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Regulation of OmpF Porin Expression by Salicylate in *Escherichia coli*

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The expression of *ompF*, the gene encoding a major outer membrane protein of *Escherichia coli*, is regulated by various environmental factors. The mechanism by which salicylate (SAL) drastically reduces *ompF* expression was studied here by means of *lacZ* fusions to *ompF*, *ompC*, and *micF*, by sodium dodecyl sulfate-gel electrophoresis of outer membrane proteins, and by measurements of outer membrane permeability. Growth of *E. coli* in LB broth containing SAL strongly reduced *ompF*-specific translation of an *ompF-lacZ* fusion. The extent of this reduction varied with the SAL concentration from 64% at 0.5 mM to 95% at 2 mM and >99% at 10 mM. *ompF-lacZ* transcription was not affected by SAL, whereas *ompC-lacZ* transcription was elevated by 70%. Since the *micF* transcript is antisense to a portion of the *ompF* transcript and is capable of decreasing the translation of *ompF*, the effect of SAL on *micF* transcription was measured in a *micF-lacZ* fusion strain. SAL-grown cells contained three- to fourfold more *micF* transcript during the logarithmic phase of growth than did the control cultures. However, *micF* was not absolutely required for the response to SAL. In *micF*-deleted strains, the effects of SAL on *ompF* translation, on OmpF in the outer membrane, and on outer membrane permeability were diminished but still evident. The effect of SAL on *ompF* expression was independent of the osmolarity of the medium and was epistatic to certain *ompB* regulatory mutations: the high levels of *ompF* expression found in *envZ3* and *ompR472* strains were greatly reduced by growth in SAL. Unexpectedly, the OmpC$^-$ phenotypes of these mutants were suppressed by SAL. Thus, growth in SAL severely decreases the translation of *ompF* while enhancing the transcription of *micF* and *ompC*. In this respect, SAL-grown cells resemble certain *marA* and *tolC* mutants that have high levels of *micF* and *ompC* transcripts and low levels of OmpF.

The growth of *Escherichia coli* and *Salmonella typhimurium* in the presence of millimolar concentrations of salicylate (SAL) decreases their sensitivity to a variety of antibiotics (16, 38), decreases the permeation of their outer membranes by cephalosporins (16), and decreases the amounts of OmpF porin in their outer membranes (39). Since the OmpF porins are largely responsible for the permeation of the outer membrane by low-molecular-mass (<600 Da) hydrophilic molecules, including those of many antibiotics (37), their absence explains, at least in part, the decreased sensitivity of *E. coli* grown in SAL to such antibiotics. The mechanism(s) by which SAL reduces *ompF* expression is the subject of this report.

ompF expression is controlled by a number of genes that are sensitive to the environment (see reference 14 for a review). The transcription of *ompF* and of *ompC* (which encodes the porin OmpC) is reciprocally regulated by the *ompR-envZ* (*ompB*)-encoded system in response to the osmolarity of the medium (19, 21, 27) or to treatment with procaine (44). The EnvZ protein is an environmental sensor that is bound to the inner membrane (12) and has kinase and phosphatase activities (2, 13, 24, 25). OmpR is a cytoplasmic DNA-binding protein that is needed to activate the transcription of *ompF* and *ompC* and can be phosphorylated by EnvZ (2, 13, 23).

The translation of *ompF* is regulated by *micF* transcripts. Seventy percent of the bases from a portion of this RNA are antisense to the 5′ portion of *ompF* mRNA, including the *ompF* promoter and the first 9 pre-OmpF codons (32, 33). The 93-nucleotide *micF* transcript (4) can form a stable duplex with *ompF* RNA in vitro at 37°C and can bind specifically with an 80-kDa protein from a *micF*-deleted strain (3). The formation of this complex is believed to reduce the amounts of *ompF* mRNA available for translation and, in some cases, to destabilize the *ompF* mRNA (8, 31). Substantial osmoregulation by the EnvZ-OmpR system can occur in the absence of the *micF* gene, but the response is slower and less complete (1). Mutations at the *tolC* and *marA* loci result in increased constitutive levels of *micF* transcripts and decreased translation of *ompF* (8, 31).

In these experiments, the effects of SAL on the transcription and translation of *ompF* and other genes was studied by means of *lacZ* fusions and by analyses of outer membrane porins and outer membrane permeation. Decreased translation of *ompF* and moderately increased transcription of *micF* and *ompC* were found for cells grown with SAL. These results, together with studies on *micF*-deleted bacteria, suggest that increased *micF* transcription in SAL-treated cells is responsible, in part, for the decreased *ompF* translation.

MATERIALS AND METHODS

Bacteria. The *E. coli* strains used are described in Table 1.

