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# Nutrient-Dependent Methylation of a Membrane-Associated Protein of *Escherichia coli*

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**Starvation of a mid-log-phase culture of *Escherichia coli* B/r for nitrogen, phosphate, or carbon resulted in methylation of a membrane-associated protein of about 43,000 daltons (P-43) in the presence of chloramphenicol and [*methyl*-$^3$H]methionine. The in vivo methylation reaction occurred with a doubling time of 2 to 5 min and was followed by a slower demethylation process. Addition of the missing nutrient to a starving culture immediately prevented further methylation of P-43. P-43 methylation is not related to the methylated chemotaxis proteins because P-43 is methylated in response to a different spectrum of nutrients and because P-43 is methylated on lysine residues. The characteristics of P-43 are similar to those of a methylated protein previously described in *Bacillus subtilis* and *B. licheniformis* (R. W. Bernlohr, A. L. Saha, C. C. Young, B. R. Toth, and K. J. Golden, J. Bacteriol. 170:4113–4118, 1988; K. J. Golden and R. W. Bernlohr, Mol. Gen. Genet. 220:1–7, 1989) and are consistent with the proposal that methylation of this protein functions in nutrient sensing.**

Bacterial cells coordinately regulate growth and cell division in response to a variety of environmental stimuli. In general, the signals generated in response to these stimuli are still poorly understood; however, it has become apparent that bacterial cells have the ability to sense their environment directly through a series of membrane-bound receptor proteins (for a review, see reference 36). Conformational changes in the receptor upon ligand binding activate the autophosphorylation of a histidine protein kinase which can reside in the receptor itself or upon a second, more centralized protein with which the receptor interacts (14, 17, 27). The signal is then relayed to the interior of the cell by transfer of the phosphate to a second set of proteins known as signal transducers. Kinase-transducer protein pairs have been identified in a variety of environmental sensing systems in a number of different organisms (36). Sequence analysis has shown that all of the histidine kinases contain homologous cytoplasmic C-terminal domains containing kinase activity, while transducer proteins contain conserved N-terminal domains, allowing for interaction of the transducer protein with the conserved domains of the histidine kinases (28, 31).

Chemotaxis is an example of a system which uses multiple receptor proteins which, when bound by a ligand, interact with a single histidine kinase (for a review, see reference 24). The use of multiple receptors allows this system to react in response to a wide variety of environmental stimuli. In *Escherichia coli*, the receptor proteins, termed methylated chemotaxis proteins (MCPs) because of their ability to become methylated in the presence of chemoattractants (11, 20) and demethylated in response to chemorepellents (19), interact with the kinase CheA resulting in autophosphorylation of CheA (5, 29). The phosphate is then transferred to CheY, which carries the signal to the flagellar motor, or to CheB, a methylesterase responsible for removing the methyl groups from the MCPs (14). Methylation of the MCPs has been implicated in both signal transduction (13) and the ability of the system to adapt to new chemoeffector concentrations (12, 36).

We have previously demonstrated that *Bacillus subtilis* and *B. licheniformis* methylate a 40,000-dalton membrane-associated protein (P-40) in response to nitrogen and phosphate levels in the medium (3, 10). Cells which are growing on good nitrogen sources elicit a strong methylation response, while cells growing on poor nitrogen sources exhibit only a weak response. In addition, *B. subtilis spo0* mutants, which grow faster than the wild type on minimal medium, presumably because of their inability to initiate the earliest steps of sporulation, were shown to be defective in demethylating the methylated P-40 protein (10). *spo0B*, *spo0E*, and *spo0F* mutants were all defective in P-40 turnover in the presence of phosphate and/or ammonia, while *spoIIJ* mutants failed to methylate P-40 at all. These observations are consistent with our hypothesis that P-40 is an integral part of a signal transduction pathway responsible for notifying cells of impending starvation and, as such, represents a second system in which methylation of a membrane-bound protein plays a role in environmental sensing.

A role for methylation has also been implicated in two other bacterial sensing systems. Inouye et al. have shown that a Tar-EnvZ chimeric protein can drive transcription of *ompF* and *ompC* in response to addition of aspartate to the medium, suggesting a role for an MCP-like protein in the EnvZ-OmpR signal transduction pathway (39). Also, mutants defective in *S*-adenosylmethionine biosynthesis demonstrate an altered heat shock response, implying a role for methylation in the heat shock response as well (26).

In this communication, we present evidence that a membrane-associated protein in *E. coli* of about 43,000 daltons (P-43) is methylated in response to carbon, nitrogen, and phosphate deprivation and that this reversible methylation has many similarities to the system reported for *B. subtilis* (10) and *B. licheniformis* (3).

