Bacterial chemotaxis controls the catabolite repression of flagellar biogenesis

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1. INTRODUCTION

Synthesis of flagellin, the composite protein of flagella, is subject to catabolite repression in enteric bacteria [1,2]. Catabolite repression, among other control mechanisms in bacteria, is in turn proposed to be regulated by the chemotaxis system [3]. In a study of flagellar biogenesis in *Salmonella typhimurium* and *Escherichia coli*, it was found [4] that benzoate and long-chain fatty acids relieved the catabolite repression of flagellum formation caused by growth on glucose. Benzoic acid, a precursor of CoQ, could act by fulfilling a specific requirement of flagellin synthesis for CoQ [4]. The role of long-chain fatty acids in flagellar growth remained to be explained.

We considered the possibility that benzoate and long-chain fatty acids, being potent chemorepellents [5], induce flagellar growth by relieving catabolite repression via a chemotaxis-controlled step. Here, we find that flagellar growth in mutants specifically defective in sensing certain repellents is not activated by these substances, whereas in isogenic, wild-type bacteria repellents activate flagellar biogenesis.

2. MATERIALS AND METHODS

Escherichia coli AW strains were provided by Dr J. Adler, University of Wisconsin. The chemotactically wild-type strain AW405 (*leu, thr, his, gal1, gal2, lac, xyl, sup, tonA, tsx, strA*) and the isogenic chemotactic mutants AW518 (*tsr*), deficient in methyl-accepting chemotaxis protein I (MCPI) and AW539 (*tar*) (MCPII-deficient) as well as AW569 (a double *tsr–tar* mutant) were used. Bacteria were grown overnight in a medium containing 1% Tripon (Difco) and 0.5% NaCl, or 1% Pepton (Chemapol), 0.5 yeast extract (Serva) and 0.5% NaCl, at 37°C. Cells from the stationary phase were inoculated into Petri dishes, containing one of the above growth media supplemented with appropriate additions of glucose and repellents, as in section 3 and solidified with 0.4% agar (Difco). Motile bacteria swarmed out of the inoculation point on semisolid agar, the diameter of the swarmer colony indicating the expression of motility. In certain cases, cells from Petri dishes were suspended in chemotaxis medium containing 10 mM potassium phosphate and 0.1 mM EDTA at pH 7.0 [6]. The fration of motile cells was counted in a phase contrast microscope.

3. RESULTS AND DISCUSSION

In bacterial chemotaxis, information from numerous attractant and repellent chemoreceptors is intregrated by several membrane proteins called methyl-accepting chemotaxis proteins (MCPs). Thus, MCPI collects information from serine and other attractant amino acids [7,8] as well as from pH that mediates the repelling response to benzoate and fatty acids [9,10]. A defect in the *tsr* gene coding for MCPI suppresses taxis to these compounds. MCPII gathers information from the attractants aspartate and maltose and repellents: Ni^{2+} and Co^{2+} [7]. Cells with a *tar* mutation lack taxis to these effectors. The aim of our study was to determine whether repellents specifically activated flagellar syntheses in the *tar* and *tsr* mutants.

Inoculation of wild-type and mutant cells of Petri dishes with semi-solid agar containing a glucose minimal medium, produced large swarmer

