Transient Repression of Erythromycin Formation in *Streptomyces erythraeus*

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(Received 2 November 1981)

The effect of D-glucose on growth and erythromycin production by *Streptomyces erythraeus* was investigated. D-Glucose stimulated growth and caused a strong but temporary suppression of antibiotic formation. Maximum specific suppression of erythromycin formation occurred at a carbohydrate concentration of 20 mg ml⁻¹. A non-metabolizable analogue of glucose, 2-deoxy-D-glucose, also suppressed antibiotic formation. Since glucose caused a decrease in erythromycin formation only when added before the stage of antibiotic production, we conclude that this sugar exerted a transient repressive effect on erythromycin biosynthesis.

INTRODUCTION

The formation of many antibiotics and other secondary metabolites is suppressed by rapidly used carbon sources (Martin & Demain, 1980). Among such carbon sources, glucose seems to be one of the most effective in exerting a negative influence on antibiotic biosynthesis (Hu & Demain, 1979).

The antibiotic erythromycin is a secondary metabolite formed by strains of *Streptomyces erythraeus* at the end of the trophophase (Smith *et al.*, 1962). Since it is a broad-spectrum antibiotic and has low toxicity for humans, erythromycin is of great medical and economic importance (Oleinik, 1975). In spite of the practical interest of erythromycin, very little has been published involving the regulatory aspects of its biosynthesis (Corcoran, 1975; Raczyńska-Bojanowska *et al.*, 1970; Spizek *et al.*, 1965).

In the present work, the effect of D-glucose on growth and erythromycin production by *S. erythraeus* was examined in a medium which eliminated changes due to pH variation. The experimental evidence obtained here indicated that this carbohydrate exerted a transient repression on erythromycin formation.

METHODS

Organisms and cultivation. *Streptomyces erythraeus* BM 21-115 wild-type strain, was obtained from the UNAM-Biomédicas Culture Collection. *Sarcina lutea* NRRL B-1018, was kindly supplied by the ARS Culture Collection, U.S. Department of Agriculture, Peoria, Ill., U.S.A.

All cultures were grown in a complex medium (CM) containing 0.4 g yeast extract and 0.3 g malt extract per 100 ml of 0.1 M-3-(*N*-morpholino)propanesulphonic acid buffer (Sigma), pH 7.0. Approximately 10⁸ *S. erythraeus* spores (from a 5 d old culture grown on a solid CM medium slant) were inoculated into CM medium (10 ml) contained in a 50 ml Erlenmeyer flask and were incubated at 29 °C for 8 h on a rotary shaker (160 rev. min⁻¹). For antibiotic production, a sample (1 ml) of the above culture was inoculated into CM medium (50 ml) contained in a 250 ml Erlenmeyer flask and was incubated at 29 °C for 72 h. D-Glucose was sterilized separately

and added to CM medium at desired concentrations just prior to inoculation. Under these conditions *S. erythraeus* BM 21-115 produced mainly erythromycin A, shown by TLC (Martin & Goldstein, 1970). Experiments were carried out in triplicate. Erythromycin production, pH and growth of *S. erythraeus* were monitored throughout the fermentation.

Assay of erythromycin. At specified intervals, the production of antibiotic was determined by a disc-agar plate technique (Aharonowitz & Demain, 1978), using *Sarcina lutea* as the assay organism. After incubation (37 °C, 15 h), zones of inhibition were measured and the amount of erythromycin was estimated using erythromycin A (Fermic Laboratories S. A., México 13, D. F.) as standard.

Growth determination. Samples of mycelia were harvested, washed with 2 vol. distilled water and placed in 2 ml of 0.3 M-trichloroacetic acid. After centrifugation, the pellet was resuspended in 1 ml of 0.4 M-NaOH and its protein concentration was determined by the Lowry method using bovine serum albumin as standard.

RESULTS

Streptomyces erythraeus BM 21-115 produced erythromycin when it was grown in CM medium at 29 °C. Under these conditions, production started at 36 h of incubation and continued linearly for approximately 50 h. When the amount of erythromycin produced was plotted as a function of growth (Fig. 1), a close relationship between these parameters was obtained. On the other hand, when D-glucose was added to the medium, this relationship was modified by an increase in the extent of growth and a delay in antibiotic production.

