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Multiple Forms of Methyl-Accepting Chemotaxis Proteins Distinguished by a Factor in Addition to Multiple Methylation

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Methyl-accepting chemotaxis proteins are central to both the excitation and adaptation phases of chemotactic behavior. Using null mutations in the genes coding for the two major methyl-accepting proteins (*tsr* and *tar*), we identified the gene products among the membrane proteins of *Escherichia coli* visualized on one- and two-dimensional gels. On two-dimensional gels, both the *tsr* and the *tar* proteins appeared as a group of multiple spots arranged in two to four diagonal arrays. The multiplicity of forms could not be completely explained by the previously documented heterogeneity of the methylated proteins resulting from different numbers of methylated glutamyl residues per polypeptide chain. We suggest that there is at least one other way besides extent of methylation in which the polypeptides of a methylated protein can differ.

Methyl-accepting chemotaxis proteins (MCPs) are central to the chemotactic behavior of enteric bacteria (28). These proteins link chemoreceptors to the common tumble regulator, which in turn controls the direction of rotation of bacterial flagella (26, 27). Upon tactic stimulation by an increase in concentration of an attractant, the sequence of events appears to be as follows: (i) binding of ligand to receptor, creating a transducer-binding site on the receptor; (ii) interaction of ligand-occupied receptor with a transducer MCP; (iii) induction of an alteration in the transducer MCP, which finally results in exclusively counterclockwise rotation of the flagella. The nature of the linkage between transducer and flagellum along this excitatory pathway is unknown, as is the nature of the alteration (excitatory signal) generated in the transducer itself.

Responses to tactic stimuli are transient. At some time after excitation (a few seconds to several minutes, depending upon the nature and magnitude of the stimulus), the original balance between the two directions of flagellar rotation is reestablished. This adaptation involves covalent modification of MCPs. Adaptations to favorable stimuli (attractant increases, repellent decreases) and to unfavorable stimuli are correlated with increases and decreases, respectively, in the extent of carboxyl methylation of the transducer MCPs through which the stimuli pass (26, 27). It is likely that the direct mechanism of adaptation is methylation and demethylation of MCPs. Thus, transducer MCPs function both in the initial excitation phase and in the gradual adaptation phase of tactic behavior.

There are approximately 25 chemoreceptors known in *Escherichia coli*, but perhaps as few as four transducers (12). Mutations in genes *tsr* (14, 23), *tar* (23), and *trg* (10, 13, 16) define three independent transductional pathways. There must be at least one additional pathway since none of the three known classes of transducer mutations eliminates responses to the sugars recognized by enzymes II (12). *tsr* mutants do not respond to gradients of serine, some other amino acids, and some repellents (14, 23, 27); *tar* mutants do not respond to aspartate, maltose, and some different repellents (23, 27); and *trg* mutants do not respond to galactose and ribose (13, 16). Thus, transducers focus information from several receptors.

Transducer MCPs accept methyl groups from *S*-adenosylmethionine to form glutamyl methyl esters (15, 31) in a reaction catalyzed by a specific methyl transferase, the product of *cheR* (29). Demethylation is catalyzed by the product of *cheB* (30). Evidence from several laboratories, including our own, indicates that an MCP molecule can be methylated at more than one site (4-6, 8). The unusual pattern of multiple bands on sodium dodecyl sulfate-polyacrylamide gels that is exhibited by each MCP can be explained at least in part by postulating that each additional methyl group increases the electrophoretic mobility of the polypeptide, probably as a result of enhanced binding of sodium dodecyl sulfate upon neutralization of a negatively charged glutamyl residue (5, 6).

