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Detergent (Sodium Dodecyl Sulfate) Shock Proteins in *Escherichia coli*

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The protein composition of *Escherichia coli* W3110 grown in the presence and absence of 5% sodium dodecyl sulfate (SDS) was examined by two-dimensional gel electrophoresis. In SDS-grown cells, at least 4 proteins were turned on, 13 were turned off, 15 were elevated, and 15 were depressed. The 19 unique and elevated SDS-induced spots constituted 7.91% of the total ³⁵S-labeled protein. There was no apparent overlap between these 19 detergent (SDS) stress proteins and those of other known bacterial stress responses. The detergent stress stimulon is a distinct and independent stimulon. Its physiological relevance probably derives from the presence of bile salts in animal gastrointestinal tracts.

Bacteria have the ability to grow in many harsh environments. Among these are extremes of temperature, pH, pressure, and salt concentration. Bacteria also have the ability to adapt to transient stress conditions by the production of an appropriate battery of shock proteins. So far shock proteins have been identified for stress due to high (9) and low (5) temperatures, anaerobiosis (11), SOS and oxidation (12), low pH (4), and ingestion by macrophages (1). For the most part, these proteins have been identified because they constitute novel or enhanced spots on autoradiograms of two-dimensional gels. Rarely have discrete functions been assigned to individual proteins. Consequently, it is difficult to develop an understanding of why a particular shock protein is induced by a particular stress condition.

Our laboratory has been studying detergent (sodium dodecyl sulfate [SDS]) resistance in enteric bacteria (6, 7). Because mammalian gut systems contain high concentrations of the bile salt detergents, we view SDS resistance as a physiologically relevant shock system. For *Enterobacter cloacae* (6, 7), we found that the bacterium tolerated the detergent (SDS) and did not metabolize or modify it. The growth rate was the same in the presence and absence of SDS. However, growth in the presence of SDS was energy dependent. In the presence of SDS, *Enterobacter cloacae* cells lysed on reaching stationary phase or when sodium azide or 2,4-dinitrophenol was added to exponential-phase cultures (6). In addition, the energy burden imposed by SDS was evident from three other characteristics of growth in SDS: cell yield was decreased by 20%, glucose utilization was increased by 30%, and oxygen consumption was increased by 50 to 70% (7).

It seems likely that the response to SDS stress is similar in other enteric bacteria. We showed (8) that 200 of 208 independent isolates of members of the family *Enterobacteriaceae*, including all strains of *Escherichia coli* K-12 tested, were able to grow in $\geq 5\%$ SDS. Consequently, we have now switched from *Enterobacter cloacae* to *E. coli* W3110 in order to utilize the extensive gene-protein index developed (10) for this strain of *E. coli*. This data base is

particularly useful for comparison of stress and shock proteins in *E. coli*. This report presents autoradiograms of two-dimensional gels depicting the proteins synthesized by *E. coli* W3110 when grown under steady-state conditions in the presence and absence of SDS (5%). In the presence of SDS, 13 proteins are turned off and 15 are significantly decreased; 4 unique proteins are turned on and 15 are significantly elevated.

MATERIALS AND METHODS

Organism and cultural conditions. *E. coli* W3110 was obtained from F. C. Neidhardt. It was grown on a defined glucose-salts medium composed of 10 g of glucose, 2 g of $(\text{NH}_4)_2\text{SO}_4$, 0.5 g of KH_2PO_4 , 4.5 g of Na_2HPO_4 , 0.28 g of MgSO_4 , and 0.2 g of thiamine per liter, adjusted to pH 6.8. This medium was used with and without 5% (wt/vol) SDS. Cells were grown at 37°C with rotary agitation at 250 rpm.

