New insights into bacterial acquisition of phosphorus in the surface ocean

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Since 1958 when Alfred C. Redfield (1) recognized the similarity between the ratios of elements in living biomass and those dissolved in the surrounding seawater, we have understood that microorganisms largely control the concentrations, distribution, and molecular makeup of nutritional resources in the ocean. The primary elemental ingredients for life, carbon (C), nitrogen (N), and phosphorus (P), are assembled, disassembled, transformed, and consumed by marine microorganisms, resulting in a steady cycling of elements between intracellular, inorganic, and organic reservoirs. Of these pools, dissolved organic matter (DOM) represents the largest C, N, and P reservoir in the surface ocean of most marine habitats, greatly exceeding the respective concentrations of inorganic pools or that found in living organisms. DOM is a source of energy and elements, fueling heterotrophic and autotrophic growth alike (2), yet we understand very little about the biomolecular strategies marine microbes employ to use organic substrates in the global ocean. What is the molecular composition of organic matter, where do these compounds originate, and how much of this is bioavailable? How do microbes hydrolyze and transport constituents of DOM into the cell? What are the factors that regulate enzyme expression and control the decomposition of organic matter? These are but a few of the questions that must be addressed to fundamentally and mechanistically understand how microorganisms assimilate, transform, and turn over elemental resources in the ocean. In this issue of PNAS, Luo et al. (3) use a bioinformatics approach to investigate the diversity and localization of bacterial phosphatases, enzymes specialized for the hydrolysis of a reactive fraction of DOM, P-linked esters. Most notably, their study indicates that a significant fraction of bacteria may transport intact organophosphate compounds across the cell membrane for intracellular depolymerization, a finding counter to the prevailing concept of phosphatases as being largely extracellular. These disparate modes of DOM hydrolysis (extracellular versus intracellular) would have fundamentally different impacts on the ratios of elements in dissolved and particulate matter.

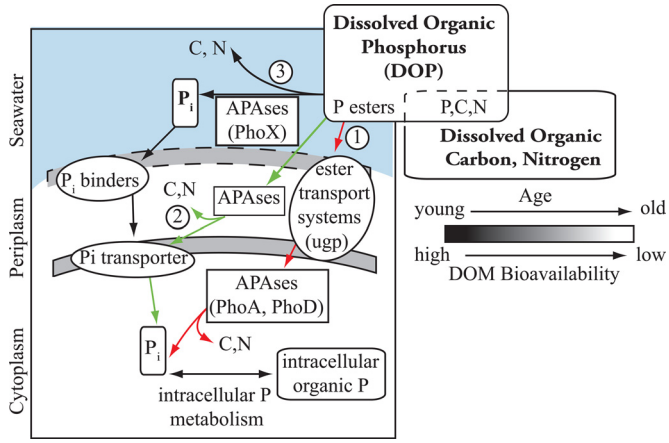

Fig. 1. In the sunlit surface ocean, DOP consists of P bound to C and N in a myriad of substrates having varying bond structures, diagentic ages, and chemical reactivities. A key step in the P–C–N cycles of the upper ocean is the hydrolysis and assimilation of organic P substrates by marine bacteria. Using the metagenomic database of the GOC and a consensus classification algorithm to predict subcellular localizations, Luo et al. (3) predict three predominant modes of DOP metabolism by marine bacteria: (*i*) small P-linked esters can be transported intact across the cell membrane and hydrolyzed in the cytoplasm by specific bacterial phosphatases (APAses, predominately PhoA and PhoD proteins). This mode of APA expression may be important for bacterial C, N, and P nutrition alike. (*ii*) Alternately, APAse associated with periplasmic, membrane-attached proteins or (*iii*) enzymes extruded from the cell (predominately PhoX proteins) result in the release of excess inorganic P (P_i) followed by the assimilation of P_i into the cell and either release or uptake of the cleaved organic moiety. The schematic builds on the conceptual models of microbial P metabolism and DOP cycling presented by Dyhrman et al. (17) and Karl (18).

Resource Management: Bacterial Hydrolysis of Dissolved Organic Phosphorus (DOP)

Particulate and dissolved P is bound to C and N in multiple forms, including monoesters, diesters, nucleotides, phosphonates, and phospholipids. Although the activity of bacterial and cyanobacterial phosphonate degrading enzymes is gaining increasing attention (4, 5), we know much more about the microbial hydrolysis of monophosphate esters via broad-spectrum alkaline phosphatases (APases). A number of excellent reviews have extensively covered the current knowledge of APase activity in heterotrophic bacteria and prokaryotic (6) and eukaryotic autotrophs (7). Moreover, molecular approaches are providing new insight into the abundance and distribution of distinct prokaryotic gene families (PhoA, PhoX, and PhoD) necessary for hydrolysis of DOP (8, 9). Despite this progress, we currently understand relatively little about the genomic diversity, regulation of APase gene expression, subcellular enzyme localization, substrate specificity, or distributions of APase in situ in natural populations.