## MATERIALS AND METHODS

**Bacterial strains and growth conditions.** Cultures of *E. coli* B/r and K-12 were grown at 37°C on minimal salts A medium

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(33), as modified by Donohue and Bernlohr (9), containing glucose (15 mM) and ammonium sulfate (10 mM) for nitrogen and carbon starvation experiments. Phosphate starvation experiments were performed by using M9 minimal medium (32) in which phosphate was added separately. *E. coli* PT17 was obtained from J. Cronan (38) and grown in nutrient broth supplemented with 10 mM ammonium sulfate. All strains were stored at −20°C in 10% glycerol, and an overnight culture was subcultured on the following morning. Growth was monitored turbidimetrically by using a Klett-Summerson colorimeter containing a green 54 filter.

**Standard methylation experiment.** For methylation experiments, a 50-ml volume of mid-logarithmic-phase cells was harvested by centrifugation at 10,000 × *g* for 5 min at 4°C in an RC-5B centrifuge (Ivan Sorvall, Inc., Norwalk, Conn.). The cells were washed in 25 mM phosphate buffer (pH 7.2) and suspended at $5 \times 10^8$/ml in two flasks containing 25 ml of minimal A salts medium lacking a nitrogen (ammonium sulfate, ammonium chloride, or glutamate) or carbon (glucose or galactose) source. For phosphate starvation, cells were washed in 25 mM MOPS (morpholinepropanesulfonic acid) and suspended in two flasks of M9 minimal medium lacking phosphate (potassium phosphate or sodium phosphate). The other two essential nutrients were always present. Unless otherwise noted, glucose, ammonium sulfate, and potassium phosphate were used as nutrients in these experiments. Cells were incubated with shaking at 37°C for 10 min to allow the cells to adapt to the medium and use any remaining nutrient not removed by the wash. Chloramphenicol was added to a final concentration of 0.3 mg/ml, and the cells were incubated for an additional 10 min. L-[*methyl*-$^3$H]methionine (80 Ci/mmol) was added to both cultures to a final concentration of 12.5 μCi/25 ml (6 nM), while the missing nutrient was added to only one. In experiments requiring [$^{35}$S]methionine incorporation, labeled methionine was added to a final concentration of 6 nM.

The cells continued to be incubated at 37°C with shaking for 30 min, after which they were placed on ice and 1.25 ml of 100% (wt/vol) trichloroacetic acid was added to stop the reaction. After incubation at 0°C for at least 15 min, the cells were sedimented at 10,000 × *g* for 5 min, washed in 100% acetone, and suspended in 1.0 ml of break buffer (25 mM MOPS, 10 mM EDTA, 0.2 mg of RNase A [protease free; R-5250; Sigma Chemical Co., St. Louis, Mo.] per ml [pH 7.2]). The cells were broken by sonic oscillation for 1 min at 0°C in a 300 sonic dismembrator (Fisher Scientific Co., Pittsburgh, Pa.) or a 200 sonifier (Branson Sonic Power Co., Danbury, Conn.). Broken cells were incubated at 37°C for 20 min and sedimented at 48,000 × *g* for 15 min at 4°C. The sedimented membrane fragments were boiled in sample buffer containing 4.52 ml of water, 1.0 ml of 0.5 M Tris hydrochloride (pH 6.8), 0.8 ml of glycerol, 1.6 ml of 10% sodium dodecyl sulfate (SDS), and 0.08 ml of 2-mercaptoethanol (3) for 7 min and sedimented at high speed in a Micro Centaur tabletop centrifuge for 5 min. The soluble fraction was retained for analysis.

The protein concentration of each sample was determined by the method of Lowry et al. (23), and equal amounts of protein were separated on SDS–12% polyacrylamide gels by the method of Laemmli (21) with a Mighty Small II gel apparatus (Hoefer Scientific Instruments, San Francisco, Calif.). After being stained with Coomassie blue, the gels were soaked in Autofluor autoradiographic image enhancer (National Diagnostics, Somerville, N.J.) for 2 h, dried in a 224 slab gel dryer (Bio-Rad Laboratories, Richmond, Calif.), and sandwiched with X-Omat AR SAR-5 diagnostic film (Eastman Kodak Co., Rochester, N.Y.). After the film was developed, exposed areas were quantitated by using a Quick Scan Jr. densitometer (Helena Laboratories, Beaumont, Tex.).

Some experiments required that samples be taken periodically after addition of L-[*methyl*-$^3$H]methionine. In each of these experiments, a single 50-ml culture was used and 5-ml samples were removed and placed in 0.25 ml of 100% (wt/vol) trichloroacetic acid at the times indicated. The samples were then treated as described above to determine P-43 methylation.

