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A pH-Sensitive Function and Phenotype: Evidence that EutH Facilitates Diffusion of Uncharged Ethanolamine in *Salmonella enterica*

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The *eutH* gene is part of an operon that allows *Salmonella enterica* to use ethanolamine as a sole source of nitrogen, carbon, and energy. Although the sequence of EutH suggests a role in transport, *eutH* mutants use ethanolamine normally under standard conditions (pH 7.0). These mutants fail to use ethanolamine at a low pH. Evidence is presented that protonated ethanolamine (Eth^0) does not enter cells, while uncharged ethanolamine (Eth^0) diffuses freely across the membrane. The external concentration of Eth^0 varies with the pH (pK = 9.5). At pH 7.0, the standard ethanolamine concentration (41 mM) provides enough Eth^0 for an influx rate that can support growth with or without EutH. When a lowered pH and/or ethanolamine concentration reduced the Eth^0 concentration below 25 μM, EutH was needed to facilitate diffusion. $EutH^+$ cells grew normally at Eth^0 concentrations above 3 μM, close to the Km (9 μM) of the first degradative enzyme, ethanolamine ammonia lyase. It is suggested that EutH facilitates diffusion of Eth^0. As predicted for a transporter, EutH contributed to the toxicity of ethanolamine seen under some conditions; furthermore, fusion of EutH to fluorescent Yfp protein provided evidence that EutH is a membrane protein.

Salmonella enterica can use ethanolamine as a sole source of carbon and nitrogen. Ethanolamine is degraded in two enzymatic steps. (i) The EutABC enzyme catalyzes cobalamin (B_{12})-dependent cleavage of ethanolamine to acetaldehyde (3, 17, 20), releasing ammonia. (ii) The EutE enzyme catalyzes NAD^+-dependent oxidation of acetaldehyde to acetyl coenzyme A (reviewed in reference 19). Despite the simplicity of the pathway (Fig. 1), 17 proteins are encoded in the *eut* operon, which supports growth on ethanolamine (6). Ethanolamine use phenotypes have been reported for six genes (*eutA*, *-B*, *-C*, *-D*, *-E*, and *-R*) (4, 16, 18), and three more were recently identified (*eutT*, *-G*, and *-J*) (19, 21a; J. Penrod, unpublished results). The remaining eight genes (including *eutH*) have no mutant phenotype under standard conditions (Penrod, unpublished) but are likely to be required under some conditions. Evidence is presented here that the EutH protein facilitates diffusion of ethanolamine and is required only at a low pH or at very low external ethanolamine concentrations.

Previous assays showed no evidence of an active ethanolamine transport system; significant amounts of isotope from ethanolamine accumulated only in cells with a functional degradative pathway (18). Deletion mutants lacking the whole *eut* operon were not more defective in uptake than simple lyase (EutBC) mutants. None of the mutations (inside or outside the *eut* operon) that impaired the use of ethanolamine owed their defect to lack of a transporter (6, 18; Penrod, unpublished). Mutants initially thought to lack an ethanolamine transporter (12) proved to be defective in transport of cobalamin, the cofactor required for growth on ethanolamine (23).

The *eutH* gene was first identified as an open reading frame in the *eut* operon sequence. Its 8 to 10 putative membrane-spanning segments (Fig. 2) suggested the possibility of a transport function (22). However, as shown here, a constructed in-frame deletion of the *eutH* gene caused no ethanolamine growth phenotype under standard conditions (pH 7). No EutH homologues were found in other bacteria, apart from those associated with genes for ethanolamine use. Here we report that EutH is needed for growth on ethanolamine at a low pH or whenever the external concentration of unprotonated ethanolamine (Eth^0) drops below 25 μM and reduces the influx rate below that needed for growth.

MATERIALS AND METHODS

Strains and crosses. All of the strains used in this study (Table 1) are derivatives of *S. enterica* serovar Typhimurium strain LT2. Transductional crosses were mediated by a high-frequency transducing mutant of phage P22 (HT105, *int*) (21). The procedures used for transduction crosses and phage lysate preparation were previously described (17).