Media and chemicals. LB broth (Difco) contained 1% tryptone, 0.5% yeast extract, and, unless stated otherwise, 0.5% (86 mM) NaCl at pH 7.4. Modified LB broth contained 50 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid) brought to pH 7.4 with NaOH and sufficient

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TABLE 1. Bacterial strains

Strain	Description (outer membrane phenotype)[a]	Source or reference
CS483	*proC24 nmpC103 ompF254 his-53 ompC171 cycA1 cycB2? xyl-14 lacY29 rpsL97 tsx-63 relA1*	Carl Schnaitman
JF568	*aroA357 ilv-277 metB65 his-53 purE41 proC24 pdxC3 cyc-1 xyl-14 lacY29 rpsL97 tsx-63 relA1*	15
JF694	JF568 *ompC263 ompF254 nmpA1* (OmpF⁻ OmpC⁻ PhoE⁺)	15
JF701	JF568 *ompC264* (OmpF⁺ OmpC⁻)	15
JF773	JF568 *ompA260 ompF254* (OmpF⁻)	15
JF797	JF568(pR471a)	RS118 × JF568
JF1044	MH2472(pR471a)	RS118 × MH2472
JF1045	TK821(pR471a)	RS118 × TK821
JF1046	MC4100 (λK) (protein K⁺)[b]	43
JF1051	RT3(pR471a)	RS118 × RT3
JF1056	MH1471(pR471a) (OmpF⁻ OmpC⁺)	RS118 × MH1471
MC4100	Δ(*argF lac*)*U169 araD139 rpsL relA thiA flbB*	6
MH225	MC4100 *malQ7* Φ(*ompC*::*lacZ*) *10-25*	18
MH513	MC4100 *araD*⁺ Φ(*ompF*::*lacZ*) *16-13*	19
MH610	MC4100 *araD*⁺ Φ(*ompF*::*lacZ*) *16-10*	19
MH621	MC4100 *araD*⁺ Φ(*ompF*::*lacZ*) *16-21*	19
MH1471	MC4100 *envZ473* (OmpF⁻ OmpC⁺)	19
MH2472	MH225 *ompR472* (OmpF⁺⁺ OmpC⁻)	41
N7208	MC4100 *lamB106 recA1 srl*::Tn*10* Φ(*ompF*::*lacZ*) *16-13* containing pPR272[c] *ompF*⁺ Km^r (OmpF⁺⁺)	30
N7209	MC4100 (λ*KO* Φ(*ompF*::*lacZ*) *16-13* (OmpF⁺)	Spencer Benson
N7420	MH621 Δ*micF* Km^r	8
RS118	*E. coli* K1(pR471a)	42
RT3	MC4100 *envZ3* (OmpF⁺ OmpC⁻)	44
SB221	MC4100(pmicB21) Φ(*micF*::*lacZ*)	33
SM3001	MC4100 Δ*micF* Km^r	28
TK821	MC4100 *ompR331*::Tn*10* (OmpF⁻ OmpC⁻)	Nancy Tron

[a] Unless otherwise stated, strains are OmpF⁺ OmpC⁺ OmpA⁺.
[b] Contains the structural gene for protein K of *E. coli* K1 in a λ_{540} vector.
[c] This pSC101 derivative carries *ompF*⁺ and has a copy number of about 6 (31).

NaCl to bring the total of added Na^+ to 86 mM or as indicated. Nutrient broth (Difco) was made up at 8 g/liter. Antibiotics, HEPES, *o*-nitrophenyl-β-D-galactopyranoside, sodium dodecyl sulfate (SDS), ultrapure urea, and SAL were obtained from Sigma Chemical Co. (St. Louis, Mo.).

β-Galactosidase assay. The method of Miller (30) was used to assay and calculate β-galactosidase activities. Overnight cultures were diluted 1:500 and grown in LB broth, supplemented as indicated, at 37°C with aeration. Samples (0.5 to 5 ml) were removed periodically, chilled in ice-water, pelleted, and resuspended in an appropriate volume of Z buffer (30). After measurement of the A_{600} with a Beckman DU-7 spectrophotometer, the cells were made permeable to *o*-nitrophenyl-β-D-galactopyranoside by treatment with SDS and $CHCl_3$ and assayed.

Assay of outer membrane permeation. The method of Zimmerman and Rosselet (48) as modified previously (35) was used to measure the rate of permeation by cephaloridine of the bacterial outer membrane. The cells contained a periplasmic TEM-1 β-lactamase (29) encoded by plasmid pR471a (42). Briefly, intact cells grown in LB broth with or without SAL were added to a buffered solution containing 1 mM cephaloridine in a total volume of 250 μl. The A_{260} of the mixture was monitored in a 1-mm-wide cuvette at 37°C, and the permeation of the cephaloridine was calculated from the measured rate of hydrolysis.

Preparation of outer membrane proteins. The bacterial cell envelopes were prepared essentially as previously described (7). Cells grown in LB broth were collected by centrifugation, washed, resuspended in Tris-HCl buffer (pH 7.8), and disrupted by passage through a French pressure cell (American Instrument Co., Silver Spring, Md.). The inner membrane fraction was solubilized by direct extraction (23°C, 30 min) of the French press lysate with 2% Triton X-100 (40), and the outer membrane materials were separated by centrifugation (180,000 × *g*, 60 min). The nearly clear pellet containing the outer membrane proteins was resuspended in 200 μl of buffer (50 mM Tris-HCl, 5 mM $MgCl_2$) and stored at −20°C before use.

