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pH Dependence and Gene Structure of *inaA* in *Escherichia coli*

SALLY WHITE, FRANKLIN E. TUTTLE, DARCY BLANKENHORN, DONALD C. DOSCH,† AND JOAN L. SLONCZEWSKI*

Department of Biology, Kenyon College, Gambier, Ohio 43022

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The weak-acid-inducible locus *inaA* in *Escherichia coli* was mapped to 48.6 min by P1 cotransduction of *inaA* Mud *lac* fusions and linked Tn*10* insertions. The *inaA1*::*lac* fusion tested negative for phenotypes characteristic of mutations in the nearby locus *ubiG*. Sequence analysis of a fragment amplified by polymerase chain reaction located the *inaA1*::*lac* fusion joint within an open reading frame 311 nucleotides downstream of *nrdB*, transcribed in the opposite direction, encoding a 168-amino-acid polypeptide. Constitutive mutant strains identified on lactose MacConkey revealed a novel regulatory locus unlinked to *inaA*, which mapped at 34 min (designated *inaR*). Expression of *inaA1*::*lac* increased slightly with external acidification; the presence of benzoate, a membrane-permeant weak acid, greatly increased the acid effect. The expression at various combinations of benzoate and external pH correlated with the decrease in intracellular pH. The uncouplers salicylate and dinitrophenol also caused acid-dependent induction of *inaA*, but substantial induction was seen at external pH values higher than the internal pH; this effect cannot be caused by internal acidification. Nondissociating analogs of benzoate and salicylate, benzyl alcohol and salicyl alcohol, did not induce *inaA*. Expression of *inaA* was inversely related to growth temperature over the range of 30 to 45°C. The *inaA1*::*lac* fusion was transferred to a strain defective for K^+ uptake (*kdpABC trkA trkD*) in which pH homeostasis was shown to depend on the external K^+ concentration. In this construct, *inaA1*::*lac* retained pH-dependent induction by benzoate but was not induced at low K^+ concentrations. Induction of *inaA* appears to involve several factors in addition to internal pH. *inaR* may be related to the nearby locus *marA*/*soxQ*, which is inducible by acidic benzyl derivatives.

Genetic response systems enable *Escherichia coli* to adapt to changes in its environment (14, 29, 48). *E. coli* grows over a wide range of external pHs (pH 5 to 9) while maintaining its internal pH within a narrow range, i.e., 7.4 to 7.8 (33, 47, 51). It survives rapid changes in external pH (47, 51) and adapts to the presence of weak acids which depress internal pH (21, 36). Nevertheless, the essential mechanisms of pH homeostasis in *E. coli* remain unclear (for reviews, see references 5 and 32).

There is a growing list of genes and regulons whose expression is regulated by pH in *E. coli* and *Salmonella typhimurium*. Expression of arginine decarboxylase (*adi*) and lysine decarboxylase (*cadA*) is induced at low pH and enhanced by anaerobiosis (4). In *S. typhimurium*, external acid induces the acid tolerance genes (11). Alkaline pH-inducible transcription is observed for the sodium proton antiporter *nhaA* (19) and for *alx* (4). The alkaline regulation of *nhaA* may play a role in alkaline pH homeostasis.

The cytoplasm of *E. coli* may be acidified without changing the external pH, for example, by addition of membrane-permeant weak acids, which are commonly found in the natural environment of the bacterium. There are separate classes of response to changes in internal and external pHs. In *Streptococcus faecalis*, expression of the proton-translocating ATPase, which regulates internal pH, is induced by internal acidification (23, 24). In *E. coli*, however, growing cells do not require ATPase for pH homeostasis, although ATPase is needed for extreme acid tolerance (11).

Many metabolic enzymes are regulated by specific weak acids, such as acetate (26), formate (3), or salicylate (40). Few genes are known to be inducible by general effects of membrane-permeant weak acids. By contrast, expression of *inaA* (previously *ina*) is induced by a range of weak acids, including the synthetic compound dimethoxazolidine dione (45).

Expression of *inaA*::*lac* fusions shows only slight external pH dependence in the absence of a weak acid, suggesting the possibility of a specific dependence on internal acidification (45). Interpretation of this result is complicated by the fact that the transmembrane pH difference (ΔpH) and proton electrochemical potential (Δp) are both depleted as the weak acid is taken up and acidifies the cell. It was of interest to identify *inaA* and determine what factors might mediate expression of this locus.

Here we report the genetic and physical mapping of *inaA* and observations of pH-dependent induction by weak acids and protonophores. We also show that internal pH can be regulated by K^+ in a strain containing multiple defects in K^+ uptake; this strain should prove useful for further studies of pH homeostasis and internal-pH-dependent gene expression.

