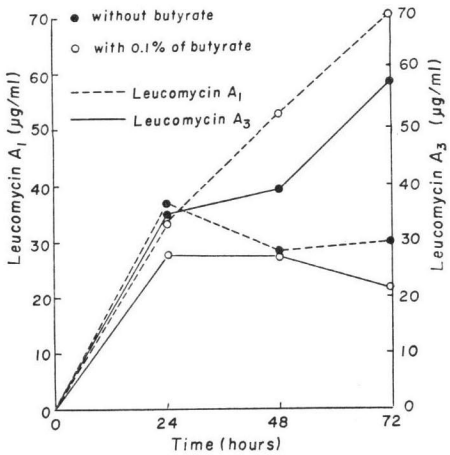
BIOCONVERSION OF LEUCOMYCINS AND ITS REGULATION BY BUTYRATE IN A PRODUCING STRAIN

Sir:

With regard to the biosynthetic study of leucomycin A_3, a 16-membered macrolide antibiotic, we have previously reported that the lactone ring of the antibiotic originates from five acetates, one propionate, one butyrate and two unknown carbons[1]. This result promoted us to examine the effects of these and other related organic acids on the biosynthesis of leucomycins.

The acids tested were as follows: formate, acetate, propionate, butyrate, isobutyrate, valerate, isovaleriate, oxalate, succinate, malonate, 2-methylmalonate, 2-ethylmalonate, 2-propionylmalonate, ethylbutyrate, butyramide and diethyl-2-ethylmalonate. Among them, only butyrate was effective for leucomycin production. As shown in Fig. 1, it was found that leucomycin (LM)A_1 was predominantly accumulated by *Streptomyces kitasatoensis* 66-14-3, one of the major producing strains of leucomycins A_1 and A_3. The structures of leucomycins A_1 and A_3 are shown in Fig. 2. The concentration at which the butyrate showed the most pronounced activity was 0.1% and it was most effective when added prior to the initiation of leucomycin formation. It had no effects on the mycelial growth and consumption of glucose.

Fig. 1. Time course of leucomycin production with or without butyrate.



The culture medium (I) used in this experiment consists of; glucose, 2%; peptone, 0.5%; meat extract, 0.5%; yeast extract, 0.3%; NaCl, 0.5% and $CaCO_3$, 0.3%. Butyrate was added at 0.1% of the final concentration at 0 time. Fermentations were carried out on a reciprocal shaker at 27°C in a 500-ml flask containing 100 ml of the medium.

Leucomycin mixture was assayed spectrophotometrically at OD_{232} using leucomycin A_3 as a standard material. The ratio of leucomycins A_1 and A_3 was determined by FID-TLC (Thinchrograph TFG-10, Iatron Labs.).

Considering the difference in the accumulation of leucomycin components between the cultures with and without butyrate, we examined the interconversion between leucomycins A_1 and A_3. As shown in Table 1, in the control culture, approximately 80% of ^{14}C-leucomycin A_1 was converted to leucomycin A_3, and in the butyrate-supplemented culture, the corresponding conversion was reduced to almost 35%. On the other hand, the conversion of leucomycin A_3 to leucomycin A_1 was not observed. It has been reported in connection with the biosynthetic pathway of platenomycins that the acylation of the lactone ring takes place prior to binding of sugars[2]. However, it has not been mentioned whether or not the acylation of the aglycone moiety in 16-membered macrolide proceeds *via* any other mechanisms. In the present study, it was concluded that leucomycin A_3 is also formed by the bioconversion of leucomycin A_1 and the extent of this reaction was reduced by butyrate.

It may be pointed out that butyrate not only serves as a precursor of the aglycone moiety of leucomycins but also acts as a regulatory factor of the bioconversion of leucomycin A_1 into leucomycin A_3. To determine whether butyrate represses or inhibits the bioconversion, mycelia grown in the presence or absence of butyrate were investigated for their ability to convert leucomycin A_1 to leucomycin A_3. In this resting cell system, activity of the *de novo* synthesis of leucomycin was suppressed by cerulenin[3], an inhibitor of β-ketoacylthioester synthase[4]. As

Fig. 2. Structures of leucomycins A_1 and A_3

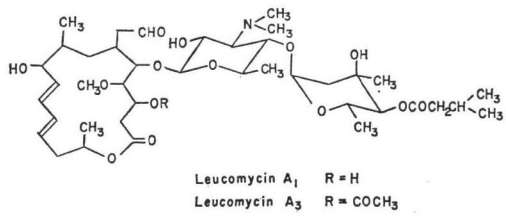


Table 1. Bioconversion of ^{14}C-leucomycin A_1 into leucomycin A_3 in growing cell culture.

Culture	Incubation time (hr)	Radioactivity (cpm)	
		LM A_1	LM A_3
^{14}C-LM A_1	0	4327	84
	24	905	4590
^{14}C-LM A_1 +Butyrate	0	4257	139
	24	3185	1956
^{14}C-LM A_3	0	65	2340
	24	57	1981
^{14}C-LM A_1 +Butyrate	0	92	1436
	24	100	1997

S. kitasatoensis 66-14-3 were grown on a medium I, with or without 0.1% of butyrate at 27°C for 24 hours, after ^{14}C-leucomycin A_1(19,000 cpm) or ^{14}C-leucomycin A_3(8,900 cpm) was supplemented into the culture and the cultivation was continued for an additional 24 hours. Condition of cultivation was as same as the footnote of Fig. 1. ^{14}C-Leucomycins were obtained from feeding of 0.2 μCi/ml of ^{14}C-acetate (54.9 mCi/mM) into the culture.

Leucomycins A_1 and A_3 were extracted with benzene and separated on alumina TLC from each other. The radioactivity was determined with a liquid scientillation counter in a toluene based scintillant.

Table 2. Bioconversion of leucomycin A_1 into leucomycin A_3 in the resting cell system

Cells	Conc. of LM A_1 added (μg/ml)	Convertant LM A_3 (μg/ml)
Control	0	0
Control	50	20.7
Control	50 +Butyrate	18.6
+Butyrate	50	3.3

Resting cells were prepared as follows; *S. kitasatoensis* 66-14-3 were grown in the medium I with or without 0.1% of butyrate for 24 hours at 27°C as same as the footnote of Fig. 1, washed twice with physiological saline and resuspended 2 g wet weight in 10 ml of the solution containing 2% of glucose, 0.5% of NaCl and 20 μg/ml of cerulenin in the presence or absence of 0.1% of butyrate. The bioconversion was carried out in a 50-ml test tube containing 10 ml of the suspension for 3 hours at 27°C on a reciprocal shaker.

shown in Table 2, about 40% of leucomycin A_1 was converted to leucomycin A_3 in the incubation of the washed myclia in the absence of butyrate when the mycelia grown without butyrate were used. The effect of butyrate was not observed in the butyrate-supplemented resting cell incubation. The bioconversion was more likely to be influenced by butyrate in the growing cell culture but seemed unaffected in the resting cell system. It was thus suggested that butyrate represses the enzyme synthesis rather than inhibits the enzyme activity[5] of which converts leucomycin A_1 into leucomycin A_3. This role of butyrate could be utilized for the selective production of leucomycin A_1 or other components which belong to Fr-group[6] in leucomycins.

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