Media and growth conditions. The rich medium used was nutrient broth (Difco) with 0.1 mM NaCl. The standard minimal medium used was NCE (2) with the indicated carbon source or nitrogen-free NCE medium (NCN) with the indicated carbon and nitrogen sources. The minimal medium used for growth rate determinations contained 5 mM KH_2PO_4, 5 mM $NaNH_4HPO_4$, and 1 mM $MgSO_4$ and was buffered at various pHs with 50 mM morpholineethanesulfonic acid (MES; pHs 5.5 and 6.0), morpholinepropanesulfonic acid (MOPS; pHs 6.5, 7.0, and 7.5), or HEPES (pH 8.0). Ethanolamine hydrochloride and sodium succinate (Sigma) served as carbon sources at the indicated concentrations. In the construction of a *eutE* mutation (below), glycerol (20 mM; Mallinckrodt) was used as a carbon source in NCN medium with ethanolamine as a nitrogen source (2). Cyanocobalamin (Sigma) was used as a supplement at 150 nM. All minimal liquid media contained biotin, Ca–*d*-pantothenic acid, nicotinamide, and pyridoxine HCl at 4×10^{-4}% (wt/vol); thiamine and riboflavin at 2×10^{-5}%; and trace metals as previously described (13). Rich medium was solidified by agar (1.5%; EM Science), and minimal medium was solidified by Noble agar (US Biological). Liquid cultures were lightly aerated by shaking standing culture tubes at 240 rpm. For measuring growth in liquid media, 3-ml nutrient broth cultures were started from single colonies and grown overnight. Cells were then pelleted, washed in pH 7.0 minimal medium, resuspended in the same medium, and used (35 μl) to inoculate 6 ml of growth medium. Growth was monitored in

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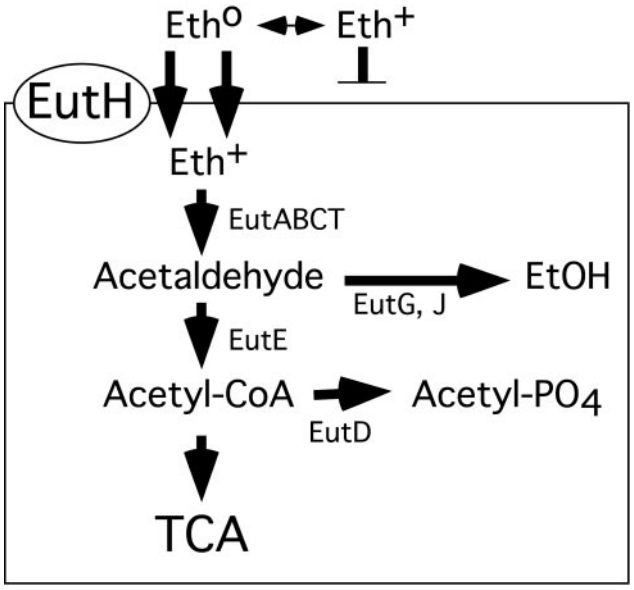

FIG. 1. Ethanolamine catabolism. The diagram describes the current understanding of ethanolamine metabolism, including conclusions drawn here for the transport mediated by the EutH protein. The functions listed are those of the EutBC (ethanolamine ammonia lyase), EutE (acetaldehyde oxidoreductase) (17, 18), and EutD (4) proteins. The EutT protein is a cobalamin adenosyltransferase (21a). On the basis of extensive homology with AdhE, the EutG protein is inferred to be an ethanol dehydrogenase, and both EutG and EutE are protected from reactive oxygen by EutJ-mediated refolding. EtOH, ethanol; CoA, coenzyme A; TCA, tricarboxylic acid cycle.

three parallel cultures by following the optical density at 650 nm (OD_{650}) on a Spectronic 20D+ spectrophotometer (Spectronic Instruments).

Construction of *eutE* and *eutH* mutations. Deletion mutations were constructed by linear transformation as described by Murphy et al. (11) and did not disturb the reading frame of the deleted *eut* gene. Final constructions were transduced by P22-mediated crosses into a wild-type LT2 background and verified by PCR and sequencing.

To make a *eutE* deletion, a chloramphenicol resistance (*cat*) cassette was amplified with primers P1 (5′ATGAATCAACAGGATATTGAACAGGTGGT GAAAGCGGTACTGAAGTTCCTATACTTTCTAGAGAATAGGAACTTC AAGCCACTGGAGCACCTCAA) and P2 (5′TTATACAATGCGAAACGCA

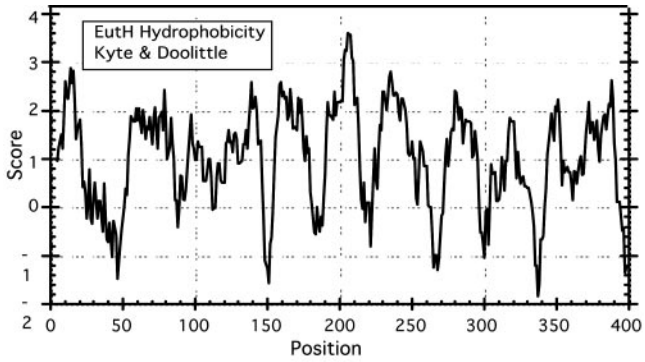

FIG. 2. Distribution of hydrophobic residues in the EutH protein. The amino acid sequence of the EutH protein (6) was analyzed for local hydrophobicity by the criteria of Kyte and Doolittle (7). The values plotted were obtained by analyzing the sequence with the ProtScale program on the SWISS-PROT website (http://us.expasy.org /tools/protscale.html).