Transducer MCPs are functionally complex; they interact with several receptors and the enzymes of methylation, and they also mediate excitation. The biochemical features of these

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proteins are correspondingly complex. We have approached the study of transducer MCPs by obtaining null mutations in the relevant genes and then constructing strains missing combinations of MCPs. Here we present a characterization of the membrane proteins of these strains, unequivocally identifying the products of *tsr* and *tar* among the proteins of *E. coli* visualized on one- and two-dimensional gels. Both the *tsr* and the *tar* proteins appeared as a group of spots on two-dimensional gels. The pattern within each group implied that the multiple species observed were the result of multiple methylation and at least one additional factor.

MATERIALS AND METHODS

Bacterial strains. The strains used in this work are all derivatives of *E. coli* K12 (Table 1). The RP strains are all related to strain B275 (1, 21) and thus are closely related to each other. *tar-52Δ1* is a deletion constructed in vitro (26), which does not affect expression of the *che* genes. Strain T49-5H, which carries a Tn*10* insertion and thus is tetracycline resistant, was found to be phenotypically Tsr and was originally thought to be *tsr*::Tn*10* (8); thus, the *tsr* mutation in strain T49-5H was used to construct mutant strains null for *tsr*. After such strains were shown to be missing the *tsr* product (see below), it became clear that the Tn*10* insertion was near, but not in, *tsr*. Strain B14, the parent of T49-5H, appears to exhibit a high frequency of mutation (10), and this may be the source of the mutation *tsr-49*.

TABLE 1. *Bacterial strains*

Strain	Relevant description	Origin/reference
RP437	F⁻ *thi thr leu is met eda rpsL*	J. S. Parkinson (21)
RP4375	*tsr-2·3·4 tar-52Δ1*	J. S. Parkinson (12)
RP4672	Like RP437, except *met⁺ cheR202*[a]	J. S. Parkinson (21)
RP4673	Like RP437, except *met⁺ cheR203*[a]	J. S. Parkinson (21)
RP4779	Like RP437, except *met⁺* cheB287	J. S. Parkinson (21)
RP4788	Like RP437, except *met⁺ cheB275*	J. S. Parkinson (21)
T49-5H	B14 *tsr-49 itt-1*::Tn*10*[b]	Tn*10* mutagenesis + a spontaneous?) mutation (10)
159λ *flaI*	*flaI*	M. Silverman (25)
HB233	RP437 *met⁺*	RP437 × P1(B14)*met⁺* transductant
HB237	HB233 *itt-1*::Tn*10 tsr-49*	HB233 × P1(T49-5H), Tsr Tet[r] transductant
HB238	HB233 *eda⁺ tar-52Δ1*	HB233 × P1(RP4375) glucuronic acid⁺, Tar transductant
HB243	HB233 *eda⁺ tar-52Δ1 tsr-49 itt-1*::Tn*10*	HB238 × P1(T49-5H), Tsr Tet[r] transductant
HB261	HB233 *eda⁺ flaI*	HB233 × P1(159λ *flaI*) glucuronic acid⁺, Fla⁻ transductant

[a] In an attempt to standardize the nomenclature of *che* genes in *E. coli* and *Salmonella typhimurium* (D. E. Koshland, Jr., personal communication), the gene of *E. coli* formerly termed *cheX* (21) has been renamed *cheR*, the corresponding gene in *S. typhimurium* (7). The names of all other *che* genes of *E. coli* are unchanged.

[b] *itt*, insertion *t*ransposon *t*en (see text).

Media. The growth media used, tryptone broth, and H1 minimal salts have been described previously (1).

Chemicals. L-[*methyl*-^{3}H]methionine (15 mCi/mmol) and L-[^{35}S]methionine (>800 Ci/mmol) were purchased from The Radiochemical Centre, Amersham, England. Ampholytes were from LKB, Stockholm, Sweden.

