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Regulation of *ompC* and *ompF* Expression in *Escherichia coli* in the Absence of *envZ*

STEVEN FORST,* JORGE DELGADO, GIRIJA RAMAKRISHNAN,† AND MASAYORI INOUYE

Department of Biochemistry, Robert Wood Johnson Medical School at Rutgers, University of Medicine and Dentistry of New Jersey, Piscataway, New Jersey 08854

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The expression of the genes encoding the major outer membrane porin proteins OmpF and OmpC in *Escherichia coli* is regulated by *ompR*, which encodes the transcriptional activator protein OmpR, and *envZ*, which encodes a receptorlike protein located in the inner membrane. To examine the role of EnvZ in the expression of the osmoregulated porin genes, we analyzed the production of OmpF and OmpC in cells that lack *envZ* function. We show that EnvZ is required for the maximal production of OmpC in cells grown in minimal medium but is not essential for the efficient induction of OmpC that occurs during a shift to a high-osmolarity medium. In contrast, the production of OmpF in cells that lack *envZ* function was similar to that of the parent strain, whereas OmpF repression during a shift to a high-osmolarity medium was incomplete in the absence of EnvZ. These results are discussed in the context of the putative role of EnvZ in the expression of *ompF* and *ompC*.

The pores in the outer membrane of *Escherichia coli* K-12, through which small hydrophilic molecules diffuse passively, are formed by the porin proteins OmpF and OmpC (22). The genes encoding OmpF and OmpC are located at 21 and 48 min, respectively, on the *E. coli* chromosome (18, 28, 29). In the *E. coli* strains most commonly used to study osmoregulation, both OmpF and OmpC are expressed when the bacterium is grown at 37°C in minimal medium, whereas in medium of high osmolarity, OmpC is preferentially expressed and OmpF is repressed. Numerous genetic studies have shown that the *ompB* operon, which consists of *ompR* and *envZ*, regulates porin expression and is located at 75 min on the chromosome (7, 8, 26). The DNA nucleotide sequence of this locus has been determined (3, 20, 21, 32). The *ompB* locus encodes two gene products, OmpR and EnvZ, which are apparently produced from a single polycistronic mRNA (20).

OmpR is a DNA-binding protein that activates the transcription of *ompF* and *ompC* by binding to sequences approximately 90 base pairs upstream of the transcriptional initiation site of these genes (9, 11, 13, 23). EnvZ, a molecule consisting of 450 amino acid residues, is an inner membrane protein whose membrane topology (4) is similar to that of the chemotactic transducer proteins Tar (25) and Tsr (2). The periplasmic domain of EnvZ is composed of 115 amino acid residues, and the cytoplasmic domain contains approximately 270 amino acid residues. It is not yet clear how EnvZ affects porin expression. Recent genetic evidence supports the hypothesis that OmpR and EnvZ interact functionally (17) and that EnvZ exerts its influence on *ompF* and *ompC* expression via OmpR (27).

At present, the role of *envZ* in the expression of *ompF* and *ompC* has been analyzed only under steady-state growth conditions. The influence of *envZ* on porin expression when the bacterium experiences an abrupt change in the osmolarity of the growth medium has not been examined. In this report, we examine both the steady-state production of OmpF and OmpC in an *envZ* amber strain of *E. coli* grown under different conditions and the rate of production of the porin proteins during a shift in the osmolarity of the growth medium.

* Corresponding author.
† Present address: Department of Molecular Biology, Princeton University, Princeton, NJ 08544.

MATERIALS AND METHODS

Bacteria and plasmids. The strains of *E. coli* and the plasmids used in this work are listed in Table 1.

Media, chemicals, and enzymes. Urea was obtained from Bio-Rad Laboratories, Richmond, Calif. Restriction enzymes and cloning linkers were obtained from New England BioLabs, Inc., Beverly, Mass. [^{35}S]methionine (1,100 Ci/mmol) was purchased from ICN Radiochemicals, Irving, Calif. Ampholines and acrylamide were obtained from LKB, Bromma, Sweden. The reagent used for immunodetection was purchased from Promega Biotec, Madison, Wis. Other reagents were obtained from Sigma Chemical Co., St. Louis, Mo.

