PHOSPHORUS CYCLING IN AQUATIC ENVIRONMENTS: ROLE OF BACTERIA

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Phosphorus is an essential nutrient for life and often
the limiting nutrient in aquatic ecosystems, especially
freshwater ones. As the most abundant component of the
aquatic biota and usually the component with the largest
surface area, bacteria have an important role to play in the
cycling of phosphorus in aquatic ecosystems, a role that
has not always been recognized. They have high cellular
phosphorus concentrations and provide an important
source of phosphorus-rich particles for grazers. Bacteria
also compete with phytoplankton for phosphorus uptake,
and they regenerate inorganic phosphate from organic
phosphate with enzymes near the cell surface, although
this regenerated phosphate is often only available to the
bacterial cell itself.

BACKGROUND

Definitions, Abbreviations, and the Aquatic Phosphorus Cycle

Varying definitions and abbreviations of environmental
phosphate pools are a significant source of confusion
for those new to phosphorus cycling studies. In this
article, these will be limited to a few essential categories.
Phosphorus (P) refers to any form of the element
and may also be called phosphate (although strictly
speaking this is the inorganic ion). Dissolved inorganic
phosphorus (DIP) is the inorganic component of the
dissolved pool; another commonly used term is soluble
reactive phosphorus (SRP). DIP includes orthophosphate
(P), and polyphosphate, composed of various polymers
of orthophosphate, is also in this fraction. Dissolved
organic phosphorus (DOP) is the organic component of the
dissolved fraction; this has also been referred to as soluble
nonreactive phosphorus (SNP). DIP and DOP together
make up the total dissolved phosphorus (TDP). Particulate
phosphorus (PP) may sometimes be separated into organic
and inorganic fractions. Unlike the nitrogen cycle, the
phosphorus cycle includes neither gaseous components
nor oxidation state changes. Most of the transformations
are from dissolved to particulate or the reverse, or
from inorganic to organic or the reverse. Different
phosphorus components dominate in different aquatic
environments. In oligotrophic low-nutrient environments
such as the open ocean and some lakes, the DOP is
dominant. In many turbid rivers, the PP may dominate.
Unlike the nitrogen cycle, the phosphorus cycle includes
no gaseous components or oxidation state changes.
Bacteria refers specifically to heterotrophic bacteria;
cyanobacteria are discussed separately. Phytoplankton
include all photosynthetic plankton, both eukaryotes and
the prokaryotic photosynthetic cyanobacteria.

Figure 1 shows a simplified phosphorus cycle. The
dissolved components are outlined in ovals, the particulate
components in squares. The abbreviations are defined
above. The arrows show the major interactions, although
not every pathway is illustrated. For this article, the
pathways to and from the bacteria are particularly
important. Phosphorus may be sequestered or released
by the sediments, depending on the redox state (see following
section).

Pho Regulon of Escherichia coli

The molecular model used to describe bacterial
transformations and assimilation of phosphorus has been the
Pho Regulon of the gram-negative bacterium E. coli. This
system has been studied for a long time and is the best
described. Under P-sufficient conditions, E. coli cells use a
low-affinity P, transport system known as the Pit system,
which is energized the cell’s proton motive force (2). At
submicromolar concentrations this system becomes ineffi-
cient, and the Pho Regulon is turned on, as signaled by
a rapid induction of alkaline phosphatase (AP) activity.
A diagram of the Pho Regulon is shown in Figure 2. The
Pho Regulon is a suite of genes that code for proteins that
facilitate P-assimilation under low-P conditions, including
the high-affinity Pst transport system for P. These
include (examples only, not comprehensive) outer mem-
brane porins (PhoE), periplasmic enzymes (AP or PhoA)
and binding proteins (PstS), membrane-associated trans-
port proteins (PstA, PstC), as well as cytoplasmic proteins
with regulatory and other functions (PhoB, PhnO).
The diagram of the Pho Regulon (Fig. 2) demonstrates that *E. coli* and most other bacteria transport phosphate largely in the inorganic form as orthophosphate (P$_i$), few organic phosphate compounds are taken up directly, most are hydrolyzed by alkaline phosphatase or other enzymes first. These enzymes remove the P$_i$ group, allowing it and remaining organic component to be assimilated separately. Among the few exceptions to the rule that only P$_i$ is directly transported, *E. coli* has a transport system for glycerol-3-phosphate, the Ugp system shown in Fig. 2, which is also considered a component of the Pho Regulon (2). *Escherichia coli* also has a UhpT system, which exchanges external glucose-6-phosphate for internal P$_i$ (3). Only bacteria that are intracellular parasites, such as *Rickettsia* and others, which live in an environment with high organic phosphate concentrations, seem to gain a large fraction of their required phosphate through transport or exchange organic phosphate compounds (3).