SDS-gel electrophoresis. Protein samples were analyzed by electrophoresis in 9% polyacrylamide gels containing 6 M urea and 0.1% SDS as described by Laemmli (26) and modified as described previously (47).

Quantitative densitometry. High-quality black-and-white photographs of the Coomassie brilliant blue-stained gels were scanned on a Bio-Rad model 620 densitometer operating in the reflecting mode. The data obtained were transferred to a personal computer and further analyzed by using the Bio-Rad 1-D analyst software (v.2.01). The baseline for a specific peak was drawn from point to point, and the fractional percentage of the stained material in a specific peak relative to the total material in the lane was determined with the software program.

RESULTS

Reduction of *ompF* translation. The effect of growth in LB medium with 5 mM SAL on *ompF* transcription and translation was assessed in various *ompF-lacZ* fusion strains. SAL had little effect on the levels of β-galactosidase synthesized in *ompF* transcriptional fusion strains whether they contained, in addition to the fusion, 0, 1, or 6 copies of the wild-type *ompF* allele (MH513, N7209, or N7209, respectively) (Table 2). However, two translational fusion strains (MH610 and MH621) showed 12- to 15-fold decreases in β-galactosidase synthesis caused by growth in 5 mM SAL. Thus, SAL strongly reduced the translation of *ompF* but not its transcription.

TABLE 2. β-Galactosidase activity (Miller units) of *ompF-lacZ* transcription and translation fusion strains grown in SAL[a]

Strain	Fusion type	β-Galactosidase (U)		
		−SAL	+SAL	+/− ratio
MH513[b]	Transcriptional	103	107	1.04
N7209[c]	Transcriptional	127	133	1.05
N7208[d]	Transcriptional	96	117	1.22
MH610	Translational	441	30	0.068
MH621	Translational	503	43	0.085

[a] The indicated strains were grown in LB broth without or with 5 mM SAL and assayed for β-galactosidase as described previously (30).
[b] No *ompF*+ present.
[c] One *ompF*+ gene present.
[d] About six copies of *ompF*+ present per cell (31).

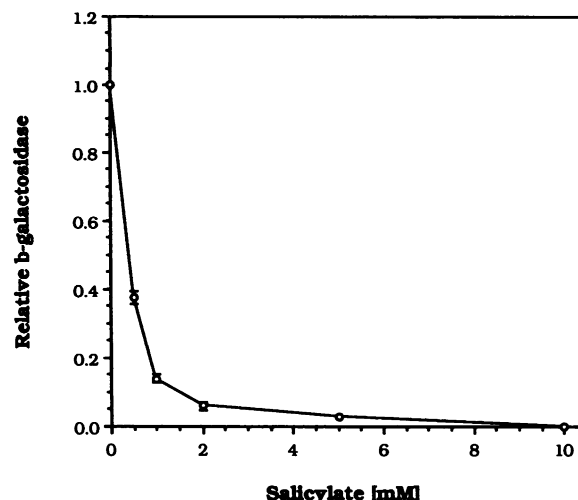

FIG. 1. Effects of SAL concentration on *ompF* translation. Cultures of the *ompF-lacZ* translational fusion strain MH621 were grown in LB broth containing 0 to 10 mM SAL and assayed for β-galactosidase. The specific activities were normalized to the activity of the control (no SAL) culture (922 Miller units).

The effect of different concentrations of SAL on the expression of β-galactosidase in the translational fusion strain MH621 is shown in Fig. 1. As little as 0.5 mM SAL reduced the β-galactosidase to 36% of the untreated value, whereas 10 mM SAL reduced the β-galactosidase to less than 1% of the control value. This compares well to the reduced amounts of OmpF found in the outer membrane of SAL-grown cells reported by Sawai et al. (39) (Table 3).

Increase in *micF* transcription. Since *micF* transcripts have been shown to reduce the translation of *ompF* (32, 33) and to be synthesized in higher amounts in certain *tolC* and *marA* mutants that also have reduced *ompF* translation (8, 31), the effect of SAL on *micF* transcription was studied in a *micF-lacZ* transcriptional fusion strain. *micF* transcription in the control culture, as reflected in β-galactosidase levels, was dependent on the growth phase of the cells (Fig. 2A). After a saturated overnight culture was diluted 500-fold into fresh LB broth, the specific activity of β-galactosidase decreased slowly for the first eight generations to a level about 15% of that of the starting culture. As the culture reentered the stationary phase of growth, the specific activity began to increase again. An apparently similar effect of cell density on *micF* expression has been observed for cells grown at 24°C (5). For cells grown in 5 mM SAL, the β-galactosidase specific activity of overnight cultures was about 40% higher than that of the controls. After dilution into fresh broth with SAL, the doubling time of the culture was 20% greater than that of the control (which is typical of growth in SAL). However, the β-galactosidase activity of this culture declined at a slower rate than that of the controls. Figure 2B shows the total β-galactosidase activity of each culture as a function of the A_{600} of the culture. During the midlogarithmic phase of growth, there was three to four times as much β-galactosidase in the SAL-grown cells as there was in the control cells. Thus, SAL enhanced the transcriptional activity of the *micF* gene, albeit to a modest extent.