The M9 minimal medium used in this study contained 160 mM Na_2HPO_4, 80 mM KH_2PO_4, 40 mM NaCl, 72 mM NH_4Cl, 0.8 mM $MgSO_4$, 0.4% glucose, 2 μg of thiamine per ml, and 0.008% biotin. For 0.3× M9 medium, the salt concentration was reduced by one-third but the $MgSO_4$, glucose, and thiamine concentrations were kept constant. Sucrose was added as required to a final concentration of 20% to obtain a high-osmolarity medium. When required, ampicillin was added at a concentration of 50 μg/ml.

Construction of pDS212, an *ompR*$^+$ Δ*envZ* plasmid. The *ompB*-containing plasmid pAT428 was constructed previously by cloning a *Pvu*II-*Hpa*I fragment (3.4 kilobases) of the *E. coli* chromosome into the *Pvu*II-*Bam*HI sites of pBR322 (12). To construct an *ompR*$^+$ Δ*envZ* plasmid from pAT428, a *Hin*dIII linker was inserted into the *Aat*II site of pBR322 and a 100-base-pair *Hin*dIII fragment was removed from the resultant plasmid, thus creating pYK12 (4). A *Hin*dIII site was introduced at the junction between *ompR* and *envZ* in pYK12 by site-directed oligonucleotide mutagenesis to create pDR201 (4). The C-terminal Ala-239 for OmpR was not altered by this manipulation, whereas the

TABLE 1. Bacteria and plasmids used

Strain or plasmid	Relevant properties	Reference or source
E. coli K-12 strains		
MC4100	F⁻ Δ*lacU169 araD rpsL relA thi flbB*	9
SG477	MC4100 *envZ22*	6
SG480Δ76	MC4100 Δ(*malT-ompB*)	5
Plasmids		
pAT428	Ap^r; vector, pBR322; cloned genes, *ompR* and *envZ*	11
pYK12	Ap^r; vector, pBR322; cloned genes, *ompR* and *envZ*; Δ*Aat*II-*Hin*dIII of pBR322	4
pDR201	Ap^r; vector, pBR322; cloned genes, *ompR* and *envZ*; *Hin*dIII introduced at junction of *ompR-envZ* of pYK12	4
pDS212	pDR201 Δ*envZ*	This study

initiation codon for *envZ* was changed to TTG to create the new *Hin*dIII site. The coding region for *envZ* was completely removed from pDR201 by digesting this plasmid with *Hin*dIII and religating the larger fragment (pDS212). The *ompB* promoter was retained in pDS212, whereas the OmpR protein thus created contained an additional three amino acids (Leu-Met-Arg) after Ala-239.

Preparation of outer membrane proteins. Mid-log-phase cells were harvested and sonicated in 20 mM sodium phosphate buffer (pH 7.1). Sodium *N*-lauryl sarcosinate was added to a final concentration of 0.5%, and the detergent extraction was carried out at room temperature for 35 min. After centrifugation (3,000 × *g*) for 1 min, the supernatant, which contained outer membrane proteins, was centrifuged at 393,000 × *g* for 14 min in a Beckman TL-100 ultracentrifuge. The resultant pellet of outer membrane protein was solubilized in buffer containing 1% sodium dodecyl sulfate (SDS), boiled for 5 min, and analyzed by urea-SDS-polyacrylamide gel electrophoresis (PAGE) as described previously (3).

Radiolabeling of *E. coli* proteins. Overnight cultures grown in M9 medium were used to inoculate subcultures of the appropriate medium, and cells were grown at 37°C to mid-log phase. For pulse-labeling experiments during shift to high osmolarity, 0.5 ml of 60% sucrose in M9 medium (37°C) was added to 1.0 ml of mid-log-phase cells grown in M9 medium. Between 50 and 100 μCi of [^{35}S]methionine was added to 200 μl of cell suspension at the indicated time points. Labeling was continued for 30 s, at which time 5 μl of 5-mg/ml unlabeled methionine was added, and the incubation was continued for an additional 30 s. To terminate the incorporation of radiolabel, sodium azide was added to a final concentration of 20 μg/ml, and the cells were transferred to 4°C, pelleted, and washed twice with 1 ml of 20 mM sodium phosphate (pH 7.1). The outer membrane proteins were obtained as described above. Radiolabeled protein was analyzed by SDS-PAGE and autoradiography as described previously (4). Coomassie blue-stained gels and autoradiographs were scanned by using a Hoeffer scanning densitometer. The quantitative analysis of the protein peaks was obtained by using the GS-360 Hoeffer Data System.