Two-dimensional gel electrophoresis. All procedures followed those recommended by Protein Databases, Inc., Huntington Station, N.Y., as developed by Garrels (2, 3). When the *E. coli* cells had grown to an optical density at 600 nm of 0.8 to 0.9, 2.2-ml aliquots were removed and incubated with 8 μl of fresh [³⁵S]methionine-cysteine (1 mCi/0.93 nmol/ml; ICN) in a 25-ml Erlenmeyer flask (37°C, 250 rpm). Radiolabeling continued for 37 min, whereupon 20 μl of nonradioactive methionine (0.6 mM) was added for 3 min. Samples (1.0 ml) were transferred to 1.5-ml microcentrifuge tubes and washed four times in a salts solution identical in composition to the salts in the growth medium. Pellets were lysed by addition of 100 μl of prewarmed (95°C) SDS- β -mercaptoethanol lysing buffer, vortexing, and incubation for 4.5 min at 95°C. The tubes were cooled on ice and then incubated on ice with 10 μl of DNase-RNase for 2 min. The lysates were frozen at -80°C and shipped in dry ice to Protein Databases for analysis.

For cells grown in the presence and absence of SDS, duplicate samples were run on separate gels (total of four gels). Approximately 395,000 dpm was loaded on each gel. The blended gels were composed of 0.2% acrylamide (pH 5 to 7) plus 1.8% acrylamide (pH 3 to 10) in the first dimension

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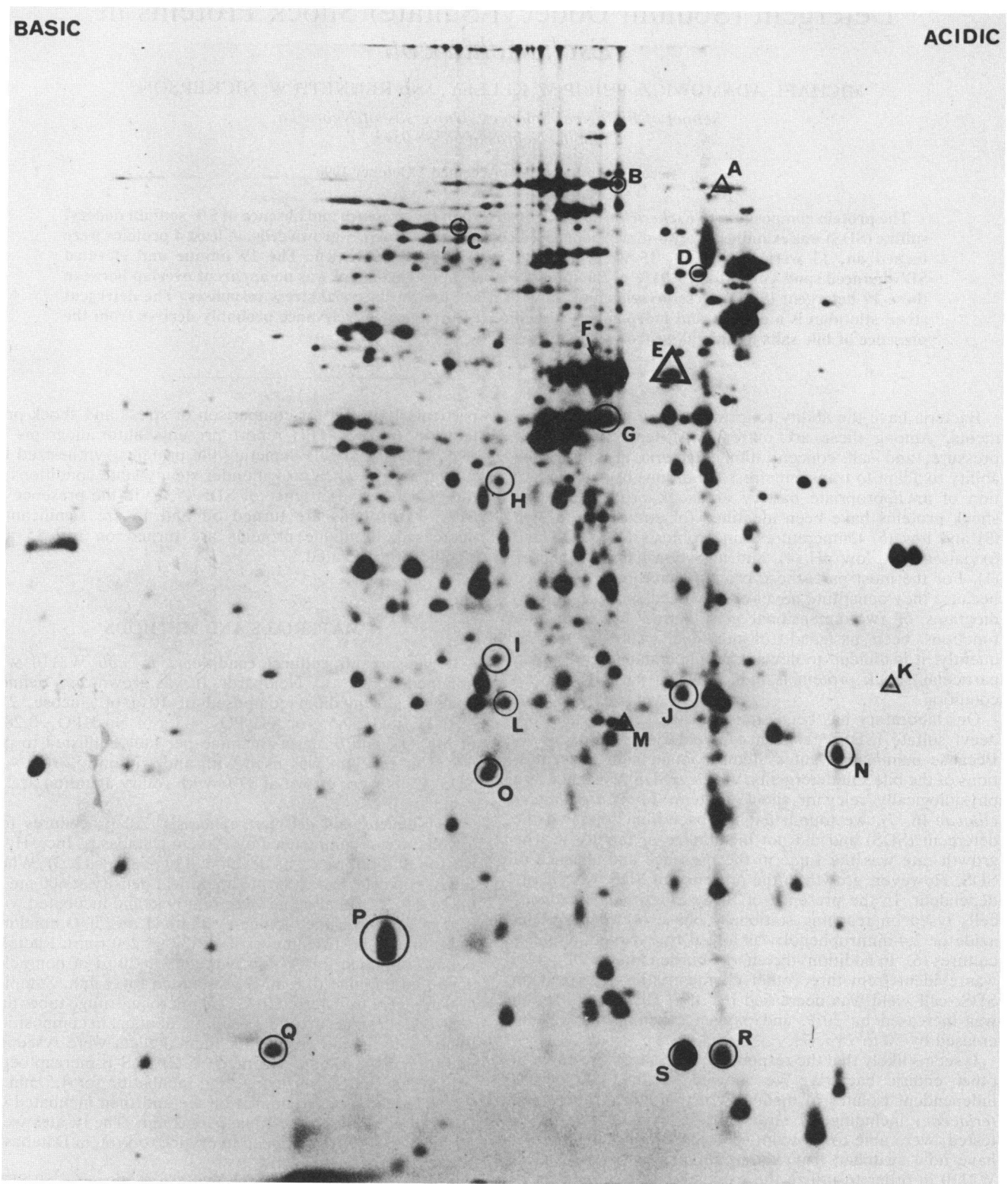


