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Current Opinion in Chemical Biology

The enzymatic conversion of phosphonates to phosphate by bacteria

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Phosphonates are ubiquitous organophosphorus compounds that contain a characteristic C–P bond which is chemically inert and hydrolytically stable. Bacteria have evolved pathways to metabolize these phosphonate compounds and utilize the products of these pathways as nutrient sources. This review aims to present all of the known bacterial enzymes capable of transforming phosphonates to phosphates. There are three major classes of enzymes known to date performing such transformations: phosphonatases, the C-P lyase complex and an oxidative pathway for C–P bond cleavage. A brief description of each class is presented.

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Current Opinion in Chemical Biology 2013, **17**:589–596

This review comes from a themed issue on **Mechanisms**

Edited by **Hung-wen Liu** and **Tadhg Begley**

For a complete overview see the Issue and the Editorial

Available online 2nd July 2013

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http://dx.doi.org/10.1016/j.cbpa.2013.06.006

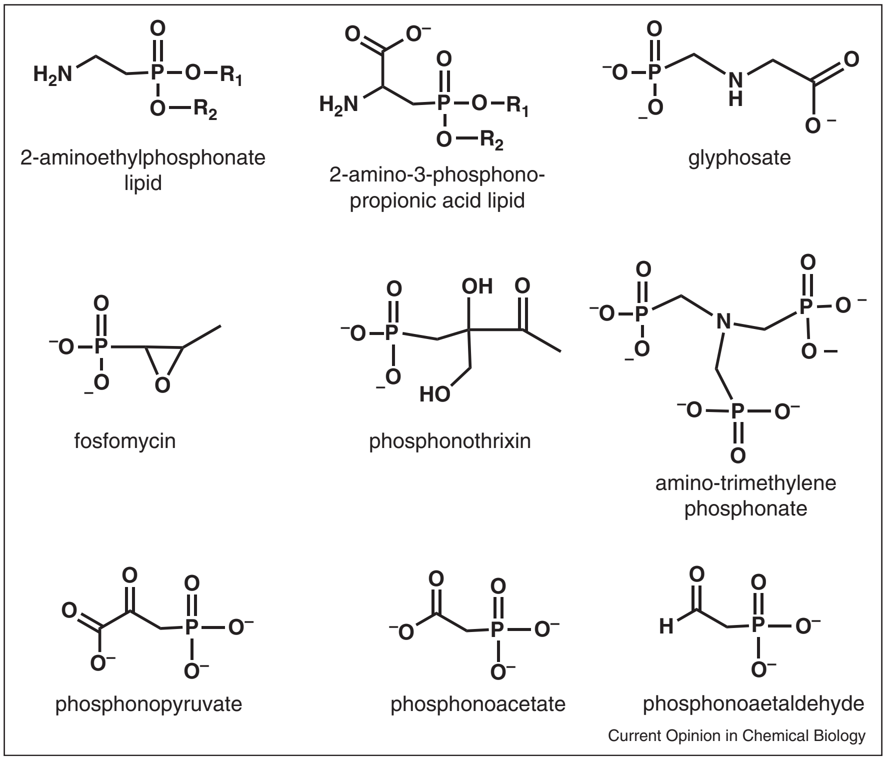
Phosphonates are organophosphorus compounds that contain a characteristic carbon–phosphorus (C–P) bond. This bond is chemically inert, hydrolytically stable and resistant to photolysis. Phosphonates are prevalent in all primitive life forms, where they can exist as integral components of membrane phosphonolipids, such as 2-aminoethylphosphonic acid and 2-amino-3-phosphonopropionic acid (Scheme 1). The presence of phosphonates in these lipid membranes confers rigidity to the membranes and protects the organisms against light and degradation from phosphatases [1–3]. Biologically relevant exopolysaccharides and glycoproteins also contain phosphonate moieties. Phosphonates are found extensively in antibiotics such as fosfomycin and phosphonothrixin, the herbicide glyphosate and the industrial detergent additive amino-trimethylene phosphonate (Scheme 1) [1–3]. It is estimated that in the US alone more than 20 000 tons of phosphonates are released annually into the environment in the form of herbicides and detergent wastes. With such large quantities of phosphonates being released into the environment, there is a significant interest in understanding the mechanisms by which phosphonates are degraded or metabolized by bacterial species [1]. The abundance and universal prevalence of phosphonates in the environment has led to the evolution of several bacterial species that are able to metabolize and utilize phosphonates as carbon and phosphorus sources [2–4]. There are three known classes of enzymes or enzymatic systems that have been mechanistically characterized which are capable of breaking the C–P bonds of phosphonate compounds. These include phosphonate hydrolases, the C-P lyase complex, and an oxidative pathway.