Preparation of soluble extracts and electrophoretic procedures. Cells grown at 37°C in either M9 or M9 plus 20% sucrose minimal medium (50 ml) were harvested at mid-log phase and washed with 20 mM sodium phosphate buffer (pH 7.1). After sonication in 400 μl of sodium phosphate buffer (4°C), the cell extracts were centrifuged (4°C) for 14 min at 393,000 × *g* (Beckman TL-100 ultracentrifuge), and the supernatants were collected and stored at −20°C. Soluble extracts were electrophoresed either on an SDS-PAGE system (15% acrylamide–0.0625% bisacrylamide) as described by Laemmli (15) or on an isoelectric-focusing gel as described below. The isoelectric-focusing gel (10 cm by 12 cm by 1.5 mm) contained 11.4 ml of 9.7% acrylamide–0.3% bisacrylamide, 2.6 ml of glycerol, 1.26 ml of ampholine (pH 3.5 to 10; 40%) and 4.8 ml of distilled water and was polymerized by the addition of 40 μl of ammonium persulfate (10% [wt/vol]) and 18 μl of *N,N,N',N'*-tetramethylethylenediamine. The gel was precooled to 4°C on a horizontal Pharmacia FBE 3000 apparatus. Soluble extracts (2 to 10 μl) were then loaded directly onto the gel surface, and electrophoresis was conducted for 2 h, first at 17 W (450 V) until the voltage reached 750 V and then at constant voltage (750 V).

Western blot (immunoblot) analysis. After electrophoresis, the gels were equilibrated in transfer buffer (48 mM Tris, 38 mM glycine, 1.3 mM SDS, 20% methanol) for 60 min at room temperature. Protein was electrotransferred onto nitrocellulose paper by using the Sartoblot semidry apparatus as described by the manufacturer (Sartorius, Göttingen, Federal Republic of Germany). The immunodetection of OmpR was accomplished by using polyclonal anti-OmpR antiserum and the alkaline phosphatase-conjugated goat anti-rabbit antibody as described by the manufacturer (Promega). Protein concentration was determined by the Bio-Rad protein assay (Bio-Rad Laboratories).

RESULTS

OmpF and OmpC production in the absence of *envZ* function. The *envZ22* mutation is recessive to all other *envZ* alleles tested (6), and the *envZ22* (amber) strain grown overnight at 37°C in tryptone-yeast extract broth does not produce OmpC and produces lower levels of OmpF (6). In our initial studies with the *envZ22* strain, we found that the pattern of outer membrane protein in cells grown in L broth was identical (S. Forst, data not shown) to that reported previously (6). We used the *envZ22* strain to study the production of OmpF and OmpC in cells grown under different conditions of osmolarity and temperature.

The pattern of outer membrane protein production in MC4100 and the *envZ22* strain grown in either M9 minimal medium or M9 minimal medium plus 20% sucrose (high osmolarity) is shown in Fig. 1. Although *ompC* was produced in MC4100 (Fig. 1, lane 1), in the *envZ22* strain, OmpC was not detectably produced in cells grown in M9 medium (Fig. 1, lane 3) but was produced in cells grown in M9 medium plus 20% sucrose (Fig. 1, lane 4). The amount of OmpF in the outer membrane of the *envZ22* strain grown in M9 medium was very similar to that found in MC4100 but was not effectively repressed by the presence of 20% sucrose (Fig. 1, lane 4). To compare the relative levels of OmpF and OmpC in the outer membranes of MC4100 and the *envZ22*