MATERIALS AND METHODS

Bacterial strains and growth conditions. All of the strains used in this study are listed in Table 1. The growth medium was Luria broth (from reference 28) containing appropriate pH buffers at 100 mM (45), including MES [2-(*N*-morpholino)ethanesulfonic acid] at pH 5.5 to 6.5, PIPES [piperazine-*N*,*N*'-bis(2-ethanesulfonic acid) at pH 6.6 to 7.0, MOPS [3-(*N*-morpholino)propanesulfonic acid] at pH 7.1 to 7.9, and TAPS [tris(hydroxymethyl)methyl-3-aminopropanesulfonic acid] at pH 8.0 to 8.7). For experiments in which the K^+ concentration was varied, MK medium [30 mM NaH_2PO_4, 15 mM $(NH_4)_2SO_4$, 0.4% Casamino Acids, 0.4% glucose, 0.1

* Corresponding author.
† Present address: Department of Biology, Carleton College, Northfield, MN 55057.

TABLE 1. Strains of *E. coli* K-12

Strain	Description or genotype	Source
MC4100	F⁻ *araD139* Δ(*argF-lac*)*U169 rpsL150 relA1 flb5301 deoC1 ptsF25 rbsR*	43
M8820	F⁻ *araD9* (*ara-leu*)*7697* Δ(*proAB-argF-lacIPOZYA*)*XIII rpsL*	7
JLS8612	M8820 *inaA1*::Mu dI1734 (Km^r *lacZYA*)	45
JLS8615	M8820 *inaA2*::Mu dI1734 (Km^r *lacZYA*)	45
JLS9052	JLS8612 *inaA52*	This study
JLS9053	JLS8612 *inaR53*	This study
CS1230	*ompC161 gyrA261 zeh-298*::Tn*10*	B. Bachmann
CAG12178	*gyrA zei-723*::Tn*10*	44
GI76	*leuB6 fhuA2 lacY1 supE4n4 zdg-299*::Tn*10 ksgB1 argG6 rpsL104 malT1 xyl-7 mtl-2 metB1* λ⁻	B. Bachmann
PLK1253	*trpA9605 zdd-230*::Tn*9 zde-234*::Tn*10 his-85 thyA714 ilv-632 deo-70 trpR55 pro-48 arg-59 tsx-84* λ⁻	B. Bachmann
TK2401	F⁻ *thi rha lacZ nadA kdpABC5 trkA409 trkD1*	W. Epstein
JLS9113	TK2401 *inaA1*::*lac*	This study

mg of thiamine per ml, 0.1 mM $MgSO_4$, 5 μM $FeSO_4$] was used. A buffer appropriate for the given pH was included at 50 mM, and KCl was at 10 to 400 mM. Assays for β-galactosidase were performed as described previously (28, 45). Each error value for enzyme assays and for internal pH represents the standard deviation based on six or more trials.

Genetic and physical mapping. Hfr matings and P1 transductions were performed by standard methods (28, 43). The transduction frequencies were based on the scoring of at least 200 colonies. The *inaA*::*lac* (Km^r) strains and constitutive derivatives were mated with the Tn*10* Hfr set of the Wanner collection (B. Bachmann) and transduced with P1 lysates of Tn*10* and Tn*9* insertion strains from B. Bachmann and C. Gross. Additional linked Tn*10* insertions were obtained from a P1 lysate grown on a Tn*10* insertion pool. The Tn*10* insertion pool (gift of T. Silhavy) was generated by tetracycline selection of cells infected with λNK561, a Tn*10* transposition vector (43).

Polymerase chain reaction was performed on genomic DNA (41) by using Amplitaq polymerase in a Perkin Elmer thermal cycler. A single colony was picked into 200 μl of reaction buffer containing 2 mM $MgCl_2$, boiled for 5 min, and prepared for reaction by the manufacturer's directions. Thirty-five cycles of 1 min at 94°C, 2 min at 55°C, and 4 min at 72°C were run. Amplified products were purified by phenol extraction and electrophoresis and extracted from gels by using an electroeluter (IBI). The following primers were made by an Applied Biosystems DNA synthesizer: 5′-GGCAAGCCAACGTTAAGCTA-3′ from the end of the Tn*10* inverted repeat (17) and 5′-TAATCCAATGTCCTC CCGG-3′, the complement of bases 31 to 50 from the Mu left end (35). Restriction enzyme analysis was performed by standard methods. Computer analysis of DNA and peptide sequences was performed by using the Wisconsin Package from the Genetics Computer Group (9). DNA sequence analysis was performed by cycle sequencing (25) with the assistance of Lark Sequencing Technologies. The codon preference plot was based on reference 16, using a table of codon frequencies derived from highly transcribed genes in *E. coli*.