Effects of SAL on a *micF*-deleted strain. To assess the role of *micF* in the SAL-mediated reduction of OmpF, the amounts of OmpF in outer membranes of a Δ*micF* strain (SM3001) were measured as a function of SAL concentration in the growth medium. Even in the absence of *micF*, SAL reduced the amounts of OmpF in the outer membrane (Fig. 3). However, greater concentrations of SAL were required for the Δ*micF* strain than for the parental strain (MC4100). Whereas growth in LB with 5 mM SAL resulted in virtually complete loss of OmpF for the parental strain, growth in 18 mM SAL did not achieve the same reduction in the Δ*micF* strain. Similar conclusions were suggested when the translation of *ompF* from *ompF-lacZ* fusions was compared for wild-type and Δ*micF* strains (MH621 and N7420, respectively) (Fig. 4). In the absence of SAL, β-galactosidase synthesis was very similar for the two strains. When the

TABLE 3. Effect of SAL on outer membrane protein composition[a]

SAL concn (mM)	Normalized % (SD) of total outer membrane protein					
	OmpF	OmpC	PhoE	Protein K	NmpC	OmpA
0	100	100	100	100	100	100
1	11.4 (8.4)	98 (16)	96 (12)	89 (13)	ND	140 (44)
5	2.9 (1.1)	85 (26)	101 (5)	71 (22)	56 (10)	204 (72)
10	0.4 (0.3)	84 (22)	90 (2)	78 (8)	ND	198 (57)

[a] Outer membrane samples were prepared from various strains grown in LB broth containing the indicated SAL concentrations. After electrophoresis of these preparations on SDS-urea-polyacrylamide gels, the quantity of each of the major proteins was determined as described in Materials and Methods. The fraction of the total protein content of the sample was determined for each of the indicated proteins and normalized to the fraction present in the cultures grown without SAL (100%). The data were then averaged from replicate experiments with different strains that synthesized the indicated protein in normal amounts when SAL was absent. The strains used (and the numbers of replicate experiments) were as follows: for OmpF, JF568 (two), JF797 (two), JF701 (one), JF1046 (one), and JF1051 (one); for OmpC, JF568 (two), JF797 (two), CS483 (one), JF773 (one), and JF1046 (one); for PhoE, JF694 (four); for protein K, JF1046 (one); for NmpC, CS483 (two); for OmpA, JF568 (two), JF797 (two), JF701 (one), JF1046 (one), JF1051 (one), and JF1056 (one). ND, not done.

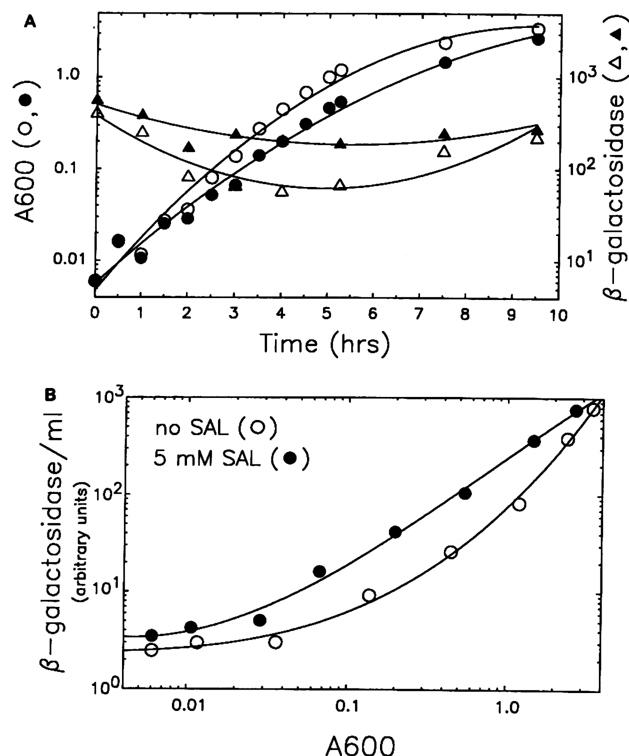

FIG. 2. Effects of SAL on *micF* transcription. Strain SB221, carrying a *micF-lacZ* fusion, was grown in LB broth with 0 or 5 mM SAL. Samples were removed at intervals to determine the A_{600} and β-galactosidase activities of the cultures. (A) A_{600} and β-galactosidase activities (Miller units) for cells grown without (○, △) or with (●, ▲) 5 mM SAL. (B) Data from panel A replotted to show the β-galactosidase activities per ml (Miller units × A_{600}) as a function of the A_{600} of the cultures grown without (○) or with (●) 5 mM SAL.

strains were grown in the presence of SAL, the amount of β-galactosidase synthesized during logarithmic growth was 20-fold lower in the *micF*$^+$ strain (MH621) but only 2-fold lower in the Δ*micF* strain (N7420). Thus, the absence of *micF* considerably diminished the inhibitory effects of SAL

