THE ROLE OF CYCLIC AMP IN CHEMOTAXIS IN ESCHERICHIA COLI

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Adler and Templeton (1967) showed that the production of flagella by E. coli and hence chemotactic motility was repressed when the organism was grown in the presence of glucose, while a normal complement of flagella was produced if the cells were grown in the presence of substrates such as glycerol, succinate, or a mixture of amino acids. These authors recognized that this repression was analogous to the regulatory phenomenon known as catabolite repression in which the formation of certain enzymes is repressed during growth in a glucose containing medium but not during growth in media containing substrates such as glycerol or succinate. Yokota and Gots (1970) recently made the interesting observation that adenosine-3'5'-cyclic phosphate (cyclic AMP) was absolutely required for flagella formation and hence motility in E. coli. Mutants lacking the enzyme adenyl cyclase produced no cyclic AMP and were unable to synthesize flagella unless provided with an exogenous supply of this cyclic nucleotide. Since it is known that catabolite repression of a number of enzymes can be abolished by the addition of exogenous cyclic AMP (Perlman and Pastan, 1969; DeCrombrugghe et al., 1969; Goldenbaum, Broman and Dobrogosz, 1970), it would logically follow that the repression of flagella synthesis by glucose should also be abolished by adding cyclic AMP to the culture medium. The studies described in this communication were designed to test this prediction by observing the effects of glucose and cyclic AMP on the chemotactic motility of E. coli in semi-solid agar media. The ability of bands of cells to migrate in response to chemotactic forces in this type of medium

is dependent on their ability to synthesize flagella (Adler and Templeton, 1967).

Materials and Methods -- Cultures of a wild type E. coli K12-701 (White, 1968) were grown overnight with vigorous aeration at 37° in 50 ml of a highly buffered basal salts medium containing (g/l): K_2HPO_4, 28 g; KH_2PO_4, 8.0 g; $MgSO_4 \cdot 7H_2O$, 0.10 g; $(NH_4)_2 SO_4$, 1 g (pH 7.2). This medium was supplemented with 0.25% casein hydrolysate (vitamin-free, acid hydrolyzed) and contained 0.02 M glucose as the substrate. Cultures were harvested by centrifugation, resuspended in 5-10 ml of 0.05 sodium phosphate buffer (pH 7.5) and used as such for inoculation into the semi-solid media described below. This suspension contained from 1-2 mg dry weight of cells/ml and 12.5 μl aliquots were inoculated just below the surface of the media using a Hamilton syringe.

Chemotaxis was measured using two types of culture medium. The first was Nutrient Broth (Difco) modified to contain 4×10^{-3}M KNO_3 and 0.15% agar (Difco). The second medium contained the basal salts combination described above and was supplemented with 4×10^{-3}M KNO_3, 0.40% casein hydrolysate, 0.20% Difco yeast extract, and 0.15% agar. Additional substrates and cyclic AMP were added as indicated in the text. The substrates, cyclic AMP and the KNO_3 solutions were sterilized by filtration and were added to freshly melted semi-solid basal agar just prior to inoculation. These media were dispensed in 20 ml amounts in 18 x 105 mm Pyrex test tubes that had been standardized for colorimetric determinations.

A simple procedure was employed for determining and recording the migration of chemotactic bands as they travelled down the tube. The culture tubes were marked at the meniscus level or 1 cm below the meniscus level with a band of 0.5 inch wide, black electrical tape. Another ring of tape was placed 8 cm down the tube. These tapes were helpful in orienting the tube in the colorimeter. The tubes were incubated at 37° in an upright position with care taken to minimize mechanical disturbances. Chemotactic bandings and other

turbidity changes in these tubes were recorded using a Spectronic 20 colorimeter that was converted into a manual densitometer by removing the shutter and a portion of the bottom plate of the instrument so that the entire length of the tubes could be passed down through the light path. The exact location of turbidity and bands in these tubes at any particular time was measured to within $\pm$ 0.10 cm using a mm scale rod fastened to the top of each tube with a rubber stopper. A tube wrapped with electrical tape was inserted in the chamber to serve as the zero adjustment and for use whenever the light source was on but the instrument was not in use. One-hundred % transmittance was obtained using uninoculated media or portions of inoculated tubes showing no turbidity. All readings were conducted at a wave length of 600 nm. All bands were recorded as described above in respect to their intensity and distance from the origin.