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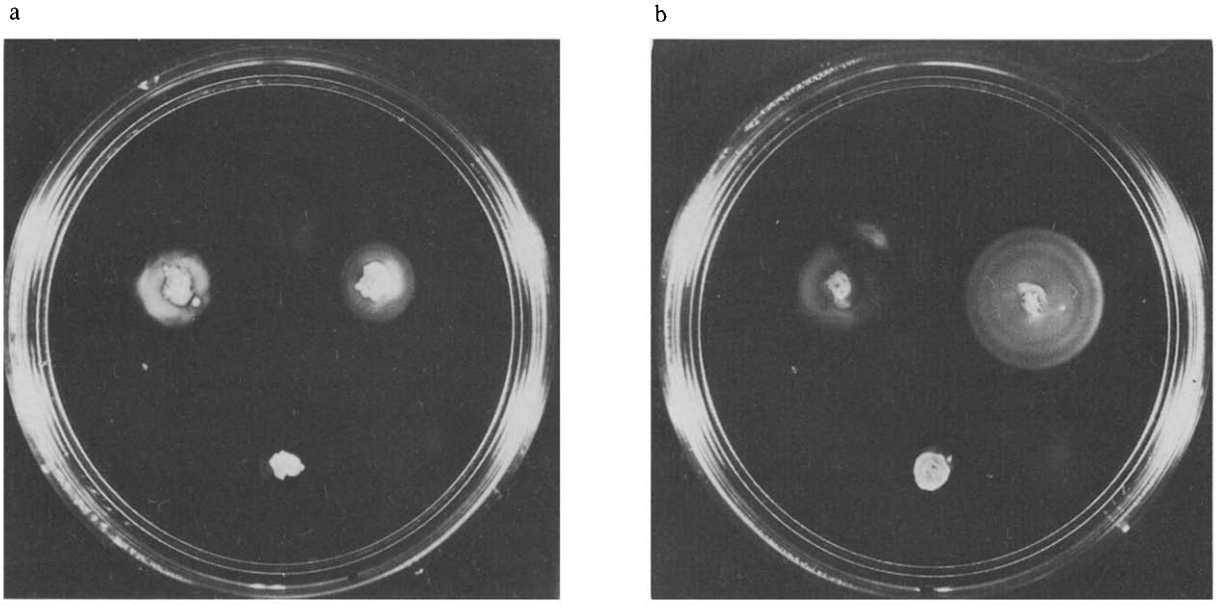

Fig.1. Catabolite repression of motility and its reversion by a repellent, Co^{2+}. (a) Cells inoculated in 0.3% agar containing 1% peptone, 0.5% yeast extract, 0.3% glucose and 0.5% NaCl were grown for 18 h. From upper left clockwise: *E. coli* AW405, chemotactically wild-type; AW518, *tsr* mutant and AW569, a *tsr–tar* mutant. (b) Conditions and cell inoculations as in (a), except that the medium was supplemented with 0.2 mM $Co(NO_3)_2$.

colonies, indicating that under these conditions flagellar synthesis was not repressed to a noticeable level. The growth medium was therefore enriched by adding tryptone or peptone + yeast extract to increase the extent of catabolite repression. In a control experiment, cells were inoculated into a medium containing peptone + yeast extract without glucose. The wild-type cells, *tar* and *tsr* mutants equally formed large swarmer colonies. Bacteria consume amino acids and move up the formed gradients [11]. A *tar–tsr* double mutant that has a severely impaired taxis [7] did not form a swarming colony (not shown), but microscopic observations revealed that the cells were fully motile. The reason why the *tsr* colony had a greater size than the wild-type is unclear, but on Tripon medium, *tsr* colonies were smaller than those of the wild-type, while the largest ones were formed by *tar* cells (not shown). A combination of glucose, peptone and yeast extract in the growth medium caused a pronounced inhibition of swarming in the wild-type and *tsr* colonies. Microscopic observations showed that wild-type, *tsr* and *tar–tsr* cells from these colonies were immotile. The *tar* cells, surprisingly, were not subject to catabolite repression. In further experiments, therefore, *tsr–tar* cells were used instead of the *tar* mutant. To create optimal conditions for the possible effect of repellents on flagellation, glucose was added at a concentration (0.3%) that produced only partial catabolite repression, allowing the cells to form small swarmer colonies (fig.1a). Addition of 1 mM benzoate to the growth medium containing 0.3% glucose, peptone and yeast extract, had no apparent effect on the size of the colonies as compared to the control without benzoate (not shown). However, microscopic observations revealed that 30% of the wild-type cells were motile, while *tsr* and *tsr–tar* cells were immotile. A dense suspension of wild-type cells showed only tumbling motion and no translational movement, thus explaining why the colony did not swarm out of the inoculation point. The observation of constant tumbling is in agreement with [9] where cells tumbled incessantly in the presence of high concentations of benzoic acid. The absence of motile cells in the *tsr* and *tsr–tar* colonies suggested that the effect of benzoate was specifically mediated by MCPI, the *tsr* gene product.

In another experiment, the same cells were inoculated into a Petri dish that contained 0.2 mM Co^{2+} in addition to the growth medium (fig.1b). The wild-type cells and the *tsr* mutant formed large swarming colonies as compared to the control without Co^{2+} (fig.1a); cells of *tsr–tar* bacteria remained immotile. Since Co^{2+} is known to signal through the MCPII protein, this experiment indicates that activation of motility by Co^{2+} is governed by MCPII, the *tar* product. The involvement of MCPII in controlling flagellation was further tested by adding α-methylaspartate together with Co^{2+} to the growth medium. α-Methylaspartate is a non-metabolizable attractant analogue that signals through MCPII [12]. In the presence of α-methylaspartate the effect of Co^{2+} was almost completely reversed (not shown). The wild-type and *tsr* cells formed very small swarmer colonies. Attractants and repellents are known to sum up in bacterial chemotaxis [13] and it appears that this rule applies to control of flagellar synthesis as well.

