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Microbial transformations in phosphonate biosynthesis and catabolism, and their importance in nutrient cycling

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Phosphorus cycling in the biosphere has traditionally been thought to involve almost exclusively transformations of the element in its pentavalent oxidation state. Recent evidence, however, suggests that a significant fraction of environmental phosphorus may exist in a more reduced form. Most abundant of these reduced phosphorus compounds are the phosphonates, with their direct carbon–phosphorus bonds, and striking progress has recently been made in elucidating the biochemistry of microbial phosphonate transformations. These advances are now presented in the context of their contribution to our understanding of phosphorus biogeochemistry and of such diverse fields as the productivity of the oceans, marine methanogenesis and the discovery of novel microbial antimetabolites.

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Introduction

The element phosphorus (P) is essential to life on Earth and in many ecosystems it is a limiting nutrient. In both the lithosphere and hydrosphere P has been widely regarded as being present almost entirely in its highest (+5) oxidation state, as phosphates and phosphate esters. In recent years, however, the importance of the phosphonates, compounds characterized by a direct C–P bond in which P is in the +3 oxidation state, as a P source, has been recognized [1,2]. For example, chemical analysis suggests that phosphonates form some 10% of dissolved and particulate P in the oceans [3–5], whilst genomic and metagenomic studies suggest that some 10% of marine microbes are capable of phosphonate biosynthesis [6,7,8••] and that a similar or greater percentage have a capacity for C–P bond cleavage [6,9]. Nor are these abilities confined to the marine environment; 10% and 38% respectively of all sequenced bacterial genomes were found to contain homologues that indicated pathways of C–P bond synthesis or cleavage [6].

Further evidence of the importance of reduced P compounds to the global biogeochemical cycle of the element comes from the fact that many environmental microorganisms are now known to have the capacity to utilize phosphite and hypophosphite [10–13]. Pasek *et al.* [14], who have recently shown that between 10 and 20% of dissolved P in a series of freshwater samples from Florida comprised phosphite and hypophosphite, suggest that these reduced species may arise from the microbial transformation of phosphonates. Strong support for these findings has come from the recent work of Van Mooy *et al.* [15••] who have demonstrated the existence of a very significant redox component in marine P cycling at a series of stations in the western North Atlantic Ocean and succeeded in quantifying the fluxes involved; as much as 16% of ^{33}P-phosphate taken up by the planktonic community was shown to be reduced to the +3 oxidation state and subsequently released into the water column.

Thus there is increasing recognition of the importance of reduced P compounds in biogeochemical P cycling, and an awareness of the interdependence between the global P cycle and those of the other biologically significant elements [2]. In addition, there is a growing realization of the potential significance of phosphonate natural products as novel antibiotics and other pharmacological agents. These drivers have combined in recent years to produce an upsurge in investigations of the enzymology of phosphonate biosynthesis and catabolism — at both the biochemical and genomic levels — and very significant breakthroughs have been made (reviewed in Ref. [16,17]). In this review we focus on further progress in the field, as reported in the years since 2012.