Studies of the marine cyanobacteria *Synechococcus* and *Prochlorococcus*, which are important components of the marine foodweb, have shown that these organisms have a P$_i$-binding protein, homologous to the PstS gene product in *E. coli*. Expression of this protein was observed in lab cultures of *Synechococcus* when the P$_i$ concentration declined below 50 nM (4). PstS expression by these organisms was also shown in field samples collected to a depth of 100 m at the Bermuda Time-series Study site (5), which is a microbially dominated, oligotrophic open-ocean station 82 km southeast of Bermuda. Others have reported evidence for phosphorus-limitation of heterotrophic bacterial growth at this site (6) where DIP concentrations in the euphotic zone can be as low as 1 to 10 nM (7) when measured with a highly sensitive method (8). Kinetics studies of P$_i$ uptake at the same location show half-saturation constants in the same range (Ammerman, unpublished).

The complete genome sequence of the freshwater cyanobacterium, *Synechocystis* sp., also reveals two genes that are highly homologous to the PstS gene in *E. coli* (9), as well as the rest of the Pst transport system. The draft genome sequences of both marine cyanobacteria discussed earlier, *Synechococcus* and *Prochlorococcus*, also clearly show the presence of the complete Pst system. These sequences are currently unpublished but are found on the website of the Institute for Genomic Research (www.tigr.org) under microbial genomes in progress.

*Vibrio cholerae*, the agent of cholera, is the first representative of this commonly isolated genus of heterotrophic marine bacteria to have its genome completely sequenced (10). It apparently has multiple high-affinity Pst phosphate transport systems on its two chromosomes, including two genes homologous to the *E. coli* PstS (10, 11). Another component of the Pho Regulon in *V. cholerae* is the Ugp transport system for glycerol-3-phosphate. However, although *V. cholerae* is an enteric bacterium closely related to *E. coli*, it surprisingly has no homolog to PhoA.
the alkaline phosphatase gene. Alkaline phosphatase is an important component of the E. coli Pho Regulon and an enzyme activity commonly measured as an indicator of phosphorus limitation in aquatic environments (discussed later). It is apparent that the E. coli model of phosphorus assimilation will not be sufficient for all aquatic bacteria, even some of those closely related to it. Fortunately, however, the increasing availability of the genome sequences of aquatic microbial species will allow us to better address these questions.

**BACTERIAL P CYCLING IN NATURE**

**Carbon to Phosphorus Ratios of Bacteria Cells**

Bacteria have large phosphorus requirements and a relatively low carbon to phosphorus (C:P) ratio of about 50 (12). Interestingly, this ratio greatly exceeds the C:P ratio of phytoplankton; it is about double the well-established Redfield ratio of 106:1 (13). The bacterial C:P ratio in cultured cells is also extremely variable (varying by a factor of 50), much more than the C:N ratio, and reflects the C:P ratio of the growth media (12). The major phosphorus-rich components of bacterial cells are nucleic acids, membrane phospholipids, and sometimes the storage product polyphosphate, whereas protein is the major nitrogen-containing component. The fraction of bacterial biomass devoted to nucleic acids (particularly RNA) and phospholipids varies significantly with growth conditions, growth rate, and cell volume. The cell volume is also inversely related to growth rate. In contrast, the fraction of biomass that is protein, varies little with growth rate. This explains why bacterial C:P is more variable than C:N (12). The small size of heterotrophic bacteria relative to phytoplankton (including cyanobacteria) may also explain why bacteria are phosphorus-rich relative to phytoplankton. In smaller cells, the surface to volume ratio is greatly enhanced, and this means more phosphorus-rich membrane phospholipid (12).