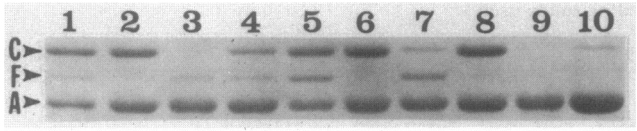

FIG. 1. Coomassie blue-stained outer membrane proteins of MC4100, the *envZ22* strain, Δ*ompB*/pAT428, Δ*ompB*/pDS212, and Δ*ompB* analyzed by urea-SDS-PAGE. Cells were grown at 37°C in either M9 medium (lanes 1, 3, 5, 7, and 9) or M9 medium plus 20% sucrose (lane 2, 4, 6, 8, and 10). Outer membrane proteins were prepared from MC4100 (lanes 1 and 2), the *envZ22* strain (lanes 3 and 4), Δ*ompB*/pAT428 (lanes 5 and 6), Δ*ompB*/pDS212 (lanes 7 and 8), and Δ*ompB* (lanes 9 and 10) by procedures described in Materials and Methods. The positions of OmpC, OmpF, and OmpA are indicated by C, F, and A, respectively.

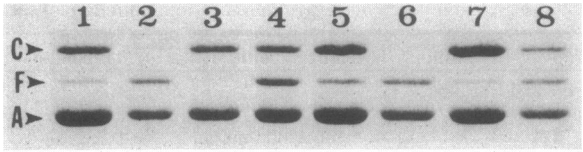

FIG. 2. Coomassie blue-stained outer membrane proteins of MC4100 and the *envZ22* strain analyzed by urea-SDS-PAGE. MC4100 (lanes 1, 3, 5, and 7) and the *envZ22* strain (lanes 2, 4, 6, and 8) were grown under the following conditions: 0.3× M9 medium (lanes 1 and 2) or 0.3× M9 medium plus 20% sucrose (lanes 3 and 4) at 37°C or M9 medium (lanes 5 and 6) or M9 medium plus 20% sucrose (lanes 7 and 8) at 22°C.

strain, densitometric measurements of the Coomassie blue-stained OmpA, OmpF, and OmpC bands were obtained and the amount of OmpF or OmpC present in the outer membrane was expressed as a percentage of the total protein absorbance of OmpA, OmpF, and OmpC (Table 2). By this analysis, OmpC production in *envZ22* reached approximately 70% of the amount found in MC4100 grown in medium of high osmolarity.

Because it is possible that an amino-terminal EnvZ fragment is produced in the *envZ22* strain that may affect the expression of *ompF* and *ompC*, we used an *ompB* deletion strain, Δ*ompB* (5) complemented with plasmids containing either *ompR envZ* (pAT428) or *ompR* Δ*envZ* (pDS212) to further study the function of *envZ* (see Materials and Methods). We first determined whether multiple copies of *ompR* and *envZ* alter *ompC* or *ompF* expression by examining the outer membrane protein composition of MC4100 containing either pAT428 or pDS212. The outer membrane protein patterns of these cells were identical to that of MC4100 carrying the control plasmid pBR322 (Forst, data not shown). The outer membrane proteins of the *ompB* deletion strain containing either the *ompR envZ* plasmid (pAT428; Fig. 1, lanes 5 and 6) or the *ompR* Δ*envZ* plasmid (pDS212; Fig. 1, lanes 7 and 8) are shown for cells grown in either M9 medium or M9 medium plus 20% sucrose. OmpC production in the *envZ* mutant cells (Δ*ompB*/pDS212) grown in M9 minimal medium was reduced by more than 70% relative to that in the Δ*ompB*/pAT428 strain and was induced in the *envZ* mutant cells when grown in the presence of 20% sucrose (Table 2). In contrast to OmpC production, the production and osmofluctuation of OmpF in the Δ*ompB*/pAT428 and the Δ*ompB*/pDS212 strains were nearly identical. It should be noted that with both pAT428 (Fig. 1, lane 5) and pDS212 (Fig. 1, lane 7) the amount of OmpF in cells grown in M9 medium was elevated relative to that found in MC4100 (Fig. 1, lane 1) and the *envZ22* strain (Fig. 1, lane 3), whereas the production of OmpF was markedly repressed in the pDS212-containing cells grown in high-osmolarity medium (Fig. 1, lane 8 versus lane 4). These differences are most likely caused by the effect of multiple copies of *ompR* in the Δ*ompB* background. The enhanced repression of OmpF has been previously observed by Matsuyama et al. (17) with another *ompR* Δ*envZ* plasmid, pMAN100, introduced into the Δ*ompB* mutant strain grown in the presence of 20% sucrose.