FIG. 1. Two-dimensional gel analysis of the proteins present in *E. coli* W3110 grown in the presence of 5% SDS. Conditions: 387,681 dpm loaded on gel; 5-day exposure time. Symbols: \triangle , uniquely present in plus-SDS cells; \circ , elevated ≥ 3 -fold in plus-SDS cells.

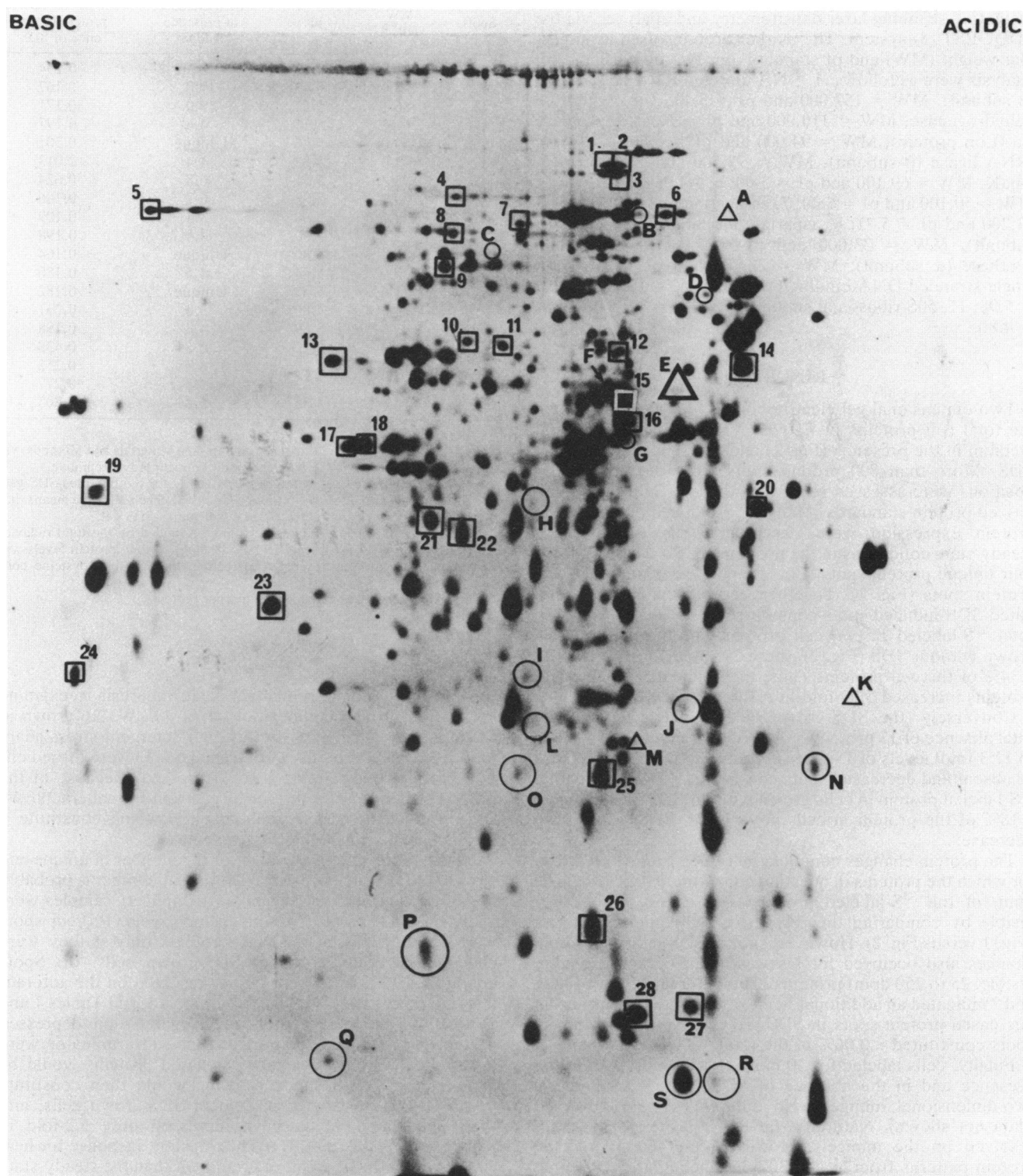


FIG. 2. Two-dimensional gel analysis of the proteins present in *E. coli* W3110 grown in the absence of SDS. Conditions: 405,060 dpm loaded on gel; 5-day exposure time. Symbols: □, proteins reduced ≥ 3 -fold by growth in SDS; ○ and △, position of corresponding protein from Fig. 1.

and 11.5% acrylamide in the second dimension. Autoradiograms were made of each gel, using three exposure times: 2, 5, and 10 days. The protein spots were quantified by high-resolution scanning laser densitometry and analyzed via the PDQUEST (3) system. The marker proteins used as molecular weight (MW) and pI standards for two-dimensional gel analysis were as follows: 1, DNA-directed RNA polymerase (β subunit), MW = 157,000 and pI = 5.43; 2, oxoglutarate dehydrogenase, MW = 110,000 and pI = 6.36; 3, protease La (Lon protein), MW = 94,000 and pI = 6.60; 4, glycine tRNA ligase (β subunit), MW = 77,500 and pI = 5.60; 5, DnaK, MW = 69,100 and pI = 5.00; 6, ATPase (α subunit), MW = 50,100 and pI = 6.20; 7, elongation factor Tu, MW = 43,200 and pI = 5.71; 8, aspartate carbamoyltransferase (β subunit), MW = 33,000 and pI = 7.00; 9, tryptophan synthase (α subunit), MW = 28,700 and pI = 5.64; 10, single-stranded DNA-binding protein, MW = 18,900 and pI = 5.98; 11, 50S ribosomal subunit L7, MW = 12,200 and pI = 4.80.

RESULTS

Two-dimensional gel electrophoresis was used to resolve the total cell proteins of *E. coli* W3110 grown in minimal medium in the presence (Fig. 1) and absence (Fig. 2) of 5% SDS. More than 800 protein spots were resolved. Their positions were assessed relative to those of 11 well-characterized protein standards. Many SDS-induced differences in protein expression were observed. Cells grown under steady-state conditions in the presence of 5% SDS exhibited four unique protein spots (Fig. 1) and 15 elevated (≥ 3 -fold) protein spots (Fig. 1). Together, these 19 unique and elevated SDS-induced spots constituted 7.91% (Table 1) of the total ^{35}S -labeled *E. coli* cell protein. For the control cells grown without SDS (Fig. 2), these 19 spots constituted only 1.34% of the cell protein. Thus, the abundance of these 19 proteins increased 5.90-fold in response to SDS stress.

Conversely, the SDS-grown *E. coli* cells exhibited the total absence of 13 protein spots (Fig. 2) and decreased (3.1- to 12.3-fold) levels of 15 other protein spots. Together, these 28 absent and decreased spots constituted 7.88% of the total ^{35}S -labeled protein in cells grown without SDS (Table 2) and 1.18% of the protein in cells grown with SDS, a 6.68-fold decrease.