To gain more information about the glucose effect, several concentrations of the carbohydrate were tested for growth and erythromycin formation (Fig. 2). D-Glucose stimulated both parameters but suppressed the specific antibiotic synthesis without significant changes in the pH of the medium. The maximum effect was reached at a concentration of 20 mg ml⁻¹. Further increase in the concentration (up to 100 mg ml⁻¹) did not significantly modify the level of suppression (not shown).

To find out whether or not glucose itself was responsible for this action, the effect on growth and erythromycin production by a non-metabolizable glucose analogue, 2-deoxy-D-glucose (Tyler *et al.*, 1967) was determined. As shown in Table 1 the analogue significantly decreased antibiotic production but had no effect on growth. In addition, higher concentrations (up to 20 mg ml⁻¹) of the analogue almost totally inhibited the production of erythromycin.

Suppression was observed when glucose was added either at the beginning of the fermentation or during the exponential growth phase, but before the stage of antibiotic synthesis (Fig. 3). Once the production of erythromycin had started, addition of glucose did not modify the linear relationship between erythromycin production and growth.

DISCUSSION

The use of D-glucose as major carbon and energy source resulted in changes in maximum growth and in production of erythromycin. This carbohydrate brought about a strong but temporary suppression of antibiotic formation. During the period of this suppression, the growth rate was increased so that the effect of glucose was not to arrest the overall synthesis of protein, but to prevent antibiotic formation specifically. Similar effects on the suppression of other secondary metabolites by glucose have been reported previously (Gallo & Katz, 1972; Redshaw *et al.*, 1976; Satoh *et al.*, 1976).

Maximum specific suppression of antibiotic formation occurred with 20 mg D-glucose ml⁻¹. The sugar, rather than a product derived from its catabolism, seemed to be responsible for this action since a non-metabolizable glucose analogue also suppressed erythromycin formation.

That glucose suppressed the specific erythromycin formation only when added before the antibiotic production phase indicated a repressive rather than an inhibitory or inactivating effect. The kinetics of this action are similar to those reported for the transient repression of

Fig. 1. Effect of D-glucose on erythromycin formation. Cultures were grown in CM medium with (Δ) or without (○) D-glucose (10 mg ml⁻¹).

Fig. 2. Effect of different D-glucose concentrations on the maximum growth (Δ), erythromycin formation (●), final pH of the medium (▲) and specific erythromycin formation (○).

β-galactosidase elicited by glucose in *Escherichia coli* (Tyler *et al.*, 1967). As pointed out by these authors, a transient repressive effect is characterized by a strong but temporary suppression in the formation of a given metabolite.

Table 1. *Effect of 2-deoxy-D-glucose on maximum growth and erythromycin formation by S. erythraeus*

2-Deoxy-D-glucose was filter-sterilized through a Millipore membrane type HAWP 02500 before addition to CM medium. Growth and erythromycin formation were determined after incubation at 29 °C for 72 h.

2-Deoxy-D-glucose (mg ml ⁻¹)	Growth (mg protein ml ⁻¹)	Erythromycin formation	
		(µg ml ⁻¹)	[µg (mg protein) ⁻¹]
None	1.8	60	33.3
10	2.0	45	22.5
20	1.8	6	3.3

Fig. 3. Effect of the time of D-glucose addition on growth and erythromycin production by *S. erythraeus*. (a) Control without glucose showing the erythromycin produced (●) and the growth (○) at the time of glucose addition (arrows). (b) Erythromycin formation obtained with glucose (10 mg ml⁻¹) being added at the beginning of the experiment (□) and 24 h (■), 48 h (△) and 60 h (▲) later. ○, Control without sugar.

On the basis of these previous observations, the experimental evidence obtained in the present investigation indicated that glucose exerted a transient repression on erythromycin formation.

We are indebted to A. Farres, A. Gonzalez-Sarabia, J. E. Herz and V. Yakoleff-Greenhouse for critical reading and helpful suggestions.

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