Mining the metagenomic database obtained from the Global Ocean Sampling (GOS) Expedition (10) for APase peptide sequences, Luo et al. (3) have identified PhoA, PhoX, and PhoD homologs in coastal and oceanic bacteria. Each homolog represents a distinct mode of DOP hydrolysis in the sense that they require different ionic activators (Zn^{2+}, Ca^{2+}, or Mg^{2+}) and appear to be differentially located within the cell itself. Whereas PhoD, an APase homolog also identified in certain strains of cultured marine picocyanobacteria (9), was found to be the most abundant APase in this global surface ocean database, multiple APases families (PhoX and PhoD) were frequently found in a single genome. Furthermore, phylogenetic analyses associated the majority of APases with uncharacterized taxonomic groups (the "known unknowns"), indicating that marine bacte-

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ria have evolved from their cultured counterparts. One would expect that these three APases differ in their substrate specificity and may be regulated and expressed in fundamentally different ways. These dissimilarities may lead to markedly different capacities for DOM utilization and niche partitioning of recognized and unclassified organisms alike.

Whole Foods

By far the most intriguing result in the article by Luo et al. (3) is the prediction that 41% of APases from the GOS database are located in the cytoplasm of oceanic and coastal bacteria alike and that each marine bacterial genome contained at least one gene homologous to known DOP transport systems of *Escherichia coli*. This finding suggests that low molecular weight DOP compounds are carried whole across the membrane, providing a source of P, C, and potentially N to bacteria (Fig. 1). The predictive metaalgorithm used by Luo et al. to determine the subcellular location of APase cannot unequivocally establish whether these cytoplasmic APases are internal regulatory enzymes involved in essential metabolic pathways or whether they are truly necessary components for hydrolysis of exogenous DOP. Yet, small hydrophilic molecules <600 Da in size can pass through the outer membrane of Gram-negative bacteria (11), and glycerophosphoric acid, AMP, and cAMP can be assimilated intact by certain bacteria (ref. 13 and references therein). So it is not exactly far-fetched to conclude, as Luo et al. have done, that marine bacteria can indeed assimilate small phosphoester compounds. In this case, there are clear implications for the elemental stoichiometry of particulate and dissolved matter in the oceans and the competitive capacity of microbes to acquire potentially limiting elements. Simply, ingesting P-esters whole rather than hydrolyzing them outside the cell will result in different ratios of P/C/N in disssolved and cellular pools. This finding also indicates that traditional measurements of the rates of hydrolysis of fluorogenic 4-methylumbelliferyl phosphate (MUF-P) as a proxy for APase activity would underestimate total enzyme potential in bacterioplankton because MUF-P cannot be transported across the cell membrane. In general, there is not a consensus as to whether bacterioplankton predominately express APase as a means of acquiring P, C, or perhaps N (12, 13). Likely, expression patterns shift with the metabolic state of cells and the abundance of substrates. However, this uncertainty coupled with the beguiling findings of Luo et al. (3) indicate that for bacterioplankton and phytoplankton alike, the warning of Cembella et al. (7) is appropriate: "The current practice of using assays of alkaline phosphatase as bioindicators of the nutritional status in natural phytoplankton populations is probably reckless and fraught with undesirable complications."

Bacterial phylotypes have several distinct tactics for hydrolyzing and transporting P-linked esters.

The Shrouded Depths: DOM Utilization Below the Euphotic Zone

Genomic studies such as that by Luo et al. (3) are powerful, but they currently rely on samples collected from the upper few meters of the water column. These analyses only scrape the bare surface of microbial metabolism in the ocean, leaving the diversity and activity of microbes residing in the depths a relative mystery. As biogenic material rains out of the surface ocean and out of the reaches of the GOS expeditions, particulate and DOM is depolymerized, hydrolyzed, assimilated, and absorbed onto living and dead particles, and refractory material begins to accumulate in the surrounding environment (13). With depth, the bioavailable and refractory DOM also becomes more C-rich, indicating the selective and preferential remineralization of P and N (14, 15). Yet elevated APase activity is observed in the mesopelagic depths despite decreased bacterial biomass, elevated inorganic P(P_i) and a decrease in the activities of other hydrolytic enzymes (16). Hypothesized to be a general feature of the deep ocean (13), enhanced APase at depth provides a mechanism for bacterial C-capture, resulting in the local regeneration of P_i and distinctly different ratios of elements in the inorganic, organic, and particulate pools relative to the surface ocean. The remineralization stoichiometry below the euphotic zone sets the ratio and concentration of inorganic pools that will be delivered to the surface ocean through diffusion, vertical mixing, and advection and thus links the activity of microbes at depth to the control of primary production in surface waters. Extension of metagenomic and biochemical analyses to the depths of the mesopelagic and beyond are needed to better understand this critical node in the resupply of inorganic nutrients to the water column.

Analyses such as those reported by Luo et al. (3) are vital for the continued advancement of our understanding of microbial metabolism in the ocean. Bacterial phylotypes have several distinct tactics for hydrolyzing and transporting P-linked esters. This bit of information should change the way we think about traditional APase activity and hence our understanding of the microbial strategies for resource utilization in the global ocean.

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