TABLE 1. Strains used in this study

Strain	Genotype
TR10000	Wild-type LT2
TT10661	*eutH25*::MudA
TT20038	*eutE181*::MudJ *eutA208*::Tn*10*dTc-P_{int}
TT22236	LT2/pTP223
TT22815	*eutE356*::FRT(Sw)
TT23230	*eutH360*Δ
TT24608	*eutE356*::FRT(Sw) *eutA208*::Tn*10*dTc-P_{int}
TT24609	*eutE356*::FRT(Sw) *eutA208*::Tn*10*dTc-P_{int} DEL750[(*cysA1585*)*MudA*(*eut-23*)]
TT24610	*eutE356*::FRT(Sw) *eutH360*Δ
TT24808	*eut-376*::SacKan; between *eutH* and *eutA*
TT24809	*eutH377*::*yfp* (protein fusion)

TCCACCAGCACGCATCGACGCAGGAAGTTCCTATTCTCTAGAAAGT ATAGGAACTTCACGGGGAGAGCCTGAGCAAA).

The 5′ end of P1 (40 bp) has a sequence identical to the first 40 bp of *eutE*. This is followed by an Flp recombination target (FRT) site and a 3′ end identical to the promoter end of the *cat* gene. The 5′ end of P2 (42 bp) is the reverse complement of the last 42 bp of *eutE* and is followed by the reverse complement of an FRT site. The 3′ end is identical to the distal end of the *cat* cassette. The linear fragment produced by PCR amplification of *cat* was used to transform strain TT22236 to CamR. Plasmid pCP20, encoding FRT recombinase, was introduced. Recombination between FRT sites removed the Cam^r cassette, leaving a *eutE* deletion; the mutant gene produces a small peptide with the first 13 and the last 13 amino acids of the EutE sequence plus an intervening in-frame sequence including a single FRT site (TT22815).

A *eutH* deletion was constructed by replacing a *eutH*::Tn*10* insertion with a short in-frame sequence unrelated to *eutH*. A short coding sequence unrelated to *eut* was amplified with primers P3 (5′ATGGGAATTAACGAAATCATCATG TACATCATGATGTTCTCACCAAACACCCCCCAAAACC) and P4 (5′TCA CGATTGCGCCTCCGCTTCGGTTTTCACCTGGGCGCCGTCCACACAAC CACACCACACCAC).

The 5′ end of P3 is identical to the first 40 bp of *eutH*, and the 3′ end is complementary to the 5′ end of the linker. The 5′ end of P4 is reverse complementary to the last 40 bp of *eutH*, and the 3′ end is a reverse complement of the 3′ end of the linker. The linker DNA has an open reading frame and therefore generates no in-frame translation stops. The DNA fragment made by amplification of the linker was used to transform a *eutH*::Tn*10* insertion mutant (TT10661). The recipient strain is phenotypically Eut$^-$ because of polar effects of the *eutH*::MudA insertion on the distal genes for lyase (*eutBC*). Cells that acquire the *eutH* in-frame deletion mutation become Eut$^+$ because they regain downstream expression and EutH function is not needed for growth on ethanolamine (at pH 7).

A double mutant carrying both the *eutE* and *eutH* in-frame deletions was constructed by a series of P22-mediated transductions. A phage lysate grown on a double-insertion [*eutA*::Tn*10*(Tc) *eutE*::MudJ(Kn)] mutant (TT20038) transduced Tc^r into the *eutE* in-frame deletion mutant (TT22815), and a Tc^r Kn^s recombinant was retained (TT24608) that carries the *eutE* deletion and *eutA*::Tn*10*. A *eut-cysA* deletion mutation with Amp^r at its join point was introduced into this double mutant to form a triple mutant (TT24609). The *eutH* deletion was introduced with selection for Cys$^+$ and loss of the *eut-cysA* deletion. Recombinants were screened to identify those that lost the recipient *eutA*::Tn*10* insertion (and are thus likely to have acquired the adjacent *eutH* deletion). These were tested to identify those that could use ethanolamine as a source of nitrogen but not carbon (a *eutE* phenotype). The structure of the *eutE eutH* double-deletion mutant (TT24610) was verified by PCR.