**Descending paper chromatography.** Methylation experiments were performed as described previously, except that the whole 50-ml culture was starved for nitrogen, carbon, or phosphate and labeled for 30 min. Membrane extracts and protein standards were separated on SDS–12% polyacrylamide gels, and lanes containing molecular weight standards (Sigma) were removed and stained with Coomassie blue. The rest of the gel was placed in 25 mM Tris hydrochloride (pH 8.2) until staining of the standards was complete. Gels were sliced between the 30,000- and 65,000-dalton molecular size standards, and the protein in these slices was eluted by incubating the slices at 37°C for 48 h in elution buffer (50 mM Tris hydrochloride [pH 6.0], 2% SDS) with gentle shaking. The protein solutions were dialyzed against double-distilled water overnight and dried under a constant nitrogen stream at 70°C. The dried protein mixture was suspended in 6 M HCl, sealed in vacuo, and hydrolyzed for 15, 30, or 45 h at 110°C. After hydrolysis, the mixture was dried as before and suspended in 100 μl of double-distilled water.

The hydrolyzed protein was subjected to descending paper chromatography by the method of Chang and Chang (8) by using a solvent system of pyridine–acetone–3 M $NH_3OH$ (50:30:25) on Whatman no. 1 paper. As reference standards, 10-μg samples of the following amino acids were tested: *O*-methyl-L-tyrosine, *S*-methyl-L-cysteine, L-glutamic acid 5-methylester, 2-aminoisobutyric acid (Aldrich Chemical Co., Milwaukee, Wis.), *N*-ε-trimethyl-L-lysine, $N^G$,$N^G$-dimethyl-L-arginine, $N^G$-monomethyl-L-arginine (Calbiochem-Behring, La Jolla, Calif.), L-methionine, *O*-methyl-DL-serine, L-*O*-methylthreonine, L-methionine sulfoxide, L-aspartic acid β-methyl ester, *N*-ε-methyl-L-lysine, L-3-methylhistidine, L-1-methylhistidine, and $N^G$-$N^G$-dimethylarginine (Sigma).

The amino acid standards were visualized by being stained with ninhydrin, while the positions of radioactive residues in the hydrolysates were assayed by cutting the chromatograph into 1-cm strips and determining the radioactivity in each. P-43 was not pure in these experiments; however, it was the only highly labeled protein extracted from the gels.

**Phase separation of membrane proteins.** Membrane proteins were phase partitioned by the method of Bricker and Sherman (6) with modifications. Membrane extracts from a methylation experiment were passed through an Extracti-Gel D column (Pierce Chemical Co., Rockford Ill.) to remove excess SDS. The extracts were dried and suspended in 1% Triton X-114–150 mM NaCl–1 mM EDTA for 10 min, and the cloudy mixture was centrifuged for 5 min at 12, 000 × *g*. This yielded a detergent phase enriched with hydrophobic proteins and a detergent-depleted aqueous phase. The detergent phase was suspended in partitioning buffer minus Triton X-114, while 1% Triton X-114 was added to the aqueous phase. A second-phase separation was then performed as a washing step, and the detergent phase was suspended in sample buffer while the aqueous phase was passed through a Centricon 10 microconcentrator (Amicon Corp., Danvers, Mass.) and washed with 2 ml of 10 mM Tris

