Spermidine Acetylation in Response to a Variety of Stresses in *Escherichia coli*  

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Heat shock, cold shock, ethanol, and alkaline shift, but not hydrogen peroxide, stimulate the accumulation of monoacetyl spermidine in *Escherichia coli*. Acetylation occurs with nearly equal frequencies at both the N² and N⁶ positions of this ubiquitous polycation. Spermidine acetylation does not appear to be associated with known stress regulons, such as htpR, oxyR, and SOS. *E. coli*, capable of acetylating spermidine, constitutively express a spermidine acetyltransferase activity during all phases of growth, and this activity is unaffected by cold shock. A mutant strain, incapable of acetylating spermidine, does not express this enzyme activity but grows at an identical rate as the parent strain at 37 °C. These results demonstrate that the monoacetylation of spermidine in *E. coli* is regulated by some mechanism other than a stress-inducible acetyltransferase and is not essential for growth of these cells. They suggest that polyamine acetylation is involved in the responses of these organisms to a variety of chemical and physical stresses.

Both pro- and eukaryotes respond to physical and chemical stresses by synthesizing unique sets of proteins whose functions appear to be related to the cellular damage produced by the specific stress (1-3). The generality of these stress responses among various species and the surprising conservation of the genes encoding certain of the stress proteins have been interpreted as evidence of the importance of these proteins to biological systems (3).

Heat shock and certain chemical stresses stimulate polyamine catabolism in mammalian cells by inducing the enzyme spermidine/spermine N⁴-acetyltransferase (4, 5). The polyamines are naturally occurring polycations and are required for optimal growth and differentiation in most cell types (6, 7). In eukaryotes, spermidine can be acetylated, thus changing its net charge at the terminal amine groups by either of two distinct acetyltransferases. The spermidine/spermine N⁴-acetyltransferase is a cytosolic enzyme, while the spermidine N⁶-acetyltransferase is found in the nucleus (see Ref. 7 for review). Given the conservation of other stress responses in various species, we wanted first, to determine if spermidine acetylation was a response of prokaryotes to heat shock and other stresses as it is in eukaryotes and second, to determine if this response belonged to one of the already described stress regulons. It had previously been reported that cold shock stimulated the accumulation of acetylated spermidine in *Escherichia coli* (8).

**Materials and Methods**  

Chemicals—[1,8-³H]spermidine (29.7 Ci/mmol), purchased as [terminal methylene-³H]spermidine, and [acetate-¹⁴C]acetate coenzyme A (48.5 mCi/mmol) were obtained from Du Pont-New England Nuclear. Putrescine, spermidine, N⁴-acetyl spermidine, and N⁶-acetyl spermidine were purchased from Sigma. All other chemicals were reagent grade.

**Bacterial Strains, Media, and Culture Conditions**—Strains used in these studies were *E. coli* K12 or its derivatives (see Table I). Cultures were grown at the indicated temperatures under aerobic conditions in either Luria-Bertani broth or MOPS medium (14) supplemented with potassium phosphate (1.32 mM), glucose (4%), thiamine, and riboflavin (each 1 mg/liter), and all 20 α-amino acids (4 mg/liter). Bacteria were subjected to a variety of stresses under the following conditions. Cells growing at 37 °C were heat or cold shocked when cultures reached mid- to late log phase of growth at an *A*_∞ of 0.5-0.8. Flasks were placed in a 42 ± 0.1 °C water bath or into an ice-water bath slurry with constant agitation for varying times as indicated. Ethanol shock was administered by adjusting the medium to a final concentration of 4% ethanol. Alkaline shock was accomplished by changing the normal medium pH of 6.5 to 8.5 by titration with 1 M NaOH buffer. Hydrogen peroxide was added to cultures at 37 °C to final concentrations ranging from 0.01 to 1 mM.

**Biochemical Techniques**—Polyamine contents were determined from acid extracts (0.2 N HClO₄) of cell lysates, using reverse phase ion pair high performance liquid chromatography (HPLC) as described elsewhere (15). Briefly, aliquots of cells were rapidly collected using suction filtration onto Millipore filter discs (HAWP 025 00, 0.45 μm). Cells were washed once with phosphate-buffered saline (137 mM NaCl, 2.7 mM KCl, 8.1 mM Na₂HPO₄, 1.5 mM KH₂PO₄) at room temperature, collected in 0.1 N HCl, sonicated to disrupt, and extracted with 0.2 N HClO₄. Acid-soluble polyamines were analyzed directly by HPLC and expressed per unit of cell protein. Protein contents were determined by the method of Bradford (16) after dissolving the acid-insoluble pellet in 0.5 N NaOH. Spermidine N-acetyltransferase activity was assayed by measuring the incorporation of [¹⁴C]acetate from radiolabeled acetyl coenzyme A into monoacetyl spermidine (4, 5).

**Results**

The predominant polyamines found in log phase *E. coli* are the diamine putrescine and the longer chain amine spermidine (Fig. 1). Heat shock (42 °C for 60 min) stimulated the accumulation of monoacetyl spermidine in these cells. Both N¹ and N⁶-acetyl spermidine accumulated with nearly equal frequencies in response to this stress. The unlabeled material on the left of each elution profile represents 16 a-amino acids and other unknown amines. Standard preparations of N-acetylputrescine elute with a retention time midway between...
Salmonella typhimurium (1). In order to determine if the oxidative stress can induce certain heat shock proteins, we measured polyamine contents in K12-derived mutant strains, which were either competent or deficient in specific stress regulon expression. As indicated in Table I, both TA4110, which efficiently expresses all of the oxyR regulon proteins, and TA4112, which is deficient in expressing these proteins in response to oxidative stress, were able to acetylate spermidine in response to heat, cold, or ethanol shock. SOS-competent, including cells with inducible (strain JL468) and constitutively (strain DM1187) expressed regulons, and deficient (DM49) strains were able to acetylate spermidine in response to cold shock. CAG2242, a strain capable of expressing heat shock genes in the htpR regulon, and strain CAG2243, which displays a reduced heat shock response, were unable to acetylate spermidine in response to any stress studied. Since CAG2242 is rpoH+ and CAG2243 is rpoH−, this locus is not involved in spermidine acetylation.