The gene for yellow fluorescent protein (*yfp* from the Clontech pEYEP vector) was fused to the distal end of the *eutH* gene by two successive linear transformations that introduced the *yfp* coding region into the *eut* operon without causing a polar effect on transcription. The first transformation introduced a cassette that includes the *sacB* and *kan* genes between the chromosomal *eutH* and *eutA* genes (8, 10). The *sacB* gene confers sucrose sensitivity, and the *kan* gene provides resistance to kanamycin. The cassette was amplified with primers EutHsacF (CGGCGCCCAGGTGAAAACCGAAGCGGAGGCGCAATCGT GATGGCGGCCGCTCTAGAACTAGTG) and EutHsacR (GTGCCGATATC GATACCGACGCTCAGTAGCTGGCGAGTGTCGACGGTATCGATAAG CTTGATATCG).

The SacKan cassette was then replaced with the *yfp* coding region with selec-

tion for sucrose resistance. For this, we used a PCR fragment created by amplifying the *yfp* gene from the pEYFP vector with primers eutHeyfpF (GACGGC GCCCAGGTGAAAACCGAAGCGGAGGCGCAATCGGGTGGAGGTGG CGGAGGCGGTGGCGGAGGCATGGTGAGCAAGGGCGAGGAGC) and eutHeypF (CGATATCGATACCGACGCTCAGTAGCTGGCGAGTGTTCA CGATTGCGCCTCCGCCTTGTACAGCTCGTCCATG).

Sucrose-resistant transformants had lost Kn^r and carried a *eutH-yfp* gene fusion that was verified by sequencing (TT24809).

Cell swelling assays. The method of Heller et al. (5) monitors the shrinkage and reswelling of cells in response to a concentrated solution of a test compound. Efflux of water (shrinking), followed by influx of ethanolamine and water (reswelling), was followed over a 15-min time course. Cells were pregrown overnight in NCE medium with 0.2% ethanolamine hydrochloride, 200 nM cyanocobalamin (B_{12}), and 1% sodium succinate. Overnight cultures were diluted 1:200 into 1 liter of the same culture medium and shaken in 2-liter Kimax flasks at 37°C until the culture reached an OD_{600} of approximately 1.0. Cells were pelleted by centrifugation, washed twice with 50 mM MOPS (pH 7), and resuspended in approximately 9 ml of the same buffer. Aliquots of this concentrated cell suspension (1.5 ml) were resuspended in 17 ml of baseline buffer to prepare the final cell suspensions used in the swelling assays. Baseline buffers, used to assay the preshrunken state of the cells, each contained 50 mM Good buffer (MOPS for pH 7.0; bicine for pHs 8.0, 8.5, and 9.0; CAPS [3-(cyclohexylamino)-1-propanesulfonic acid] for pHs 9.5, 10.0, and 10.5) and were all adjusted to the desired pH with NaOH. Ethanolamine solute buffers contained 375 mM ethanolamine hydrochloride but were otherwise identical to the baseline buffers. Swelling assays were initiated by mixing 1 ml of cell suspension with 2 ml of baseline or ethanolamine buffer and monitoring the OD_{600} every 6 s for 15 min with a Beckman Instruments DU series 600 spectrophotometer.

RESULTS

Ethanolamine crosses the cell membrane in a pH-dependent manner. The cell swelling assay (5) measures solute entry into cells indirectly through changes in the turbidity of a cell suspension that result from plasmolysis (shrinkage) and deplasmolysis (reswelling) of cells after solute addition. Shrinkage results from water loss caused by exposure of cells to an external hypertonic solution, and reswelling results from entry of the solute accompanied by water (1). These assays (Fig. 3) show that, at pH 7, ethanolamine (250 mM) caused shrinkage but no reswelling, suggesting no entry of the solute. At a higher pH, the amount of shrinkage decreased (smaller ΔOD_{600} between the baseline and the ethanolamine curve at time zero), and the rate of reswelling increased (slope of OD_{600} curve returning to the baseline). The highest reswelling rate seen was at pH 10.5. At this pH, ethanolamine is mostly unprotonated since the pK for protonation is 9.5. A *eutH* mutation had no effect on this assay (data not shown), suggesting that the massive ethanolamine flux required to reswell cells is not dependent on this channel. These data indicate that the charged species, which predominates at low pH, does not enter cells at a rate that can be seen in this assay. These results suggested that EutH may not be needed at pH values sufficiently high to allow unaided diffusion. More detailed implications of these results are discussed later.

