STUDIES ON THE BIOSYNTHESIS OF FOSFOMYCIN

2. CONVERSION OF 2-HYDROXYPROPYLPHOSPHONIC ACID TO FOSFOMYCIN BY BLOCKED MUTANTS OF *Streptomyces wedmorensis*†

Sir:

Several antibiotics containing a unique C-P bond have been reported to occur in nature. These include bialaphos[2] (BA), fosfomycin[3] (FM), FR-33289[4], phosphazinomycin[5], phosalacine[6] and fosfonochlorin[7]. FM which possesses a C_3 skeleton with an epoxide is known to be the simplest and smallest member of this class, and is produced by various species of *Streptomyces*[3,8]. FM is effective against Gram-positive and Gram-negative bacteria, and now is in clinical use. Its adenylated derivative, fosfadecin[9], was reported to be produced by *Pseudomonas viridiflava*. ROGERS and BIRNBAUM reportd that the methyl moiety of FM was derived from a methionyl methyl group, and suggested that the C-2 unit next to the phosphonic acid group was derived from phosphoenolpyruvic acid (PEP)[8].

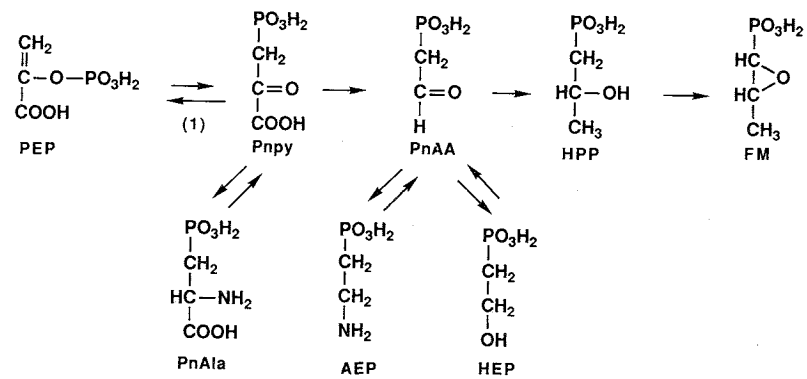
BA is a tripeptide produced by *Streptomyces hygroscopicus* which contains a unique C-P-C bond[2]. During studies on the biosynthesis of BA, we determined that one of the C-P bonds in BA was formed by intramolecular rearrangement of PEP to phosphonopyruvic acid (Pnpy) catalyzed by phosphoenolpyruvate phosphomutase[10] (PEPPM, Fig. 1 (1)). Other findings are also in agreement with the assumption that PEPPM is common to all of the organisms producing C-P compounds[10]. In further support of this hypothesis, we proved the presence of PEPPM in *Tetrahymena* sp., a protozoa producing 2-aminoethylphosphonic acid (AEP) in collaboration with the Harvard group[11], and in *Pseudomonas gladioli* B-1 that produced 2-hydroxyethylphosphonic acid (HEP)[12]. We have recently succeeded in detecting this enzymatic activity in cell free extracts of FM producing *Streptomyces wedmorensis*††.

As reported previously, both AEP and HEP, which were proven to be intermediates in BA biosynthesis[13], were converted to FM presumably *via* phosphonoacetaldehyde (PnAA) by a FM non-producing mutant NP-7[1]. Thus, we concluded that the pathway leading from PEP to HEP was common to both the BA and FM biosyntheses including the unique PEPPM catalyzed reaction (Fig. 1, (1)).

The final stage of FM biosynthesis including epoxide formation remained unresolved, however, epoxidation of *cis*-propenylphosphonic acid (PPOH) seemed to be the most likely route to FM. In fact FM is commercially produced by making use of this reaction. To our surprise, however, all attempts to

Fig. 1. Proposed biosynthetic pathway of fosfomycin (FM).

PEP: Phosphoenolypyruvic acid, Pnpy: phosphonopyruvic acid, PnAA: phosphonoacetaldehyde, HPP: 2-hydroxypropylphosphonic acid, PnAla: phosphonoalanine, AEP: 2-aminoethylphosphonic acid, HEP: 2-hydroxyethylphosphonic acid.



† See ref 1.

†† Previously, we reported the failure to detect PEPPM activity in a FM producing strain of *S. wedmorensis*[10]. Recently, we succeeded in detecting this activity using a high FM producing strain, *S. wedmorensis* 144-91. Details will be reported elsewhere.

Table 1. Transformation of phosphonic acid derivatives to fosfomycin (FM) by blocked mutants of *Streptomyces wedmorensis* 144-91.

Phosphonic acid derivatives (μg/ml)		FM produced by mutants (μg/ml)	
		NP-7	A16
None		0	0
Vinylphosphonic acid	200	0	0
cis-Propenylphosphonic acid	100	0	0
2-Hydroxypropylphosphonic acid	100	8.4	2.2
2-Hydroxypropylphosphonic acid	500	24.5	9.0

convert PPOH to FM using blocked mutants were unsuccessful (Table 1). This result is in striking contrast to the findings that several FM-non-producing microorganisms could stereospecifically transform PPOH to FM[14].

In agreement with our experimental results, HAMMERSCHMIDT and KÄHLIG observed the failure of the incorporation of $^{18}O_2$ into FM by *Streptomyces fradiae*, and proposed that the most plausible mechanism for the formation of the epoxide ring of FM is dehydrogenation of 2-hydroxypropylphosphonic acid (HPP)[15].

In order to reveal the pathway from HEP to FM, we have attempted to convert two putative intermediates, vinylphosphonic acid and HPP to FM by FM non-producing mutants of *S. wedomorensis* 144-91 designated as A16 and NP-7. NP-7 could transform HEP to FM as previously reported[1]. A16 is a newly isolated FM non-producing mutant, and when hydroxocobalamin (1 μg/ml) was added to the production medium, A16 could produce FM in an approximately equal amount to that produced by the parent strain. Thus, it was proposed that A16 is defective in the biosynthesis of vitamin B_{12} required in the methylation of a C_2 biosynthetic intermediate (possibly a PnAA analog).

These mutants were cultivated as reported previously[1] in the production medium containing starch 4%, salad oil 1.5%, Sungrain 5%, wheat germ 2%, K_2HPO_4 0.1% and $CoCl_2$ 0.0001% (pH 8.0). Chemically synthesized HPP (racemic form)[16] and vinylphosphonic acid[17] were added separately at the beginning of the fermentation. After 5 days, the yield of FM was determined by biological assay using *Proteus* sp. MB838. As shown in Table 1, NP-7 produced FM by the addition of HPP, but not through the addition of vinylphosphonic acid. Formation of FM was confirmed by bioautography against *Proteus* sp.[1] These results clearly showed that the epoxide ring of FM is formed by dehydrogenation of HPP excluding the involvement of PPOH.

Since A16 defective in the biosynthesis of vitamin B_{12} converted HPP to FM, incorporation of the methyl group into the FM molecule is suggested to occur during a stage between HEP and HPP. However, no C-P compound was detected in the fermentation broth of A16 so far tested (data not shown). This may be due to the instability of the intermediate which seems to be most likely PnAA formed by decarboxylation of Pnpy. Fig. 1 shows the biosynthetic pathway of FM based on the results described. Further investigation using blocked mutants is now under way.

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