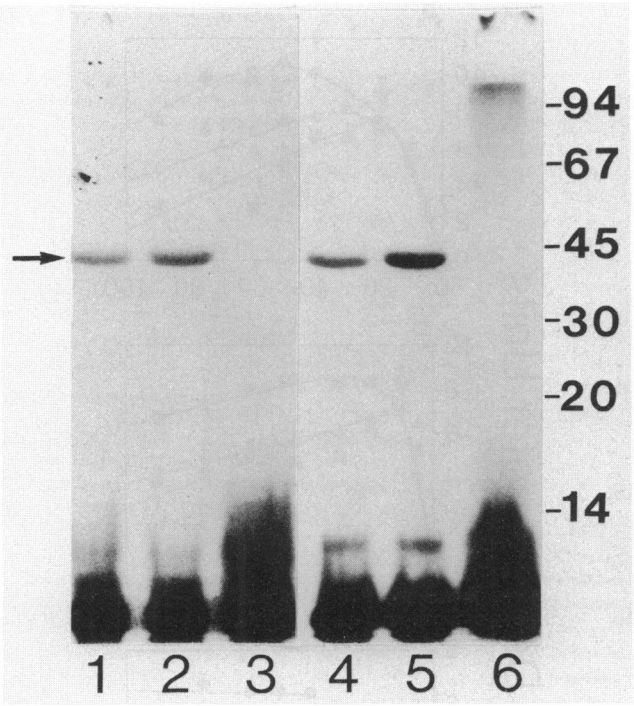


FIG. 1. Autoradiograph of a standard methylation experiment in which cells were starved for glucose (lanes 1 to 3) or ammonia (lanes 4 to 6) as described in Materials and Methods. Lanes: 1 and 4, membrane extracts from controls in which nutrient was introduced at the same time as tritiated methionine; 2 and 5, membrane extracts from cultures in which cells were allowed to starve for 30 min after introduction of tritiated methionine; 3 and 6, membrane extracts from cultures in which nutrient was added immediately after the initial centrifugation step. Densitometeric analysis showed that P-43 contained three times more label in lane 2 than in lane 1. The numbers on the right indicate molecular sizes in kilodaltons. Arrow, P-43.

hydrochloride (pH 7.6). The aqueous phase was dried and suspended in sample buffer, and both fractions were boiled. The proteins were then separated on 12% polyacrylamide gels. The gels were dried and autoradiographed as previously described.

**Materials.** The materials and instruments used were previously described (3).

## RESULTS

**Identification of P-43 methylation as a nutrient-dependent signal.** Starvation of a mid-exponential-phase culture of *E. coli* B/r or K-12 was accomplished by centrifugation and suspension of the culture in medium lacking one of the essential nutrients (carbon, nitrogen, or phosphate). The original culture was divided in half to provide a control, and protein synthesis was stopped with chloramphenicol. After 10 minutes of incubation, tritiated methionine was added to both cultures while the missing nutrient was added back to only the control. The methylation reaction was stopped after 30 min with 5% trichloroacetic acid, and membrane extracts were prepared as described in Materials and Methods. Autoradiographs of SDS–12% polyacrylamide gels of membrane extracts (Fig. 1) identified a 43,000-dalton protein which was labeled to at least a threefold greater extent in starved cultures (lanes 2 and 5) than in control cultures to which the nutrient had been added (lanes 1 and 4). Labeling

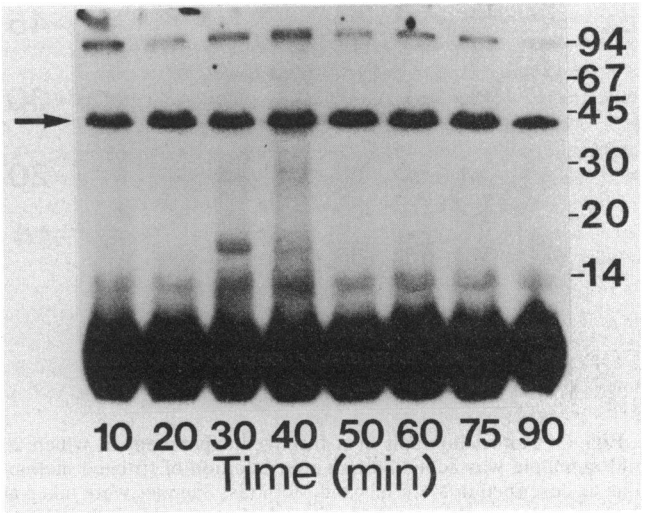


FIG. 2. Autoradiograph of a turnover experiment in which cells were starved for ammonia throughout the time course as described in Materials and Methods. Samples were taken at the times shown. Arrow, P-43. The numbers on the right indicate molecular sizes in kilodaltons.

of this protein, hereafter referred to as P-43, was observed when cells were starved for nitrogen (ammonium sulfate, ammonium chloride, or glutamate), phosphate (sodium phosphate or potassium phosphate), or carbon (glucose or galactose). With carbon starvation, acetate, a poor carbon source, was required at all times, presumably to act as an energy source for the reaction.

Because a small yet significant amount of P-43 was methylated in the culture to which nutrient had been added at the time of [*methyl*-$^3$H]methionine addition, experiments were performed to determine whether methylation could be prevented by provision of nutrients throughout the entire experiment. In these experiments, cultures were suspended directly in complete medium after the initial centrifugation. Membrane extracts from these cells showed no visible labeling of P-43 (Fig. 1, lanes 3 and 6) at normal exposure times. Both *E. coli* B/r and K-12 have been shown to methylate P-43 equally under conditions of nitrogen starvation (data for K-12 not shown).

In addition to P-43, a large mass of labeled material that migrated to a molecular mass of 0 to 12,000 daltons was observed on autoradiographs of gels from methylation experiments (Fig. 1 to 3). Most of this material appeared to be the result of lipid methylation. In bacteria, cyclopropane fatty acids are formed by transfer of the methyl group of *S*-adenosyl-L-methionine to the double bond of an unsaturated fatty acid moiety of a phospholipid (37). Mutant PT17, which is defective in cyclopropane fatty acid synthesis, was shown to reduce the amount of label in this area by >95%. When RNase was not added to extracts after cell breakage, an area between 20 and 30 kilodaltons was extensively methylated. Presumably, once cut with RNase, residual label also migrated to the dye front. Consequently, P-43 was the only protein identified on gels which had altered methylation rates upon addition of nutrient to the medium.