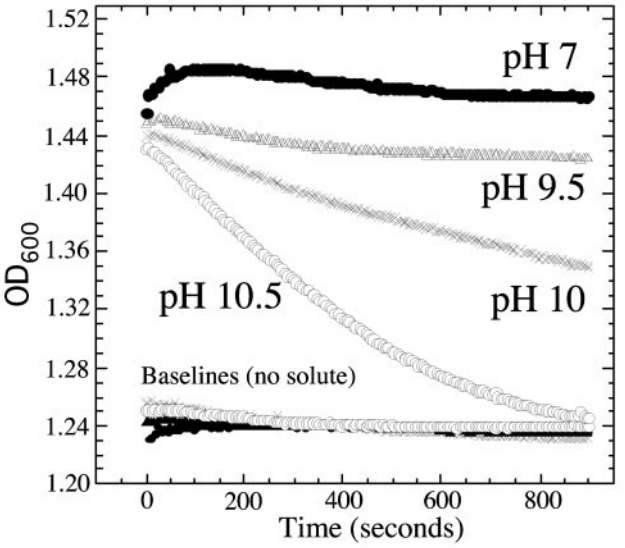

FIG. 3. Cell shrinking and swelling caused by ethanolamine at various pH values. Wild-type cells were grown on succinate in the presence of ethanolamine and cobalamin (to induce the *eut* operon). Washed cells were added to buffered ethanolamine solutions at various pH values to give a final external ethanolamine concentration of 250 mM. Parallel suspensions were made in buffer set at the various pH values but with no ethanolamine (bottom traces). Shrinking and reswelling were monitored by following the OD_{600}. Results identical to those presented were seen for uninduced eut^+ cells and for *eutH* mutant cells whether induced or uninduced.

The ethanolamine growth phenotype of a *eutH* mutation is pH dependent. The swelling assay suggested that uncharged ethanolamine enters cells by diffusion at a rate that might be sufficient to support growth at standard pH without a specific transporter. That is, a *eutH* mutant might show a growth defect only at a pH and ethanolamine concentration for which the influx rate is limited by a low external Eth^0 concentration. This was tested initially by scoring growth on solid medium.

Table 2 shows the effects of pH and ethanolamine concentration on the growth of wild-type and *eutH* mutant cells with ethanolamine as the carbon source. For each set of conditions, the calculated concentration of Eth^0 is indicated. While the concentration of the charged species is not significantly affected by changes in pH, the concentration of Eth^0 varies

TABLE 2. Conditions under which a *eutH* mutant has a growth phenotype

Strain	Genotype	Growth[a] on ethanolamine as a carbon source at the indicated pH and concns of total ethanolamine[b] and Eth^0[c]								
		pH 5.5			pH 6.0			pH 7.0		
		20 mM (2 μM)	41 mM (4 μM)	82 mM (8 μM)	20 mM (6 μM)	41 mM (13 μM)	82 mM (26 μM)	20 mM (63 μM)	41 mM (0.13 mM)	82 mM (0.26 mM)
TT22524	*eutH*Δ	0	0	2+	0	2+	4+	4+	4+	4+
TR10000	Wild-type LT2	4+	4+	4+	4+	4+	4+	4+	4+	4+

[a] Growth was scored qualitatively on an increasing scale from no growth (0) to strong growth (4+).
[b] Total concentration of ethanolamine in assay plates, regardless of charge.
[c] The concentration of Eth^0 (in parentheses) was calculated for the indicated pH by using the Henderson-Hasselbach equation.

TABLE 3. Minimum ethanolamine for wild type

pH	Growth[a] on ethanolamine as a carbon source at the indicated concn of total ethanolamine (mM)[b]					
	0.25	0.5	1	2.5	5	10
5.5	0 (0.026)	0 (0.05)	0 (0.10)	1+ (0.26)	1+ (0.52)	2+ (1.0)
6.0	0 (0.10)	0 (0.20)	1+ (0.41)	1+ (0.81)	2+ (1.6)	3+ (3.2)
6.5	0 (0.26)	1+ (0.51)	1+ (1.0)	2+ (2.6)	3+ (5.2)	4+ (10)

[a] Growth on ethanolamine as the carbon source was scored qualitatively on an increasing scale from no growth (0) to strong growth (4+).

[b] Total concentration of ethanolamine in plates regardless of charge. The concentrations (in micromolar units) of Eth^0 at the indicated pHs were calculated by using the Henderson-Hasselbach equation and are presented in parentheses.

widely. Mutants lacking EutH were impaired for growth at Eth^0 concentrations below about 25 μM. The concentration required by a *eutH* mutant is about 10-fold higher than that of wild-type cells (Table 3). The concentration required by $EutH^+$ cells—about 3 μM ethanolamine—is close to the K_m (9 μM) of the first enzyme, ethanolamine ammonia lyase (24). The growth ability of both wild-type and *eutH* mutant strains correlates with the external Eth^0 concentration.

