pH-Dependent Catabolic Protein Expression during Anaerobic Growth of Escherichia coli K-12

Joan Slonczewski
Kenyon College, slonczewski@kenyon.edu

Elizabeth Yohannes

D. Michael Barnhart

Follow this and additional works at: https://digital.kenyon.edu/biochem_publications

Part of the Biochemistry, Biophysics, and Structural Biology Commons, and the Biology Commons

Recommended Citation

This Article is brought to you for free and open access by the Biochemistry and Molecular Biology at Digital Kenyon: Research, Scholarship, and Creative Exchange. It has been accepted for inclusion in Faculty Publications by an authorized administrator of Digital Kenyon: Research, Scholarship, and Creative Exchange. For more information, please contact noltj@kenyon.edu.
pH-Dependent Catabolic Protein Expression during Anaerobic Growth of Escherichia coli K-12

Elizabeth Yohannes, D. Michael Barnhart, and Joan L. Slonczewski*

Department of Biology, Kenyon College, Gambier, Ohio 43022

Received 24 June 2003/Accepted 23 September 2003

During aerobic growth of Escherichia coli, expression of catabolic enzymes and envelope and periplasmic proteins is regulated by pH. Additional modes of pH regulation were revealed under anaerobiosis. E. coli K-12 strain W3110 was cultured anaerobically in broth medium buffered at pH 5.5 or 8.5 for protein identification on proteomic two-dimensional gels. A total of 32 proteins from anaerobic cultures show pH-dependent expression, and only four of these proteins (DshA, TnaA, GatY, and HdeA) showed pH regulation in aerated cultures. The levels of 19 proteins were elevated at the high pH; these proteins included metabolic enzymes (DhaKLM, GapA, TnaA, HisC, and HisD), periplasmic proteins (ProX, OppA, DegQ, MalB, and MglB), and stress proteins (DshA, Tig, and UspA). High-pH induction of the glycolytic enzymes DhaKLM and GapA suggested that there was increased fermentation to acids, which helped neutralize alkalinity. Reporter lac fusion constructs showed base induction of sdaA encoding serine deaminase under anaerobiosis; in addition, the glutamate decarboxylase genes gadA and gadB were induced at the high pH anaerobically but not with aeration. This result is consistent with the hypothesis that there is a connection between the gad system and GABt metabolism of 4-aminobutanoate. On the other hand, 13 other proteins were induced by acid; these proteins included metabolic enzymes (GatY and AckA), periplasmic proteins (ToIC, HdeA, and OmpA), and redox enzymes (GuaB, HmpA, and Lpd). The acid induction of NikA (nickel transporter) is of interest because E. coli requires nickel for anaerobic fermentation. The position of the NikA spot coincided with the position of a small unidentified spot whose induction in aerobic cultures was reported previously; thus, NikA appeared to be induced slightly by acid during aeration but showed stronger induction under anaerobic conditions. Overall, anaerobic growth revealed several more pH-regulated proteins; in particular, anaerobiosis enabled induction of several additional catabolic enzymes and sugar transporters at the high pH, at which production of fermentation acids may be advantageous for the cell.

The pH response is important for growth and survival of Escherichia coli in an environment such as the human gastrointestinal tract, in which the pH fluctuates over the range from pH 6 to 8 (14, 18). The role of pH in gene expression in E. coli and related enteric bacteria has been studied extensively, but it has been studied largely under aerobic conditions (7, 11, 59, 64, 66; for reviews see references 15 and 54). Relatively few studies have addressed the relationship between pH and anaerobiosis, the predominant condition of enteric growth (2); the best-studied cases include anaerobic acid induction of amino acid decarboxylases (1, 6, 61) and a limited two-dimensional (2-D) gel study of protein profiles (7). However, enteric bacteria behave very differently under anaerobic and aerobic conditions; for example, in microarrays more than one-third of the genes expressed during aerobic growth are altered when E. coli cells are shifted to anaerobic conditions (49). The differences between aerobic and anaerobic conditions become even more complex during intracellular pathogenesis (1).

