Available online at www.sciencedirect.com

SciVerse ScienceDirect

Current Opinion in Chemical Biology

Phosphonate biosynthesis and catabolism: a treasure trove of unusual enzymology

Spencer C Peck[1,2] and Wilfred A van der Donk[1,2]

Natural product biosynthesis has proven a fertile ground for the discovery of novel chemistry. Herein we review the progress made in elucidating the biosynthetic pathways of phosphonate and phosphinate natural products such as the antibacterial compounds dehydrophos and fosfomycin, the herbicidal phosphinothricin-containing peptides, and the antimalarial compound FR-900098. In each case, investigation of the pathway has yielded unusual, and often unprecedented, biochemistry. Likewise, recent investigations have uncovered novel ways to cleave the C–P bond to yield phosphate under phosphorus starvation conditions. These include the discovery of novel oxidative cleavage of the C–P bond catalyzed by PhnY and PhnZ as well as phosphonohydrolases that liberate phosphate from phosphonoacetate. Perhaps the crown jewel of phosphonate catabolism has been the recent resolution of the longstanding problem of the C-P lyase responsible for reductively cleaving the C–P bond of a number of different phosphonates to release phosphate. Taken together, the strides made on both metabolic and catabolic fronts illustrate an array of fascinating biochemistry.

Addresses
[1] Institute for Genomic Biology, University of Illinois at Urbana-Champaign, 1206 West Gregory Drive, Urbana, IL 61801, USA
[2] Department of Chemistry and Howard Hughes Medical Institute, University of Illinois at Urbana-Champaign, 600 S. Mathews Ave, Urbana, IL 61801, USA

Corresponding author: van der Donk, Wilfred A (vddonk@illinois.edu)

Current Opinion in Chemical Biology 2013, **17**:580–588

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 17th July 2013

1367-5931/$ – see front matter,

http://dx.doi.org/10.1016/j.cbpa.2013.06.018

Introduction

With resistance to commonly deployed antibiotics on the rise, new compounds are urgently needed to tackle pathogenic bacteria. Phosphonate and phosphinate natural products have been attractive as they are stable mimics of carboxylic acids and/or phosphate esters, thereby allowing them to potently inhibit enzymes in a variety of metabolic processes. Whereas phosphonates have only a single C–P bond, the less common phosphinates have either two C–P bonds or one C–P bond and a P-H bond [1,2]. The C–P bond can be broken in pathways dedicated to phosphonate catabolism as described in the second part of this review, but it is resistant to acid/base hydrolysis as well as the action of phosphatases and phosphodiesterases. Recent investigations of the pathways responsible for construction and breakdown of organophosphonates/phosphinates have yielded many novel enzymatic transformations [2–4]. This review will focus on pathways and enzymatic mechanisms that have been reported in the period 2009–2013, with particular emphasis on unusual chemistry.

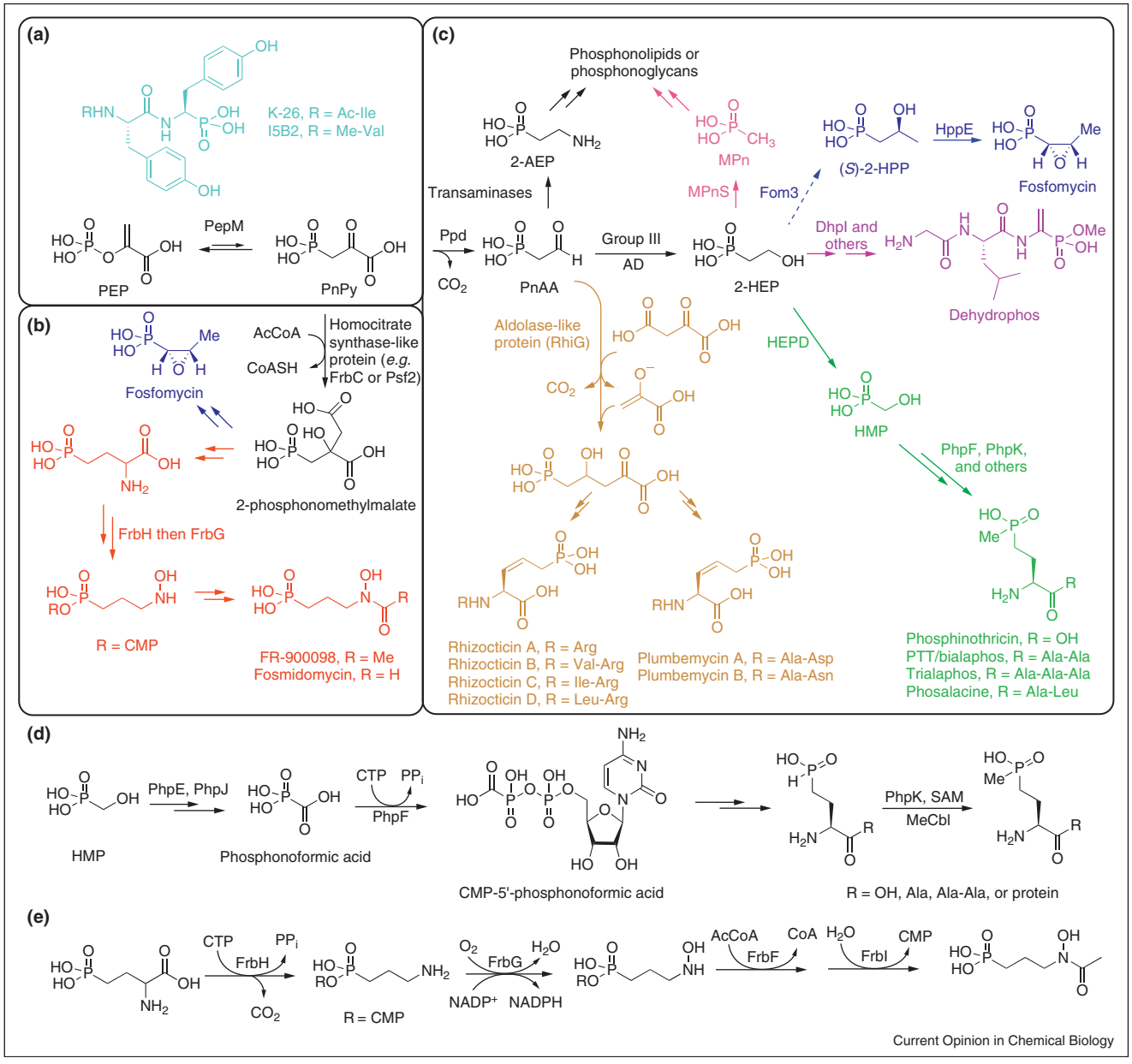
Initial steps in the biosynthesis of phosphonates: PEP mutase and phosphonopyruvate decarboxylase