These results suggest that the absence of *envZ* function does not affect the level of OmpF production in cells grown in M9 medium. Because cells grown in M9 medium produce relatively low levels of this porin, the influence of EnvZ on OmpF production may remain undetected under these conditions. Therefore, we grew MC4100 and the *envZ22* strain in either medium of lower osmolarity (0.3× M9) or at 22°C (16), both of which are conditions that increase the relative levels of OmpF in the outer membrane. The results of the densitometric scanning analysis of the Coomassie blue-stained gel (Fig. 2) containing the outer membrane proteins of MC4100 and the *envZ22* strain grown under these conditions are shown in Table 3. The results are very similar to those obtained for MC4100 and the *envZ22* strain grown at 37°C in M9 medium (Table 2). Taken together, these results indicate that the *envZ* function is not essential for OmpF production in cells grown at 37°C in either M9 or 0.3× M9 medium or in cells grown at 22°C in M9 medium but is required for the complete repression of OmpF. In contrast, *envZ* is required for OmpC production in cells grown under these conditions but is not essential for the induction of OmpC by 20% sucrose.

OmpF and OmpC production during shift in osmolarity. Because the effects of *envZ* on *ompF* and *ompC* expression have been examined solely under steady-state growth conditions, we analyzed the time course of OmpF and OmpC production in both the MC4100 and the *envZ22* strains when the cells were shifted from M9 medium to high-osmolarity conditions (Fig. 3). At the time points indicated, the cellular proteins were pulse-labeled for 30 s with [^{35}S]methionine, and the radiolabeled outer membrane proteins were analyzed by SDS-PAGE as described in Materials and Methods. The time courses of the repression of OmpF production for MC4100 and the *envZ22* strain were similar, but OmpF was not fully repressed in the *envZ22* strain (Fig. 3). OmpC

TABLE 2. Steady-state production of OmpF and OmpC in M9 medium by various strains[a]

Medium	MC4100		*envZ22*		Δ*ompB*/pAT428		Δ*ompB*/pDS212	
	% OmpF	% OmpC	% OmpF	% OmpC	% OmpF	% OmpC	% OmpF	% OmpC
M9	15	48	18	0	22	34	38	10
M9 + sucrose	3	44	18	34	0	36	2	43

[a] The values represent the amount of OmpF or OmpC expressed as a percentage of the total absorbance of OmpA, OmpF, and OmpC, as determined by scanning densitometry, of the outer membrane proteins (Fig. 1).

TABLE 3. Steady-state production of OmpF and OmpC in 0.3× M9 medium at 22°C[a]

Medium	MC4100		envZ22	
	% OmpF	% OmpC	% OmpF	% OmpC
0.3× M9	20	37	28	5
0.3× M9 + sucrose	6	38	23	37
M9 (22°C)	22	32	36	9
M9 (22°C) + sucrose	4	32	22	25

[a] Given are percentage values of OmpF and OmpC analyzed as shown in Fig. 2.

production was effectively induced by sucrose in the *envZ22* strain (Fig. 3). The rate of OmpC production increased noticeably by 10 min after sucrose addition (Fig. 3, lanes 3) and increased to levels that were comparable to that found in MC4100. Densitometric scanning revealed that the induction of OmpC in the *envZ22* strain, at 120 min after sucrose addition, had reached approximately 75% of the amount found in MC4100 (data not shown). Because OmpC production is already high in MC4100 grown in M9 medium, an induction by sucrose was not seen (see also reference 1). The low levels of OmpC that were apparent in the *envZ22* strain grown in M9 medium (Fig. 3B, lane 1 versus Fig. 1, lane 3) most likely reflect the greater sensitivity of autoradiography compared with Coomassie blue staining of protein.

