INDUCTION OF THE BIOCONVERSION OF LEUCOMYCINS BY GLUCOSE IN A PRODUCING STRAIN

Sir:

In the course of our biosynthetic studies on leucomycin, a 16-membered macrolide antibiotic, it was found that leucomycin A_3 is formed by the bioconversion of leucomycin A_1 and that this reaction is repressed by butyrate[1]. However, since butyrate is a precursor of leucomycin[2], it is difficult to evaluate whether it is directly involved in the repression of enzyme synthesis. In fact, the effect of butyrate was found to depend on glucose in the medium. In the present paper, we describe the effect of glucose on the bioconversion of leucomycin A_1 (LM A_1) into leucomycin A_3 (LM A_3) which is the 3-O-acetyl derivative of leucomycin A_1[3].

Leucomycin A_3 accumulation was predominantly extracellular in *Streptomyces kitasatoensis* 66-14-3, one of the major producing strains of leucomycins A_1 and A_3. When the culture was grown in medium I containing 2% glucose, 0.5% peptone, 0.5% meat extract and 0.5% NaCl (pH 7.0), the level of leucomycin A_3 accumulated was reduced by the addition of butyrate[1]. The production of leucomycin A_3 was also reduced in medium II, in which the glucose component of medium I is replaced by 2% soluble starch. The composition of the leucomycin mixture remained unchanged upon addition of butyrate to medium II. When glucose was added to medium II, the production of leucomycin A_3 was the same as in medium I, but it was reduced by the addition of butyrate.

We then examined the effect of glucose on the bioconversion of leucomycin A_1 into leucomycin A_3 in a resting cell system. In this resting cell system, the *de novo* synthesis of leucomycin is inhibited by cerulenin[4], an inhibitor of β-ketoacyl-thioester synthase. As shown in Table 1, the bioconversion did not occur when mycelia had been grown in medium II irrespective of whether glucose was present or absent from the resting cell system. On the other hand, even in the absence of glucose in the resting cell system, the bioconversion took place if the mycelia had been grown in the presence of glucose. The bioconversion was therefore dependent on glucose in the growing cell culture but not in the resting cell system. This indicates[5] that glucose is acting as an inducer of enzyme synthesis, not as an activator of the enzyme.

Other sugars and acids were tested to examine their inducing activity on the bioconversion of leucomycin A_1 into leucomycin A_3. As shown in Table 2, the bioconversion was observed when the mycelia were grown in the presence of valerate. It was thus established that both glucose and valerate possess inducing activity.

In our previous report, it was mentioned that butyrate represses the bioconversion of leucomycin A_1 into leucomycin A_3[1]. As shown in Table 3, such bioconversion did not take place when mycelia were grown in medium II in the presence or absence of butyrate. Butyrate repressed the bioconversion when mycelia were grown in medium I[1] or in medium II with glucose added. These observations indicate that the butyrate tends to inhibit the induction of the bioconversion by glucose.

It is often observed that the biosynthesis of some secondary metabolites begins after the depletion of certain rapidly utilized sugars. Phenoxazinone synthase in actinomycin biosynthesis is known to be repressed by glucose[6].

Table 1. Effect of glucose on the bioconversion of leucomycin A_1 into leucomycin A_3

Addition to medium II	Conc. of glucose in the resting cell system (%)	Conversion of LM A_1 into LM A_3 (%)
none	2	0
none	0*	0
Glucose (2%)	2	39.8
Glucose (2%)	0*	41.5

S. kitasatoensis were grown for 24 hours on a reciprocal shaker at 27°C in a 500-ml flask containing 100 ml of medium II. The mycelia were washed twice with physiological saline and resuspended in 10 ml of the solution (2 g wet weight) containing 2% glucose, 0.5% NaCl, 20 μg/ml of cerulenin and 50 μg/ml of leucomycin A_1. 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. The suspension was filtered and extracted with benzene at pH 8.0. The extract was applied to a silica gel TLC plate and developed with benzene-acetone (1:1). The ratio of leucomycins A_1 and A_3 was determined by Dual Wavelength RLC Scanner CS-910 (Shimadzu Seisakujo) at the wavelength of 232 nm.

* Glucose was removed in the resting cell system.