These growth responses were also seen in liquid growth tests (Fig. 4). At pH 7.5, wild-type and *eutH* mutant cells grow similarly at a range of ethanolamine concentrations (Fig. 4A and B). A reduced pH (Fig. 4C and D) impaired the ability of a *eutH* mutant to grow on lower ethanolamine concentrations but had no effect on the growth of wild-type cells (data not shown). It should be noted that at pH 7.5, the growth ability of parallel *eutH* mutant cultures was rather variable. We suggest that this reflects the frequent appearance of suppressor mutants that allow faster entry of ethanolamine.

A second test of EutH as a facilitator of ethanolamine uptake. It was previously suggested that ethanolamine catabolism releases sufficient acetaldehyde to inhibit the growth of cells that lack protective functions such as glutathione or the repair protein DNA polymerase I (14, 15). This suggested that conditions might be found under which added ethanolamine inhibits the growth of cells on an unrelated carbon source. If

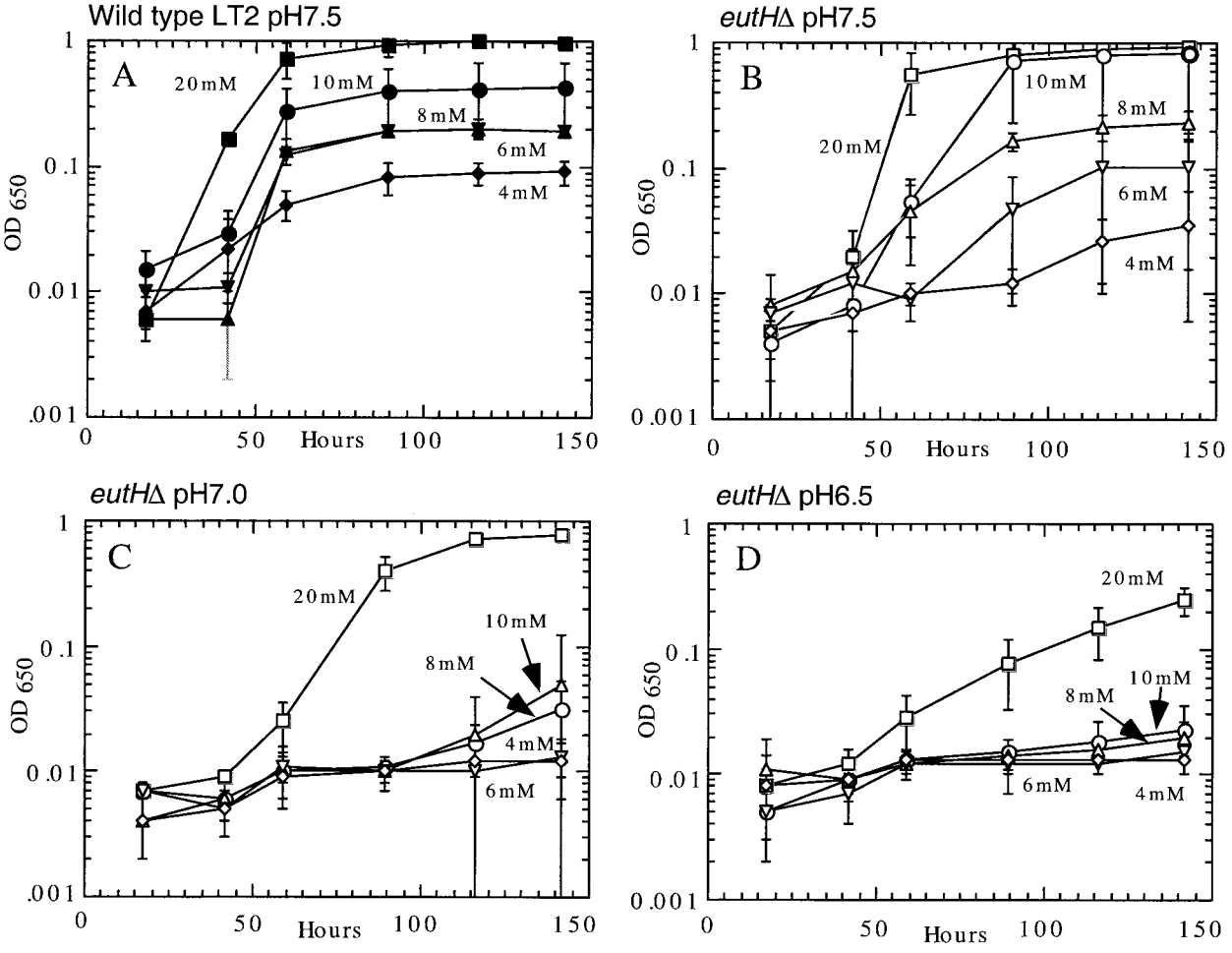

FIG. 4. Effect of pH on growth on ethanolamine as the sole carbon source. Solid and open symbols represent growth of wild-type LT2 and the *eutH*Δ mutant, respectively. Individual curves indicate growth on different initial concentrations of added ethanolamine. Symbols: squares, 20 mM; circles, 10 mM; triangles, 8 mM; diamonds, 6 mM. Parts A and B describe the growth of wild-type and *eutH* mutant cells at pH 7.5. Parts C and D describe the growth of a *eutH* mutant at pHs 7 and 6.5. Wild-type cells show the same growth response at pHs 6.5 and 7 (not shown) as at pH 7.5 (above).

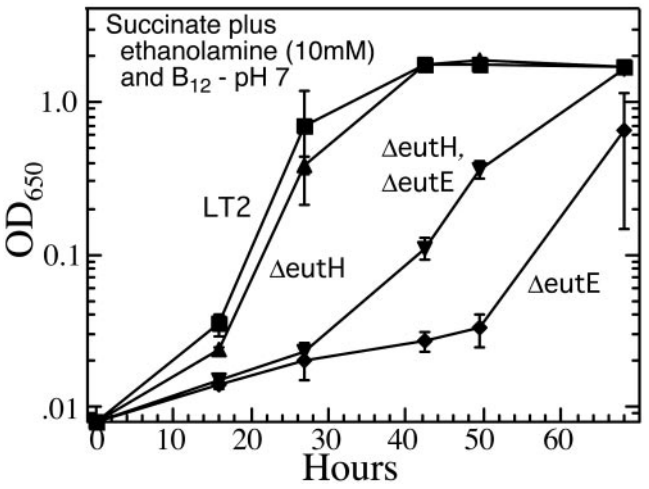

FIG. 5. Ethanolamine inhibits the growth of *eutH* mutants on succinate. Wild-type LT2 is indicated by squares, the *eutH*Δ mutant is indicated by triangles pointing up, the *eutE*Δ mutant is indicated by diamonds, and the *eutEH*Δ double mutant is indicated by triangles pointing down. The results presented (for pH 7) are intermediate between the result seen at pH 6.5 (complete correction) and that seen at pH 7.5 (no correction). Both the wild type and the *eutH* single mutant grow normally on succinate at all three pH values.

EutH contributes to ethanolamine influx, it should also contribute to this sensitivity under appropriate conditions.