Respiration on succinate. The ability to respire on succinate (requiring *ubiG*) was determined by aerobic growth overnight at 37°C in M63 medium (43) containing 0.2% Casamino Acids and 0.2% sodium succinate.

Measurement of cell volume and internal pH. Radiochemicals were obtained from ICN. Internal cell volume was determined by exclusion of [^{14}C]sucrose. A 3-ml culture volume was grown to an optical density at 625 nm of 0.2 to 0.3 and then centrifuged and concentrated in 200 μl of buffered LB or MK containing 5 mM sucrose. [U-^{14}C]sucrose (10.3 mCi/mmol) and ^3H_2O (100 mCi/ml) were added to final concentrations of 0.5 and 16 μCi/ml, respectively. A 100-μl sample volume was pipetted onto 100 μl of silicon oil (density, 1.03; Petrarch Systems). Samples were centrifuged at 1,000 × *g* for 5 min. The pellets were frozen and cut out into Ecolume, and their radioactivity was counted in a liquid scintillation counter. The ^{14}C-labeled external volume was subtracted from the ^{3}H-labeled total pellet volume to obtain the internal cell volume. The total intracellular volume was determined to be 1.4 μl for a 10-ml culture grown to an optical density at 600 nm of 0.2.

Measurement of ΔpH and internal pH was based on transmembrane equilibration of a radiolabeled weak acid or weak base (20, 50). Cells were grown as for internal volume measurement at 30°C in LB medium or at 37°C in MK medium. A 20-ml culture was incubated for 75 s with 0.6 μM [^{14}C]benzoic acid (7 μCi/μmol) or with 0.6 μM [^{14}C]methylamine (7 μCi/μmol) to observe inverted ΔpH. The culture was then filtered by vacuum through a 25-mm-diameter GF/C glass microfiber filter. Absorption of radiolabeled benzoate (or methylamine) by the filter and cells at zero ΔpH was determined by inclusion of 100 μM carbonyl cyanide-*m*-chlorophenylhydrazone in parallel samples. The transmembrane ΔpH and internal pH were calculated by using the Nernst equation, on the basis of the internal cell volume determined as stated above.

RESULTS

Genetic mapping of *inaA* and constitutive mutants. The map location of *inaA1*::*lac* was determined by conjugation with Hfr strains containing Tn*10* insertions and by P1 transduction of different Tn*10* insertions into JLS8612. The wild-type allele of the *inaA1*::*lac* fusion cotransduced 90% with *zei-723*::Tn*10* (48.5 min), 80% with *gyrA* (48.3 min), and 33% with *zeh-298*::Tn*10* (47.75 min). Similar results were obtained for *inaA2*::*lac*, confirming the proximity of the two fusions. The possibility that *inaA1* maps in *ubiG* just to the right of *gyrA* (53) was tested phenotypically. The product of *ubiG*, which functions in ubiquinone biosynthesis, is required for respiration on succinate, and its expression is repressed by glucose (13). Strain JLS8612 was tested for respiration on succinate and for the effect of glucose on *inaA1*::*lac* expression. The *inaA1*::*lac* strain grew on succi-

TABLE 2. *inaA1*::*lac* expression in regulated and constitutive strains

inaA1::*lac* strains	Additional genotype	Mean expression in buffered LB (pH 6.7)[a] ± SD	
		Plain	10 mM benzoate
JLS8612		70 ± 6	483 ± 26
JLS9052	*inaA-52*	1,125 ± 226	1,287 ± 145
JLS9053	*inaR-53*	609 ± 343	1,353 ± 157
JLS9113[b]	*kdpABC5 trkA409 trkD1*	82 ± 10	557 ± 162

[a] Expression of β-galactosidase is shown in Miller units (n = 6). Growth conditions were as for Fig. 3.
[b] 100 mM KCl was added to the growth medium.

nate at the same rate as parental strain M8820; glucose had no effect on *inaA1*::*lac* expression (data not shown). Therefore, *inaA* was not an allele of *ubiG*.