Phosphonate hydrolases

Phosphonate hydrolases have been generically referred to as 'phosphonatases'. The characteristic feature of the substrates for the phosphonatases is the presence of an electron withdrawing β-carbonyl group that facilitates bond delocalization and allows the heterolytic cleavage of the C–P bond. The phosphonate substrates hydrolyzed by this group of enzymes include phosphonopyruvate (PnPy), phosphonoacetate (PAA) and phosphonoacetaldehyde (Pald) (Scheme 1). Proteins belonging to the phosphonatase class of enzymes have evolved from different enzyme superfamilies, and have been characterized mechanistically and structurally.

The first reported phosphonopyruvate hydrolase (PPH) was identified from cell free extracts of an environmental isolate capable of utilizing phosphonoalanine as a carbon, nitrogen and phosphorus source, from *Burkholderia cepacia* Pal6 [5]. The PPH reaction is presented in Figure 1a, where phosphonopyruvate is converted to pyruvate and orthophosphate. On the basis of amino acid sequence identity, the gene for PPH has also been identified in *Variovorax* sp. Pal2, another environmental sample obtained from a soil isolate [6]. PPH belongs to the phospho(enol)pyruvate (PEP) mutase/isocitrate lyase superfamily of enzymes [7•]. PPH has a 40% amino acid sequence identity to PEP mutase and has a $(\beta/\alpha)_8$-barrel structural fold. The monomers associate as a tetramer and the 8th α-helix is swapped between two dimers [7•]. There are three available structures for PPH from *Variovorax* sp. Pal2: apo-enzyme (PDB id: 2HRW), PPH complexed with Mg^{2+} and phosphonopyruvate (PDB id: 2HJP), and PPH complexed with Mg^{2+} and oxalate (PDB id: 2DUA). The active site Mg^{2+} anchors the

Scheme 1



Structures of important phosphonates.

phosphonopyruvate substrate. On the basis of the available structures of PPH and mechanistic experiments, a consensus catalytic mechanism analogous to that of PEP mutase has been proposed (Figure 2) [5,6,7•,8]. PPH does not utilize any other phosphonate compounds besides phosphonopyruvate [6].

Phosphonoacetate is biogenically available to bacteria from the degradation of 2-aminoethylphosphonate (2AEP) [9•]. The first phosphonoacetate hydrolase (PAH) activity was found in *Pseudomonas fluorescens* 23F, a bacterial isolate from the sludge of a laundry waste treatment plant in Ireland [10]. PAH catalyzes the hydrolysis of phosphonoacetate to yield acetate and orthophosphate (Figure 1b). PAH belongs to the alkaline phosphatase superfamily [11]. Members of this superfamily have an active site consisting of a binuclear metal center with a highly conserved serine or threonine residue that is utilized to form a covalent phospho-enzyme adduct as an integral part of the catalytic mechanism [12]. There are two PAH superfamily enzymes that have been extensively characterized: PAH from *P. fluorescens* 23F [9•] and *Sinorhizobium meliloti* 1021 (phnA) [13•]. Both of these enzymes have high specificity for zinc, but the PAH from *S. meliloti* can be activated with Mn^{2+} or Fe^{2+}. The structure of PAH from *P. fluorescens* has been determined in a complex with phosphonoformate (PDB id: 1EI6). The structure of PAH from *S. meliloti* has been determined for the apo-enzyme (PDB id: 3SZY), complexed with phosphonoacetate (PDB id: 3T02), acetate (PDB id: 3SZZ), vanadate (PDB id: 3T00) and phosphonoformate (PDB id: 3T01). On the basis of these crystal structures, PAH possess a catalytic core of the alkaline phosphatase superfamily and uses a conserved threonine residue to form the phospho-enzyme adduct in the catalytic cycle. However, this enzyme possesses a unique capping domain analogous to the nucleoside pyrophosphatase/phosphodiesterase enzyme family that is critical for shielding the substrate from solvent during turnover. A detailed reaction mechanism for the PAH has been proposed (Figure 3) [9•,13•].

Phosphonoacetaldehyde (Pald) is formed biologically by the action of 2AEP-pyruvate transaminase, which uses 2AEP and pyruvate as substrates and yields Pald and L-alanine as products. Pald is subsequently hydrolyzed by phosphonoacetaldehyde hydrolase (PaldH) to form acetaldehyde and orthophosphate (Figure 1c). PaldH belongs to the haloalkanoic acid dehydrogenase (HAD) superfamily of enzymes [14,15]. Members of the HAD superfamily perform diverse set of transformations that require the Mg^{2+} dependent formation of a covalent intermediate to an active site aspartate residue [16]. PaldH from *Bacillus cereus* is the most extensively