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Altered Osmoregulation of *ompF* in Integration Host Factor Mutants of *Escherichia coli*

PING TSUI, VICTOR HELU, AND MARTIN FREUNDLICH*

Department of Biochemistry, State University of New York, Stony Brook, New York 11794-5215

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Osmoregulation of the porin protein OmpF was strongly altered in integration host factor (IHF) mutants. These mutants produced approximately 15-fold more OmpF than did the parent strain when grown in media of intermediate osmolarity. At high osmolarity IHF mutants continued to produce considerable amounts of OmpF, although this protein was undetectable in the parent grown under these conditions. Experiments with an *ompF-lacZ* chromosomal fusion strain suggested that these changes in osmoregulation in large part involve alterations in transcriptional activity of the *ompF* promoter. These results add to the growing list of genes whose expression is modified in IHF mutants.

Integration host factor (IHF) is a protein composed of two dissimilar subunits encoded by the *himA* and *hip* (*himD*) genes of *Escherichia coli* (31). IHF is a DNA-binding protein that is involved in a variety of site-specific recombination events, including integration of bacteriophages λ (23, 30) and φ80 (21), transposition by IS*1* (10) and Tn*10* (28), and phase variation in *E. coli* (3, 5). Other studies have shown that IHF also participates in the expression of a number of phage (6, 12, 15, 20, 33) and bacterial genes (3, 7, 8, 9, 24). In many of these cases IHF function has been correlated with a 13-base-pair DNA binding consensus sequence (2, 21) located in proximity to the proposed site of IHF action (10, 11, 20–22). We have recently identified this consensus sequence in many operons in *E. coli* K-12, including the promoter regions of *ompB*, *ompC*, *ompF*, and *micF* (P. Tsui and M. Freundlich, unpublished observations; see Table 3). These operons are necessary for the expression and osmoregulation of the major outer membrane proteins OmpF and OmpC (13, 14, 19, 26, 37). The location of these sequences suggested to us that IHF might play a role in the expression of these proteins. In the present study we examined the effect of mutations in IHF on the formation of the major outer membrane proteins. The results show that osmoregulation of OmpF is strongly altered in the mutants and that the alteration, at least in part, involves changes in transcription at the *ompF* promoter.

The strains used in this study are listed in Table 1. We compared the levels of the major outer membrane proteins in three different IHF mutants and their isogenic parent. The bacteria were grown in nutrient broth (low osmolarity) supplemented with various concentrations of sucrose, and outer membranes were prepared and analyzed as described by Ramakrishnan et al. (36). Very little differences were found between the mutants and parent in the levels of the major outer membrane proteins when the strains were grown in medium of low osmolarity (Fig. 1, lanes a to d). However, a marked difference in OmpF levels was observed in the mutants when they were grown in nutrient broth supplemented with 5 and 10% sucrose. Under these conditions the amount of OmpF in the parent, as has been shown previously (13, 19, 37), was sharply reduced (Fig. 1, lanes a, e, and i). This reduction was much less severe in the mutants. There was considerably more OmpF made by the mutants during growth with 5 and 10% sucrose (Fig. 1). This difference in OmpF synthesis was even more apparent when the cells were grown in nutrient broth supplemented with 15 or 20% sucrose. Under these conditions, OmpF was essentially undetectable in wild-type *E. coli* K-12 (Fig. 2; 19, 35), but it was still made in appreciable amounts in the mutant. The data shown in Fig. 2 was quantified with a Shimadzu densitometer. The results (Table 2) clearly demonstrate that the IHF mutant is strongly altered in osmoregulation of OmpF. The addition of 15% sucrose to nutrient broth reduced OmpF production 115-fold in the parent and only 4-fold in the mutant. No OmpF was detectable in the parent during growth in medium with 20% sucrose. Under these conditions the mutant produced approximately as much OmpF as was found in the parent grown with 5% sucrose (Table 2). The levels of the other major outer membrane proteins, OmpC and OmpA, were altered to a small extent in

TABLE 1. Strains used

Strain	Relevant genotype	Source or reference
N99	Wild type	7
K634	N99 *himA42*	7
K5185	N99 Δ*himA82*	7
K5248	N99 *himD157*	7
MH513	Δ*lac* φ(*ompF-lacZ*$^+$)	13
MF2750	MH513 Δ*himA82*	This study