Environmental sensing systems are characterized by covalent modifications which are capable of turning over. In this way, a cell can return to its original state when the stimulus is no longer present. To determine whether the methyl label on P-43 could turn over, time course experi-

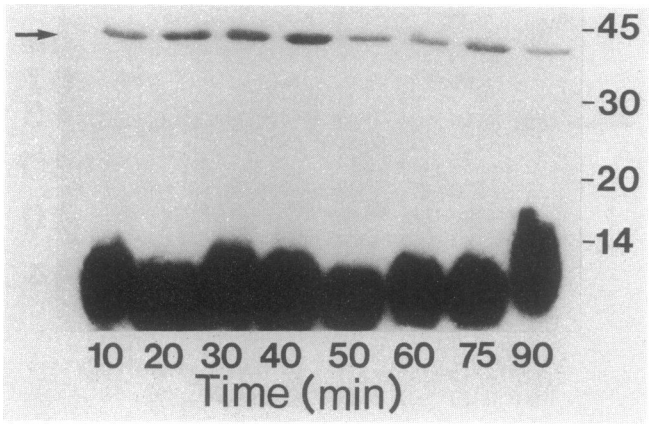


FIG. 3. Autoradiograph of a turnover experiment in which 20 mM ammonia was added 30 min after addition of tritiated methionine as described in Materials and Methods. Samples were taken at the times shown. Arrow, P-43. The numbers on the right indicate molecular sizes in kilodaltons.

ments were performed in which 50-ml cultures of log-phase cells were allowed to starve and methylate P-43. At various times, 5-ml samples were removed from the culture and treated as in standard methylation experiments. First, control experiments in which no nutrient was added throughout the time course were performed to determine the kinetics of the in vivo methylation reaction. In a second set of experiments, unlabeled methionine or the missing nutrient was introduced into parallel cultures at a time when a significant amount of P-43 methylation had occurred. Figure 2 shows a typical autoradiograph of a control experiment in which cells were starved for ammonia, and Fig. 3 shows an autoradiograph of a typical time course experiment in which ammonia was added 30 min after labeled methionine. The radioactivity incorporated in P-43 was quantitated at each time point by densitometry of autoradiographs and integration of the resulting exposed bands (Fig. 4). It was calculated on the basis of these densitometer tracings that P-43 was methylated with a doubling time of 2 to 5 min whether the cells were starved for ammonia, phosphate, or glucose (Fig. 4A to C, ●). After a time (presumably to exhaust the labeled *S*-adenosylmethionine pool), a slower demethylation process was observed. When the missing nutrient (Fig. 4A to C, ■) or unlabeled methionine (Fig. 4A to C, ▲) was added to the cultures after 10 (Fig. 4B) or 20 (Fig. 4A or C) min, further methylation of P-43 was rapidly inhibited, followed by the same slow demethylation process evident in the control experiments. These results showed that P-43 methylation was controlled by the independent actions of the carbon, nitrogen, and phosphate sources in the external medium.

**Labeling of P-43 is not due to methionine incorporation.** To demonstrate that P-43 labeling was due to a methylation event and not methionine incorporation, two experiments were performed. In the first, methylation experiments were conducted in which [$^{35}$S]methionine was substituted for tritiated methionine. A small amount of methionine incorporation into protein occurred, even after chloramphenicol treatment; however, no significant labeling above the background was observed at 43,000 daltons, corresponding to P-43 (Fig. 5). This was true for both nitrogen starvation and phosphate starvation (a $^{35}$S experiment for carbon sensing was not performed). Labeling with [*methyl*-$^{3}$H]methionine was typical and showed extensive methylation under ammonia (lane 1) or phosphate (lane 3) starvation conditions.