The markers *gyrA* (Nal^r) and *zei-723*::Tn*10* (48.5::Tn*10*), both closely linked to *inaA*, were transduced into JLS8612 (*inaA1*::*lac*). The Nal^r 48.5::Tn*10* *inaA1*::*lac* construct was used to perform a three-point cross with MC4100, which is Nal^s Tet^s Lac$^-$. Tet^r transductants were scored on nalidixic acid and on MacConkey plates containing 5 mM benzoate. The results were as follows: Nal^r Lac$^+$, 56%; Nal^r Lac$^-$, 19%; Nal^s Lac$^+$, 13%; Nal^s Lac$^-$, 2%. The overall cotransduction of 48.5::Tn*10* and *inaA1*::*lac* (69%) was lowered, as expected, given the extra length of the Mu d-*lac* insertion element in the P1 lysate. Of the four phenotypic classes, Nal^s Lac$^-$ had the lowest frequency, indicating that 48.5::Tn*10* lies between the two markers. Thus, *inaA* appeared to be a newly identified locus mapping at about 48.6 min, near *nrdAB* (12, 53).

Twenty-seven constitutive isolates of JLS8612 (*inaA1*::*lac*) were obtained by picking red papillae which appeared out of cultures grown on lactose MacConkey plates for 5 days at 30°C. These strains expressed high levels of β-galactosidase in the absence of added weak acid.

P1 lysates of 48.5::Tn*10* *inaA1*::*lac* were used to transduce the constitutive isolates of *inaA1*::*lac* to Tet^r. In 19 of the 20 isolates, only Tet^r Lac$^+$ transductants were obtained in the absence of benzoate; thus, the constitutive mutation in these strains appeared unlinked to *inaA*.

A Tn*10* insertion pool (43) was used to transduce one of the unlinked constitutive strains (JLS9053) to Tet^r. In one isolate, the Tn*10* had 80% linkage to the locus conferring constitutive expression, designated *inaR*. A P1 phage lysate of a construct containing *inaA1*::*lac* and the *inaR*-linked Tn*10* (but wild type for *inaR*) was used to transduce each of the other unlinked constitutive strains to Tet^r. All such transductions yielded some Lac$^-$ colonies in the absence of benzoate (that is, reversion of *inaR*). Thus, all of the constitutive mutations unlinked to *inaA* that were isolated were mapped in or near *inaR*.

The *inaR* mutation in JLS9053 (Table 1) cotransduced 11% with *zdd-230*::Tn*9* (33 min), 83% with *zde-234*::Tn*10* (34 min), and 14% with *zdg-299*::Tn*10* (36 min). These crosses placed *inaR* at 34 to 35 min. Interestingly, the locus *marA*, which maps at 34 min, is induced by salicylate and related weak acids (8, 39). *marA* confers low-level resistance to several antibiotics, including nalidixic acid and tetracycline. Strain JLS9053, however, showed no growth in the presence of either of these antibiotics at a concentration of 5 μg/ml.

Expression of *inaA1*::*lac* in linked and unlinked (*inaR*) constitutive isolates was compared (Table 2). Both the linked mutant (JLS9052) and the *inaR* mutant (JLS9063) showed high β-galactosidase activity in the presence or absence of benzoate. The high standard deviations observed represent variability which we find to be typical for constitutive mutants of *lacZ* fusion strains.

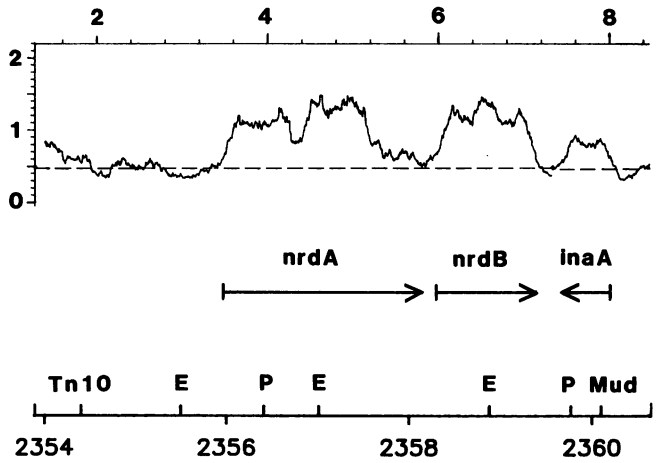

FIG. 1. Physical map of the Tn*10*-Mu dl polymerase chain reaction segment. The genomic locations of restriction sites in kilobases (lower scale) are based on reference 27; the *nrdAB* reading frames are aligned (upper scale) on the basis of reference 6. The codon preference plots used the appropriate reading frames for *nrdAB* (1.5 to 7.4 kb) and *inaA* (7.4 to 8.3 kb). The codon preference window size was 75. The broken line indicates the average level of codon preference compared with the random sequence. Restriction sites: *Eco*RV, E; *Pvu*II, P.