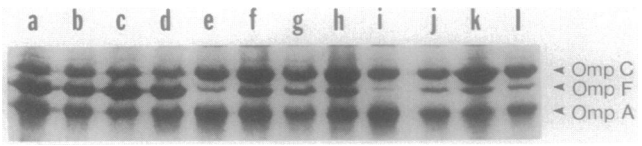

FIG. 1. Osmoregulation of outer membrane proteins in IHF mutants. One milliliter of a 16-h nutrient broth culture was added to 25 ml of fresh medium, and growth was continued for 5 h at 37°C. The cells were centrifuged at 10,000 × *g* and suspended in 4 ml of sodium phosphate (10 mM, pH 7.0). The outer membranes were prepared as described by Ramakrishnan et al. (36) and analyzed by 8 M urea–sodium dodecyl sulfate–polyacrylamide gel electrophoresis (27). Strains N99 (lanes a, e, and i), K5185 (lanes b, f, and j), K634 (lanes c, g, and k), and K5248 (lanes d, h, and l) were grown in nutrient broth supplemented with the following amounts of sucrose: 0%, lanes a to d; 5%, lanes e to h; 10%, lanes i to l.

* Corresponding author.

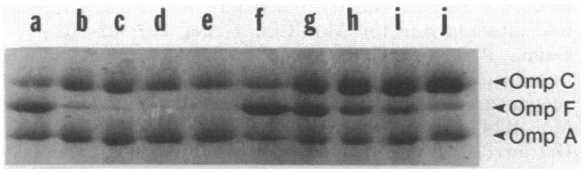

FIG. 2. Level of outer membrane proteins in an IHF mutant grown in medium of high osmolarity. Strains N99 (lanes a to e) and K634 (lanes f to j) were grown in nutrient broth supplemented with the following amounts of sucrose: 0%, lanes a and f; 5%, lanes b and g; 10%, lanes c and h; 15%, lanes d and i; 20%, lanes e and j. For other conditions see the legend to Fig. 1.

the mutant. At high osmolarity the amount of OmpC in the mutant was somewhat increased compared with that in the parent, whereas the amount of OmpA was slightly reduced (Table 2).

Osmoregulation of OmpF and OmpC is thought to occur primarily at the level of transcription (13, 14). We used strain MH513, which has a chromosomal fusion between the *lacZ* structural gene and the *ompF* promoter, to investigate whether changes in osmoregulation of OmpF in IHF mutants involves changes in transcription. We transduced a *himA* mutation into this strain, and β-galactosidase was measured after growth of the cells in nutrient broth supplemented with various concentrations of sucrose. The data shown in Fig. 3 show a strong reduction in β-galactosidase activity in the parent strain grown in media with increasing amounts of sucrose. In contrast, under these conditions there was only a small decrease in β-galactosidase activity in the IHF mutant. In addition, β-galactosidase levels under all conditions were higher in the mutant. This ranged from about 1.6-fold in cells grown without sucrose to about 6-fold when the cultures were grown in medium supplemented with 20% sucrose (Fig. 3). These results suggest that altered osmoregulation of OmpF in IHF mutants is due, at least in part, to a strong reduction in the negative osmoregulation of *ompF* transcription normally seen in wild-type cells (Fig. 3; 13, 14).

Osmoregulation of *ompF* is a complex process that is thought to mainly involve transcriptional control mediated by OmpR and EnvZ, the protein products of the *ompB* operon (13, 14, 29). OmpR, in addition to its role in osmoregulation, is a positive activator of *ompF* (14, 19). Recent evidence indicates that OmpR binds to the *ompF* promoter (18) in a region between −105 and −60 (32). This region, as well as other upstream and downstream sequences, have been reported to be necessary for *ompF* osmoregulation mediated by OmpR (16, 34). In addition, evidence suggests that this regulation also involves translational control by *micF* RNA (1, 26) and OmpR-independent osmoregulation (16, 36). Our results suggest that IHF mutants are deficient in

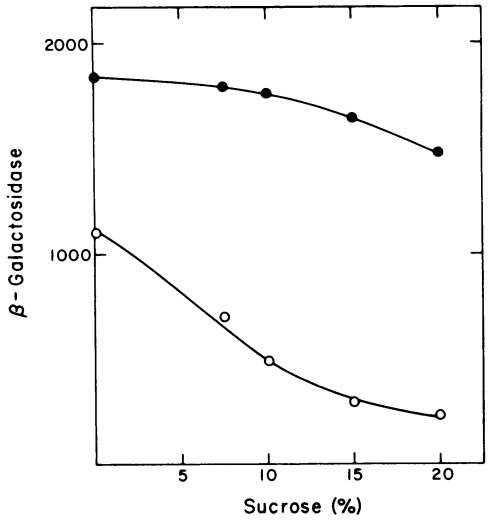

FIG. 3. Effect of an IHF mutation on β-galactosidase activity in an *ompF-lacZ* fusion strain. Cells were grown in nutrient broth containing various amounts of sucrose. Samples were removed during mid-exponential growth, and β-galactosidase activity was measured as described by Miller (25). Symbols: ●, strain MF2750 (IHF^-); ○, strain MH513 (IHF^+). The data are averages of three separate experiments.

