Note

Studies on the Biosynthesis of Fosfomycin. Conversion of 2-Hydroxyethylphosphonic Acid and 2-Aminoethylphosphonic Acid to Fosfomycin

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In a previous paper, we described that two contiguous carbons adjacent to the phosphorous atom in the herbicide, bialaphos, originated from glucose *via* phosphoenolpyruvate.[1] Similar results were also reported for the biosynthesis of fosfomycin[2] and FR-33289.[3] More recently, we isolated 2-hydroxyethylphosphonic acid from the fermentation broth of a blocked mutant of *Streptomyces hygroscopicus* SF-1293, a producing organism of bialaphos, and proved the metabolite to be a true intermediate located in the early stage of the biosynthesis of bialaphos.[4]

This finding suggested that 2-hydroxyethylphosphonic acid may also be involved in the biosynthesis of other C-P compounds (see Fig. 1) such as fosfomycin, FR-33289 and plumbemycin.[5] In order to prove this hypothesis, we tried to transform 2-hydroxyethylphosphonic acid and 2-aminoethylphosphonic acid to fosfomycin by a blocked mutant of *Streptomyces wedmorensis* ATCC 21239, the results being reported here.

The blocked mutant NP-7 obtained by treating with nitrosoguanidine did not produce any fosfomycin. This microorganism was cultivated in S-1 medium for one day as reported previously[6] and then 0.6 ml each of the broth was transferred to 250 ml Erlenmeyer flasks containing 20 ml of the production medium consisting of 4% starch, 1.5% salad oil, 5% sungrain, 2% wheat germ, 0.1% K_2HPO_4 and 0.0001% $CoCl_2 \cdot 6H_2O$, the pH being adjusted to 8.0. Fermentation was carried out on a rotary shaker at 28°C. 2-Aminoethylphosphonic acid and 2-hydroxyethylphosphonic acid were separately added to the flasks at the beginning of fermentation. After 5 days, the amount of fosfomycin was determined by a biological assay against *Proteus* sp. MB 838.

As shown in Table I, the addition of substrates at zero time caused the production of fosfomycin at a level of 14～70 μg/ml, compared with the control (no addition) being 0 μg/ml. This result suggests that both 2-hydroxyethylphosphonic acid and 2-aminoethylphosphonic acid were converted to fosfomycin. The identity of the active substance as fosfomycin was confirmed by comparing with an authentic sample by TLC-bioautography (silica gel, MeOH–2% aq. NaCl solution = 75 : 25, *Rf* 0.39; cellulose, BuOH–AcOH–H_2O = 3 : 1 : 1, *Rf* 0.37 against *Proteus* sp. MB 838).

Thus, 2-hydroxyethylphosphonic acid and 2-aminoethylphosphonic acid, which may be interchanged by the producing organism, turned out to be common biosynthetic intermediates for bialaphos and fosfomycin. It will be interesting to investigate whether these phosphonic acids are also biosynthetic intermediates for FR 33289 and plumbemycin.

Although 2-hydroxyethylphosphonic acid was converted to fosfomycin more efficiently than 2-aminoethylphos-

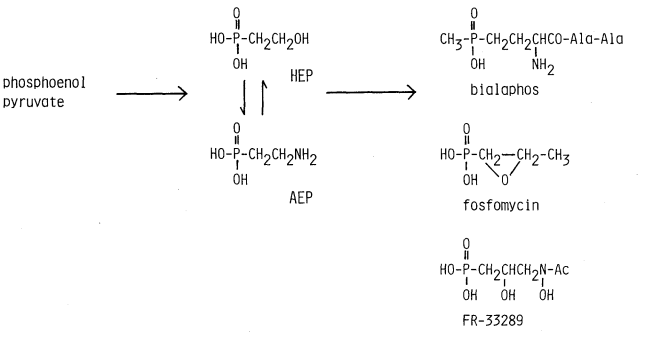

FIG. 1. The Structures of Bialaphos and Related C-P Compounds.
HEP, hydroxyethylphosphonic acid; AEP, aminoethylphosphonic acid; ala-ala, alanylalanine.

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TABLE I. TRANSFORMATION OF 2-HYDROXYETHYLPHOSPHONIC ACID AND 2-AMINOETHYLPHOSPHONIC ACID TO FOSFOMYCIN BY BLOCKED MUTANT NP-7 OF *Streptomyces wedmorensis*

Precursor	Concentration (μg/ml)	Fosfomycin produced[b] (μg/ml)
None	—	0
HEP[a]	200	21
HEP	1000	70
AEP[a]	200	14
AEP	1000	55

[a] HEP, 2-hydroxyethylphosphonic acid; AEP, 2-aminoethylphosphonic acid.

[b] Determined by biological assay against *Proteus* sp. MB 838. The precursors were added to the medium at zero time.

phonic acid as shown in Table I, it cannot necessarily be concluded that the former is an advanced precursor for the antibiotic, since the availability of these two precursors may be affected by the difference in their permeability into the cells of the producing organism. There is a possibility that prior to the incorporation into fosfomycin, these two compounds are transformed to phosphonoacetaldehyde, a well-known metabolite of phosphonopyruvic acid,[7] which is believed to be formed by the intramolecular rearrangement of phosphoenolpyruvate. The detailed mechanism for the conversion of these two metabolites to fosfomycin still remains to be clarified.

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