Western blot analysis of OmpR. Although the available genetic evidence derived from the *envZ22* strain suggests that the lack of *ompC* transcription and apparent decrease in *ompF* transcription are not caused by reduced levels of OmpR (6), a comparison of the levels of OmpR found in MC4100 and the *envZ22* strain has not been reported. To this end, we used the Western immunoblotting method to determine the amount of OmpR found in equivalent amounts of soluble cellular extracts applied to either an SDS-polyacrylamide gel or an isoelectric-focusing gel. The intracellular levels and the isoelectric points (pI 6.8) of OmpR in extracts of MC4100 and the *envZ22* strain grown in M9 medium plus

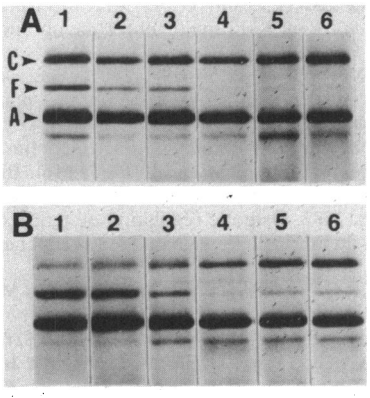

FIG. 3. Autoradiograph of radiolabeled outer membrane proteins of MC4100 (A) and the *envZ22* strain (B) pulse-labeled following a shift to high-osmolarity growth conditions. Cells were grown in M9 medium and pulse-labeled with [^{35}S]methionine during steady-state growth (lanes 1). At time = 0, sucrose (20% final concentration) was added to the growth medium, and the cells were pulse-labeled at 5 min (lanes 2), 10 min (lanes 3), 30 min (lanes 4), 60 min (lanes 5), and 120 min (lanes 6). Pulse-labeling, preparation, and electrophoresis of outer membrane proteins were carried out as described in Materials and Methods.

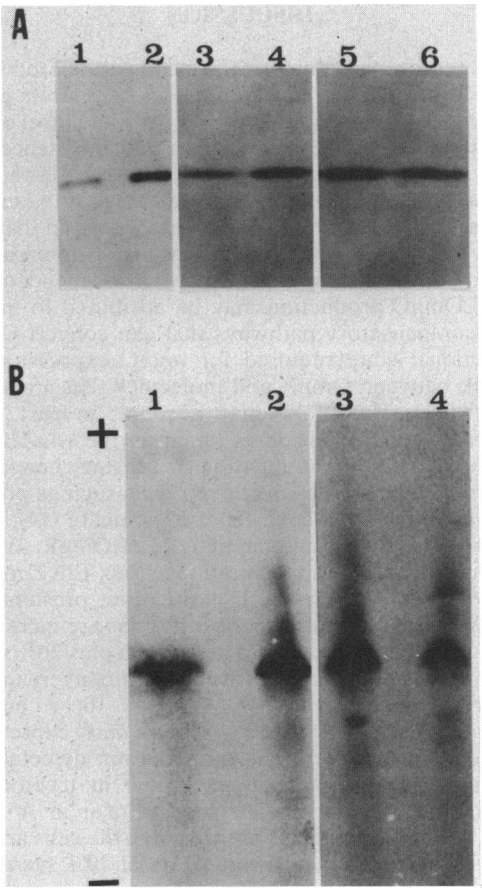

FIG. 4. Western blot analysis of OmpR in soluble extracts. (A) Soluble extracts (60 μg) from MC4100 (lanes 3 and 4) or the *envZ22* strain (lanes 5 and 6) grown at 37°C in either M9 medium (lanes 3 and 5) or M9 medium plus 20% sucrose (lanes 4 and 6) were applied to an SDS-polyacrylamide gel, and OmpR was detected by the immunoblot procedure described in Materials and Methods. Purified OmpR was applied to lanes 1 and 2 at 7.5 and 15 ng, respectively. (B) Extracts (60 μg) from MC4100 (lanes 1 and 2) or the *envZ22* strain (lanes 3 and 4) grown at 37°C in either M9 medium (lanes 1 and 3) or M9 medium plus 20% sucrose (lanes 2 and 4) were applied to an isoelectric-focusing gel, and OmpR was detected by the immunoblot procedure described in Materials and Methods. The anodal solution (+) was 40 mM aspartic acid and the cathodal solution (−) was 1 M sodium hydroxide. Samples were applied approximately 1 cm from the cathodal electrode strip.