**Bacterial Role in Phosphorus Uptake and Regeneration**

Phosphorus cycling has long been studied in lakes because of the importance of phosphorus as a limiting nutrient in freshwater environments. There have been fewer studies of phosphorus cycling in marine and estuarine environments because of the focus on nitrogen, not phosphorus, as the limiting nutrient. Most early studies of aquatic phosphorus cycling, at least those in the water column, emphasized the role of phytoplankton, rather than bacteria. Water column studies of phosphorus cycling by phytoplankton through the early 1980s have been extensively reviewed (14,15).

In the past, bacteria in aquatic ecosystems were considered to be important only in phosphorus regeneration, or mineralization of the organic to the inorganic form, followed by release to the environment. Only a few early papers suggested that they might be important in phosphorus uptake, see references in (16). However, since the development of the microbial food web paradigm in the early 1980s (17), many studies have examined the role of aquatic bacteria in phosphorus cycling, including the following reviews (16,18,19). Among other important roles in aquatic food webs, bacteria are very effective competitors with phytoplankton for P, as shown more than 15 years ago (20). Heterotrophic bacteria are now known to be responsible for about 60% of the inorganic phosphate uptake in various aquatic ecosystems (19), as determined by the radiolabeled-P uptake taken up by the plankton fraction passing through 0.8 or 1.0 μm polycarbonate filter. Some of this uptake may be due to cyanobacteria or other small phytoplankton found in the same size fraction. Studies that were specifically corrected for phytoplankton found a somewhat lower fraction of uptake by the heterotrophic bacteria (19). Phosphorus-limited bacteria and cyanobacteria are also capable of "luxury uptake" of P beyond the immediate cellular requirements when additional P is provided (5,16).

In addition to competing directly with phytoplankton for uptake of DIP, heterotrophic bacteria can be important in the regeneration of phosphate and other nutrients (12), the role that they have been traditionally assigned. However, in P-limited systems, bacteria are probably not net mineralizers at all, but rather a sink for inorganic phosphorus (16). Any DOP regenerated to DIP by bacteria would be immediately taken up by the same cell. In this scenario, the bacteria would still be an important link in the regeneration process, as a major phosphorus-rich source of particles for grazers (particularly bacterivores) in aquatic ecosystems. Because bacterivory accounts for a large fraction of the bacterial production in many aquatic environments (21), this is an important connection. In non-P-limited systems, however, DIP that has been regenerated from DOP by bacterial "ectoenzyme" activity (discussed later) is made available for other organisms in the food web (22).

Bacteria have high-affinity transport systems, presumably because of the presence of the P$_1$-binding proteins discussed above. Vadstein (16) reviewed P$_1$ uptake parameters for bacteria from his and other studies. In seven different bacterial strains or communities analyzed, the median half-saturation constant ($K_m$) for P$_1$ uptake was 90 nM, with a range of 10 to 250 nM. This contributes to their effective competition with phytoplankton for P$_1$. However, they also have large cellular phosphorus requirements, as discussed earlier (12). Although bacteria are often considered more efficient at P$_1$ uptake than phytoplankton (20), others suggest that this competitive ability varies with a uniform or pulsed nutrient supply (16). None-the-less, phosphorus-limitation of bacterial growth is common during summer stratification in lakes (16), and also occurs in marine systems (6,23,24).

**Enzymatic Hydrolysis of DOP**

Cell-surface enzymes of aquatic microbes together with "extracellular" enzymes that are dissolved or adsorbed to other than their original particles (25), are important catalysts in the decomposition of dissolved organic matter (DOM) and particulate organic matter (POM). In aquatic science these are sometimes collectively referred to as "ectoenzymes" to distinguish them from other types. Polymer hydrolysis can be the rate-limiting step in microbial DOM and POM utilization (26). Efficient hydrolysis and
utilization of these polymers is important to the rapid microbial growth seen in many aquatic ecosystems (27). In a review, Christ (28) lists 18 different microbial ectoenzymes whose activities have been measured in natural waters and sediments. A major component of these are phosphatases, such as alkaline phosphatase, enzymes that hydrolyze phosphate groups from organic phosphate compounds (specifically phosphate esters) making the phosphate group available for assimilation.