Results -- The data presented in Figure 1 were obtained when E. coli K12 was inoculated on semi-solid Nutrient Broth without (graphs A, B and C) and with 0.04 M glucose added (graphs D, E and F). Cyclic AMP (2.5×10^{-3}M)

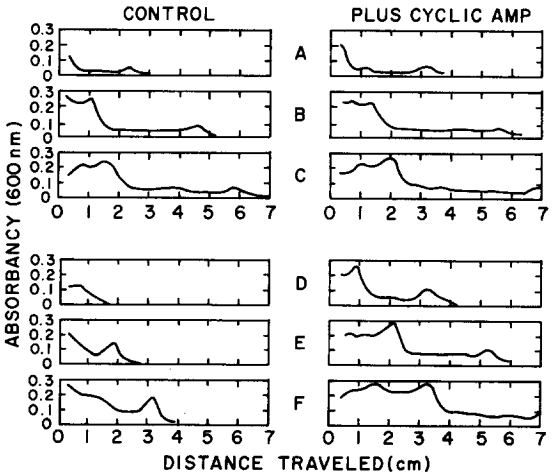

Figure 1. Effect of glucose and cyclic AMP on chemotaxis in Nutrient Broth. Cells were inoculated on the surface of semi-solid Nutrient Broth without (graphs on the left) and with (graphs on the right) 2.5×10^{-3}M cyclic AMP added to the medium. These data show the absorbancy reading taken after 4 hr (graph A), 5 hr (graph B) and 6 hr (graph C) of incubation. Graphs D, E and F are the reading taken at 4 hr, 5 hr and 6 hr respectively with cultures grown in the same Nutrient Broth medium supplemented with 0.04 M glucose.

was added to one set of cultures in each case. Although readings were taken at hourly intervals for 10 hr only the results obtained at 4, 5 and 6 hr are shown here. It can be seen that in the absence of added glucose this medium supported the development of a rapid chemotactic migration. This response was unaffected by cyclic AMP. When glucose was present on the other hand chemotaxis was repressed, but the addition of cyclic AMP abolished this repression and produced a chemotactic response similar to that observed with the cultures grown in the absence of glucose.

A similar series of experiments were conducted using a highly buffered mineral salts medium supplemented with nitrate, yeast extract and casein

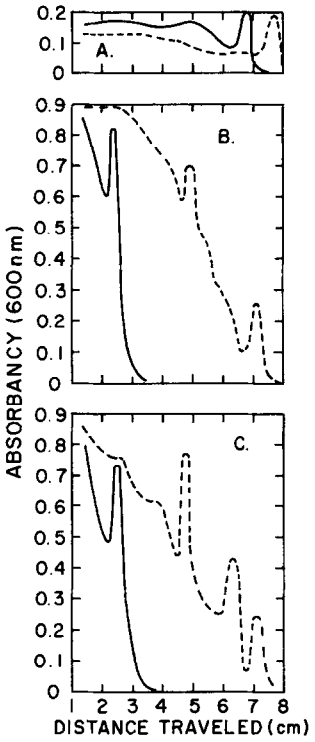

Figure 2. The effect of cyclic AMP on repression of chemotaxis by glucose and fructose. The highly buffered basal salts medium supplemented with nitrate, casein hydrolysate and yeast extract was used in these experiments. Graph A: no further addition; Graph B: 0.04 M glucose added; Graph C: 0.04 M fructose added. The solid lines represent the measurements obtained in the absence of cyclic AMP. The dashed lines represent the measurements obtained with 2.5 x 10^{-3}M cyclic AMP added. The absorbancy readings were obtained 10 hr post inoculation.

hydrolysate (Figure 2). Cultures grown in this medium were highly motile as indicated by their rapid migration down the tubes, and cyclic AMP had no appreciable effect on this pattern (graph A). If glucose (graph B) or fructose (graph C) were added, the development of a chemotactic response was seriously retarded. The concomitant addition of cyclic AMP, however, prevented this repression. All of these experiments were repeated a number of times with essentially identical results obtained each time. Similar responses to cyclic AMP were obtained when the nitrate and yeast extract were eliminated from the medium although the patterns of development were slower. When pyruvate which does not cause catabolite repression was added to the medium the chemotactic response was unaffected by cyclic AMP. Preliminary studies with an adenyl cyclaseless mutant of E. coli K12 isolated in our laboratory have shown that these cells are not motile unless grown in the presence of cyclic AMP, as would be expected from the findings of Yokota and Gots (1970).

Discussion -- Our findings at the present time indicate that chemotactic movements and hence flagella production are in large measure subject to classical catabolite repression which can be overcome by addition of exogenous cyclic AMP. In the presence of substrates such as glucose, catabolite sensitive enzymes are thought to be repressed because their synthesis and consequent function would serve no purpose other than to augment already large metabolic pools of carbon and energy (Neidhardt and Magasanik, 1956). We feel that this teleological description of catabolite repression of sensitive enzyme systems is sufficient to also describe the observed effect on repression of flagella synthesis. The formation of these organelles in a glucose-rich environment can be viewed as an unnecessary expenditure of energy and materials. Their synthesis would become crucial when the glucose supply becomes depleted and the cells must undergo chemotactic migrations to more suitable locations. It is likely that these de-repression-repression cycles are a response to the availability and the lack of availability respectively of endogenously produced cyclic AMP (Makman and Sutherland, 1965; DeCrombrugghe et al., 1969; Broman, Goldenbaum and

Dobrogosz, 1970). The regulation of endogenous production of this cyclic nucleotide is thus clearly indicated as a key factor in understanding chemotaxis in E. coli.

Acknowledgments

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