20% sucrose were the same (Fig. 4). In contrast, the amount of OmpR in extracts of the *envZ22* strain grown in M9 medium was elevated relative to the amount found in MC4100, although the isoelectric points of OmpR in the respective extracts were identical. The relatively lower level of OmpR in extracts of MC4100 grown in M9 medium compared with M9 medium plus 20% sucrose contributed to the differences observed between MC4100 and the *envZ22* strain. That the intracellular level of OmpR per se does not play a primary role in osmoregulation is evident from the fact that the elevated levels of OmpR in MC4100(pDS212) did not alter porin expression in this cell (data not shown). Taken together, these results suggest that EnvZ does not regulate the intracellular levels of OmpR or modulate the charge properties of OmpR.

DISCUSSION

In this report, we show that the steady-state production of OmpC is dramatically reduced in the *envZ22* strain grown in M9 minimal medium and that the steady-state level of OmpC produced in the *envZ22* strain grown in the presence of 20% sucrose increases to about 70% of that found in the parent strain (MC4100). Our results also indicate that the increased rate of production of OmpC in the *envZ22* strain that occurs during a shift to a growth medium of high osmolarity is not dependent on *envZ* function. This *envZ*-independent stimulation of OmpC production may be attributed to the existence of compensatory pathways that can convert OmpR to the functional state required for *ompC* expression. Such cross-talk between OmpR and molecules that are structurally similar to EnvZ (24) may account, in part, for the sucrose-induced expression of *ompC* in the *envZ22* strain. Alternatively, EnvZ may function by sensing changes in the cytoplasmic concentrations of osmolytes such as potassium ion (10) and glycine betaine (10) or glutamate (14), thereby modulating the transcriptional activity of OmpR. When the intracellular osmolyte concentration is low, EnvZ may have a potentiating affect on *ompC* expression, presumably via OmpR. When the osmolyte concentrations are increased, as would occur in cells grown in M9 medium plus 20% sucrose, OmpR may have a greater propensity to convert to a form that preferentially activates *ompC*. Under these conditions, *envZ* would not play a primary role in *ompC* expression.

Others have observed that OmpC is not detectably produced in various *envZ* mutants grown in tryptone-yeast broth (6), in M63 minimal medium (30), or in A minimal medium (17) but could be produced when the cells are grown in the presence of sodium chloride (30) or 20% sucrose (17). The steady-state level of OmpC observed when the *envZ* mutant cells were grown in the high-osmolarity medium mentioned in the previous reports appear to be considerably lower than the higher level of production we report in this study. This difference could be caused by differences in the growth medium used and the concentration of the sodium chloride or sucrose used to increase the osmolarity of the growth medium. In this regard, it is noteworthy that in the *envZ* deletion strain AT142, there is substantial production of OmpC in cells grown in A minimal medium containing 20% sucrose (17) although OmpC production is barely detectable in cells grown in the presence of 15% sucrose (19).

Unlike the situation for OmpC, we found that *envZ* is not essential for the steady-state production of OmpF in cells grown in M9 medium. Furthermore, we found that although the rate of OmpF production decreased to a similar extent in MC4100 and the *envZ22* strain during a shift to M9 medium plus 20% sucrose, OmpF repression was incomplete in the absence of *envZ* function. These results suggest that, like *ompC* regulation, the putative *envZ* homologs may be able to partially compensate for *envZ* function. That OmpF production is not completely repressed in the *envZ22* strain and that the induction of OmpC is usually only 70% of the levels observed in the parent cells may indicate that, if compensatory pathways do function in *E. coli*, these pathways are not as efficient as the primary pathway with *envZ*.

Our results are consistent with those of Villarejo and Case (30), who showed that for cells grown in M63 minimal medium the amount of OmpF produced in the *envZ22* strain is only slightly less than that in MC4100, after results are normalized for differences in the total protein applied to the gel, and that OmpF production is not repressed by the addition of sodium chloride to the growth medium. However, although a twofold dilution of the M63 minimal medium greatly accentuated the differences in OmpF production, as well as *ompF* transcription, in the two strains we did not find such differences when the M9 minimal medium was diluted threefold. This discrepancy may be caused either by differences in the osmolarity of the medium or by factors other than osmolarity. The latter possibility was addressed by Villarejo et al. (31), who showed that the production of OmpF can vary considerably in cells grown in different media even though the osmolarities of the growth media are very similar.

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