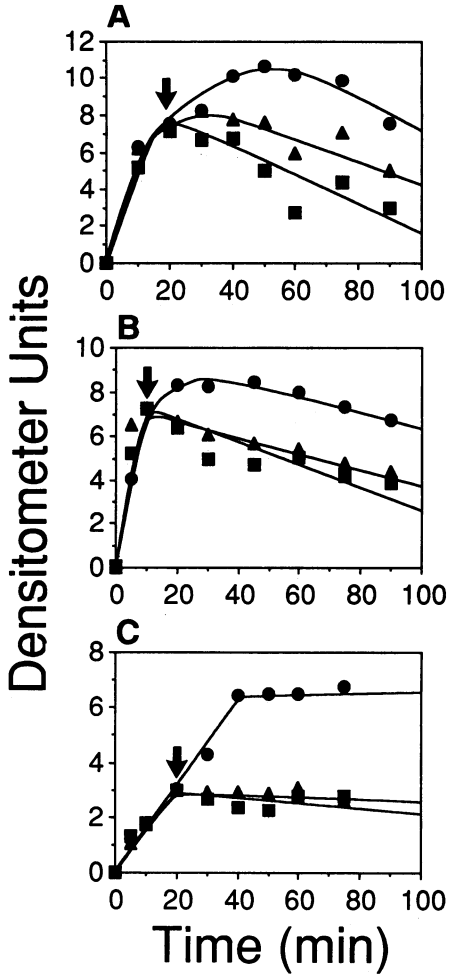


FIG. 4. Plot of turnover experiments showing incorporation of [*methyl*-$^{3}$H]methionine into P-43. Densitometer tracings of autoradiographs, such as those shown in Fig. 2 and 3, in which the integral of the peak height of labeled P-43 is expressed as arbitrary units, are shown. Cells were starved for ammonia (A), phosphate (B), or glucose (C). In each case, a control experiment (●) was run to determine the kinetics of the in vivo methylation reaction under starvation conditions. Methionine (▲) or the missing nutrient (■) was added to identical starving cultures at the time shown by the arrow.

In the second experiment, P-43 was hydrolyzed and the resulting amino acids were separated by descending paper chromatography using a solvent system of pyridine–acetone–3 M NaOH (50:25:10) (8). Results of a typical experiment are shown in Fig. 6. Four major areas of radioactivity were observed. The radioactivity in fraction 1 comigrated with the trimethyllysine standard, and the size of this fraction varied greatly from one experiment to the next. Fraction 2, which contained the largest amount of radioactivity, migrated in an area corresponding to methyl-L-lysine. Since dimethyllysine was unavailable commercially, it was not chromatographed as a standard, but it presumably would migrate between the other two lysine derivatives. Fraction 3 comigrated with the methionine standard and is presumably due to small levels of methionine incorporation into protein. Fraction 4 corresponded to none of the methylated amino acids tested and increased in size as hydrolysis times were increased (data not shown). Since the sizes of the fractions

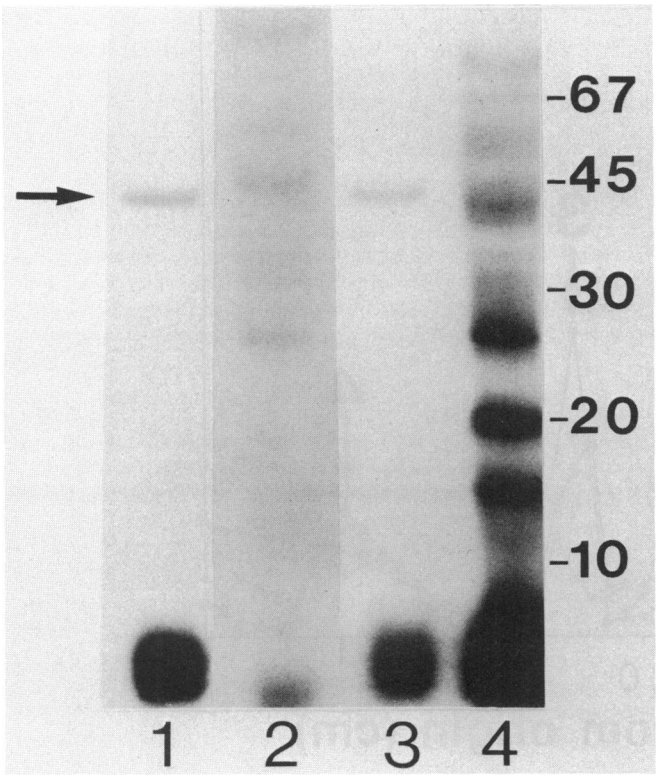


FIG. 5. Autoradiograph of a polyacrylamide gel of membrane proteins from cells starved for ammonia or phosphate. Lanes: 1, cells starved for ammonia and labeled with [*methyl*-$^3$H]methionine; 2, cells starved for ammonia and labeled with [$^{35}$S]methionine; 3, cells starved for phosphate and labeled with [*methyl*-$^3$H]methionine; 4, cells starved for phosphate and labeled with $^{35}$S-methionine. Lane 2 was exposed for one-third as long as the other lanes. The numbers on the right indicate molecular sizes in kilodaltons. Arrow, P-43.

corresponding to methionine and methyllysine decreased as hydrolysis times were increased, we believe that fraction 4 is a breakdown product produced during hydrolysis. The same results were obtained whether the cells were starved for glucose (Fig. 6, ■), ammonia (Fig. 6, ●), or phosphate (Fig. 6, ▲). Since a large majority of the radioactivity of hydrolysates chromatographed in fractions other than methionine, it was concluded that P-43 was labeled by a transmethylation event on lysine residues. This was the same result that had been obtained for protein P-40 in *B. subtilis* (10).