The protein changes compiled in Tables 1 and 2 are those for which the proteins in question constitute $\geq 0.06\%$ (ca. 250 dpm) of the ^{35}S -labeled protein. The changes are easily visible by comparing the respective two-dimensional gels (Fig. 1 versus Fig. 2). However, similar SDS-induced protein changes also occurred for less prevalent (i.e., not readily visible, 25 to 250 dpm) proteins. Computer analysis of Fig. 1 and 2 indicated an additional 12 elevated protein spots and 32 decreased protein spots in SDS-grown cells. Each of these spots constituted $\leq 0.06\%$ of the total ^{35}S -labeled protein.

Finally, cells labeled for 30 min versus 3 min, both in the presence and in the absence of SDS, were compared by two-dimensional minigels. No differences were observed (data not shown). Naturally, far fewer protein spots were resolved on the minigels. However, the identity of the protein patterns from 3- and 30-min labeling argues against the possibility that the smaller SDS shock proteins (Fig. 1) were proteolysis products. Because we labeled the cells under steady-state growth conditions, any shock proteins that arose via proteolysis had to be either stable themselves or the result of an equilibrium by which they were produced as rapidly as they were further degraded.

TABLE 1. Proteins induced or uniquely expressed in *E. coli* W3110 grown in the presence of 5% SDS

Spot ^a	MW (10 ³) ^b	pI ^b	Fold increase in presence of SDS ^c	% of ^{35}S -labeled protein in presence of SDS ^d
A	101.4	5.03	Unique	0.294
B	98.0	5.43	11.8	0.167
C	86.1	5.91	9.0	0.171
D	67.2	5.16	4.6	0.197
E	49.0	5.26	Unique	0.301
F	48.3	5.63	4.4	2.013
G	45.3	5.51	6.8	0.624
H	40.1	5.83	6.7	0.106
I	29.7	5.86	3.4	0.109
J	27.9	5.30	4.4	0.198
K	27.4	<4.80 ^e	Unique	0.164
L	27.0	5.84	4.5	0.180
M	25.6	5.51	Unique	0.182
N	23.3	<4.80 ^e	3.0	0.231
O	23.0	5.89	4.8	0.198
P	16.0	6.22	6.6	0.438
Q	13.4	6.68	6.3	0.252
R	12.9	5.22	4.7	0.277
S	12.8	5.35	5.2	1.805

^a From Fig. 1.

^b Extrapolated from the 11 marker proteins (see Materials and Methods) via PDQUEST data analysis (3). Values should be regarded as tentative.

^c Difference between the average spot intensity for the two plus-SDS gels and the average spot intensity for the two minus-SDS gels. Unique means that the indicated spot was not detected on either minus-SDS gel.

^d Spot intensity (disintegrations per minute) divided by the total radioactivity in all protein spots in Fig. 1 (383,853 dpm). Actual protein levels will vary depending on their respective percent methionine-plus-cysteine contents.

^e Outside the range covered by the marker proteins.

DISCUSSION

We used two-dimensional gel electrophoresis to examine the altered protein composition of *E. coli* W3110 grown in the presence of 5% SDS. In this high-detergent-stress adaptation, at least 4 proteins were turned on, 13 were turned off, 15 were elevated, and 15 were depressed. Because of the presence of bile salts in animal gastrointestinal tracts, we suggest that these detergent shock proteins constitute a physiologically relevant stress response.

