JOURNAL OF BACTERIOLOGY, Apr. 1978, p. 356-358
0021-9193/78/0134-0356$02.00/0

Vol. 134, No. 1
Printed in U.S.A.

Cyclic AMP-Dependent Synthesis of Fimbriae in *Salmonella typhimurium*: Effects of *cya* and *pts* Mutations

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Received for publication 12 December 1977

Synthesis of bacterial fimbriae (group 1, subtype 1) was shown to be dependent on cyclic AMP and was subject to catabolite repression by many carbohydrates. Mutations in the genes coding for the energy-coupling protein constituents of the phosphoenolpyruvate:sugar phosphotransferase system prevented repression of fimbrial production by the sugar substrates of this enzyme system.

Bacterial fimbriae (group 1, subtype 1) consist primarily of a single, α-mannopyranoside-specific, carbohydrate-binding protein (lectin). These surface appendages are peritrichously arranged helical filaments, approximately 7 nm in width, which are responsible for the adhesion of a variety of enterobacteria to both procaryotic and eucaryotic cell types (5). If intercellular adhesion serves the bacterial cell in nutrient acquisition, one might expect that fimbrial synthesis would be subject to repressive control by a mechanism that depends on the intracellular concentration of cyclic AMP (M. H. Saier, *In* L. M. Orston and J. R. Sokatch [ed.], *The Bacteria*, vol. 6, in press). In this report, evidence is presented which suggests that this is, in fact, the case.

The fimbriated parental strains of *Salmonella typhimurium* used in the present study were S7471φF (LJ266) and S625φF (LJ267). These and isogenic *fim* strains were obtained from J. P. Duguid, Department of Bacteriology, University of Dundee, Dundee, Scotland (3, 4). Mutants in which the activity of adenylate cyclase was reduced (i.e., strain LJ303 *cya-866*) were isolated from strain LJ266 by positive selection with fosfomycin (1). Strains with mutations in the *ptsH* and *ptsI* genes, coding for the heat-stable protein HPr and enzyme I of the phosphoenolpyruvate:sugar phosphotransferase system, respectively, were isolated by a similar procedure (2). All mutant strains were characterized with respect to their fermentation properties on eosin-methylene blue fermentation agar media as well as by in vivo and in vitro enzyme assays (7).

Bacterial strains were grown at 37°C without shaking in 25 ml of medium contained within 125-ml Erlenmeyer flasks. The medium used for most of the studies reported here consisted of medium 63 (7) supplemented with nutrient broth (16 g/liter). Cells grown in tubes, in flasks with shaking, or in minimal media without nutrient broth were poorly fimbriated. Growth of the parental strains in nutrient broth without additional salts resulted in fimbriation comparable to that obtained with the medium described above, but *ptsH* and *ptsI* mutants were poorly fimbriated under these conditions. *cya* mutants were poorly fimbriated after growth in either medium. The inoculum size was 0.2 ml, and cells were routinely grown for 48 h before assay of fimbriation.

The standard assay used to quantitate fimbriae depended on the ability of these organelles to agglutinate animal erythrocytes. The assay, based on the procedures of Old et al. (3) and Rosen et al. (6), was as follows. Droplets (25-μl volume) of the following solutions were dispensed with disposable pipette droppers into wells of multititer plates (Cooke Laboratory Products, Alexandria, Va., catalog no. 1-220-35; catalog no. 1-221-25) in the following order: (i) phosphate-buffered saline; (ii) 1% bovine serum albumin in phosphate-buffered saline; (iii) the bacterial cell preparation to be assayed after serial dilution to an appropriate concentration; (iv) 3.3% Formalin-treated rabbit erythrocytes. The suspensions were mixed, wells were covered with microtiter plate sealers (catalogue no. 1-220-30), and agglutination was recorded after an incubation period, at room temperature, in excess of 2 h. A degree of fimbriation of 1 corresponds to detectable hemagglutination in an undiluted cell suspension.

Hemagglutination by the bacterial suspensions was completely inhibited by 1 mM mannose or methyl α-mannopyranoside, partially inhibited by 12 mM fructose, but not inhibited by any of the other sugars listed in Table 1 at a concentration of 12 mM. Isogenic *fim* strains were non-agglutinating. These results attested

to the specificity of the lectin assayed and established that the assay measures group 1, subtype 1 fimbriae (5).

