Osmotic Regulation of Uropathogenic *Escherichia Coli fim* Genes

Brian T. Matthews

Faculty Sponsor: William Schwan, Department of Microbiology

ABSTRACT

Uropathogenic *Escherichia coli* (UPEC) cause most urinary tract infections. The UPEC adhere to epithelial cells via structures like type 1 pili encoded by the *fimA* gene. Upstream of *fimA* is a 314 bp invertible element containing its promoter. Positioning of the promoter is affected by two site-specific recombinases encoded by the *fimB* and *fimE* genes. Single copy number plasmids containing either *fimB::lacZ* or *fimE::lacZ* were transformed into strains of *E. coli* with mutations in the *hns* gene or *ompR* gene. By comparing ß-galactosidase activity between the mutants and their parental strains grown in neutral pH-low osmolarity and low pH-high osmolarity media, the role of each gene in osmoregulation was ascertained. A mutation in the *hns* gene resulted in a 2-fold derepression of *fimB* expression, but only a slight derepression of *fimE*. A similar derepression of *fimB* was noted in the *ompR* mutant. H-NS and OmpR appear to repress *fimB* transcription in a low pH-high osmolarity environment, but the effect on *fimE* is unclear. This suggests that the levels of *fimB* and *fimE* are affected in an environment that mimics the human urinary tract and could explain *in vivo* regulation of type 1 pili.

INTRODUCTION

The bacterial species *Escherichia coli* is the leading cause of urinary tract infections (5). One crucial factor for infection is the ability to adhere to epithelial cells in the urinary tract, typically via long filamentous appendages called pili (1, 3). One variety of pili are type 1 pili, which mediate binding to bladder epithelial cells (1). Type 1 pilus expression can undergo a process called phase variation in which the cell can switch between a piliated state to a non-piliated state (3). The type 1 pili are encoded by several *fim* genes, including *fimA*, *fimB*, and *fimE* (5). FimA is the major structural subunit for type 1 pili, whereas FimB and FimE are two recombinase proteins that affect expression of the *fimA* gene. Recent data suggests that the pH and the level of osmolarity in the environment can affect expression of *fimB* and *fimE* (6). Two genes involved in sensing osmotic changes in the environment are *bglY (*H-NS), encoding for a DNA-binding protein affecting the winding of DNA, and *ompR*, which serves as a transcriptional activator of other genes during osmotic stress.

The goal of this study was to determine the relationship of H-NS and OmpR in the regulation of *fimB* expression under different environmental conditions. H-NS was found to repress *fimB* expression, especially under high osmotic conditions, but did not have any considerable effect on *fimE* expression. OmpR was also found to repress *fimB* expression. A mutation in *ompR* appeared to deregulate the osmoregulation of *fimE*, but the data were statistically insignificant and require further study.

MATERIALS AND METHODS

Bacterial strains and plasmids. An unmarked *E. coli ompR* mutant strain MH1160 and parental strain MC4100 were provided by Linda Kenney, Oregon Health Sciences University. The *E. coli hns* mutant strain RR1 *bglY* and parental strain RR1 were provided by Staffan Normark, Karolinska Institute. The single-copy number plasmid pPP2-6 contains a multiple-cloning site and a gene for chloramphenicol resistance. The plasmid pUJ9 contains a promoterless *lacZ* gene downstream of a multiple-cloning site, and contains a gene that encodes for ampicillin resistance. To construct the plasmids pJLE4-3 (*fimE*::*lacZYA*) and pJB5A (*fimB*::*lacZYA*), the *fim* gene promoters were each ligated into the multiple-cloning site of pUJ9. The *fim*::*lacZ* gene fusions of these plasmids were then ligated into the multiple-cloning site of pPP2-6 to incorporate the gene fusion into a single-copy number plasmid.

Transformation. Competent cells of RR1, RR1 *bglY*, MC4100, and MH1160 were prepared by exposing the bacteria to cold $CaCl_2$ solution. These cells were then transformed with DNA plasmids pPP2-6, pJB5A, or pJLE4-3. The transformed cells were then grown on LB agar with chloramphenicol (12.5 ?g/mL).

Assay Media. To obtain pH variations in the culture media in vitro, LB medium was adjusted to pH 5.5 or 7.0 using 0.1 M Na_2HPO_4-NaH_2PO_4 buffer combined with 1% (vol/vol) glycerol. The osmolarity of Luria broth was adjusted by adding NaCl to a final concentration of 400 mM to test different combinations of osmolarity and pH.

