Effect of Actinomycin D and Chloramphenicol on the Induction of the Malate Decarboxylase System in *Lactobacillus thermobacterium* 5 (CNRZ) 313

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SUMMARY

This paper presents data on the induction of malate decarboxylase in *Lactobacillus thermobacterium* and the effect of chloramphenicol and actinomycin D on the induction.

INTRODUCTION

Experimental data and basic information on inducible enzyme systems in micro-organisms are available (Jacob & Monod, 1961; Pollock & Kramer, 1958; Englesberg *et al.* 1965; Schwartz, 1967; Cove, 1966) but no specific information has been given concerning the *Lactobacilli* species. We present data on the induction of the malate decarboxylase (MDC) system in *Lactobacillus thermobacterium* 5 (CNRZ) 313.

It is of interest that the decarboxylation of the L-malic acid has been used as a taxonomic characteristic for the classification of certain groups of *Lactobacilli* (Keddie, 1959).

METHODS

Organism and growth conditions. *Lactobacillus thermobacterium* 5 (CNRZ) 313 (Raibaud *et al.* 1973) was used. For good growth, transfer to LAPTg medium (see below) every 12 h was necessary. After 8 h incubation at 37 °C, 0.2 ml of the resulting culture was added to 5 ml of fresh LAPTg medium and stored at 4 °C.

Medium. LAPT medium, and LAPTg medium, i.e. LAPT medium + glucose (Raibaud *et al.* 1961) were used. When required, 0.5 % L-malic acid and other supplements were added.

Chemicals. Tryptone, yeast extract and peptone were obtained from Oxoid, actinomycin D and mercaptoethanol from Sigma, glucose from Carlo Erba, L-malic acid from NBC, Tween 80 from Fluka and chloramphenicol from Parke-Davis.

Preparation of cell suspension. *Lactobacillus thermobacterium* was grown in LAPTg with and without L-malic acid at 37 °C for 8 h. Cells were removed by centrifugation at 3020 g for 20 min, washed with 0.1 % mercaptoethanol and finally resuspended in mercaptoethanol to a cell concentration of 10 % transmittance measured at 560 nm with a Spectronic 20 spectrophotometer.

Enzyme activity. The activity was determined by measuring the CO₂ released at 37 °C with a Gilson differential respirometer at regular intervals, using the reaction systems shown in Table 1.

Accumulation of transcription intermediates. LAPTg medium containing L-malic acid and 2 μ g chloramphenicol (Cm)/ml was inoculated with a suspension of cells grown without the inducer, and incubated at 37 °C. Cm is thought to block translation on bacterial 70S ribosomes, but not transcription; therefore since samples were drawn after 10, 20, 40 and 60 min of incubation, we assumed that in each case different amounts of transcription intermediates were accumulated in the cells. The cells were immediately collected, washed and resuspended as described.

Translation of the previously accumulated transcription intermediates. Each of the resulting cell suspensions obtained as indicated above was used for the inoculation of LAPTg medium supplemented with L-malic acid and 2 μ g actinomycin D (Am)/ml, which blocks transcription but not translation. The systems were incubated and the enzyme activity measured.

RESULTS

Induction of the malate decarboxylase system

Experiments were performed as shown in Table 1, using cells grown without inducer. The results (Fig. 1) can be summarized as follows: besides the inducer, glucose and LAPT medium were necessary for induction; the induction curve shows an absolute lag period of 10 min followed by an acceleration lag period of 30 min; the addition of Cm inhibited the joint action of glucose, LAPT and L-malic acid on the induction.

Experiments performed as shown in Table 1, using cells grown in the presence of the inducer (Fig. 2): the absence of the absolute lag period is apparent; up to approximately 30 min of incubation no substantial difference in activity appears in the systems, even if Cm is present; the system containing LAPT and glucose, but not Cm, shows a marked increase in activity after approximately 30 min.

Influence of Am on the induction – cells grown without the inducer (Table 1 and Fig. 3): Am added along with the other components of the system completely inhibits the enzyme activity from the beginning; the inhibitory effect of Am when added to the systems at different intervals appears immediately after the addition of the antibiotic.