Physical mapping sequence of the *inaA1*::*lac* insertion site. To locate *inaA1*::*lac* on the *E. coli* restriction map (27), the region between 48.5::Tn*10* and the Mu d fusion joint was synthesized by polymerase chain reaction amplification using a primer from the end of the inverted repeat of Tn*10* and a second primer from the left end of the Mu d vector. (Had the relative orientation of the two transposons been reversed, a right-end Mu primer would have been needed.) A 5.8-kb product was obtained. Digestion with restriction enzymes *Bam*HI, *Eco*RI, *Eco*RV, and *Pvu*II located this DNA in the region of 2354 to 2360 kb on the genomic map (27), partly overlapping *nrdAB* (6). The *Eco*RV and *Pvu*II sites are shown in Fig. 1. On the basis of the genetic analysis (above), the Tn*10* end maps upstream of *nrdA*, between *gyrA* and *inaA*.

The insertion of the Mu d fusion in *inaA* was defined approximately by a 300-bp *Pvu*II fragment adjacent to the *Pvu*II site at 2,359.77 kb. Analysis of the DNA sequence placed the fusion joint at 779 bp (Fig. 2), within an open reading frame of 168 amino acids, transcribed opposite *nrdAB* (6). Possible −10 and −35 promoter sequences are indicated for the putative *inaA* gene. A codon preference plot (16) showed a significant degree of biased codon usage for this sequence, consistent with a translated peptide (Fig. 1). The amino acid sequence of the *inaA* peptide appears moderately hydrophilic and contains regions of probable alpha helix.

Induction of *inaA* as a function of external and internal pHs. The effects of benzoate, salicylate, and dinitrophenol on *inaA1*::*lac* expression are shown in Fig. 3. Benzoate and salicylate are weak acids which partially uncouple the proton potential at high concentration (42), whereas dinitrophenol is

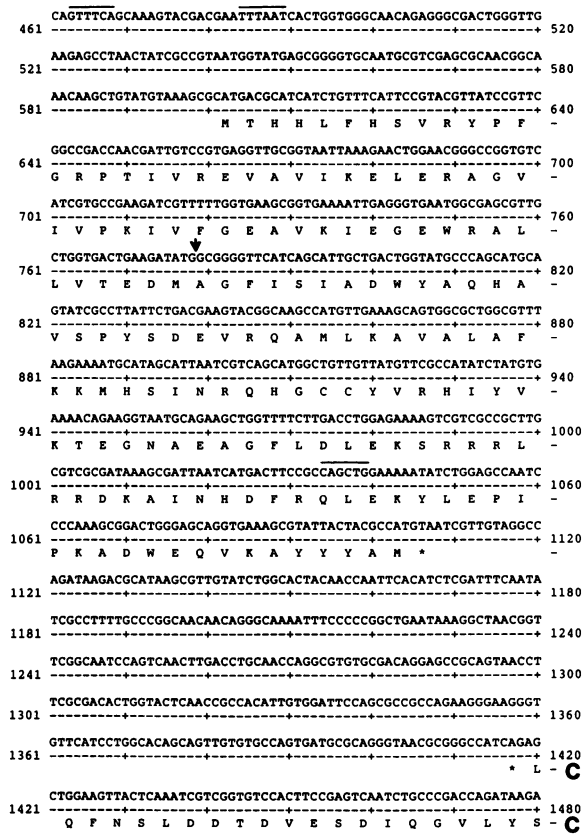

FIG. 2. DNA sequence and translation of the proposed *inaA* reading frame. The sequence shown is the reverse complement of a published sequence (6), downstream from *nrdAB*. Possible −35 and −10 promoter sequences for *inaA* are overlined. The arrow marks the fusion joint for *inaA1*::*lac*. The *Pvu*II site at base 1037 maps at 2,359.77 kb in the genome (Fig. 1). The 3′-terminal end of *nrdB* is indicated in a complementary reading frame, labeled C.