Aerated E. coli cultures respond to pH changes by selective expression of numerous stress proteins, redox modulators, and envelope proteins (21, 59, 65). The acid stress chaperones HdeA and HdeB enhance survival in extreme acid conditions (5, 16). The membrane-bound Na⁺H⁺ antiporter NhaA protects the cell from excess Na⁺ at a high external pH (26, 43). Genes that show pH dependence are often coinduced by other environmental factors, such as growth phase, carbon source, and anaerobiosis (33, 56, 57). External acids and membrane-permeant acids, whose uptake is amplified by the pH gradient, induce heat shock and oxidative stress proteins, as well as the RpoS regulon (5, 7, 30, 32, 50).

The response to pH includes modulation of catabolism, particularly in the presence of complex carbon sources, such as the tryptophan and yeast components of Luria-Bertani medium (LB). Tryptone consists of primarily tryptic peptides and 7.7% (wt/ wt) carbohydrates (primarily lactose), whereas yeast extract contains peptides plus 17.5% carbohydrates (primarily glyco
gen and trehalose) (68; Difco manual, 11th ed., Difco Labo-
datories, Detroit, Mich.). Peptides from casein and yeast extract can be taken up by transporters such as OppA and then catabolized via pathways that begin with removal of CO₃ or NH₃ (39). Whether decarboxylation or deamination occurs is influenced by pH: external acid conditions induce decarboxylation (6, 11, 38, 53) and production of alkaline amines, whereas external base conditions induce deamination and production of fermentation acids (7, 15, 54, 59). The carbohydrate in LB is predominantly lactose from casein and glycogen and trehalose from yeast extract. These sugars are taken up by specific transporters and then catabolized by pathways that produce variable amounts of fermentation acids (8, 28, 35).

During early-log-phase growth, even well-oxygenated E. coli cells initially produce fermentation products such as acetate...
and formate, which at a low external pH can reenter the cell and reach deleterious concentrations (29, 46, 47). For this reason, fermentation pathways respond to pH; for instance, ldhA is induced severalfold by acid in order to produce lactate instead of acetate plus formate (9). A number of proteins induced by acetate and by short-chain fatty acids (5, 7, 30) are also induced by growth at low pH, and the pH gradient drives the fermentation acids back into the cell. For example, the low-oxygen pyruvate-formate lyase YfdD, induced by acetate or formate (7, 30), is also induced during growth on LB at low pH, whereas several acetate-repressible proteins, such as tryptophanase (TnaA) and high-affinity maltose binding protein (MalE), are repressed at low pH (3, 7, 59).

Anaerobiosis amplifies induction of several acid-regulated pathways of catabolism, such as the cadAB, 1ysU, and adl pathways (34, 48, 53, 61). The absence of oxygen limits the metabolic options available to cells, necessitating increased excretion of weak-acid fermentation products that stress the cell. At the same time, anaerobiosis makes new enzymatic pathways available, such as the pathway for anaerobic beta-oxidation of fatty acids (10). Thus, one would expect anaerobiosis to favor additional pH responses not seen during growth with oxygen.

We describe here a proteomic 2-D gel comparison of E. coli protein profiles at low pH and at high pH for cells grown under anaerobiosis. New patterns of gene expression were obtained that substantially augment our picture of pH-dependent protein expression, especially for pathways of catabolism.

### MATERIALS AND METHODS

#### Growth conditions.

E. coli K-12 strain W3110 (Table 1) was grown overnight in unbuffered potassium-modified Luria broth (LBK) (10 g of tryptone per liter, 5 g of yeast extract per liter, 7.45 g of KCl per liter). For aerobic growth, cultures were diluted 500-fold in 2 ml of buffered medium with aeration at 37°C. For anaerobic growth, each overnight culture was diluted 500-fold in 9 ml of buffered medium and transferred to a Pyrex screw-cap tube whose volume was exactly 9 ml to avoid an air space. The buffers used were homopiperazine-N,N′-bis(2-ethanesulfonic acid) (HOMOPIPES) (pKₐ 4.55), 2-(N-morpholino)ethanesulfonic acid (AMPSO) (pKₐ 9.10). All buffers were obtained from Research Organics or Sigma. The pH values of media were adjusted by using KOH to avoid extra sodium ions, which stress cells at high pH (26, 43). For all cultures, the pH was adjusted to the appropriate value with KOH; thus, the potassium ion concentration of the high-pH medium was approximately 50 mM higher than the potassium ion concentration of the low-pH medium. All cultures were grown anaerobically in closed tubes without an air space, which were rotated end over end at 37°C until the optical density at 600 nm (OD₆₀₀) reached 0.15.

