Biochimica et Biophysica Acta 1844 (2014) 2135–2144

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbapap

Spectroscopic characterization and mechanistic investigation of P-methyl transfer by a radical SAM enzyme from the marine bacterium *Shewanella denitrificans* OS217

Kylie D. Allen [1], Susan C. Wang *

School of Molecular Biosciences, College of Veterinary Medicine, PO Box 647520, Washington State University, Pullman, WA 99164-7520, USA

ARTICLE INFO

Article history:
Received 26 July 2014
Received in revised form 5 September 2014
Accepted 7 September 2014
Available online 16 September 2014

Keywords:
Radical *S*-adenosyl-L-methionine
Methyltransferase
Cobalamin
Phosphinate

ABSTRACT

Natural products containing carbon–phosphorus bonds elicit important bioactivity in many organisms. L-Phosphinothricin contains the only known naturally-occurring carbon–phosphorus–carbon bond linkage. In actinomycetes, the cobalamin-dependent radical *S*-adenosyl-L-methionine (SAM) methyltransferase PhpK catalyzes the formation of the second C–P bond to generate the complete C–P–C linkage in phosphinothricin. Here we use electron paramagnetic resonance and nuclear magnetic resonance spectroscopies to characterize and demonstrate the activity of a cobalamin-dependent radical SAM methyltransferase denoted SD_1168 from *Shewanella denitrificans* OS217, a marine bacterium that has not been reported to synthesize phosphinothricin. Recombinant, refolded, and reconstituted SD_1168 binds a four-iron, four-sulfur cluster that interacts with SAM and cobalamin. In the presence of SAM, a reductant, and methylcobalamin, SD_1168 surprisingly catalyzes the P-methylation of N-acetyl-demethylphosphinothricin and demethylphosphinothricin to produce N-acetyl-phosphinothricin and phosphinothricin, respectively. In addition, this enzyme is active in the absence of methylcobalamin if the strong reductant titanium (III) citrate and hydroxocobalamin are provided. When incubated with [methyl-^{13}C] cobalamin and titanium citrate, both [methyl-^{13}C] and unlabeled N-acetylphosphinothricin are produced. Our results suggest that SD_1168 catalyzes P-methylation using radical SAM-dependent chemistry with cobalamin as a coenzyme. In light of recent genomic information, the discovery of this P-methyltransferase suggests that *S. denitrificans* produces a phosphinate natural product.

© 2014 Elsevier B.V. All rights reserved.

1. Introduction

Phosphonate and phosphinate natural products contain carbon–phosphorus (C–P) bonds and have noteworthy bioactivities due to their structural similarities to phosphate esters, carboxylic acids, and various tetrahedral intermediates in enzymatic reactions [1]. These compounds are widely used as antimicrobial, antifungal, and herbicidal agents [2–4]. Such applications, as well as the extraordinary biochemical reactions thought to be involved in the biosynthesis of C–P compounds, have inspired a wealth of research. The majority of C–P compound biosynthetic pathways begin with the reaction catalyzed by phosphoenolpyruvate mutase (PepM), which forms the initial C–P bond via isomerization of phosphoenolpyruvate to generate phosphonopyruvate. This energetically unfavorable reaction is usually driven by decarboxylation catalyzed by phosphonopyruvate decarboxylase (Ppd) to generate phosphonoacetaldehyde [1].

L-Phosphinothricin (PT; L-2-amino-4-hydroxymethylphosphinylbutanoate) is an unusual amino acid that contains the only known naturally occurring C–P–C bond sequence (Fig. 1A). PT is a L-glutamate analog and has herbicidal and antimicrobial activities through inhibiting plant and bacterial glutamine synthetases [5–7]. PT is produced as part of a tripeptide by *Streptomyces hygroscopicus*, *Streptomyces viridochromogenes*, and *Kitasatospora phosalacinea* [8–10]. In the two *Streptomyces* species, at least 24 genes are required for biosynthesis of the PT tripeptide, L-PT-Ala–L-Ala (PTT) [11,12]. Although the biosynthetic pathway for phosalacine (L-PT–L-Ala–L-Leu) in *K. phosalacinea* has not been investigated, it is likely to be similar. These tripeptides are readily absorbed by target cells, where intracellular peptidases release the active PT antibiotic. In the latter stages of PT biosynthesis, the P-methyltransferase PhpK is thought to append a methyl group to the