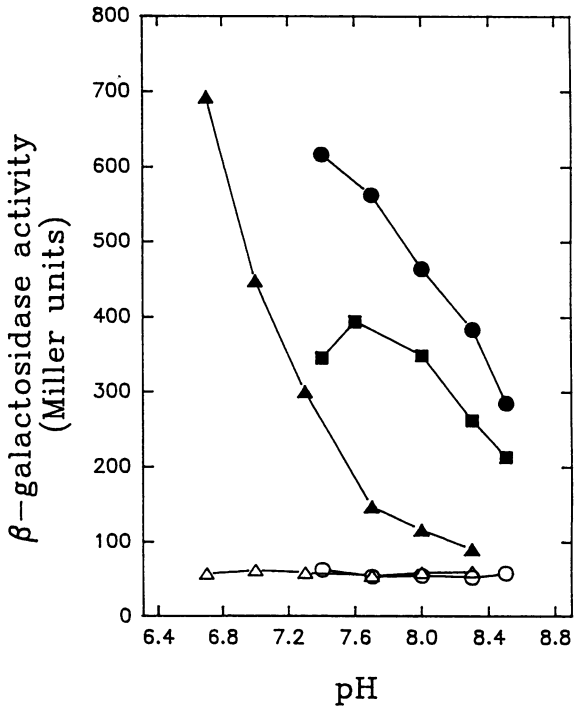

FIG. 3. External pH dependence of β-galactosidase expressed by *inaA1*::*lac* in the presence of organic acids. Cultures grown overnight in buffered Luria broth were diluted 1:50 and grown at 30°C to an optical density at 600 nm of 0.2. All growth media included buffers appropriate for the pH, at 100 mM, as previously described (45), except that TAPS was used at pH 8.0 to 8.5. Media contained 0.5 mM dinitrophenol in 0.5% ethanol (●), 10 mM salicylate (■), 15 mM benzoate (▲), 0.5% ethanol (○), and 15 mM NaCl (△).

a strong uncoupler at the concentration used. The expression of β-galactosidase in the presence of these acids increased as the external pH decreased. In the absence of weak acid or uncoupler, expression showed no dependence on external pH over the pH range of 6.8 to 8.5. At lower pH (Fig. 4, pH 5.5), a small but significant increase in expression was observed, as reported previously (45).

A possible explanation for the pH-dependent effect of weak acids is that their uptake, and their effect on internal pH, is increased at lower external pH. The expression of *inaA1*::*lac* as a function of internal pH is shown in Fig. 4. The internal pH was determined at various combinations of external pH and benzoate concentration. The expression of *inaA1*::*lac* showed a correlation with internal acidity.

On the other hand, when salicylate or dinitrophenol was used to induce *inaA* (Fig. 3), high levels of expression could be obtained at external pH values considerably higher than the expected range of internal pH (7.5 to 7.9). For example, cells grown with 15 mM salicylate at an external pH of 8.3 had an internal pH of 8.1 but the *inaA* expression was increased under this condition. This effect cannot be explained on the basis of internal acidification.

K^+-dependent internal pH and *inaA* expression. Potassium transport may be a requirement for pH homeostasis (2, 5, 37). Strain TK2401 contains null alleles of transporters *kdpABC*, *trkA*, and *trkD*, a construct in which K^+ transport is virtually absent (10). We found that the requirement for K^+ was strongly dependent on external pH (Fig. 5). At high pH, TK2401 grew nearly as well as K-12 over the range of 20 to 200 mM K^+. At pH 6.0, however, growth was greatly diminished and decreased to zero below 40 mM K^+. By contrast, growth of *E. coli* K-12 was virtually constant over the range of 10 to 200 mM K^+ (data not shown).

At an external pH of 5.9, at which *E. coli* normally maintains an internal pH of about 7.4 to 7.6, the pH homeostasis of TK2401 was strongly dependent on the K^+ concentration (Table 3). At 100 mM K^+, TK2401 maintained an internal pH that was about half a unit less than at 400 mM K^+ whereas the internal pH of JLS8612 was unaffected by K^+. Results for K-12 were comparable to those obtained with JLS8612 (data not shown).

When *inaA1*::*lac* was transduced from JLS8612 into TK2401 (JLS9113), the usual induction by benzoate was observed at an external pH of 6.7 in the presence of 100 mM added K^+ (Table 2). Table 3 compares JLS8612, TK2401, and JLS9113 with respect to internal pH and β-galactosidase activity at an external pH of 6.0, with 100 or 400 mM K^+. Despite the lowered internal pH, *inaA1*::*lac* was not induced at 100 mM K^+.

Other effects on *inaA* expression. The effects of nondissociating analogs of benzoate and salicylate on *inaA1*::*lac* expression were tested. Benzyl alcohol and salicyl alcohol, at concentrations of up to 20 mM at an external pH of 7.0,