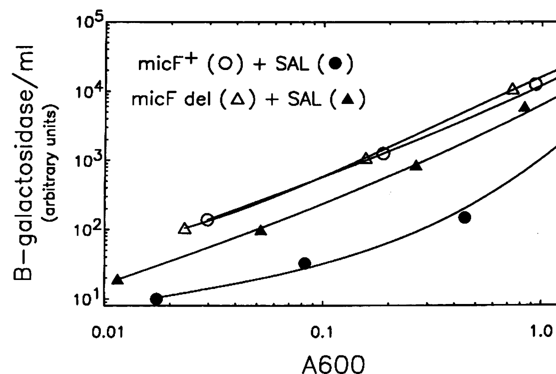

FIG. 4. *ompF* translation in wild-type (MH621) and Δ*micF* (N7420) *ompF-lacZ* translational fusion strains grown in SAL. The A_{600} and β-galactosidase activities were determined and plotted as in Fig. 2B for wild-type or Δ*micF* cells during growth in LB broth containing 0 or 5 mM SAL.

on *ompF* translation and on the OmpF content of the outer membranes.

A major consequence of the loss of OmpF protein in the outer membrane of *E. coli* is decreased outer membrane permeation by small hydrophilic molecules, such as the β-lactam antibiotics (36). Indeed, cells grown in 5 mM SAL showed three- to fivefold decreases in outer membrane permeability to various β-lactams (16). The influence of *micF* on outer membrane permeability to cephaloridine in SAL-treated cells is shown in Fig. 5. The antibiotic permeation of the Δ*micF* strain MS3001(pR471a) decreased by 25% when it was grown in only 1 mM SAL and by 70% when it was grown in 10 mM SAL. However, the decrease for the *micF*$^+$ strain MC4100(pR471a) was more extensive and required about twofold-lower SAL concentrations: a 60% decrease for 1 mM SAL cultures and an almost 90% decrease for 5 to 10 mM SAL cultures. Thus, *micF* enhanced the ability of SAL to reduce *ompF* translation, OmpF in the outer membrane, and outer membrane permeability. However, *micF* was not absolutely required for these effects.

Independence of osmolarity. The relationship between the effect of SAL and the effect of osmolarity on the porin content of strain JF797 was studied as a function of the NaCl

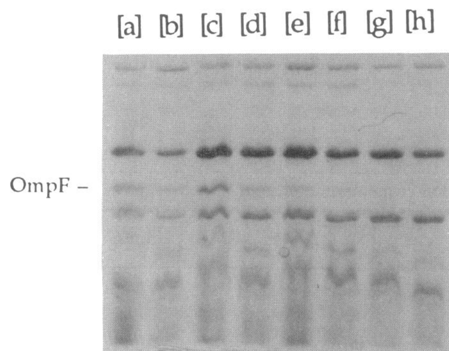

FIG. 3. Effects of SAL on the outer membrane protein composition of strain SM3001 (Δ*micF*). SM3001 was grown in modified LB broth containing the indicated concentrations of SAL. Outer membranes prepared from these cultures were subjected to electrophoresis on an SDS-polyacrylamide gel, and the gel was stained with Coomassie blue. Lanes and SAL concentrations (millimolar): a, 0; b, 1; c, 3; d, 6; e, 9; f, 12; g, 15; h, 18.

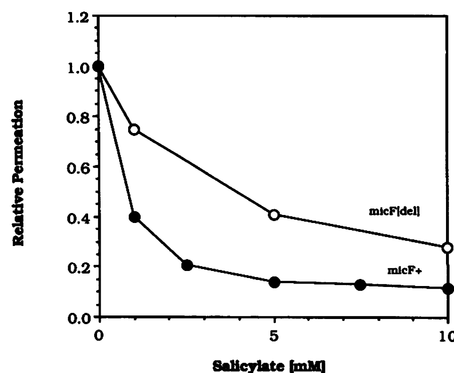

FIG. 5. Relative cephaloridine permeation of wild-type and Δ*micF* cells grown in SAL. The cephaloridine permeation of *micF*$^+$ [MC4100(pR471a)] and *micF*Δ [SM3001(pR471a)] cells grown in LB broth containing SAL (0 to 10 mM) was determined and normalized to the control rate at 0 mM SAL (4×10^{-4} cm/s).

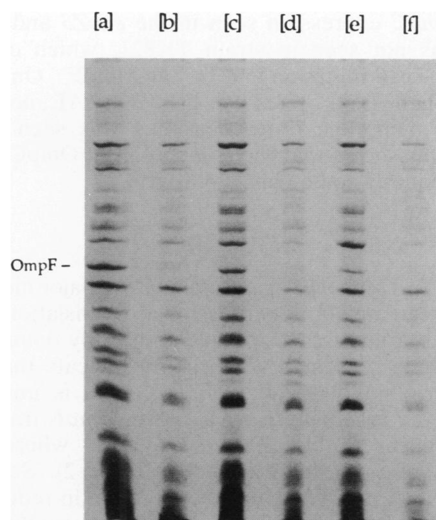

FIG. 6. Outer membrane proteins of cells grown in SAL with different concentrations of NaCl. Outer membrane fractions were prepared from strain JF797 grown in modified LB broth containing 0 or 5 mM SAL with 0, 86, or 300 mM NaCl. Electrophoresis of the proteins was then carried out as described in the legend to Fig. 3. Lanes: a, 5 mM NaCl, 0 mM SAL; b, 0 mM NaCl, 5 mM SAL; c, 86 mM NaCl, 0 mM SAL; d, 81 mM NaCl, 5 mM SAL; e, 300 mM NaCl, 0 mM SAL; f, 295 mM NaCl, 5 mM SAL.