The involvement of taxis in the regulation of flagellar assembly suggested an alternative:

(i) Repellent taxis either acts at the level of cAMP, thus modifying the expression of all catabolite operons; or

(ii) There exists a specific pathway of information processing from the taxis system to *fla* genes.

A simple way of solving this problem would be to see whether flagellar growth would be activated by repellents in a mutant lacking cAMP cyclase activity. To this end, *E. coli cya* mutant was grown in liquid medium containing tryptone, supplemented with various concentrations of cAMP ± a repellent mixture (benzoate + Co^{2+}). Flagellar growth was observed neither in the presence nor in the absence of repellents in the samples without cAMP. In the samples containing 0.2 mM cAMP, 5% of the cells were motile and at 0.5 mM cAMP, motility increased to 80%; yet, once again, the presence of repellents had no influence on the fraction of motile cells. We conclude that repellents do not act through a seperate regulatory pathway. Rather, taxis and catabolite repression intersect at the level of cAMP. Our experiments further indicate that the action of repellents on catabolite repression is not mediated by cGMP. Theoretically, cGMP should compete with cAMP for CRP binding and thus increase the extent of catabolite repression [14]. Repellents decrease the amount of cGMP [15] which might lead to a decrease in catabolite repression. In the presence of external cAMP, repellents could have decreased catabolite repression in *cya* mutants as well, if their action was mediated via cGMP. However, this was not the case. This means that some other component of chemotaxis (an MCP, for instance) interacts with the catabolite repression system.

The adaptive role of catabolite repression of flagellin synthesis is quite apparent. There is no reason for a cell to complete movement if it has already reached an optimal environment. However, the conditions may deteriorate due to an increase of toxic substances and the rich medium will turn into a trap. To be able to escape, cells must be able to take advantage of catabolite repression in the presence of repellents. As our present work indicates, this is indeed the case: repellents acting through specific proteins involved in chemotaxis (MCP) activate flagellar growth. The supression of catabolite repression by repellents might have a broader significance than simply activating motility; other catabolite operons would also be expected to become activated, helping the cell to survive in an unfavorable environment.

The total inability of the *tar* mutant to be catabolically repressed in our experiments may indicate a tight coupling between MCPII and the repression. The *tar* mutation may thus provide a good tool for further experimentation.

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REFERENCES

[1] Komeda, Y., Suzuki, H., Ishidsu, J. and Iino, T. (1975) Mol. Gen. Genet. 142, 289–298.

[2] Silverman, M. and Simon, M.I. (1974) J. Bacteriol. 120, 1196–1201.

[3] Sherman, M.Yu. (1982) submitted.

[4] Bar-Tana, J., Howlett, B.I. and Hertz, R. (1980) J. Bacteriol, 143, 637–643.

[5] Tso, W.-W. and Adler, J. (1974) J. Bacteriol. 118, 560–576.

[6] Adler, J. (1973) J. Gen. Microbiol. 74, 77–91.

[7] Reader, R.W., Tso, W.-W., Springer, M.S., Goy, M.F., Adler, J. (1979) J. Gen. Microbiol. 111, 363–374.
[8] Hedblom, M. and Adler, J. (1980) J. Bacteriol. 144, 1048.
[9] Kihara, M. and Macnab, R.M. (1981) J. Bacteriol. 145, 1209–1221.
[10] Repaske, D.R. and Adler, J. (1981) J. Bacteriol. 145, 1199–1208.
[11] Adler, J. (1966) Science 153, 708–716.
[12] Springer, M.S., Goy, M.F. and Adler, J. (1977) Proc. Natl. Acad. Sci. USA 74, 3312–3316.
[13] Tsang, V., Macnab, R.M. and Koshland, D.E. jr (1973) Science 181, 60–63.
[14] Botsford, J.L. (1981) Mikrobiol. Rev. 45, 620–642.
[15] Black, R.A., Hobson, A.C. and Adler, J. (1980) Proc. Natl. Acad. Sci. USA 77, 3879–3883.