Tests of this prediction were done with an in-frame *eutE* deletion mutant that lacks the second step of the ethanolamine pathway (Fig. 1) and should accumulate acetaldehyde produced from ethanolamine. This mutant grew as well as the wild type on succinate. Unlike the wild type, this mutant was inhibited for growth on succinate by added ethanolamine. The toxicity of ethanolamine required the first enzyme of the pathway. That is, toxicity was eliminated by lack of the lyase cofactor B_{12} and by mutations that inactivate lyase (*eutBC*), suggesting strongly that acetaldehyde is responsible. We have also demonstrated that acetaldehyde is released by strains growing on ethanolamine (Penrod, unpublished). This allowed a second test of the suggested role of EutH in transport.

If EutH is involved in ethanolamine transport, it should contribute to the toxic effect of ethanolamine (described above) on a *eutE* mutant. That is, under conditions that require EutH for transport, a *eutH* mutation should reduce the toxic effect of ethanolamine. Conversely, if EutH acts at some later step in ethanolamine metabolism or if it facilitates efflux of toxic acetaldehyde, one might expect a *eutH* mutation to increase the toxicity of ethanolamine.

Rates of growth in liquid support the idea that EutH contributes to ethanolamine influx (Fig. 5). A *eutH* mutation did not impair the growth of a *eutE* mutant on succinate. However, as shown in Fig. 5, a *eutH* mutation substantially relieved the toxic effect on an *eutE* mutant of 10 mM ethanolamine at pH 7.0. As predicted if only Eth^0 enters cells, the same *eutH* mutation had no effect at pH 7.5 and completely corrected ethanolamine sensitivity at pH 6.5 (data not shown).

EutH protein is localized in the cell membrane. The EutH sequence included 8 to 10 putative transmembrane segments (Fig. 2), suggesting that the protein might be inserted in the membrane, as expected for a transporter. The predicted location of EutH was tested with a fusion of yellow fluorescent protein (Yfp) to EutH. The *yfp* coding sequence was fused to the *eutH* gene within the *eut* operon as described in Materials and Methods. This chimeric gene produces a hybrid protein with the Yfp sequence linked to the C-terminal end of the EutH protein with a linker of 10 glycine residues. The EutH-Yfp fusion strain grew like the wild type in the assay described in Table 2, indicating that the fusion protein supplied normal EutH function and the construction caused no polar effects on downstream genes in the operon. Fluorescence microscopy revealed the localization of the EutH-Yfp protein near the cell periphery (Fig. 6). The specificity of this labeling is clear since the same Yfp sequence showed uniform labeling across the cell when fused to other proteins and labeled only points within the cell (thought to be carboxysomes) when fused to carboxysome shell proteins.