concentration of the LB broth. A marked decrease of OmpF due to growth in 5 mM SAL was seen in outer membranes of cells grown in LB with 5, 86, or 300 mM NaCl (Fig. 6). A similar effect on outer membrane permeation by cephaloridine was found for cells grown in low-osmolarity nutrient broth medium (Fig. 7). Permeability was very high for these cells, as expected, when they were grown in low-osmolarity medium, but permeability was reduced more than eightfold by the addition of 5% (or more) sucrose. In the presence of 5 mM SAL, however, permeation was reduced 17-fold even in the absence of sucrose. Thus, the effect of SAL on *ompF*

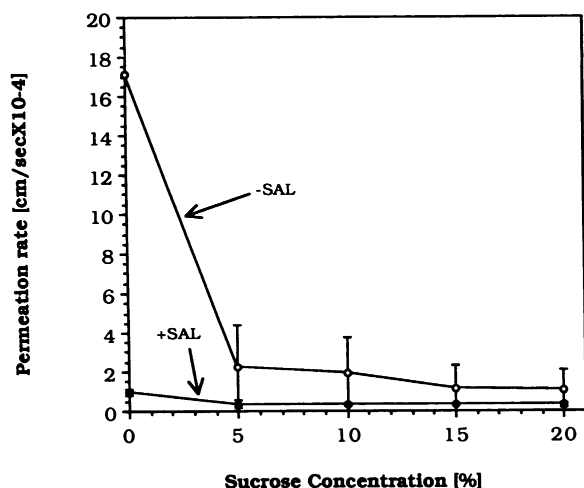

FIG. 7. Cephaloridine permeation of cells grown in SAL and sucrose. The permeation by cephaloridine was determined for strain JF797 grown in nutrient broth containing 0 or 5 mM SAL and sucrose at the indicated concentrations.

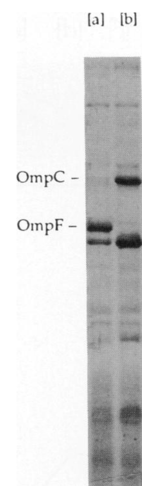

FIG. 8. Outer membrane proteins of an *envZ3* strain grown in SAL. Outer membranes prepared from strain JF1051 (*envZ3*) grown in LB broth containing 0 mM (lane a) or 5 mM (lane b) SAL were subjected to SDS-urea-gel electrophoresis and stained as described in the legend to Fig. 3. The prominent band under OmpF that increased with increasing SAL concentration is OmpA.

expression was essentially independent of the osmolarity of the medium.

Effects of SAL on OmpC and other outer membrane proteins. Sawai et al. (39) had observed a decrease in outer membrane OmpC due to growth in Penassay broth with SAL. To investigate this result, outer membranes were prepared from strains grown in LB broth with several concentrations of SAL. After electrophoresis on SDS-urea-polyacrylamide gels, quantitative measurements of the amounts of outer membrane proteins were made (Table 3). The amounts of OmpC decreased by less than 20% for cells grown in up to 10 mM SAL, whereas the amounts of OmpF decreased by 89 and 99% due to growth in 1 and 10 mM SAL, respectively. Thus, the effect of SAL on OmpC was less than that seen by Sawai et al. (39), perhaps because of differences in the media or strains used. Unexpectedly, when the effect of SAL on the β-galactosidase synthesis of an *ompC-lacZ* transcriptional fusion strain (MH225) was measured, an increase in transcription was observed. For cells grown in LB (86 mM NaCl), the addition of 5 mM SAL increased the synthesis of β-galactosidase by 70%, from 159 to 267 Miller units. For the same cells grown in LB with low NaCl (7 mM), only 81 U of β-galactosidase was measured, as expected for growth in a low-osmolarity medium. Nevertheless, the addition of 5 mM SAL to this medium increased the β-galactosidase synthesis threefold to 252 U. Thus, similarly high levels of *ompC* transcription were attained during growth in the presence of SAL at high or low osmolarity. However, as stated above, this increased transcription was not reflected in increased OmpC in the outer membrane preparations of wild-type cells (Table 3). Whether this is due to inhibition of *ompC* translation or assembly is not known.

The results in Table 3 also show that growth in SAL had no marked effect on outer membrane amounts of protein K or PhoE, whereas it increased OmpA levels about twofold and decreased NmpC levels about twofold. More substantial increases in OmpA can be seen in the mutant strains *envZ3* and *ompR472* (Fig. 8 and 9). These results were not further examined.