Other ectoenzymes include those that hydrolyze polymeric carbohydrates or proteins, such as glucosidases and peptidases. Most of these ectoenzymes are found on the cell surface or in the periplasmic space of gram-negative heterotrophic bacteria (29), and appear to be ubiquitous in aquatic isolates (27). Certain ectoenzymes, such as alkaline phosphatase (AP) and leucine aminopeptidase, are also found in some species of cyanobacteria and eukaryotic phytoplankton (30-32). Alkaline phosphatase is typically induced by phosphorus deficiency, and in E. coli and some other prokaryotes, this enzyme is part of the Pho regulon discussed earlier. Alkaline phosphatase activity in eukaryotic phytoplankton is also a response to phosphorus-deficiency, as has been known for some time (14,33), however, phosphatase regulation in eukaryotic phytoplankton is not as well understood as in prokaryotes (5).

Marine field studies of phosphatases using high-sensitivity fluorescent substrates date back more than twenty-five years (34). In the last fifteen years there has also been increased interest in many different ectoenzymes in a variety of aquatic environments (28), particularly since the introduction of the fluorescent methyleneumbelliferyl and related derivatives (35). These derivatives offer the advantages of low background fluorescence and are available as substrates for many different enzymes. There have also been recent improvements in the analysis methods for the fluorescent hydrolys products, such as the use of flow-injection (36) or of fluorescence microplate readers (37,38). Recently, alkaline phosphate activity has been measured by continuous flow methods aboard ship, allowing its surface distribution to be mapped in detail (39).

Alkaline phosphatase activity is easier to interpret than many other ectoenzyme activities, because it usually is a response to phosphorus-deficiency. Many of the early AP studies, mostly in phytoplankton, have been extensively reviewed (14,15). Although there is often a positive relationship between AP activity and biomass, it is probably not the controlling factor, as suggested by some (40). Very high AP activities were found in three different estuarine and coastal ecosystems only when DIP concentrations were less than 200 nM (15). Criteria for P-limitation based on AP activity normalized to chlorophyll a have been developed for freshwater, although not widely applied (41,42). The use of this or similar measures, corrected for factors such as dilution, depth, etc., can determine the AP activity is dominated by “hot spots”. Nonetheless, “alkaline phosphatase” activity, as commonly measured in the field, is a mix of different enzyme activities from different organisms that can hydrolyze the substrate, which is usually a fluorescent or chromogenic analog of natural substrates. These different enzymes probably also have different kinetic properties. Aggregate kinetic properties for AP activities measured in the field, typically range from 1 to 10 μM for the half-saturation (Km), and 0.1 to 1.0 μmoll-1h-1 for the maximum activity (Vmax) (38,39). Activity is also found in different size fractions, including phytoplankton greater than 2 μm, bacteria and cyanobacteria less than 2 μm (often referred to as “picoplankton”), and sometimes a “dissolved” fraction less than 2 μm. In a recent example from the oligotrophic Red Sea (42), most of the AP activity was associated with Synechococcus or dissolved. In contrast, in the eutrophic Mississippi River plume, about half of the AP activity is in the size fraction greater than 10 μm (Ammerman, unpublished). The function of dissolved activity is difficult to understand, but occurs regularly enough such that methodological or functional explanations are needed.

Clearly, new approaches are needed to better understand the sources of AP activity and its regulation. As discussed earlier, the genome sequence for V. cholerae does not even have a gene homologous to the E. coli PhoA gene for alkaline phosphatase, although we assume that most aquatic bacteria have this enzyme. An example of a novel approach to these issues is the use of the fluorescent ELF substrate to label the alkaline phosphatase of phosphate-stressed dinoflagellate, Prorocentrum minimum, in the lab and the field (43,44). ELF (Enzyme-Labeled Fluorescence; Molecular Probes, Eugene, Oregon) is an enzyme substrate that forms an insoluble precipitate at the site of the activity.