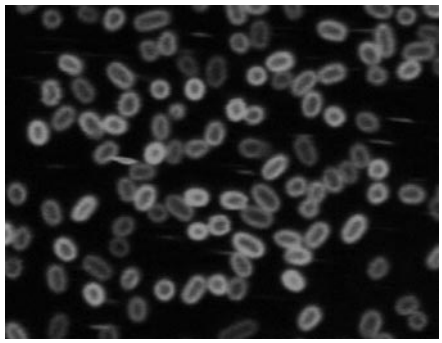

FIG. 6. Distribution of fluorescence in cells expressing a EutH-Yfp fusion protein. The *eutH-yfp* fusion allele was constructed in the chromosomal *eut* operon by linear transformation as described in Materials and Methods. This allele does not disturb the transcription of downstream genes, and cells with the fusion allele show wild-type growth under conditions that impair the growth of *eutH* null mutants.

DISCUSSION

Cell swelling experiments showed a pH-dependent diffusion of ethanolamine into cells of *S. enterica*, indicating that the uncharged form Eth^0 diffused freely across the membrane while the protonated form was excluded. This suggested that the putative ethanolamine transporter EutH might facilitate influx of the uncharged species and thus be needed only when the Eth^0 concentration was reduced by a low pH and ethanolamine.

Evidence that EutH is a diffusion facilitator for uncharged ethanolamine. (i) In previous experiments, isotopes from labeled ethanolamine did not accumulate in cells without an intact degradative pathway (18). (ii) Wild-type cells use ethanolamine only when the Eth^0 concentration is above 3 μM, a concentration near the K_m of ethanolamine ammonia lyase (9 μM). (iii) A *eutH* mutant failed to grow normally at Eth^0 concentrations below 25 μM, suggesting that at lower concentrations the gradient provided insufficient flux for growth without facilitation. (iv) At physiological pH values, wild-type cells require an Eth^0 concentration that approximates the K_m of the first enzyme.

Selective maintenance of the *eutH* gene in natural popula-

tions suggests that *S. enterica* frequently encounters ethanolamine at concentrations or under pH conditions that limit the external Eth^0 level to between 3 and 25 μM—the range over which EutH enhances the ability to utilize ethanolamine.

The lack of an active transport system for ethanolamine may have several explanations. (i) The small amount of energy obtained from a two-carbon compound may make the cost of active transport prohibitive. Much of the derived energy is needed to generate larger carbon-containing molecules. Because ethanolamine is metabolized via the tricarboxylic acid cycle and glyoxalate shunt, formation of large carbon-containing molecules requires formation of phosphoenolpyruvate either from oxaloacetate (at a cost of one ATP) or from malate through pyruvate (at a cost of two ATPs). (ii) Much of the transported ethanolamine is lost as the volatile intermediate acetaldehyde (Penrod, unpublished), and some is likely to be lost as ethanol (formed to balance redox). (iii) Perhaps most importantly, actively transported ethanolamine would be subject to immediate loss because the uncharged form can cross the membrane by nonspecific diffusion.

Interpretation of cell swelling assays. The cell swelling assays in Fig. 3 have some curious features. While increased pH allowed more rapid swelling, the major effect is seen at pH values (pHs 9.5 to 10.5) that are above the pK for ethanolamine (pH 9.5), a range in which the external Eth^0 concentration changes less than twofold; a much smaller effect is seen in the pH range of 7.5 to 9.5, over which the Eth^0 concentration increases about 50-fold. Furthermore, even at the highest pH tested, the influx rate is slow compared to that seen for uncharged solutes such as glycerol (5, 9) and is not affected by the presence of EutH. Generally these assays have been used to study the influx of nonionic solutes while here an ionizable compound is used. We suggest that EutH is a low-capacity channel and at these high concentrations, Eth^0 enters at a slow rate, primarily through general unfacilitated routes. After entry, ethanolamine is reprotonated within the small cell volume, causing an increase in the internal pH that limits protonation of ethanolamine and reswelling. Full swelling requires reduction of the internal pH to that of the outside, which may be easier to achieve when the external pH is higher. During growth, the pH problems may be solved in part by metabolism of ethanolamine and attendant release of excess ammonia from the cell.

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