Table 2. Effect of sugars and organic acids on the bioconversion of leucomycin A_1 into leucomycin A_3*

Addition to medium II** (%)		Conversion of LM A_1 into LM A_3 (%)
Soluble starch	(1)	0
Maltose	(1)	0
Lactose	(1)	0
Sucrose	(1)	0
Glucose	(1)	22.3
Mannose	(1)	0
Galactose	(1)	0
Fructose	(1)	0
Xylose	(1)	0
Glycerol	(1)	0
Sodium succinate	(1)	0
Sodium citrate	(0.5)	0
Acetic acid	(0.1)***	0
Propionic acid	(0.1)***	0
Butyric acid	(0.1)***	0
iso-Butyric acid	(0.1)***	0
Valeric acid	(0.05)***	57.5
iso-Valeric acid	(0.05)***	0
2-Propyl malonic acid	(0.1)***	0

* See legend of Table 1.
** Mycelia grown under these conditions were tested for the bioconversion of leucomycin A_1 into leucomycin A_3 in the resting cell system.
*** pH adjusted to 7.0 with 2 N NaOH.

Table 3. Effect of butyrate on the bioconversion of leucomycin A_1 into leucomycin A_3*

Addition to medium II**		Conversion of LMA_1 into LM A_3 (%)
none		0
Butyric acid***	(0.1%)	0
Glucose	(2%)	39.8
Glucose + butyric acid***	(2%) (0.1%)	10.9

* See legend of Table 1.
** Mycelia grown under these conditions were tested for the bioconversion of leucomycin A_1 into leucomycin A_3 in the resting cell system.
*** pH adjusted to 7.0 with 2 N NaOH.

In the case of the biosynthesis of streptomycin, it is known that α-D-mannosidase is repressed by glucose and induced by mannan[7]. Acetylhydrolase which converts cephalosporin C to desacetylcephalosporin C is also repressed by glucose[8]. In contrast to these earlier observations, we have shown that glucose induces the bioconversion of leucomycin A_1 into leucomycin A_3. This is the first report, to our knowledge, of glucose induction of any enzyme related to secondary metabolite biosynthesis.

SATOSHI ŌMURA
JUN MIYAZAWA
HIDEO TAKESHIMA
CHIAKI KITAO
MINORU AIZAWA*
Kitasato University and the Kitasato Institute, Minato-ku, Tokyo 108, Japan
*Tōyō Jōzō Co., Ltd., Ōhito-cho, Tagata-gun, Shizuoka-ken, Japan

(Received November 13, 1976)

References

1) ŌMURA, S.; J. MIYAZAWA, H. TAKESHIMA, C. KITAO, K. ATSUMI & M. AIZAWA: Bioconversion of leucomycins and its regulation by butyrate in a producing strain. J. Antibiotics 29: 1131~1133, 1976
2) ŌMURA, S.; A. NAKAGAWA, H. TAKESHIMA, K. ATSUMI, J. MIYAZAWA, F. PIRIOU & G. LUKACS: Biosynthetic studies using ^{13}C-enriched precursors on the 16-membered macrolide antibiotic leucomycin A_3. J. Amer. Chem. Soc. 97: 6600~6602, 1975
3) ŌMURA, S. & A. NAKAGAWA: Chemical and biological studies on 16-membered macrolide antibiotics. J. Antibiotics 28: 401~433, 1975
4) ŌMURA, S.: Antibiotic cerulenin, a novel tool for biochemistry as the inhibitor of fatty acid synthesis. Bacteriol. Rev. 40: 681~697, 1976
5) DEMAIN, A. L.: Regulatory mechanisms and the industrial production of microbial metabolites. Lloydia 31: 395~418, 1968
6) GALLO, M. & E. KATZ: Regulation of secondary metabolite biosynthesis: Catabolite repression of phenoxazinone synthase and actinomycin formation by glucose. J. Bact. 109: 659~667, 1972
7) DEMAIN, A. L. & E. INAMINE: Biochemistry and regulation of streptomycin and mannosidostreptomycinase (α-D-mannosidase) formation. Bacteriol. Rev. 34: 1~19, 1970
8) HINNEN, A. & J. NÜESCH: Enzymatic hydrolysis of cephalosporin C by an extracellular acethylhydrolase of *Cephalosporium acremonium*. Antimicr. Agents & Chemoth. 9: 824~830, 1976