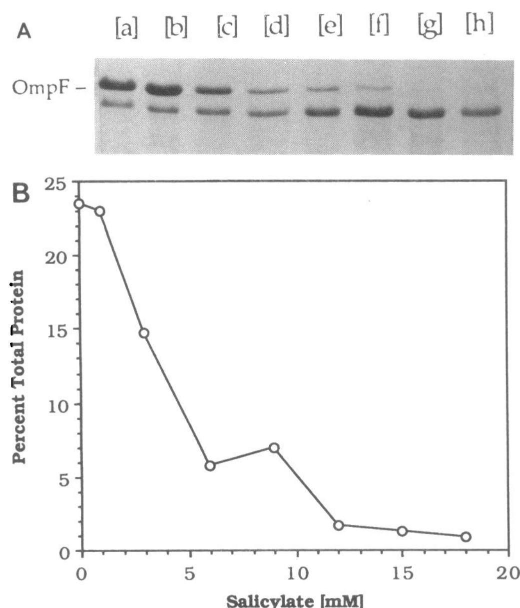

FIG. 9. Outer membrane proteins of an *ompR472* strain grown in SAL. (A) Outer membranes prepared from strain MH2472 (*ompR472*) after growth in LB broth containing 0 to 18 mM SAL were analyzed by electrophoresis as described in the legend to Fig. 3. OmpC is not observed because of the *ompC-lacZ* fusion present in this strain. The prominent band under OmpF is OmpA. Lanes and SAL concentrations (millimolar): a, 0; b, 1; c, 3; d, 6; e, 9; f, 12; g, 15; h, 18. (B) The amounts of OmpF determined from densitometer tracings of each sample shown in panel A are expressed as percentages of the total protein in these outer membrane samples.

Epistatic effects of SAL. SAL affected *ompF* and *ompC* expression even in certain *ompR* and *envZ* mutants. Most dramatic was the effect of SAL on an *envZ3* mutant (strain RT3), which has an $OmpF^+$ $OmpC^-$ phenotype. This mutant is insensitive to transcriptional downregulation of *ompF* by procaine but remains sensitive to osmoregulation (44). Growth in the presence of 5 mM SAL severely reduced the amount of OmpF in outer membranes of RT3 (Fig. 8). Most surprising, however, was that the amount of OmpC was restored virtually to normal ($OmpC^+$) levels. A similar effect was seen with the *ompR472* (*ompR2*) mutant MH2472, which has a hyper-$OmpF^+$($OmpF^{++}$) $OmpC^-$ phenotype and is relatively insensitive to osmoregulation (20). Growth in SAL strongly reduced the amounts of OmpF in the outer membrane of this strain, but higher than normal concentrations of SAL were required (compare Fig. 9 with Table 3). Corresponding to the $OmpF^{++}$ phenotype of this strain was a very high rate of cephaloridine permeation (12.3×10^{-4} cm/s). This rate decreased to 5.36×10^{-4} cm/s with 5 mM SAL and to 0.41×10^{-4} cm/s with 15 mM SAL. Compared with the concentrations required for wild-type *E. coli* (Fig. 5), higher SAL concentrations were required to fully decrease the cephaloridine permeation of strain MH2472. Since this strain has an *ompC-lacZ* fusion, the effect of growth in SAL on *ompC* transcription was measured. The β-galactosidase activity (average of two experiments) increased from 9 U for growth in the absence of SAL to 80 and 186 U for growth in the presence of 5 and 10 mM SAL, respectively. These increases in activity are substantial even in comparison to the activity of wild-type *ompB* cells (MH225) grown in the absence of SAL (159 U). The ability to turn on *ompC* expression seen in the *envZ3* and *ompR472* strains was not seen in strain TK821, which carries the *ompR331*::Tn*10* mutation and has an $OmpF^-$ $OmpC^-$ phenotype. When strain TK821 was grown in SAL, no change in the outer membrane protein profiles was seen (data not shown). This shows that the stimulation of OmpC synthesis by SAL requires some OmpR activity.

DISCUSSION

Effect of SAL on *ompF* translation. Two major mechanisms known to regulate *ompF* expression are translational control by *micF* RNA and transcriptional control by *ompB* proteins (14). The data presented here clearly indicate that translational control involving the *micF* system is important in mediating the response to SAL. First, *ompF* transcription did not appear to be affected by SAL, whereas *ompF* translation was markedly reduced (Table 2). Second, the concentrations of SAL that were effective in reducing both the amounts of OmpF in the outer membrane (39) (Table 3) and outer membrane permeability (Fig. 5) were similar to those that inhibited *ompF* translation (Fig. 1). Third, *micF* transcription was greater in cells grown in SAL (Fig. 2), and *micF* played an important, although not absolute, role in the reduction by SAL of OmpF outer membrane proteins (Fig. 3), *ompF* translation (Fig. 4), and outer membrane permeability (Fig. 5).

It is also clear that SAL does not act as an osmoregulator or like procaine. The effects of SAL on *ompF* expression were visible over a range of NaCl (Fig. 6) and sucrose (Fig. 7) concentrations. Moreover, the effects of SAL were epistatic to certain mutations at the *ompB* locus that render the transcription of *ompF* insensitive to either procaine or osmolarity (Fig. 8 and 9).