TABLE 1. Effect of an *hns* mutation on *fim*B and *fim*E expression compared to expression in a wild-type strain under pH and osmotic conditions that mimic the range of the human urinary tract

Strain	Plasmid	pH	NaCl (mM)[a]	ß-gal activity[b] (Miller units)
RR1	pJB5A[c]	5.5	0	258 ± 30
RR1	pJB5A	5.5	400	236 ± 26
RR1	pJB5A	7.0	0	352 ± 12
RR1	pJB5A	7.0	400	307 ± 90
RR1 *bglY*	pJB5A	5.5	0	534 ± 18
RR1 *bglY*	pJB5A	5.5	400	486 ± 15
RR1 *bglY*	pJB5A	7.0	0	453 ± 123
RR1 *bglY*	pJB5A	7.0	400	698 ± 156
RR1	pJLE4-3[d]	5.5	0	139 ± 13
RR1	pJLE4-3	5.5	400	176 ± 26
RR1	pJLE4-3	7.0	0	294 ± 40
RR1	pJLE4-3	7.0	400	318 ± 37
RR1	*bglY* pJLE4-3	5.5	0	151 ± 27
RR1	*bglY* pJLE4-3	5.5	00	190 ± 13
RR1	*bglY* pJLE4-3	7.0	0	313 ± 34
RR1	*bglY* pJLE4-3	7.0	400	336 ± 27

[a] Concentration of NaCl added to Luria-Bertani broth.

[b] Expressed as mean ± standard deviation for three separate experiments.

[c] Single-copy number plasmid with a *fimB*::*lacZ* gene fusion.

[d] Single-copy number plasmid with a *fimE*::*lacZ* gene fusion.

ß-galactosidase assays. Cultures were grown to mid-logarithmic phase, permeabilized with sodium dodecyl sulfate and $CHCl_2$, and ß-galactosidase assays were done according to the method of Miller (2). Measurements were done after 20 min of exposure to the substrate. Assays were performed at least three times on different days and the data were expressed as mean ± standard deviations.

RESULTS

As shown in Table 1, an *hns* mutation resulted in a derepression of *fimB* expression that was strongest under high osmotic conditions and neutral pH. This mutation did not result in any significant change in *fimE* expression. Similarly, a smaller derepression was observed for *fimB* expression in an *ompR* mutant strain as demonstrated in Table 2.

TABLE 2. Effect of an *omp*R mutation on *fim*B and *fim*E expression compared to expression in a wild-type strain under pH and osmotic conditions that mimic the range of the human urinary tract

Strain	Plasmid	pH	NaCl (mM)[a]	ß-gal activity[b] (Miller units)
MC4100	pJB5A[c]	5.5	0	119 ± 22
MC4100	pJB5A	5.5	400	3 ± 6
MC4100	pJB5A	7.0	0	8 ± 36
MC4100	pJB5A	7.0	400	163 ± 30
MH1160	pJB5A	5.5	0	151 ± 12
MH1160	pJB5A	5.5	400	312 ± 50
MH1160	pJB5A	7.0	0	283 ± 53
MH1160	pJB5A	7.0	400	252 ± 64
MC4100	pJLE4-3[d]	5.5	0	101 ± 23
MC4100	pJLE4-3	5.5	400	158 ± 21
MC4100	pJLE4-3	7.0	0	148 ± 21
MC4100	pJLE4-3	7.0	400	195 ± 42
MH1160	pJLE4-3	5.5	0	26 ± 18
MH1160	pJLE4-3	5.5	400	124 ± 30
MH1160	pJLE4-3	7.0	0	212 ± 30
MH1160	pJLE4-3	7.0	400	213 ± 57

[a] Concentration of NaCl added to Luria-Bertani broth.
[b] Expressed as mean ± standard deviation for three separate experiments.
[c] Single-copy number plasmid with a *fimB*::*lacZ* gene fusion.
[d] Single-copy number plasmid with a *fimE*::*lacZ* gene fusion.

DISCUSSION

Osmotic conditions in the environment may affect two systems, the OmpR-EnvZ and H-NS systems. High osmotic conditions activate the EnvZ protein, an inner membrane protein that phosphorylates OmpR (6). Once phosphorylated, OmpR can bind to DNA sequences to activate or repress transcription of other genes. We observed a three-fold increase in expression of *fimB* in an *ompR* mutant grown in low-osmolarity neutral-pH conditions. The effect of this mutation on *fimE* expression was not as clear. It may be neutralizing the repressive

effect of an acid response regulator on *fimE*. The repression of *fimB* and the possible effect on *fimE* by OmpR favors *fimE* expression, which would shift the cell to a phase-OFF state.

Another system that may be affected by osmotic conditions is H-NS. An increase in osmolarity increases DNA supercoiling. H-NS is a histone-like DNA-binding protein that uses this increased curvature as a template, and can activate or repress transcription (6). Our results show that *fimB* is derepressed in an *hns* mutant, but variations in *fimE* expression were minimal and may be explained by strain differences.

This data provides a better understanding of how uropathogenic *E. coli* regulate a critical virulence factor needed to infect the urinary tract. This information may be used to develop drugs that target pili expression or to provide dietary guidelines to prevent urinary tract infections.

ACKNOWLEDGMENTS

I would like to thank the University of Wisconsin – La Crosse Undergraduate Research Committee for providing funding for this project, and the University of Wisconsin – La Crosse Summer Undergraduate Fellowship Committee for giving me the opportunity to pursue this research extensively. I would like to thank Dr. William Schwan for the guidance and direction he has provided me in this endeavor.

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