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Short Communication

Methionine inhibition of thienamycin formation

Masaru Uyeda* and Arnold L. Demain

Fermentation Microbiology Laboratory, Department of Applied Biological Sciences, Massachusetts Institute of Technology, Cambridge, MA, U.S.A.

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SUMMARY

Methionine interference in the formation of thienamycin by *Streptomyces cattleya* is due, to a major extent, to inhibition of enzyme activity.

INTRODUCTION

The biosynthesis of the carbapenem antibiotic, thienamycin (Fig. 1) has been studied by Albers-Schönberg et al. [1] and Williamson et al. [5]. They found the precursors of the molecule to be acetate, methionine, cysteine and glutamate. Despite the fact that the methyl group of methionine is the precursor of the two carbons of the hydroxyethyl group attached to C-6, methionine interferes with the production of the antibiotic by resting cells [5]. In the present work, we show that this interference is mainly due to inhibition of the activity of one or more thienamycin synthetases.

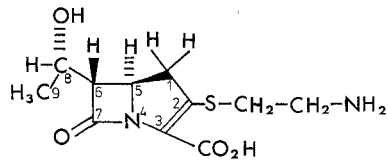

Fig. 1. Structure of thienamycin.

MATERIALS AND METHODS

The strain of *Streptomyces cattleya* used was NRRL-8057 (MA 4297). The media, growth of the organism and preparation of resting cells were as previously described [4].

Resting cell incubations were carried out in Erlenmeyer flasks (250 ml) on a rotary shaker (250 rpm, 5 cm radius) at 30°C for 6 h. Each flask contained the following (in a total volume of 4.0 ml): cells (40–50 mg dry cell weight), 50 mM sodium MES buffer (pH 7.0), 0.1 mM $CoCl_2$, 0.625 mM ATP, 1.25 mM phospho*enol*pyruvate, 0.1 mg pyruvate kinase, 5 mM $MgSO_4$, 2 mM sodium acetate, 2 mM monosodium glutamate and 0.5 mM

* Present address: Laboratory of Medicinal Microbiology, Faculty of Pharmaceutical Sciences, Kumamoto University, Kumamoto, Japan.
Correspondence: A.L. Demain, Fermentation Microbiology Laboratory, Department of Applied Biological Sciences, Massachusetts Institute of Technology, Cambridge, MA 02139, U.S.A.

cystine. Aliquots (0.5 ml) of the incubation mixture were sampled at 0, 3 and 6 h of incubation. After incubation, the cells were removed by centrifugation. The amount of thienamycin produced was determined by the disk assay method. *Staphylococcus aureus* ATCC 6538P was seeded into the upper layer of a double layer of nutrient agar supplemented with 0.2% yeast extract. The agar plates were incubated overnight at 37°C. Data are expressed as average values of duplicate experiments. The standard for the assay was imipenem (*N*-formimidoylthienamycin; MK-787).

RESULTS

The ability of methionine to interfere with thienamycin production by resting cells [5] was confirmed by us. Suppression amounted to 50% at 1 mM methionine and 100% at 4 mM methionine. Since methionine showed no inhibitory effect on the assay organism when tested with authentic antibiotic or with culture filtrates, the effect was real and not due to an assay artifact. In contrast to methionine, cystine showed no effect at concentrations up to 5 mM (data not shown).

Since resting cell experiments were performed over a 6 h period, thienamycin production might have been catalyzed by synthetases present at the time of cell harvest as well as by newly formed synthetases. If true, the above experiments and those of Williamson et al. [5] could not have differentiated between negative effects of methionine on synthetase action (inhibition) or on synthetase formation (repression). To determine whether enzyme synthesis plays a role in resting cell production of thienamycin, we added chloramphenicol to the system at 50 μg/ml. (This level did not inhibit the assay organism.) Indeed, we found this protein synthesis inhibitor to inhibit thienamycin production by 30–40%, thus showing that newly synthesized enzymes were contributing to thienamycin formation in the resting cell system. To eliminate newly synthesized enzymes from consideration, the methionine effect was tested in the presence of 50 μg of chloramphenicol per ml. As shown in Fig. 2, methionine showed major inhibitory effects at 1 and 2 mM concentrations.

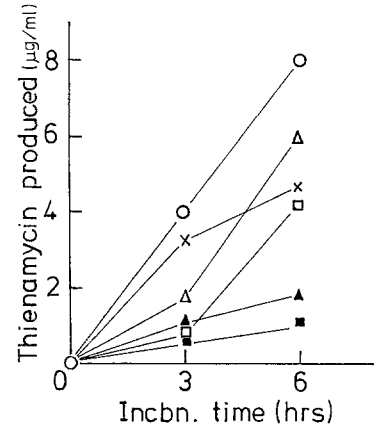

Fig. 2. Inhibition of resting cell production of thienamycin by L-methionine in the presence of chloramphenicol. To the incubation mixture, the following were added: ○, none (control); △, 1 mM methionine; □, 2 M methionine; ×, chloramphenicol (50 μg/ml); ▲, 1 mM methionine plus chloramphenicol; ■, 2 mM methionine plus chloramphenicol.

When compared to methionine, the inhibitory effect of *S*-adenosylmethionine was rather weak (Fig. 3). The *S*-adenosylmethionine was reported to be over 80% pure, and we found by TLC that it was contaminated with only traces of methionine.

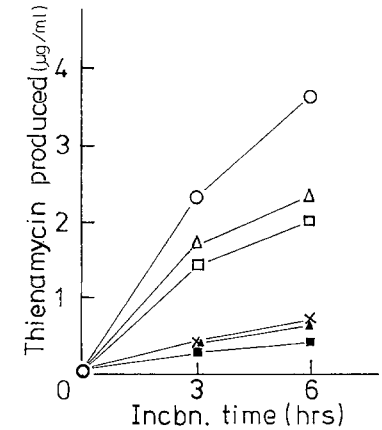

Fig. 3. Inhibition of resting cell production of thienamycin by *S*-adenosylmethionine and methionine in the presence of chloramphenicol. To the incubation mixture containing chloramphenicol, the following were added: ○, none (control); △, 2 mM *S*-adenosylmethionine; □, 5 mM *S*-adenosylmethionine; ×, 2 mM methionine; ▲, 2 mM methionine plus 2 mM *S*-adenosylmethionine; ■, 2 mM methionine plus 5 mM *S*-adenosylmethionine.

It thus appears that the inhibition observed with *S*-adenosylmethionine was not due to methionine contamination. Fig. 3 also shows that *S*-adenosylmethionine does not reverse methionine inhibition. The latter observation makes unlikely the possibility that methionine inhibition is due to an interference in the formation of *S*-adenosylmethionine from methionine. Such interference is known in *Escherichia coli*, but it is due to methionine repression of *S*-adenosylmethionine synthetase rather than inhibition [2]. It should be noted that methyl transfer in thienamycin biosynthesis is a complicated set of reactions possibly involving a corrin intermediate carrier [3]. Any one of these reactions could be the site of methionine inhibition.

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