Other stimuli that increase the levels of *micF* RNA and decrease *ompF* translation are elevated growth temperatures and exposure to ethanol (5). (High osmolarity also stimulates *micF*, but this is usually masked by the more salient transcriptional regulation of *ompF* [1].) These stimuli differ in the extent to which their effect on *ompF* translation is *micF* dependent (5): the thermal effect requires a $micF^+$ allele, whereas the ethanol effects more nearly resemble those of SAL (Fig. 3, 4, and 5) in that they are only partially decreased by a deletion of *micF*. Thus, in addition to *micF*, a second anti-*ompF* translational activity is implicated in the effects of SAL (and ethanol). One candidate for such an activity is the newly identified *micF* RNA-binding protein, which can also bind to *ompF* mRNA in vitro (3).

Effect of SAL on *ompC* expression. An increase in *ompC-lacZ* transcription was noted for wild-type bacteria grown in SAL (see Results), but, in fact, less-than-normal amounts of OmpC were found in the outer membranes of such bacteria (39) (Table 3). Unexpectedly, growth in SAL stimulated the expression of *ompC* (Fig. 8 and Results) in bacteria that are defective in *ompC* transcription due to *envZ3* (44) or *ompR472* (20, 31–33) mutations. This effect of SAL appears to be OmpR dependent, since SAL did not stimulate *ompC* expression in the *ompR331* (R1) mutant, which is defective in both *ompF* and *ompC* transcription (17).

Facilitated transcription of *micF* and *ompC*. Whereas growth in SAL increased transcription of *micF* and of *ompC* (particularly in the *ompR472* and *envZ3* mutants), transcription of *ompF* was unaffected. It is not surprising that *ompC* and *micF* are similarly regulated by SAL, since transcription of *ompC* and *micF* is normally coordinated by OmpR (9, 31, 33), perhaps through the common OmpR binding sequences

present in their transcriptional control regions (46). It is also reasonable to expect that the putative *micF*-independent, anti-*ompF* translational activity would be under the same control. However, the lack of an effect on *ompF* transcription is harder to understand, since osmoregulation of *ompF* and *ompC* by OmpR is usually reciprocal.

To explain these results, we propose that growth in SAL specifically facilitates transcription of both *ompC* and *micF* by altering some component of the *ompC-micF* transcriptional complex, e.g., the conformation of the DNA or the activity of transcriptional factors such as OmpR. We suggest that this facilitation obviates the normal requirement for modification of OmpR by EnvZ and thereby allows even the OmpR present in *envZ3* cells or the OmpR472 protein to transcribe *micF* and *ompC*. Studies on the role of integration host factor (IHF) in *ompC* transcription provide a conceptual basis for how facilitation may work. The binding of IHF to a region upstream of the *ompC* promoter was found to have a negative effect on *ompC* transcription in vitro (22). Furthermore, *ompC* was transcribed in either an *ompR472* or *envZ* mutant when the strain had a *himA* (IHF subunit A) mutation (22). Thus, it is possible that growth in SAL enables OmpR472 to transcribe *ompC* (and *micF*) by overcoming the inhibitory effects of IHF. However, it should be noted that OmpF synthesis is very high in IHF^- mutants even when they are grown in high-osmolarity media (45). Thus, the role of SAL cannot be simply to antagonize IHF activity or synthesis, since that alone would be expected to increase OmpF synthesis. Accordingly, it must be argued that the facilitation is specific for *ompC* and *micF*.

Several consequences of growth in SAL need further exploration. Does the observation that *ompC* transcription increased only 70% while *micF* transcription increased severalfold indicate asymmetric regulation of these transcripts by SAL? Is the decreased OmpC synthesis in $ompB^+$ bacteria due to decreased translation of *ompC*? What is the nature of the putative non-*micF*, anti-*ompF* translational activity, and what controls its synthesis? Finally, how does SAL induce these changes? The isolation and study of mutants that do not respond to SAL may help answer these questions.

Resemblance to *tolC* and *marA* mutants. Facilitated transcription of *ompC* may also explain the porin phenotypes of *tolC* (31) and *marA* (8) mutants, which resemble those of SAL-grown cells: decreased *ompF* translation, relatively normal *ompF* transcription, and increased *micF* transcription. Furthermore, in *tolC* mutants *ompC* transcription is increased and the *ompR472* mutation is suppressed with regard to both *micF* and *ompC* transcription (31). However, *marA* and *tolC* mutants have considerably different antibiotic resistance phenotypes. *marA* mutants (8), like SAL-treated cells (11, 16, 38), are resistant to β-lactams, chloramphenicol, quinolones, and tetracycline, whereas *tolC* mutants are hypersensitive to ampicillin and nalidixic acid and to several detergents and dyes (10). This distinction may not prove to be relevant, since the hypersensitivity of *tolC* mutants appears to be due to effects that are unrelated to the porin phenotypes (34). This raises the possibility that the effects of SAL on porin expression are due to the regulation of *marA* or *tolC* expression.

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