#### Gel electrophoresis.

2-D gel analyses were performed by using a previously described procedure (59), which is updated online (biology.kenyon.edu/labtools/2d_method.html). Cells from three independent cultures were harvested for each pH. Each culture was pelleted by centrifugation at 4°C, resuspended in unbuffered LBK, and recentrifuged. The cell pellets were then treated with sample buffers and rehydration solution (55) in order to extract the proteins.

The protein mixtures were first separated by isoelectric focusing by using 18-cm polyacrylamide gel strips with an immobilized pH 4 to 7 gradient according to the protocol of the manufacturer (AP Biotech). For each gel, 50 μg of cell protein was loaded onto an IPG strip. For the second dimension, an electrophoretic gel slab containing 11.5% acrylamide was prepared as described previously (55, 59). The gels were silver stained by a procedure compatible with matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) analysis, and the patterns were scanned and digitized. Protein spots were analyzed both qualitatively and quantitatively by using the Compugen Z3 v.3.0 software (Compugen, Tel Aviv, Israel).

The differential expression ratio (DE) of the spot densities for each growth condition (pH 5.5 or pH 8.5) was computed by pairwise comparisons of a set of three gels from pH 8.5 cultures and a set of three gels from pH 5.5 cultures. A protein spot was considered a candidate for significant induction if seven of nine pairwise comparisons produced a DE greater than or equal to 2 or less than 0.5. Proteins observed at pH 8.5 that had no matching proteins on the pH 5.5 gels were scored as having a DE of 0.1. For each protein, the log₁₀ of all nine DE values was computed, and the mean log₁₀ DE (LDE) was considered a measure of induction (positive values) or repression (negative values) (Table 2).

#### Identification of proteins.

Proteins having a significant LDE were identified either by MALDI-TOF analysis at the Proteomic Mass Spectrometry Laboratory at the University of Massachusetts (http://www.umassmed.edu/proteomic/) or by protein sequence comparison with previous gels in which proteins had been identified by MALDI-TOF analysis or N-terminal sequencing (30, 59). The differentially expressed proteins were further identified by using a Kratos Axima CFR MALDI-TOF mass spectrometer. The mass spectrometer data were obtained by using tryptic peptide mixtures, as well as postsource decay analysis of individual peptides. For database searches of MALDI-TOF masses the Protein Prospector site was used (prospector.ucsf.edu).

#### Strain construction.

To describe the sdaA::lac strain JLS0711, a sequence containing the putative sdaA promoter (positions −154 to 55 from the AUG start site) was PCR amplified from E. coli W3110 genomic DNA by using the following corresponding primers: right primer 5'- CGCGAATTCGCTTGAGACAAT CATTGCAATAA-3' and left primer 5'- CCGGGAATCTGAGGAAATAGTG GACCA-3'. The product was digested with EcoRI and BamHI and inserted upstream of the β-galactosidase gene in the plasmid vector pRS415, generating a transcriptional fusion (52). The plasmid constructs were transformed into E. coli strain MC4100 and sdaA::lac clones were selected by growth on ampicillin and X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside). The sdaA::lac fusion was then recombined into the genome by using a λRS45 phage and lysate selection process as described by Hand and Silhavy (22). Genomic recombinant of the sdaA::lac fusion was confirmed by sequencing across the fusion joint (Ohio State Plant-Microbe Genomic Facility).