Abbreviations: 2-HEP, 2-hydroxyethylphosphonate; 2-HPP, 2-hydroxypropylphosphonate; [4Fe-4S], four-iron, four-sulfur; Ado-CH_2•, 5′-deoxyadenosyl radical; Ado-CH_3, 5′-deoxyadenosine; Cbl, cobalamin; CH_3Cbl, methylcobalamin; DMPT, L-2-amino-4-hydroxyphosphinylbutanoate or demethylphosphinothricin; NAcDMPTT, NAcDMPT tripeptide; NAcPT, N-acetylphosphinothricin; NAcPTT, NAcPT tripeptide; NMR, nuclear magnetic resonance; PT, L-2-amino-4-hydroxymethylphosphinylbutanoate or L-phosphinothricin; PTT, phosphinothricin tripeptide; SAH, *S*-adenosylhomocysteine; SAM, *S*-adenosyl-L-methionine; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; UV–vis, ultraviolet–visible

* Corresponding author at: Washington State University, School of Molecular Biosciences, BLS 202, PO Box 647520, Pullman, WA 99164-7520, USA. Tel.: +1 509 335 7714.

E-mail addresses: kdallen@vt.edu (K.D. Allen), susan_wang@wsu.edu (S.C. Wang).

[1] Present address: Department of Biochemistry, Virginia Polytechnic Institute and State University, 111 Engel Hall, Mail Code 0308, Blacksburg, VA 24061, USA.

http://dx.doi.org/10.1016/j.bbapap.2014.09.009
1570-9639/© 2014 Elsevier B.V. All rights reserved.

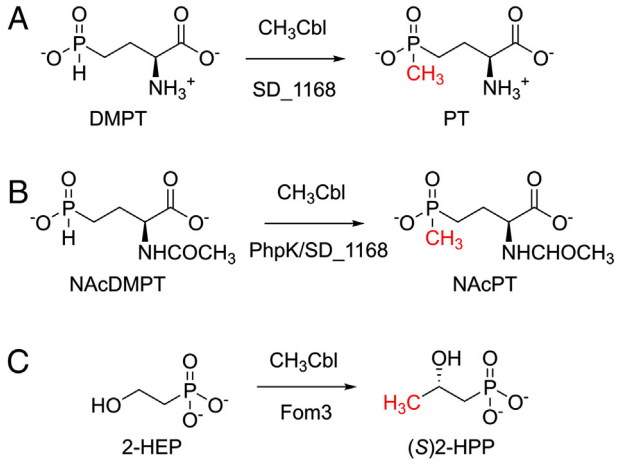

Fig. 1. P-methylation and C-methylation reactions of interest.

phosphinate precursor 2-acetylamino-4-hydroxyphosphinylbutanoate (N-acetyldemethylphosphinothricin or NAcDMPT) to produce 2-acetylamino-4-hydroxymethylphosphinylbutanoate (N-acetylphosphinothricin or NAcPT), which contains the final C–P–C bond sequence (Fig. 1B) [11,13–15]. In a randomly-generated *S. hygroscopicus* mutant that could not catalyze P-methylation, NAcDMPT and its tripeptide, N-acetyldemethylphosphinothricin tripeptide (NAcDMPTT) accumulated, suggesting that these two N-acetylated metabolites were substrates for PhpK (Fig. 1B) [14]. Furthermore, only the N-acetylated precursors were methylated by *S. hygroscopicus* cell lysates while the corresponding non-acetylated precursors, demethylphosphinothricin (DMPT; 2-amino-4-hydroxyphosphinylbutanoate) and DMPT tripeptide (DMPTT), were not methylated. Isotopic labeling studies demonstrated that methylcobalamin (CH_3Cbl) was the methyl group donor for the P-methylation reaction [5,13].

In 2001, PhpK was identified as a radical *S*-adenosyl-L-methionine (SAM) superfamily member based on the presence of a highly conserved CxxxCxxC motif whose cysteine residues coordinate a four-iron, four-sulfur ([4Fe–4S]) cluster [16]. The reduced $[4Fe–4S]^{+1}$ cluster and SAM are used to generate a 5′-deoxyadenosyl radical (Ado-CH_2•) that abstracts a hydrogen atom from a substrate as the first step of catalysis (Fig. 2) [17]. PhpK belongs to a subset of radical SAM enzymes that contain a cobalamin (Cbl)-binding domain [16,18]. In vitro studies in our laboratory demonstrated that PhpK from *K. phosalacinea* catalyzes the P-methylation of NAcDMPT to produce NAcPT in a SAM-, sodium dithionite-, and CH_3Cbl-dependent manner (Fig. 1B) [15]. Three related Cbl-dependent radical SAM methyltransferases, TsrM, GenK, and Fom3, have been reported upon since the initial PhpK work [19–21]. These enzymes are found in bacterial biosynthetic pathways for the antibiotics thiostrepton, gentamicin, and fosfomycin, respectively. Although some similarities exist between known members of the Cbl-dependent radical SAM family, a variety of differences have been reported, and many mechanistic details remain unresolved.