Another periplasmic ectoenzyme involved in phosphorus cycling, that has also been measured in aquatic ecosystems is 5'-nucleotidase, which is specific for 5'-nucleotides (45-48). This enzyme is involved in nucleotide and nucleoside salvage and metabolism, and is sometimes included in the Pho Regulon (2). However, at least in aquatic systems it apparently responds more to the demand for carbon rather than phosphorus (18), and is largely independent of the DIP concentration. Although it can regenerate significant amounts of DIP from nucleotides, its primary function may be to remove the Pi group from nucleotides, allowing further metabolism of the remaining nucleoside (18). Although apparently a prokaryotic enzyme, there is at least one report of 5'-nucleotidase activity in a eukaryotic phytoplankton (49). Both the V. cholerae and Synechocystis sp. genome sequences contain multiple proteins homologous to the 5'-nucleotidase gene (UshA) in E. coli (11).

Figure 3 shows the major steps in the breakdown of DOP polymers and monomers into P, and an organic carbon moiety. Nucleases first hydrolyze nucleic acid polymers to monomers, AP and 5'-nucleotidase enzymes then hydrolyze the monomers, and finally enzymes from other transport systems take the diphosphate form of the monomers (and other organic compounds) across the plasma membrane. Each of these steps is potentially important in the transfer of DOP in the sea, and the transfer of these nutrients and electron donors to other cells. The fate of these groups apparently depends on the current nutritional status of the cell in terms of phosphorus and organic carbon.
a general review of marine phosphorus cycling (1) and a review of marine DOP, including its properties and transformation processes (40). Also, there is a new monograph on marine microbial ecology (53), and a review of bacterial phosphorus cycling in lakes from a quantitative and modeling perspective (16). Finally, there is a review of the application of molecular methods to the assessment of P-limitation in marine bacteria and phytoplankton (5).

**BIBLIOGRAPHY**


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**PHOTOSYNTHETIC BACTERIA IN SOILS.** See *Soil Bacteria*

**PHOTOSYNTHETIC BACTERIA IN WASTE STABILIZATION PONDS.** See *Wastewater Stabilization Ponds*

**PHOTOSYNTHETIC PIGMENTS IN MARINE ALGAE AND BACTERIA**

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Photosynthetic organisms have evolved a diversity of chemically distinct chlorophylls (Chls) and bacteriochlorophylls (BChls). In most marine photoautotrophs, Chl a represents the major light-harvesting pigment and no plant without it is known to be able to trap light energy for photosynthesis. Recently, a new class of photoactive protein pigment compounds (rhodopsin family) has been discovered in marine bacteria, which may represent the basis of a new phototrophic pathway (1, 2). It is not known at this time, however, whether rhodopsin-containing marine bacteria can fix CO₂.

Light-harvesting antenna pigments may also include Chl b and Chl c type pigments. Other types of Chl molecules observed in marine algae are more obscure (e.g., Chl d in Rhodophyta; 3). In addition to Chls, photosynthetic organisms have evolved two main types of nonchlorophyllous pigments: carotenoids and phycobilins. Xanthophylls (the oxygenated derivatives of carotenoids) are present in all marine algae and have been used to chemotaxonomically identify major algal classes present in oceanic waters (4, 5). Various xanthophylls can serve either a photoprotective function (e.g., diatoxanthin, zeaxanthin) or a light-harvesting function (e.g., fucoxanthin). Phycobiliproteins occur as the main light-harvesting pigments in cyanobacteria and some eukaryotic algae (Cryptophyta and Rhodophyta). They are important in harvesting energy in the low Chl-absorbing region of the spectrum and transferring this energy to

**PHOSPHORUS IN MARINE MICROORGANISMS.** See *Inorganic Nutrient Use by Marine Microorganisms*

**PHOTOSYNTHETIC BACTERIA.** See *Phototrophic Purple and Green Bacteria in Marine and Hypersaline Environments*