#### β-Galactosidase assays.

For assays of promoter-lac fusion expression, strains were grown either anaerobically or aerobically in LBK buffered at different pHs ranging from 5.5 to 9.0. The buffers used were HOMOPIPES at pH 5.0, MES at

### TABLE 1. E. coli K-12 strains used in this study

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>W3110</td>
<td>F− λ prototroph</td>
<td>57</td>
</tr>
<tr>
<td>MC4100</td>
<td>F− araD139 Δ(arg-lac)U169</td>
<td>51</td>
</tr>
<tr>
<td>EF614</td>
<td>F− sdaA::lacT74::lacZ</td>
<td>11</td>
</tr>
<tr>
<td>JLS0214</td>
<td>MC4100 trpDC::putPA1303::km</td>
<td>This study</td>
</tr>
<tr>
<td>EF615</td>
<td>F− sdaA::lacT74::lacZ</td>
<td>11</td>
</tr>
<tr>
<td>JLS0215</td>
<td>MC4100 trpDC::putPA1303::km</td>
<td>This study</td>
</tr>
<tr>
<td>JLS0711</td>
<td>MC4100 sdaA::lacZ</td>
<td>This study</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>JLS0214</td>
<td>MC4100 trpDC::putPA1303::km</td>
<td>This study</td>
</tr>
<tr>
<td>EF615</td>
<td>F− sdaA::lacT74::lacZ</td>
<td>11</td>
</tr>
<tr>
<td>JLS0215</td>
<td>MC4100 trpDC::putPA1303::km</td>
<td>This study</td>
</tr>
<tr>
<td>JLS0711</td>
<td>MC4100 sdaA::lacZ</td>
<td>This study</td>
</tr>
</tbody>
</table>

Vol. 186, 2004 pH-DEPENDENT PROTEIN EXPRESSION IN E. COLI 193
method described previously (40, 51, 59).

were grown aerobically or anaerobically, as described above. Cultures
containing different buffers at each pH in order to minimize the
buffer strategies were used. In experiment A, the media con-
tenance varied greatly. Because pH stress caused growth
Acid induced

Base induced

RESULTS

For proteomic 2-D gels, E. coli K-12 strain W3110 was
grown anaerobically in LBK buffered at pH 5.5 or 8.5. The
log-phase doubling times were observed to be 31 min (pH 5.5) and
34 min (pH 8.5). At more extreme pH values, such as those
favored expression of 13 proteins, but only 2 of these 13 pro-
tended different buffers at each pH in order to minimize the
difference in the K+ concentrations, and in experiment B, the
media included the same buffers at each pH. The composite
protein profiles are shown in Fig. 1, and the results of a quan-
titative analysis of pairwise comparisons are shown in Table 2.
The overall patterns of differentially expressed proteins in ex-
periments A and B were largely the same. Six proteins had
significant LDE values in experiment A but not in experiment
B (Tig, MglB, GapA, GatY, Tsf, and HdeA), whereas two
proteins had significant LDE values in experiment B but not in
experiment A (MalB and AccB). These differences could re-

to assess the effects of buffers and counte-
rion concentrations under anaerobic growth conditions, as
shown previously for aerobic cultures (59). Two alternative
buffer strategies were used. In experiment A, the media con-

pH 6.0, MOPS at pH 7.0, TAPS at pH 8.0, and AMPSO at pH 8.7 to 9.0. Cultures
were grown aerobically or anaerobically, as described above. β-Galactosidase activities were determined for E. coli strains carrying

hαlacZ, gadA::lacZ, and gadB::lacZ (Table 1) by using the microtiter plate
method described previously (40, 51, 59).