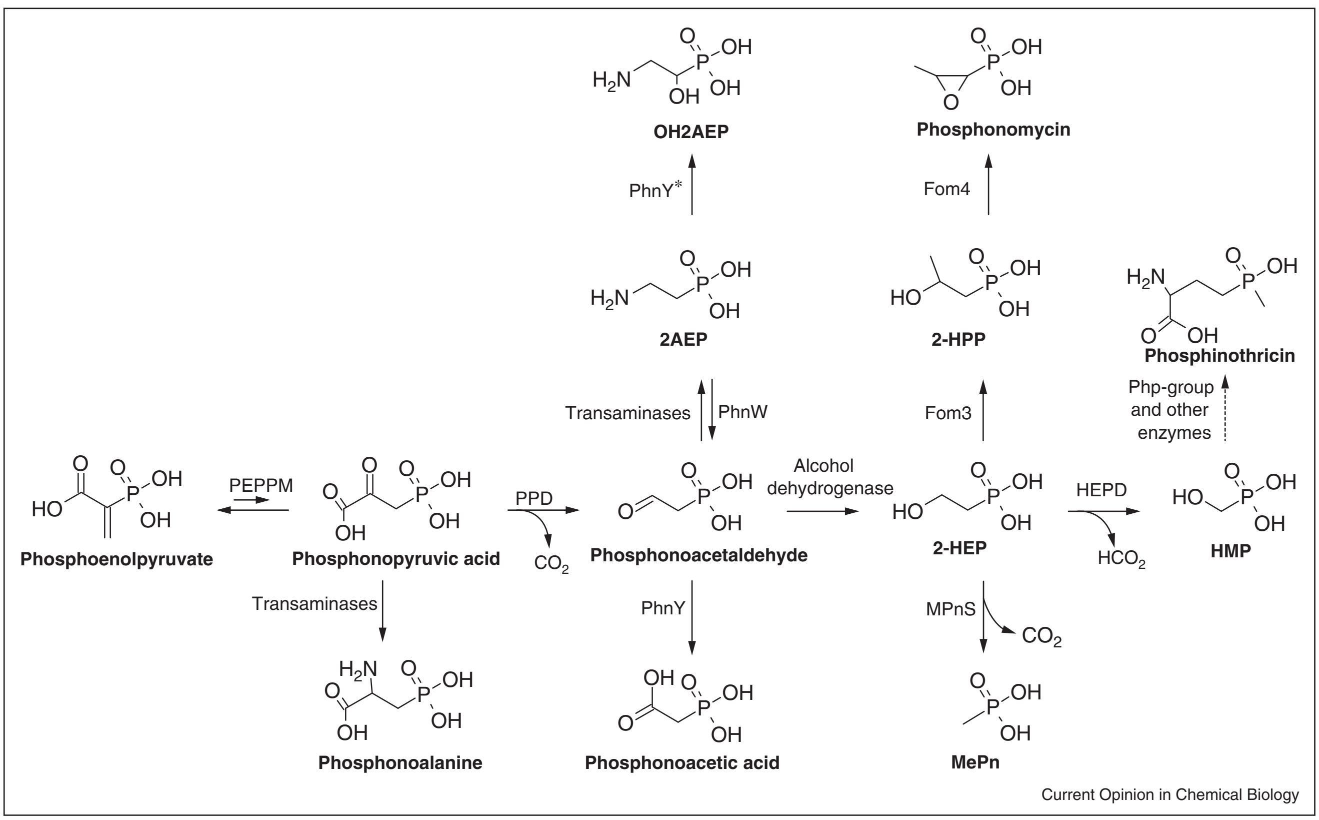
Phosphonate biosynthesis

It is well-established that the initial step in the biosynthesis of the phosphonates is common to virtually all of the known pathways and involves a reversible intramolecular rearrangement of the intermediary metabolite phosphoenolpyruvate (PEP) to phosphonopyruvate by the enzyme PEP phosphomutase (PepM). In most instances this conversion is followed by the irreversible decarboxylation of phosphonopyruvate to produce phosphonoacetaldehyde, which forms the central metabolite for a wide range of subsequent biosynthetic sequences

[18]. These pathways lead to the formation of a variety of low molecular weight molecules (Figure 1) that often possess potent biological activities; their biosynthesis has revealed several previously unreported enzymatic transformations (recently reviewed in [16]). C–P biosynthetic pathways also lead to the formation of macromolecules such as phosphonolipids, phosphonoglycans and phosphonylated glycoproteins. These perform structural functions that remain unclear [19], but their wide distribution in microorganisms and lower eukaryotes means that they are nonetheless likely to constitute the largest source of phosphonate-P in the biosphere.

Some 30 natural products that contain the C–P bond have been described to date, and the pace of discovery is accelerating as new high-throughput protocols for their detection, purification and characterization continue to be developed [19–22]. These techniques will neatly complement recently developed genome-mining strategies in which *pepM* has been used as a molecular marker to identify those genomes that encode C–P biosynthetic capacity [8••]. Thus the range of known phosphonate producers will be extended far beyond those microorganisms that can be readily isolated and cultivated, and specialized C–P metabolites appropriate to exotic ecological niches and microbial lifestyles are likely to be identified. In fact Yu *et al.* [8••] have inferred — on the basis of the strong correlation they observed between PepM phylogeny and the *pepM* genomic context, and on the diversity of *pepM* sequences they obtained — that

Figure 1



Biosynthetic pathways of a range of common natural phosphonates. The biosynthesis of almost all natural phosphonates begins with the conversion of phosphoenolpyruvate to phosphonopyruvic acid by phosphoenolpyruvate phosphomutase (PEPPM). The equilibrium of this reaction strongly favours the phosphate, and so the phosphonate must be rapidly converted to a more stable molecule such as phosphonoalanine by transaminases or phosphonoacetaldehyde (PnAcHyde) by the phosphonopyruvate decarboxylase enzyme (PPD). Phosphonoacetaldehyde can then be converted into many different phosphonates depending on which pathways are present in the organism. 2-aminoethylphosphonic acid (2AEP) can be produced by the transamination of PnAcHyde, and subsequent oxidation by the 2AEP dioxygenase PhnY* will produce 1-hydroxy-2-aminoethylphosphonic acid (OH2AEP). Phosphonoacetaldehyde dehydrogenase (PhnY) can convert PnAcHyde into phosphonoacetic acid. Alternatively, the conversion of PnAcHyde to 2-hydroxyethylphosphonic acid (2-HEP) by alcohol dehydrogenases is required for the production of a different group of phosphonates. 2-HEP can be converted directly to methylphosphonic acid by methylphosphonate synthase (MPnS), or to 2-hydroxypropylphosphonic acid (2-HPP) by the Fom3 methyltransferase and subsequently to the antibiotic phosphonomycin by 2-HPP epoxidase (Fom4). Finally, 2-HEP dioxygenase produces hydroxymethylphosphonic acid (HMP) from 2-HEP which, after several other transformations, leads to the production of phosphinothricin [[16], and references within]. The most recently described findings related to these transformations are discussed in this review.