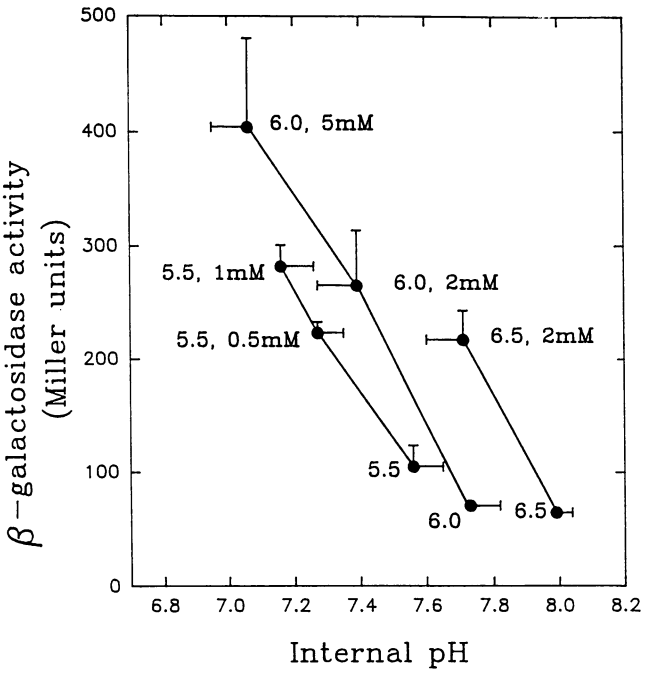

FIG. 4. Internal-pH dependence of *inaA1*::*lac* expression. Cultures were grown as for Fig. 3. Internal pH was measured by uptake of [^{14}C]benzoate as described in Materials and Methods; the mean of seven or more trials is shown. The β-galactosidase values shown are the means of nine trials. All error bars represent standard deviations. For each condition, the external pH and the concentration of benzoate, if any, are shown.

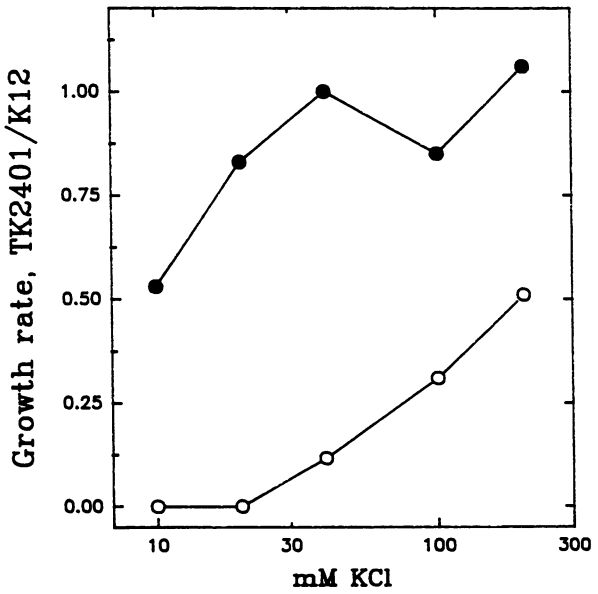

FIG. 5. K^+ dependence of growth for TK2401. The relative growth rates of TK2401 divided by those of the parent, K-12, at pHs 8.5 (●) and 6.0 (○) are shown. Overnight cultures were grown in MK–100 mM KCl, pH 7.7, diluted 1:200 in MK medium over a range of K^+ concentrations, and grown at 37°C.

caused no significant induction of *inaA1*::*lac*. This finding eliminates chemical interaction with the hydrophobic moiety of weak acids as the direct cause of *inaA* induction.

A number of *E. coli* proteins show temperature-dependent expression (18, 30), and this parameter was tested for *inaA*. Benzoate-induced expression of *inaA1*::*lac* decreased sharply as the temperature increased from 35 to 45°C, whereas basal expression decreased gradually over the range of 25 to 45°C (Fig. 6).

DISCUSSION

The *inaA* gene appears to be a novel locus, mapping just outside the well-documented region of *gyrA*, *ubiG*, and *nrdAB* (53). While its function is not known, its product might assist the cell in coping with internal stress on pH or on proton motive force, analogous to the acid-inducible decarboxylases which alkalinize the growth medium in response to external acid.

The regulator gene *inaR*, which may encode a repressor, was located in a largely unmapped region near the termination site of replication. One nearby locus of interest is *marA* (8), whose mutations confer resistance to various antibiotics; the closely linked *soxQ* confers resistance to antibiotics as well as to superoxide-generating drugs (15). Transcription of *marA* is induced specifically by acidic benzyl derivatives (39). Therefore, there may be some connection between *inaR* and *marA*, although *inaR* mutations did not confer antibiotic resistance in the *inaA* background.

The expression of *inaA* appears to be induced by some common effect of various membrane-permeant weak acids and uncouplers. While the functions of *inaA* and its regulator *inaR* are not known, they may involve a significant response to changes in pH or proton potential. In *E. coli* chemotaxis, the detection mechanism for weak acid repellents has been correlated with detection of internal acidification (36, 46).