TABLE 2. Proteins showing differential expression as a function of pH

<table>
<thead>
<tr>
<th>Spot</th>
<th>Proteina</th>
<th>Expt A</th>
<th>Expt B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>LDE</td>
<td>SE</td>
</tr>
<tr>
<td>1</td>
<td>OppA</td>
<td>0.24</td>
<td>0.02</td>
</tr>
<tr>
<td>2</td>
<td>DhaM (YcgC)</td>
<td>0.71</td>
<td>0.12</td>
</tr>
<tr>
<td>3</td>
<td>Tig</td>
<td>0.64</td>
<td>0.13</td>
</tr>
<tr>
<td>4</td>
<td>HisD</td>
<td>0.81</td>
<td>0.10</td>
</tr>
<tr>
<td>5</td>
<td>DegQ</td>
<td>0.58</td>
<td>0.22</td>
</tr>
<tr>
<td>6</td>
<td>MalB</td>
<td>0.06</td>
<td>0.15</td>
</tr>
<tr>
<td>7</td>
<td>TnaA</td>
<td>0.41</td>
<td>0.10</td>
</tr>
<tr>
<td>8</td>
<td>DhaK (YcgT)</td>
<td>0.55</td>
<td>0.07</td>
</tr>
<tr>
<td>9</td>
<td>HdeA</td>
<td>0.26</td>
<td>0.04</td>
</tr>
<tr>
<td>10</td>
<td>MalB</td>
<td>0.06</td>
<td>0.15</td>
</tr>
<tr>
<td>11</td>
<td>TnaA</td>
<td>0.41</td>
<td>0.10</td>
</tr>
<tr>
<td>12</td>
<td>DhaK (YcgT)</td>
<td>0.55</td>
<td>0.07</td>
</tr>
<tr>
<td>13</td>
<td>ProX</td>
<td>0.80</td>
<td>0.03</td>
</tr>
<tr>
<td>14</td>
<td>MglB</td>
<td>0.49</td>
<td>0.04</td>
</tr>
<tr>
<td>15</td>
<td>HisD</td>
<td>0.64</td>
<td>0.12</td>
</tr>
<tr>
<td>16</td>
<td>DhaL (YcgS)</td>
<td>0.30</td>
<td>0.10</td>
</tr>
<tr>
<td>17</td>
<td>DsbA</td>
<td>0.33</td>
<td>0.06</td>
</tr>
<tr>
<td>18</td>
<td>DhaK</td>
<td>0.30</td>
<td>0.10</td>
</tr>
<tr>
<td>19</td>
<td>DhaK</td>
<td>0.30</td>
<td>0.10</td>
</tr>
<tr>
<td>20</td>
<td>DhaK</td>
<td>0.30</td>
<td>0.10</td>
</tr>
<tr>
<td>21</td>
<td>DsbA</td>
<td>0.33</td>
<td>0.06</td>
</tr>
<tr>
<td>22</td>
<td>DhaK</td>
<td>0.30</td>
<td>0.10</td>
</tr>
<tr>
<td>23</td>
<td>DhaK</td>
<td>0.30</td>
<td>0.10</td>
</tr>
<tr>
<td>24</td>
<td>DhaK</td>
<td>0.30</td>
<td>0.10</td>
</tr>
<tr>
<td>25</td>
<td>DhaK</td>
<td>0.30</td>
<td>0.10</td>
</tr>
<tr>
<td>26</td>
<td>DhaK</td>
<td>0.30</td>
<td>0.10</td>
</tr>
<tr>
<td>27</td>
<td>DhaK</td>
<td>0.30</td>
<td>0.10</td>
</tr>
<tr>
<td>28</td>
<td>DhaK</td>
<td>0.30</td>
<td>0.10</td>
</tr>
<tr>
<td>29</td>
<td>DhaK</td>
<td>0.30</td>
<td>0.10</td>
</tr>
<tr>
<td>30</td>
<td>DhaK</td>
<td>0.30</td>
<td>0.10</td>
</tr>
</tbody>
</table>

a Proteins were identified by MALDI-TOF, unless indicated otherwise.

b Protein identified by position, based on the method described previously (30, 59).

For proteomic 2-D gels, E. coli K-12 strain W3110 was
grown anaerobically in LBK buffered at pH 5.5 or 8.5. The
log-phase doubling times were observed to be 31 min (pH 5.5) and
34 min (pH 8.5). At more extreme pH values, such as those
tested previously with aerated cultures (59), the growth rate
was low and varied greatly. Because pH stress caused growth
problems for anaerobic cultures, we focused on comparing
proteins had significant LDE values in experiment A but not in experiment
B (Tig, MglB, GapA, GatY, Tsf, and HdeA), whereas two
proteins had significant LDE values in experiment B but not in
experiment A (MalB and AccB). These differences could re-

to assess the effects of buffers and counte-
rion concentrations under anaerobic growth conditions, as
shown previously for aerobic cultures (59). Two alternative
buffer strategies were used. In experiment A, the media con-