Figure 1 shows the effect of cyclic AMP on the synthesis of fimbriae in an adenylate cyclase-deficient strain (LJ303) and its wild-type parent (LJ266). When cyclic AMP was not present in the growth medium, fimbriation of the mutant strain was not observed, but low concentrations of cyclic AMP (less than 1 mM) permitted synthesis of these organelles. Higher concentrations of cyclic AMP were inhibitory. These effects appeared to be specific to cyclic AMP, since cyclic GMP, adenosine, and 5'-AMP (1 mM concentrations) did not stimulate synthesis of fimbriae in this strain. The parental strain (LJ266) synthesized fimbriae in the absence of cyclic AMP, and no stimulatory effects by low concentrations of cyclic AMP were observed. Cyclic AMP concentrations in excess of 0.5 mM inhibited fimbrial production, as it did for the adenylate cyclase-deficient mutant.

TABLE 1. *Repression of fimbrial synthesis by carbohydrates in wild-type and pts mutant strains of S. typhimurium*[a]

Carbon source in growth medium	Relative fimbriation of strain:			
	LJ266 (wild type)	LJ269 (*ptsH323*)	LJ267 (wild type)	LJ271 (*ptsI324*)
No sugar	8	10	8	8
Fructose	<1	<1	<1	4
Glucose	1	4	1	16
Mannitol	<1	12	<1	8
N-acetylglucosamine	<1	8	<1	10
Methyl α-glucoside	12	12	8	8
Ribose	<1	<1	<1	<1
Glycerol	<1	<1	<1	<1

[a] Cells were grown for 48 h in the presence of the carbon source indicated at a concentration of 1%, as described in the text. Subsequently, serial dilutions of the cell preparations were assayed for fimbriation. Since fructose interfered with the hemagglutination assay, fructose-grown cells were washed by centrifugation and resuspension before assay. Strain LJ266 is the parent of strain LJ269; strain LJ267 is the parent of LJ271.

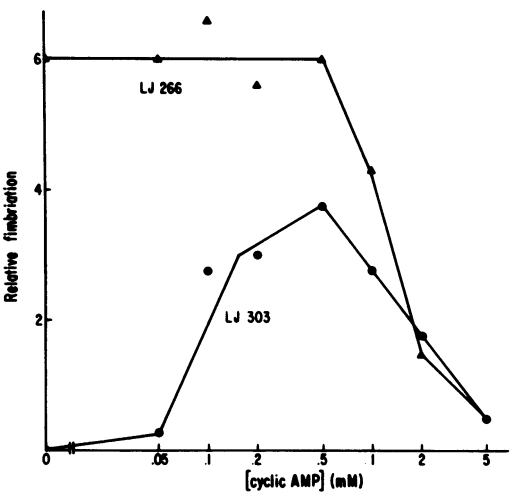

FIG. 1. *Effect of cyclic AMP on the synthesis of fimbriae in wild-type (LJ266) and adenylate cyclase-negative (LJ303) strains of S. typhimurium. Strains were grown in the presence of various concentrations of cyclic AMP (Sigma Chemical Co.) as described in the text. Fimbriation was estimated after a 48-h growth period. Results represent averages of triplicate determinations. Cyclic AMP concentration is plotted on a logarithmic scale.*

Table 1 summarizes the effects of various sugars on the extent of fimbriation, both in wild-type strains and in mutants deficient in one of the energy-coupling proteins of the phosphoenolpyruvate:sugar phosphotransferase system, HPr or enzyme I (7). All of the metabolizable sugars tested strongly repressed fimbrial synthesis in the wild-type strains. The non-metabolizable glucose analog methyl α-glucoside, which is a substrate of the glucose phosphotransferase, did not repress synthesis of fimbriae in either the parental or mutant strains. Mutations that reduced the activity of either enzyme I or HPr of the phosphotransferase system permitted fimbrial synthesis in the presence of the sugar substrates of the phosphotransferase system. These mutations did not abolish repression by carbohydrates that were not substrates of the phosphotransferase system (ribose and glycerol). The only exception to these generalizations was that fructose repressed fimbrial production in the HPr-deficient strain LJ269 *ptsH323*. This observation is in accordance with expectations since *ptsH* mutants of *S. typhimurium* can utilize fructose (7). The effects of other *pts* mutations on the repression of fimbrial synthesis were similar to those recorded in Table 1.

The preliminary results presented above suggest that the synthesis of bacterial fimbriae (group 1, subtype 1) is subject to catabolite repression by a mechanism that depends on the metabolism of the repressing sugar. Moreover, involvement of cyclic AMP is suggested by the observation that adenylate cyclase-negative strains were nonfimbriated. These observations are in accord with the possibility that bacterial adhesion, mediated by cell surface fimbriae, functions, at least in part, in nutrient acquisition (Saier, in press).

We thank J. P. Duguid for the parental bacterial strains used in this study and S. H. Barondes for advice concerning the hemagglutination assay and for the formalized rabbit erythrocytes.

This work was supported by Public Health Service grant 1 R01 AI 14176-01 from the National Institute of Allergy and Infectious Diseases and grant PCM76-81899 from the National Science Foundation.

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