Nineteen is a minimum value for the number of unique and elevated SDS shock proteins. The actual number is probably higher for the following reasons. (i) Duplicate samples were analyzed on separate two-dimensional gels. Protein spots were identified as SDS shock proteins only if they were elevated on both gels from SDS-grown cells. (ii) Spots containing ≤ 250 dpm were poorly resolved on the autoradiograms (Fig. 1 and 2) and were not included in Tables 1 and 2. (iii) The threefold cutoff for elevated and depressed proteins was chosen arbitrarily. If the discriminator were lowered to 2.5-fold, five more elevated proteins would be included. The SDS shock proteins would then constitute 9.70% of the ^{35}S -labeled protein in SDS-grown cells, and their abundance would have increased only 5.2-fold in response to SDS stress. (iv) The transient response for heat shock proteins is often more prominent than the steady-state response (9). However, the steady-state response seems the appropriate model for bacterial growth in detergent-containing gastrointestinal tracts, wherein the detergent stress would presumably be continuous.

The detergent stress stimulon appears to be an independent entity rather than a subset of the heat shock stimulon

TABLE 2. Proteins repressed or absent in *E. coli* W3110 grown in the presence of 5% SDS

Spot ^a	MW (10 ³) ^b	pI ^b	Fold decrease in presence of SDS ^c	% of ³⁵ S-labeled protein in absence of SDS ^d
1	138.7	5.54	Absent	0.295
2	138.5	5.56	4.4	0.273
3	131.7	5.59	Absent	0.127
4	108.7	6.04	Absent	0.054
5	102.3	>7.00 ^e	Absent	0.112
6	97.6	5.32	Absent	0.108
7	94.4	5.85	3.5	0.083
8	91.7	6.05	3.1	0.158
9	82.1	6.00	Absent	0.158
10	57.1	6.00	4.4	0.076
11	56.0	5.90	4.0	0.102
12	53.9	5.55	Absent	0.120
13	53.5	6.56	6.9	0.302
14	50.8	5.02	5.8	0.761
15	46.8	5.51	Absent	0.330
16	46.4	5.50	12.3	0.177
17	44.8	6.47	5.9	0.361
18	44.8	6.41	4.9	0.309
19	41.8	>7.00 ^e	Absent	0.313
20	39.3	4.97	6.5	0.475
21	38.8	6.16	3.7	0.252
22	37.4	6.04	Absent	0.215
23	33.0	7.00	Absent	0.653
24	30.2	>7.00 ^e	Absent	0.130
25	22.6	5.64	4.4	0.493
26	16.5	5.68	4.5	0.466
27	14.5	5.30	Absent	0.141
28	14.4	5.54	10.1	0.833

^a From Fig. 2.^b Extrapolated from the 11 marker proteins (see Materials and Methods) via PDQUEST data analysis (3). Values should be regarded as tentative.^c Difference between the spot intensity for the plus-SDS gel (Fig. 1) and minus-SDS gel (Fig. 2). Absent means that the indicated spot was not detected on the plus-SDS gel.^d Spot intensity (disintegrations per minute) divided by the total radioactivity in all protein spots in Fig. 2 (485,622 dpm). Actual protein levels will vary depending on their respective percent methionine-plus-cysteine contents.^e Outside the range covered by the marker proteins.

(9), the oxidation stress stimulon (12), or the anaerobic stimulon (11). That is, the four unique SDS-induced proteins (Table 1) are truly unique. Stress-induced proteins are often cross-related (12). However, the known gene-protein index for *E. coli* (10) is heavily weighted toward stress proteins, and so far we have been unable to detect any overlap between the unique and threefold-elevated detergent shock proteins and other known stress responses. The existence of a discrete detergent stress stimulon fits the pattern established by the separately inducible heat shock, SOS, and oxidation stress stimulons (12).

Our future work on detergent stress in enteric bacteria will focus on (i) where the detergent shock proteins are located in the cell, (ii) whether SDS stress is indeed identical to bile salt stress, (iii) a comparison of the steady-state response to

detergent stress (this report) versus the transient response, (iv) whether the protein changes observed represent differential gene expression or posttranslational modification, and (v) the identities and functions of the 4 unique and 15 elevated SDS shock proteins. In this regard, we note that the proteins turned off by the growth in SDS are generally of higher MW and neutral to basic pI, whereas the proteins turned on are generally of lower MW and acidic pI. This shift is easily seen by contrasting the positions of the circles and triangles in Fig. 2 with those of the squares.

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