TABLE 2. Proteins showing differential expression as a function of pH

Acid induced

<table>
<thead>
<tr>
<th>Spot</th>
<th>Protein</th>
<th>Expt A</th>
<th>Expt B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>LDE</td>
<td>SE</td>
</tr>
<tr>
<td>5</td>
<td>TolC</td>
<td>0.58</td>
<td>0.10</td>
</tr>
<tr>
<td>6</td>
<td>NikA</td>
<td>0.62</td>
<td>0.10</td>
</tr>
<tr>
<td>10</td>
<td>Hmp</td>
<td>0.60</td>
<td>0.09</td>
</tr>
<tr>
<td>15</td>
<td>GatY</td>
<td>1.00</td>
<td>0.00</td>
</tr>
<tr>
<td>16</td>
<td>Lpd</td>
<td>0.21</td>
<td>0.04</td>
</tr>
<tr>
<td>17</td>
<td>AckA</td>
<td>0.45</td>
<td>0.07</td>
</tr>
<tr>
<td>19</td>
<td>0.25</td>
<td>0.02</td>
<td>0.34</td>
</tr>
<tr>
<td>22</td>
<td>0.82</td>
<td>0.18</td>
<td>0.34</td>
</tr>
<tr>
<td>26</td>
<td>Ppa</td>
<td>0.12</td>
<td>0.03</td>
</tr>
<tr>
<td>28</td>
<td>OmpA</td>
<td>0.42</td>
<td>0.05</td>
</tr>
<tr>
<td>31</td>
<td>HdeA</td>
<td>0.07</td>
<td>0.10</td>
</tr>
<tr>
<td>32</td>
<td>GuaB</td>
<td>0.71</td>
<td>0.03</td>
</tr>
</tbody>
</table>
FIG. 1. pH-dependent protein profiles after anaerobic growth. The horizontal axis represents the approximate pH range of the isoelectric focusing first dimension, and the vertical axis represents the molecular weight (Mw). In the layered view shown two composite images, one representing growth at pH 8.5 (pink) and one representing growth at pH 5.5 (green), are superimposed. Each composite image is based on three 2-D gels from independent replicate cultures. All cultures of *E. coli* W3110 were grown at 37°C to an OD₆₀₀ of 0.15 in LBK with buffer of the appropriate pH at a concentration of 100 mM as described in Materials and Methods. (A) Cultures grown in LBK buffered with 100 mM MES (pH 5.5) or 100 mM TAPS (pH 8.5). (B) Cultures grown in LBK buffered with a mixture of 50 mM MES and 50 mM TAPS for both pH 5.5 and pH 8.5.
was reported previously to be acid induced aerobically but whose concentration too low for MALDI-TOF identification (59). The acid-induced proteins observed under anaerobiosis included catabolic enzymes (GatY and AckA), periplasmic proteins (TolC, HdeA, and OmpA), and redox proteins (GuaB, HmpA, and Lpd).

**Strain construction.** Our growing picture of pH-regulated catabolism predicts that pH regulates expression of additional pathways of amino acid catabolism. For example, one of the most strongly base-induced proteins in *E. coli* is TnaA (7, 59), which deaminates tryptophan, cysteine, and serine (58, 60). Therefore, we predicted that other enzymes that degrade cysteine or serine, such as the degradative serine deaminase encoded by *sdaA* (62), would also show base induction. A lac reporter fusion to *sdaA* was constructed as described in Materials and Methods. PCR sequence analysis of the fusion strain showed that the *sdaA* promoter, located at positions −154 to 55 in the *E. coli* K-12 genome, was inserted 17 hp from the start of the *EcoR*I restriction site and 260 bp from the *lacZ* sequence in the pRS415 vector. The fusion was then moved into the MC4100 genome (strain JLS0711).