a major transcriptional component of *ompF* osmoregulation. One explanation for these results is that IHF mutants may have quantitative or qualitative alterations in OmpR or changes in the ability of OmpR to interact with the *ompF* promoter. In this regard, there is an IHF-binding consensus sequence within the *ompB* promoter region and within the proposed OmpR-binding site (32) in *ompF* (Table 3). Therefore, binding of IHF to these sites could directly effect *ompR* expression and/or the interaction of OmpR with the *ompF* promoter. Alternatively, IHF may affect osmoregulation indirectly by influencing the expression of a factor that in turn affects a component of this regulation.

Although osmoregulation of OmpF was strongly reduced in the IHF mutants, there was still an appreciable decrease in the amount of this protein when these strains were grown in media of high osmolarity (Fig. 1 and 2). Since *ompF* transcription does not appear to have been greatly reduced under these conditions in the mutants (Fig. 3), this decrease may have been due to a mechanism occurring after transcription initiation. This may reflect the normal functioning in the mutants of additional mechanisms that participate in *ompF* osmoregulation at the level of transcription, translation, secretion, or membrane assembly. For example, IHF mutants may be deficient in the major mechanism of osmoreg-

TABLE 2. Effect of osmolarity on formation of outer membrane proteins in a *himA* mutant

Protein	Level (%)[a] of protein in % sucrose:									
	0		5		10		15		20	
	N99	K634	N99	K634	N99	K634	N99	K634	N99	K634
OmpC	12.5	17.6	33.6	37.1	33.2	47.5	26.7	51.0	27.7	54.8
OmpF	47.5	53.8	5.3	33.4	1.1	18.9	0.4	12.9	0	6.9
OmpA	40.0	28.6	61.1	29.5	65.7	33.6	72.9	36.1	72.3	38.3
OmpF/OmpC	3.8	3.1	0.16	0.9	0.03	0.4	0.02	0.25		0.13

[a] The levels of the outer membrane proteins on the polyacrylamide gel (Fig. 2) were quantified with a Shimadzu densitometer. The values shown are the percentages of the total outer membrane proteins obtained by adding the values for OmpA, OmpC, and OmpF for each sample. This was done to reduce fluctuations in the levels of these proteins due to differences in the amounts of sample loaded on the gel.

TABLE 3. IHF consensus sequences in the *ompF* promoter and related regulatory gene promoters

Promoter[a]	Sequence[b]	Location[c]	Reference
ompF	TAAtctaTTTATA	−177	17
ompF	TAAagatTTGGTT	−68	17
ompB	CATctcgTTGATT	−227	38
micF	TAAgttaTTGATT	+4	26
micF	TAAgtatTTGACA	+21	27
Consensus	C/TAAnnnnTTGATA/T		21

[a] The *ompB* promoter controls the expression of the *ompR* and *envZ* genes.
[b] The sequences are aligned with the IHF consensus sequence taken from Leong et al. (21). Bases that differ from the consensus are underlined.
[c] Except for *ompB*, the sequences are located at their 5′ ends in relation to the transcription initiation site at +1. This site is not known in *ompB*. Therefore, the location of the IHF consensus is given in relation to the start of *ompB* translation at +1.

ulation mediated by the OmpR protein (13, 14, 29) but other reported components of this system, such as OmpR-independent osmoregulation (16, 36) or control by *micF* (1, 26), may operate normally. It should be noted that the *OmpF-lacZ* fusion strain used in the present study may not have contained all of the determinants reported to be necessary for OmpR-mediated transcriptional osmoregulation of *ompF* (16, 34). Therefore, some aspects of this transcriptional mechanism may still be functional in IHF mutants.

The preliminary results in this report show that, directly or indirectly, IHF has an important role in the osmoregulation of *ompF*. While the physiological role and mechanism of action of IHF in *E. coli* have not been established, the present report adds to the growing volume of data (4) that suggests that this protein may have a major function in gene expression in this organism.

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