**Relationship between P-43 labeling and chemotaxis.** No labeled protein corresponding to the methylated chemotaxis proteins (approximately 60,000 daltons) was observed (20) (Fig. 1 and 3). Phosphate is not a chemotactic agent in *E. coli* and would not be expected to elicit a chemotactic response (24). Phosphotransferase system sugars, such as glucose, are chemoattractants and utilize one of the MCPs as a secondary receptor. However, no phosphotransferase system sugar stimulation of methylation has ever been observed (1, 2, 22, 24). Ammonia has also been reported to be a chemotactic agent, but its response is reportedly due to changes in intracellular pH and proton motive force (30). As with glucose, cells do not elicit a chemotactic response by using MCP methylation to ammonia. Although none of the nutrients used in our turnover experiments caused methylation of chemotaxis proteins, all prevented methylation of P-43, indicating that the two systems are functionally different.

The MCPs form γ-carboxymethyl derivatives of glutamic acid when methylated, and these are sensitive to hydrolysis in base (38). The methylated residue on P-43 is alkali stable (data not shown) and is located primarily on lysine residues. These facts establish P-43 methylation as a unique signal separate from the chemotaxis system. In this regard, the physical characteristics of the *E. coli* P-43 molecules are almost identical to those of the *Bacillus* P-40 molecule. In that system, it was shown genetically that nutrient-dependent methylation of a membrane protein was not related to chemotaxis (10).

**Localization of P-43 in a cell.** Methylated P-43 protein could not be extracted from unbroken cells by boiling in SDS-containing buffers and was found only in very small amounts in the cytosolic fraction (approximately 10% compared with membrane extracts). Radioactivity in the cytosolic fraction could represent a second, soluble form of P-43 or may have been due to the presence of very small membrane fragments which were not recovered during centrifugation. Although most P-43 was isolated with membrane fragments, it was possible that the trichloroacetic acid treatment used to stop the reaction might precipitate cytosolic proteins to make them appear to be membrane bound. To show that this was not true for P-43, a standard methylation experiment was performed without treating the cells with trichloroacetic acid. After methyl group incorporation, the cells were washed and broken as quickly as possible. In this experiment (data not shown), P-43 remained in the membrane fraction and was not observed in the soluble fraction after centrifugation at 48,000 × *g* for 15 min.

To show that P-43 was truly hydrophobic and membrane associated, it was partitioned between hydrophobic and hydrophilic fractions by using Triton X-114 micells as described by Bricker and Sherman (6). The proteins from each of these fractions were separated on SDS-polyacrylamide gels. In this experiment, methylated P-43 was found exclusively in the detergent phase, indicating that it contained a significant hydrophobic character capable of associating with membranes (data not shown).

## DISCUSSION

We have identified and partially characterized a membrane-associated protein in *E. coli* of approximately 43,000 daltons which became methylated when cells were starved for glucose, galactose, ammonia, glutamate, or phosphate. This protein was similar in many respects to a membrane protein from *B. subtilis* and *B. licheniformis* which we identified previously (3, 10). The *E. coli* protein was methylated rapidly during starvation, with a doubling time of 2 to 5 min. Maximum incorporation of label occurred 40 min after labeled methionine was added to ammonia- or glucose-starved cultures and after 20 min when cells were starved for phosphate. Reintroduction of the missing nutrient to the culture immediately prevented further methylation of P-43 in all cases and allowed a slower demethylation process to be observed. P-43 appears to be methylated on lysine residues. These data are consistent with our hypothesis that the methylation reaction is responsible for initiation of the signal for nutrient availability.