The degradative glutamate decarboxylases, GadA and GadB, have been extensively studied to determine their role in acid resistance, and expression of these proteins is induced by acid compared with expression in cultures grown at pH 7 (11, 12, 65). Nevertheless, expression of the GadA protein is also elevated at pH 9 under anaerobiosis (7). To investigate *gad* expression at the transcriptional level, *gadA::lac* and *gadB::lac* constructs were obtained (11), and the fusion loci were transduced by phage P1 into MC4100.

**Expression of sdaA, gadA, and gadB.** The gene fusions *sdaA::lac*, *gadA::lac*, and *gadB::lac* were tested for expression as a function of pH by using cultures grown with and without aeration. All of the fusions were induced at high pH under anaerobiosis, and induction was enhanced at a higher cell density (Fig. 2). Growth curves indicated that the data for the cultures assayed were obtained during early- or mid-log-phase growth (Fig. 3); growth of cultures at the low and high pHs stopped at a lower cell density than growth of cultures at pH 7. The curves shown in Fig. 3 are for strain JLS 0711; other strains assayed produced similar results (data not shown).

The enhancement of high-pH induction at a higher cell density parallels previous reports of cell density enhancement of expression of *maaA*, *cysK*, and *gabT* (59). The *sdaA::lac* construct also exhibited slight induction by base with aeration. The *gadA::lac* and *gadB::lac* constructs, however, showed high-pH induction only during anaerobic growth at the mid- to late stationary phase.

**DISCUSSION**

Our 2-D gel analysis of anaerobic protein profiles revealed a substantial number of pH-dependent proteins that were not observed with aeration. Most of these proteins were catabolic enzymes or catabolite transporters. These new observations may have several explanations. (i) In the absence of oxygen, catabolism generates greater quantities of organic products whose buildup threatens the cell, especially permeant acids at low pH; therefore, greater regulation of catabolism is needed. (ii) Some proteins that show pH regulation with or without oxygen may fail to show up under aeration conditions if their overall expression level is repressed by oxygen; an example is NikA, whose high-pH induction is barely detectable when aeration is used (59) but appeared more strongly in an anaerobic culture. (iii) During anaerobic growth, a number of proteins expressed at high levels when aeration is used may be repressed; the repression of these proteins may reveal the presence of protein spots previously undetected in the gels prepared from aerated cultures.

**High-pH-induced proteins during anaerobic growth.** The high-pH induction of several more catabolic enzymes fits into our growing picture of pH-regulated catabolism during growth in complex medium under conditions that may resemble the growth conditions in the intestine (Fig. 4). Our general model is that low pH favors production of alkaline amines that counteract acidification plus CO₂, an acid that readily diffuses and is removed rapidly by the host circulation, whereas high pH favors production of fermentation acids plus NH₃, which diffuses and is removed. This model is consistent with acidic induction of amino acid decarboxylases and alkaline induction of deaminases and sugar breakdown.
We show here that high pH induces the three major components of the dihydroxyacetone (Dha) kinase system (DhaK, DhaL, and DhaM) (20, 44). The Dha kinase system transfers phosphate from the phosphotransferase system to DhaM and then through Dha kinase (DhaLM) to Dha, a catabolic product of sugars and amino acids. The phosphorylated Dha is then converted to glyceraldehyde 3-phosphate by GapA, which is also induced at high pH; from there, breakdown leads to fermentation products. Unlike other phosphotransferase systems, Dha kinase acts entirely within the cytoplasm, without involving vectorial transport; thus, the catabolite and its acidic fermentation products are maintained in the cytoplasm. Besides enzymes, two sugar transporters were induced at high pH, the maltose oligosaccharide porin MalB (4, 17, 36) and the galactose binding protein MglB (25). These transporters should be useful for uptake of the hydrolyzed glycogen and lactose in LBK. In general, glycolysis and fermentation of available sugars should proceed more rapidly at high pH, at which the fermentation acids either buffer the internal pH or exit the cell down the pH gradient. Interestingly, several sugar porins, including MalB and OmpF, exhibit channel closure at pH values below 5, at which even low concentrations of fermentation acids can endanger the cell (4, 42).

