar function to ubiquitin in eukaryotes; the organelles of eukaryotic cells may possess analogous proteases
D1: a core protein in the reaction centre of Photosystem II
Endopeptidases: proteases that cleave in the middle of proteins
Exopeptidases: proteases that cleave from the terminal ends of proteins
Glycyl-betaine: a peptide used as a compatible solute for osmoregulation in algae
GOGAT: glutamine-oxoglutarate aminotransferase
Half-saturation constant: value of substrate concentration at which the uptake rate is half the maximum uptake rate
Heterocyst: a specialized cell in the filaments of cyanobacteria where N fixation occurs
N: nitrogen
Necrosis: a process of cell death involving a disorganized disintegration and usually associated with cell injury
Nitrogen fixation: reduction of N_2 gas to an inorganic form, which can be assimilated; this

ability is limited to prokaryotes
NP: new production, based mainly on nitrate
NR- nitrate reductase: the enzyme involved in reducing nitrate to nitrite and a key step in nitrate assimilation
OG: oxoglutarate
Phagotrophy: ingestion of particles
Photoautotrophs (or photolithotrophs): light-dependent self-feeders; organisms using light as sole energy source and inorganic nutrients for biomass synthesis
Phycobiliproteins : the key photosynthetic accessory pigments in red algae and cyanobacteria
Phytoplankton: photoautotrophs that are free-floating in the water; unicellular algae which can sometimes form chains
Pinocytosis: ingestion of surrounding fluid by cell
Proteases: generic term for protein degrading enzymes
Proteasome: a large protease found in eukaryotic cells, which has multiple protease activities and participates in degradation of proteins that have been ubiquitin-labeled
RP: regenerated production, based mainly on ammonium
Rubisco: Ribulose biphosphate carboxylase
T: tera (10^{12})
UBQ: ubiquitin- a small, highly conserved protein that is covalently attached to proteins that have been targeted for degradation; found in eukaryotic cells

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