Alternatively, the induction mechanism might involve a structural element of the hydrophobic moiety of these compounds, as was shown for the potentiation of *ara* expression by benzyl derivatives (22). In contrast to *inaA* induction, however, derivatives lacking the acidic groups show a comparable effect on *ara*. The multiple drug resistance induced by salicylates also is induced by nonacidic derivatives (38).

Several possible signals for induction of *inaA* have been sorted out, although questions remain. The effect of benzoate at various combinations of concentration and pH (Fig. 2) was consistent with internal pH as a signal, as is the small increase in basal expression at low external pH (45), when the internal pH decreases significantly (47). The effect of salicylate and dinitrophenol was also pH dependent, but the strong response seen at high external pH requires some other explanation, possibly the depletion of proton potential as a signal for *inaA*. On the other hand, basal *inaA* expres-

TABLE 3. Internal pH and *inaA1*::*lac* expression as a function of K^+ concentration at an external pH of 5.9[a]

Strain	Mean internal pH ± SD		Mean β-galactosidase activity ± SD (Miller units)	
	400 mM[b]	100 mM	400 mM	100 mM
JLS8612	7.39 ± 0.06	7.48 ± 0.03	53 ± 6	50 ± 22
TK2401	7.57 ± 0.08	6.82 ± 0.27	ND[c]	ND
JLS9113	7.50 ± 0.08	7.10 ± 0.27	61 ± 7	38 ± 10

[a] Cultures were grown at 37°C to an optical density at 625 nm of 0.2 to 0.4.
[b] MK medium included the K^+ concentrations shown.
[c] ND, not done.

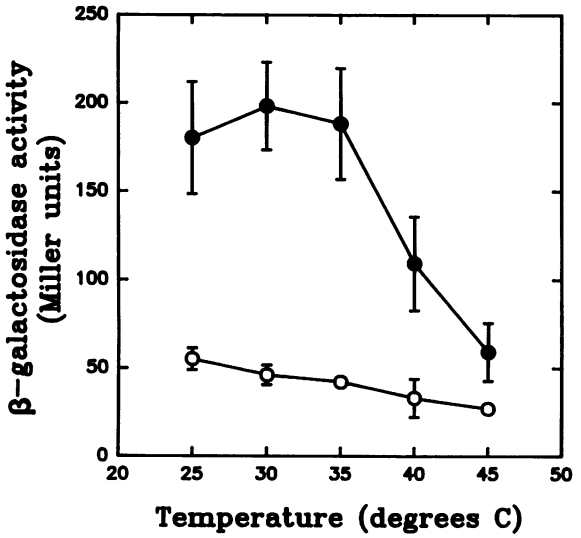

FIG. 6. Temperature dependence of *inaA1*::*lac* expression. Cultures were grown as described for Fig. 3, at pH 7.0. The medium contained 10 mM benzoate (●) or 15 mM NaCl (○).

sion did not increase at high external pH, when the proton potential is significantly diminished (34, 51).

In the K^+ uptake-defective background, *inaA* expression was little affected at high K^+ concentrations and was not induced by the lowered internal pH in the presence of low K^+. These findings suggest that *inaA* does not detect internal pH directly. Another possibility is that *inaA* activity requires sufficient intracellular K^+ for its function; for example, this locus might have a role in uncoupler-dependent K^+ efflux (37).

The *inaA* gene may be an environmental sensor responsive to several stimuli, including internal pH, proton motive force, temperature, and possibly other unknown factors. Given the emerging picture of pH-dependent gene expression, it would not be surprising if *inaA* detected a combination of stimuli. For example, expression of lysine decarboxylase (*cadA*/*exa*) shows induction by external acid, enhanced by anaerobiosis (1, 45). The linked *cadC* mediates acid induction (52); the unlinked regulator CadR mediates lysine induction (49); and yet another unlinked regulator, ExaR, mediates aerobic repression (31). Another example of multiple regulation is the Na^+/H^+ antiporter NhaA, which shows pH-dependent induction by Na^+ (19).

We are now seeking to identify loci which show clearer evidence of direct dependence on internal pH. K^+ uptake-defective TK2401 is a strain in which the internal pH can be set by the K^+ concentration in the growth medium. This strain should be a useful tool for study of internal pH-regulated genes and pH homeostasis.

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ADDENDUM IN PROOF

Preliminary data indicate that *inaR* confers resistance to low levels of chloramphenicol and nalidixic acid (J. Rosner and J. Slonczewski, unpublished data). This finding supports a possible connection between *inaR* and *marA*/*soxQ*.

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