*ntr* and *pho*, two systems which use a set of proteins belonging to the two-component regulatory system motif, are responsible for activating the nitrogen- and phosphate-scavenging pathways of the cell, respectively (for reviews, see references 25 and 35). The signal to which the *pho* system responds has yet to be identified (16), while the signal for *ntr* has been proposed to be a change in the ratio of

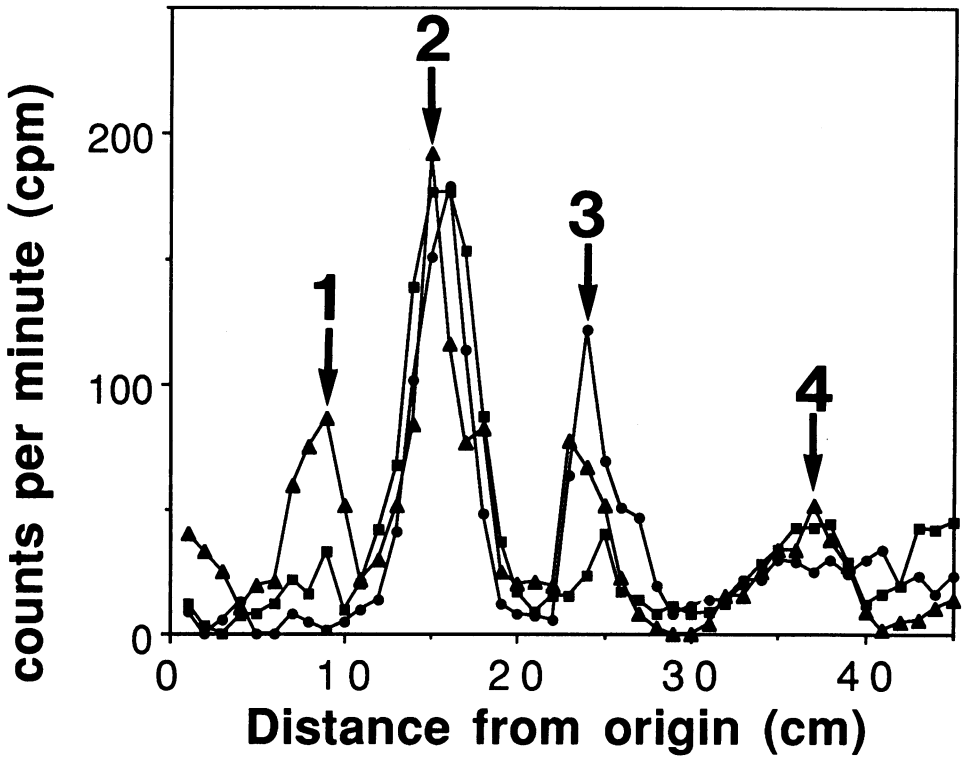


FIG. 6. Radioactivity recovered from a paper chromatograph of 15-h hydrolysates of P-43 prepared as described in Materials and Methods. The arrows indicate the locations of the following standards: 1, trimethyllysine; 2, methyllysine; 3, methionine; 4, unknown. Hydrolysates were from standard methylation experiments in which cells were starved for ammonia (●), phosphate (▲), or glucose (■).

intracellular glutamine to α-ketoglutarate when ammonia concentrations drop below 1 mM in the external environment (34, 35). However, some bacterial species, such as bacilli, do not respond to glutamine/α-ketoglutarate ratios in this fashion and must sense an alternative signal (4). Keener and Kustu have shown that the $P_{II}$ protein of *E. coli* is required only for rapid dephosphorylation of NtrC and has no effect on NtrB autophosphorylation, indicating that $P_{II}$ is not a nitrogen-dependent signal transmitter (18). This is consistent with the results of Bueno et al., which indicated that a *glnB* deletion strain of *E. coli* showed normal nitrogen regulation of histidase activity (7). By using *glnB*:Tn*5* insertions which caused loss of $P_{II}$ function, Holtel and Merrick have shown that the $P_{II}$ protein of *Klebsiella pneumoniae* is not essential for regulation of the phosphorylation or dephosphorylation of NtrC by NtrB in response to changing nitrogen status(15). Since NtrB itself does not appear to be sensitive to the nitrogen status of cells (18), these data suggest that another regulatory element is present within the cell to respond as a nitrogen-dependent signal transmitter (15). It is possible that methylation of P-43 serves as the initial signal in these and other environmentally sensitive systems.

Bacterial cells in the last generation of logarithmic growth apparently recognize that one or more essential nutrients are being exhausted and that the next cycle of cell division should not be initiated. The mechanism that controls this entrance into the stationary phase of growth is not known, but is is possible that the nutrient-dependent, reversible methylation system reported here initiates the signals for growth control that are interpreted by members of the homologous two-component regulatory systems known to be involved in nitrogen and phosphate starvation. We propose that P-43 is the protein responsible for sensing nutrient availability and transferring this information to intracellular growth control systems. The membrane location of P-43 could allow it to communicate with the cellular environment directly or allow for monitoring of a set of membrane receptors. This second possibility is appealing, as we have observed only a single protein band corresponding to P-43 on SDS-polyacrylamide gels. However, we cannot rule out the possibility that there is a set of closely related P-43 proteins which are similar enough that they could not be distinguished in our experiments.

## ACKNOWLEDGMENTS

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