The Effect of Cyclic AMP on Catabolite Repression of Isocitrate Lyase in *Nocardia salmonicolor* (NCIB9701)

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INTRODUCTION

In *Escherichia coli*, cyclic AMP is thought to play an essential role in the initiation of transcription of those genes subject to catabolite repression; this involves interaction of the cyclic nucleotide with an activator protein which then binds to the promoter site of the operon (De Crombrughe *et al.*, 1971). During catabolite repression of inducible enzymes by glucose in *E. coli*, the sugar or its metabolic products probably cause a lowering of the level of cyclic AMP in the bacterium. Indeed, in *E. coli* repression by glucose of β -galactosidase synthesis can be overcome by adding cyclic AMP (Pastan & Perlman, 1968) as also can be the weak catabolite repression by glucose of tryptophanase (Botsford & De Moss, 1971). In *Pseudomonas aeruginosa*, weak repression by lactate of the aliphatic amidase is partially relieved by cyclic AMP (Smyth & Clarke, 1975*a*) and it is thought that amidase synthesis in the pseudomonad may be under the same type of control as some catabolic enzymes in *E. coli* (Smyth & Clarke, 1975*b*).

The regulation of isocitrate lyase (EC. 4.1.3.1) biosynthesis in *Nocardia salmonicolor* most probably involves induction by acetate and catabolite repression by a C₄ acid of the tricarboxylic acid cycle, possibly fumarate (Higgins & Sariaslani, 1973; Sariaslani, Westwood & Higgins, 1975). In *E. coli*, however, isocitrate lyase synthesis is regulated only by a repression mechanism involving the intracellular levels of phosphoenolpyruvate or a closely related metabolite (Kornberg, 1966). Takahashi (1975) found that repression of *E. coli* isocitrate lyase by glucose was unaffected by 3 mM-cyclic AMP. We have therefore determined whether cyclic AMP affects repression of isocitrate lyase by fumarate in *N. salmonicolor*.

METHODS

Micro-organism and growth. *Nocardia salmonicolor* (NCIB9701) was maintained and grown as previously described (Sariaslani *et al.*, 1975). A continuous culture of the bacterium was grown at 30 °C in a magnetically-stirred culture vessel (1.2 l culture volume; air flow rate, 100 ml min⁻¹) in minimal medium, described previously (Sariaslani *et al.*, 1975), under acetate limitation (dilution rate, 0.029 to 0.034 h⁻¹). The concentration of acetate in the dilution medium was 0.36 % (w/v). This culture vessel was connected to a second one (70 ml capacity) which was temperature-regulated, magnetically-stirred and vortex-aerated (Pirt & Callow, 1964). For reasons of economy, experiments were done using this secondary vessel. At the beginning of each experiment, steady-state culture (70 ml) was fed from the parent vessel into the secondary one and a new steady-state (dilution rate, 0.01) was established in the parent vessel, its overflow being via the secondary vessel. Fumarate or fumarate plus cyclic AMP were added aseptically to the secondary culture, the accompanying volume increase being less than 2 %.

Fig. 1. Effect of sodium fumarate and cyclic AMP on isocitrate lyase biosynthesis by *N. salmonicolor*. Additions were made at zero time to a continuous culture of the bacterium growing on acetate as described in Methods. ○, ● and □, 0.5, 0.2, 0.1 % (w/v) sodium fumarate respectively; △, 0.5 % (w/v) sodium fumarate plus 3 mM-cyclic AMP; ▲, 0.5 % (w/v) sodium fumarate plus 30 mM-cyclic AMP.

Measurement of growth. Growth was determined by measuring the absorbance of cultures in 1 cm pathlength cuvettes at 540 nm.

Other procedures and source of chemicals. These were as described previously (Sariaslani *et al.*, 1975). Additional biochemicals were from Sigma.

RESULTS

Adding sodium fumarate (0.1 to 0.5 %, v/v) to a continuous culture of *N. salmonicolor* growing on acetate caused a rapid decrease in the specific activity of isocitrate lyase in the bacterium, the kinetics being similar to those previously reported when the effect was studied in batch culture (Sariaslani *et al.*, 1975). The degree of inhibition was related to the amount of fumarate added (Fig. 1). When sodium fumarate (0.5 %, v/v) was added with cyclic AMP (3 or 30 mM) there was a dramatic but incomplete release of the inhibition of isocitrate lyase synthesis (Fig. 1). Dibutyl cyclic AMP (3 mM) had no significant effect. In all cases, the initial specific activity of isocitrate lyase before any additions was 150 to 160 nmol min⁻¹ (mg of extract protein)⁻¹ and the growth rate did not change appreciably during the period of the experiment.

DISCUSSION

Cyclic AMP supplied exogenously to growing *N. salmonicolor* releases catabolite repression of isocitrate lyase by fumarate. Failure to achieve total release may well be due to low permeability of the bacterial membrane to cyclic AMP. The true magnitude of the catabolite repression by fumarate will be greater (by approximately 16 %) than that shown

in Fig. 1 because in the culture system used there is a continuous, slow inflow of organisms that have not been exposed to fumarate. These results suggest that a similar mechanism of regulation of transcription for isocitrate lyase biosynthesis may be involved in *N. salmonicolor* to that thought to be involved for some catabolic enzymes of *E. coli* (De Crombrugghe *et al.*, 1971) and *P. aeruginosa* (Smyth & Clarke, 1975*a*). Although cyclic AMP levels have not been measured in *N. salmonicolor*, adenyl cyclase has been detected in the related bacterium *Nocardia erythropolis* (Ide, 1971).

The involvement of cyclic AMP in bacterial transcription is thought to be concerned mainly in the regulation of catabolic enzymes (Rickenberg, 1974). Partial release of catabolite repression of isocitrate lyase in *N. salmonicolor*, but not in *E. coli* (Takahashi, 1975), on adding cyclic AMP, suggests that the two bacteria differ in the regulation of biosynthesis of this enzyme at the transcriptional level. This concurs with our previous findings (Sariaslani *et al.*, 1975).

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