Here, we describe the characterization of a Cbl-dependent radical SAM methyltransferase encoded by the *sden_1168* gene from the denitrifying marine bacterium *Shewanella denitrificans* OS217 [22]. We will refer to the resulting protein as SD_1168. *S. denitrificans* OS217 has not been reported to biosynthesize known C–P compounds. However, its genome encodes phosphoenolpyruvate mutase (PepM; *sden_1161*) and phosphonopyruvate decarboxylase (Ppd; *sden_1162*, annotated as a thiamine pyrophosphate-dependent enzyme) (Fig. 3) near the *sden_1168* gene, suggesting that this organism has the capacity to produce C–P compounds [23].

Although the biological function of SD_1168 is currently unknown, the protein shares significant identity and similarity with both Fom3 and PhpK (34% and 53%, and 13% and 31%, respectively), methyltransferases involved in C–P compound biosynthesis [15,21]. Fom3 is required for the penultimate step of fosfomycin biosynthesis, where it adds a methyl group to the sp^3-hybridized C-2 carbon of 2-hydroxyethylphosphonate (2-HEP) to generate *S*-2-hydroxypropylphosphonate (*S*-2-HPP) (Fig. 1C) [21,24,25]. The significant sequence similarity between SD_1168 and Fom3 led us to hypothesize that SD_1168 might catalyze a similar, or even the same, C-methylation as Fom3. To date, we have not observed SD_1168 C-methylation activity upon 2-HEP. Instead, we found that SD_1168 unexpectedly catalyzes P-methylation upon both NAcDMPT and DMPT to generate the final C–P–C bond sequence found in PT (Fig. 1A and B). We show that SD_1168 is likely to perform radical SAM chemistry using a Cbl coenzyme for this difficult reaction. Since PT and its derivatives are the only naturally-occurring phosphinates discovered to date, our results suggest that *S. denitrificans* synthesizes an as-yet undiscovered phosphinate natural product.

2. Materials and methods

2.1. Materials

Reagents were obtained from typical suppliers unless otherwise indicated. Titanium(III) (Ti) citrate and [methyl-^{13}C]Cbl were synthesized as described elsewhere [26,27]. SAM was acquired from Safeway and was purified as described elsewhere [15]. 2-HEP was synthesized as described elsewhere [21].

2.2. Cloning of sden_1168 from S. denitrificans OS217 (GenBank accession ABE54454.1)

S. denitrificans OS217 was obtained from the American Type Culture Collection (ATCC) (ATCC-BAA 1090), was reconstituted in liquid media according to ATCC recommendations, and was streaked onto marine broth 2216 (Difco, Sparks, MD) agar plates to obtain isolated colonies. A single colony was used to inoculate 5 mL of marine broth, and the culture was incubated with shaking at 30 °C overnight. Genomic DNA was isolated from the overnight culture using the Wizard Genomic DNA purification kit (Promega, Madison, WI). The *sden_1168* gene was amplified from *S. denitrificans* genomic DNA using PCR. The forward primer was 5′-TATATACATATGCGACCAAATTTTTA-3′ and the reverse primer

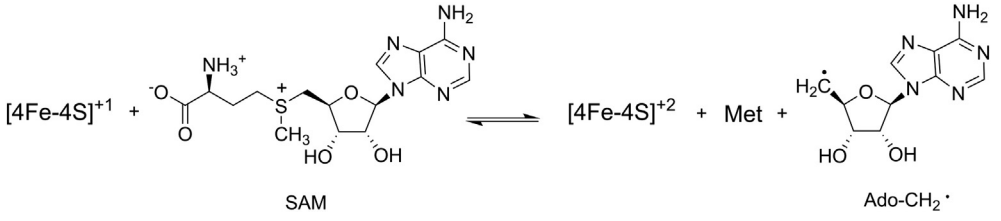

Fig. 2. Radical SAM cleavage.

Download English Version:

https://daneshyari.com/en/article/7560833

Download Persian Version:

https://daneshyari.com/article/7560833

Daneshyari.com