With the exception of the related phosphonotripeptides K-26 and I5B2 [5••] (Figure 1a, light blue), the first step of all known phosphonate biosynthetic pathways is the reversible conversion of phosphoenolpyruvate (PEP) to phosphonopyruvate (PnPy) by PEP mutase (PepM) (Figure 1a). Because the isomerization equilibrium greatly favors PEP [6], PnPy formation is always connected to an essentially irreversible second step. One strategy is the addition of an acetate anion equivalent to the ketone of PnPy during the biosynthesis of FR-900098 and the fosfomycin biosynthetic pathway in pseudomonads (Figure 1b). A second, more common strategy involves decarboxylation of PnPy by phosphonopyruvate decarboxylase (ppd) to produce phosphonoacetaldehyde (PnAA), which is subsequently functionalized in myriad ways (Figure 1c). These transformations include reduction by metal dependent alcohol dehydrogenases (ADs) to generate 2-hydroxyethyl phosphonate (2-HEP) [7] and transamination to produce the polar head group for phosphonoglycans or phosphonolipids [8] (Figure 1c).

Dehydrophos

The antibiotic dehydrophos is currently the only known naturally occurring phosphonate that is esterified; it also contains an unusual aminovinylphosphonate moiety [10] (Figure 1c, purple). Both functionalities are critical for its antimicrobial activity [11]. Like many phosphonopeptides, dehydrophos is a Trojan horse antibiotic that is taken up by peptide permeases and activated by intracellular proteolysis, which unmasks methyl acetylphosphonate that potently inhibits pyruvate dehydrogenase [11,12] and 1-deoxy-D-xylulose-5-phosphate synthase [13]. Production of dehydrophos in a heterologous host identified sixteen genes that were required for dehydrophos production [12]. Comparison to homologous

Figure 1



Overview of phosphonate biosynthesis. Frequently encountered reactions are shown in black in panels **(a–c)**. Unique transformations towards various family members are shown in color. Because of space constraints, the rhizocticin/plumbemycin pathway (light brown) [9] will not be discussed in this review. (a) All known pathways start with the conversion of PEP to PnPy except for the biosynthesis of K-26 and I5B2. (b) During the biosynthesis of FR-900098 in actinomycetes and fosfomycin in pseudomonads, an acetate group is added to the ketone of PnPy. (c) A range of products are accessed from PnAA after decarboxylation of PnPy. AD = alcohol dehydrogenase. **(d)** Biochemically intriguing steps in phosphinothricin biosynthesis. The physiological substrate for the *P*-methylase is currently unknown. **(e)** The late stages of FR-900098 biosynthesis.

proteins of known function, construction of genetic knockouts, and *in vitro* characterization of nine proteins allowed construction of a pathway that involves the use of aminoacylated tRNA for non-ribosomal peptide bond formation [7,12,14•,15]. The *S*-adenosyl methionine (SAM) dependent protein responsible for *O*-methylation of dehydrophos, DhpI, is intriguing for both its unusual 'domain-swapped dimer' architecture as well as its relaxed substrate specificity [14•].

Phosphinothricin

Another class of Trojan horse compounds is the phosphinothricin (PT) containing peptides (Figure 1c, green) such as phosphinothricin tripeptide (PTT). PT is the