One of the possible mechanisms underlying the stimulation of monoacetylspermidine pools in E. coli is the induction of the acetylating enzyme (8). We measured spermidine N-acetyltransferase activity in both C600 cells and its derivative CAG2242 as a function of growth (Fig. 3). The C600 cells contained an active spermidine N-acetyltransferase activity that was constitutively expressed throughout log and plateau phases of growth. The CAG2242 strain did not express a detectable spermidine acetyltransferase activity. When either C600 or CAG2242 cells were cold shocked, no change in spermidine N-acetyltransferase activity was observed (Table II).

**DISCUSSION**

Tabor (8) initially reported that cold shock stimulated spermidine acetylation in E. coli B, but she did not determine the structure of the monoacetylspermidine. Matsui and co-workers (21) reported the existence of a spermidine N′-acetyltransferase activity in E. coli and found that this activity increased early in growth and then decreased. We now show that E. coli acetylate spermidine at both the N′ and N⁸ positions in response to a variety of stresses including cold and heat shock, alkaline shift, and ethanol treatments. Oxidative stress does not stimulate spermidine acetylation, and the enzymes/proteins regulating stress-induced spermidine acetylation do not appear to belong to either oxyR, SOS, or htpR, the major stress regulons in bacteria. Rather than displaying an inducible spermidine acetyltransferase activity, E. coli competent to form monoacetylspermidine constitutively express this enzyme activity.

Tabor (8) reported that E. coli B converted nearly 60% of radiolabeled spermidine to monoacetylspermidine during a 2-h interval at 0.4 °C. We found that E. coli K12 also converted radiolabeled spermidine to N′- and N⁸-acetylspermidine during periods of stress (data not shown). These results demonstrate that the monoacetylspermidine that accumulates following stresses is derived from spermidine.

We have shown that a wide variety of stresses stimulates the accumulation of monoacetylspermidine in E. coli. N′-Acetylspermidine formation is also stimulated by certain chemical and physical stresses in rodent and human cells (4, 5). The regulation of this stress response, however, is quite different in bacteria and animal cells. In animal cells, stimulation of monoacetylation of spermidine is regulated by the actinomycin D-sensitive induction of spermidine/spermine N′-acetyltransferase (5), while this type of enzyme activity is constitutively expressed and not stress inducible in E. coli, as shown here.

![Fig. 1. Heat shock stimulates the accumulation of both N′- and N⁸-acetylspermidine in E. coli C600 cells.](image1)

![Fig. 2. Effects of various stresses on polyamine acetylation in E. coli K12 cells.](image2)
Spermidine Acetylation in E. coli

TABLE I
Accumulation of N\(^1\)- and N\(^4\)-acetyl spermidine in E. coli stress regulon mutants during exposure to various stresses

Incubation times with all stresses were 90 min. Heat shock was at 42 °C. Cold shock was ice bath temperature (−0.4 °C). Ethanol treatments were 4% in the medium concentration. Alkaline shock was accomplished by changing the pH from 6.5 to 8.5 by titration with NaOH, and \( \text{H}_2\text{O}_2 \) was up to 1 mM.

| Strain          | Ref. | Stress regulon | Accumulation of monoacetyl spermidine | No accumulation of monoacetyl spermidine |
|-----------------|------|----------------|---------------------------------------|------------------------------------------|
| K12             | 9    | Wild type      | Heat shock                            | \( \text{H}_2\text{O}_2 \)               |
| TA4110 (oxyR2)  | 1    | oxyR\(^+\)     | Cold shock                            | Ethanol                                  |
| TA4112 (oxyR3)  | 1    | oxyR\(^-\)     | Heat shock                            | Ethanol                                  |
| JL468           | 11   | SOS\(^+\)      | Cold shock                            | Ethanol                                  |
| DM49            | 12   | SOS\(^-\)      | Cold shock                            | Ethanol                                  |
| DM1187          | 13   | SOS\(^-\)      | Cold shock                            | Ethanol                                  |
| C600            | 9    | Wild type      | Heat shock                            | Ethanol                                  |
| CAG2242 (C600 rpoH\(^+\)-Tn10) | 10   | htpR\(^+\)     | Cold shock                            | Ethanol                                  |
| CAG2243 (C600 rpoH112-Tn10) | 10   | htpR\(^-\)     | Heat shock                            | Ethanol                                  |

Derivative E. coli strains CAG2242 and CAG2243, which are incapable of acetylating spermidine under any conditions reported here, do not express this acetyltransferase activity. Since these latter strains proliferate at 37 °C with nearly identical kinetics as do strains that express the acetyltransferase activity (Fig. 3), we conclude that spermidine acetylation is not essential for E. coli growth at normal temperatures.

Rather, we suggest that this aspect of polyamine metabolism may be an important facet of both bacterial and mammalian responses to certain physical and chemical stresses.

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