Amino acid catabolism at high pH favors deaminases, such as TnaA (7). The high-pH induction of TnaA, which deaminates Trp, Cys, and Ser, led us to test the pH dependence of expression of serine deaminase. The SdaA protein did not show up on our 2-D gels, which separated only a subset of E. coli proteins. Nevertheless, an sdaA::lac fusion showed strong induction at high pH. The high-pH induction required anaerobiosis, which is consistent with our prediction that anaerobic conditions turn on modes of pH regulation of catabolism that are not seen under aerobic conditions. Serine deamination may also play a role in the stationary phase, when the pH of LB rises above pH 9 (53), since mutants with increased stationary-phase survival show enhanced catabolism of serine (69).

The gadA and gadB reporters showed increased expression as the pH increased across the pH range (Fig. 2). The high-pH induction of gadA::lac and gadB::lac required a high cell density and anaerobiosis. These results confirmed the previous report of elevated GadA levels at pH 9 (7). In other studies, expression of gadA and gadB may have been induced by acid in the early stationary phase (12), although a gadX mutant actually showed acid repression of gadA and gadB (37). The high-pH induction of gad is interesting in view of the role of this gene in resistance to acid (11, 12, 38, 63, 65). However, gadC mutants show defective acid resistance only when they are grown at pH values above 7; thus, the role of gad in acid resistance appears to be especially important for cultures grown at high pH before exposure to extreme acid conditions (24).

The complexity of the gad response may be related to the fact that unlike the other acid-induced decarboxylases (CadA, Adi, and SpeF), which generate amines, GadA and GadB generate an amino acid, 4-aminobutanoate (GABA), which can be directed into alternative pathways (Fig. 4). At high pH, GABA is directed into production of succinate by GabT (59). Succinate is a nonpermeant acid that could neutralize internal alkalization or be converted to other fermentation acids.

Also induced at high pH were the histidine biosynthesis components HisC and HisD. HisC catalyzes amino transfer from L-histidinol-phosphate to 2-oxoglutarate, forming glutamate (19, 23). The role of HisC during high-pH induction may be related to its interaction with the pH-dependent GABA-glutamate system.

The DegQ periplasmic endoprotease (31) cleaves misfolded proteins by recognizing specific peptide folds usually buried within the three-dimensional protein structure. Other protein-folding agents induced at high pH include UspA and Tig. Both base stress and acid stress cause problems with protein folding, which are addressed by different chaperones and proteases; at low pH, HdeA was induced, as observed previously in aerobic cultures (59).

Low-pH-induced proteins during anaerobic growth. Fewer catabolic proteins were induced under acidic conditions than at
high pH. Several proteins induced by acid under anaerobiosis are known to be induced by acetate (GalT, Lpd, HdeA, and Ppa), whereas proteins which we found to be induced at high pH anaerobically are repressed by acetate (MgLb and TnaA) (30). These results are consistent with the prediction that low pH amplifies the response to reuptake of membrane-permeant fermentation acids.

The increase in the level of the nickel transporter NiaK in acid conditions may be related to the requirement for nickel for hydrogenase activity during anaerobic fermentation (13, 67). Another metal that may influence acid-dependent protein expression is zinc. Several of the acid-induced proteins (AckA, LpD, Ppa, and TsF) are known to have higher affinity for zinc(II) (27). The structural and functional roles of zinc in these zinc binding proteins are poorly understood.

The flavohemoglobin Hmp is also known to be induced by various oxidative signals, including oxygen, NO and nitrate, and iron depletion (41, 45). Hmp may provide protection against NO and other reactive nitrogen species. Previously, the hmp gene was reported to be negative for pH-dependent expression (45), but only aerobic growth was tested. Other anti-oxidant species induced in acid include AhpC and SodB (7, 59).

Overall, we observed several new effects of pH on catabolic pathways and other proteins in E. coli under anaerobiosis. These effects are largely consistent with our model that E. coli regulates catabolism so as to counteract environmental acidity or alkalinity and that anaerobiosis increases the need for pH regulation of catabolism.

ACKNOWLEDGMENTS

This work was supported by grant MCB-0234732 from the National Science Foundation. We thank J. W. Foster and R. Simons for the generous gift of strains and R. Dawson for valuable discussions.

REFERENCES