Cells grown in the presence of the inducer (Table 1, Fig. 4): enzyme activity is detected even if Am is added from the beginning; when Am is added to the system after 10 or 20 min of incubation the enzyme activity increases accordingly.

Cells with different amounts of accumulated transcription intermediates: there is a direct correlation between enzyme activity and amount of transcription intermediates accumulated in the cells; once the pool of transcription intermediates is exhausted the synthesis of the enzyme(s) stops.

DISCUSSION

Under the experimental conditions tested L-malic acid is able to produce induction of the MDC system only in the presence of glucose and LAPT medium, the latter probably because of the amino acids (Halvorson & Spiegelman, 1953; Monod & Cohn, 1952) contained in yeast extract. The results show that the inhibitory effect of Cm is apparent immediately after the absolute lag in cells without previous induction (Fig. 1). If the antibiotic is added after an adequate period of induction (Fig. 2), Cm has no effect on the activity of the enzyme(s) already formed in the cells but in some way it blocks enzyme synthesis.

Similar conclusions may be drawn when the effect of Am on the induction is tested (Figs. 3 and 4), suggesting that inhibition occurs at an early stage of protein synthesis. This

Fig. 1

Fig. 2

Fig. 1. Induction of the MDC system. Influence of different reaction mixtures (Table 1) on the induction of cells grown on LAPTg medium without the addition of L-malic acid (inducer). Enzyme activity measured by means of a differential respirometer and expressed as the CO_2 released. ●, Reaction mixture 1, with glucose; △, reaction mixture 2, with LAPT medium; ○, reaction mixture 3, with LAPT medium and glucose; ▲, reaction mixture 4, with LAPT medium, glucose and Cm.

Fig. 2. Induction of the MDC system. Influence of different reaction mixtures (Table 1) on the induction of cells obtained using LAPTg medium containing L-malic acid. For further details and symbols see the legend to Fig. 1.

Table 1. *Composition of the reaction mixtures used in the experiments whose results are presented in Figs. 1 and 2*

	Composition (ml) of reaction mixture nos.			
	1	2	3	4
0.1 M-phosphate buffer, pH 5.5	0.5	0.5	0.5	0.5
0.24 M L-malic acid	0.5	0.5	0.5	0.5
0.24 M-glucose	0.5	—	0.5	0.5
LAPT medium	—	0.2	0.2	0.2
Chloramphenicol (2 $\mu\text{g}/\text{ml}$ final concn)	—	—	—	0.1
Water	0.5	0.8	0.3	0.2
Cell suspension	0.5	0.5	0.5	0.5

hypothesis has further support from the experimental data displayed in Fig. 5, which show a direct correlation between the amount of transcription intermediates, probably mRNA, accumulated in the cells and the resulting enzyme activity measured after the addition of Am. Thus, it seems likely that when the pool of transcription intermediates is exhausted the synthesis of the enzyme(s) is no longer possible due to the inhibitory effect of Am on transcription. These data suggest that the induction of the MDC system in *L. thermobacterium* 5 (CNRZ) 313 occurs at the transcription level, probably through the same or similar general mechanisms as have been described in other micro-organisms.

Fig. 3

Fig. 4

Fig. 3. Effect of Am on the induction of cells obtained using LAPTg medium without the addition of L-malic acid. Reaction mixture, containing LAPT medium, glucose, L-malic acid and Am, was added at various times. Taking the time that incubation commenced in the differential respirometer as time zero, Am was added at the following times: Δ , 0 min; $\blacktriangle$, 10 min; $\bullet$, 20 min. $\circ$, Control without Am.

Fig. 4. Effect of Am on the induction of cells obtained using LAPTg medium containing L-malic acid. For further details and symbols see the legend to Fig. 3.

Fig. 5. Determination of the possible induction level. Cells grown without the inducer were used to inoculate LAPT medium containing glucose, L-malic acid and Cm (to inhibit translation but not transcription). Samples were taken after 10, 20, 40 and 60 min of incubation and the cells were collected, washed, transferred to LAPT medium supplemented with glucose, L-malic acid and Am (to inhibit transcription but not translation), and the enzyme activity was measured as usual. Taking into account the times of incubation in the presence of Cm, the results were as follows: $\blacktriangle$, 10 min; $\bullet$, 20 min; Δ , 40 min; $\circ$, 60 min.

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