Labeling with *methyl*-^{3}H. Cells were prepared for methylation experiments essentially by the method of Kort et al. (17). They were grown in tryptone broth at 35°C with rotary shaking to a density of approximately 2.5×10^8 cells per ml, harvested by centrifugation, washed three times in 10 mM potassium phosphate (pH 7.0)–0.1 mM EDTA (chemotaxis buffer), and suspended at a density of 2.5×10^8 cells per ml in the same buffer containing 200 μg of chloramphenicol per ml and 25 mM sodium succinate. The suspensions were kept on ice until use. Portions of the cell suspensions were shaken at 30°C for 10 min before the addition of L-[*methyl*-^{3}H]methionine (15 Ci/mmol) to a final concentration of 2 μM. Experiments in which whole cells were analyzed on one-dimensional gels were terminated at 50 min by adding 10% trichloroacetic acid. Samples were placed on ice for 15 min, pelleted by centrifugation, and suspended in electrophoresis sample buffer containing 1% sodium dodecyl sulfate and bromophenol blue. The pH was adjusted to the color transition of the indicator (pH 4.0), and samples were boiled for 2 min before they were applied to the gels.

For experiments in which membranes were analyzed on two-dimensional gels, cell growth was terminated by placing samples on ice for 15 min. The cooled cell suspensions (usually 2 to 5 ml) were disrupted by ultrasonic vibration (three bursts of 5 s each from a standard 8-mm diameter tip). After dilution with chemotaxis medium to approximately 9 ml, the suspensions were centrifuged for more than 4 h at 100,000 × *g* to pellet the membranes. The pelleted material was suspended in chemotaxis buffer and either used immediately or stored frozen at −20°C for no more than a few days.

Labeling with ^{35}S. Cells were grown in H1 ribose (0.2%) medium containing the required amino acids under the conditions described above. When the cells reached a density of approximately 2.5×10^8 cells per ml, 20 to 30 μCi of L-[^{35}S]methionine was added to 5 × 10^8 cells. Cultures were shaken at 35°C for 1 h and centrifuged for 10 min at 8,000 × *g* in an Eppendorf model 5412 centrifuge; the cells were then suspended in 1 ml of chemotaxis buffer, sonicated, centrifuged, and resuspended as described above.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Samples corresponding to 10^8 cells or less were applied to a discontinuous gel containing 9 or 10% polyacrylamide in the separation gel, which was prepared by the method of Laemmli (18) with the modifications described by Randall and Hardy (22). Gels prepared for fluorography were treated by the method of Bonner and Laskey (3) or with En^{3}Hance (New England Nuclear Corp., Boston, Mass.) and exposed to X-ray film at −70°C. Otherwise, gels were dried and analyzed by autoradiography directly.

Two-dimensional gel electrophoresis. The procedure used was essentially that of O'Farrell (20), as modified for membrane proteins by Ames and Nikaido (2). The electrofocusing gel was a 0.5-mm slab; tracks were cut out of this slab and then laid on top of a 1.0-mm slab gel identical to the gels described above. Ampholytes were 1.4% pH 4 to 6 and 0.6% pH 6 to 8. The polyacrylamide concentration in the second dimension was 10 or 12.5%.

Determination of the relative amount of MCPs among the membrane proteins. The total lengths of the autoradiograms (for MCP regions, see Fig. 4) were traced by using a Joyce-Loebl microdensitometer. The relative amount of the MCPs was determined by cutting out and weighing the total trace and the MCP peaks. The trace did not completely resolve the two MCP bands visible on the autoradiogram, so a single combined area was cut out. The absolute amount of MCPs per cell was calculated by using a value of 18 fg of membrane protein per cell.

RESULTS

Transducer MCPs are easily visualized as *methyl*-^{3}H-labeled bands on fluorograms of sodium dodecyl sulfate-polyacrylamide gels of whole cells or isolated membranes (26, 27). An example of such a fluorogram is shown in Fig. 1A; this figure illustrates that MCP I, which is missing in *tsr* mutants, and MCP II, which is missing in *tar* mutants, constitute the great majority of the MCPs. After a longer exposure MCP III appears just below the MCP II region (16). MCP I and MCP II were identified previously as the products of *tsr* and *tar*, respectively, by demonstrating that the *methyl*-^{3}H-labeled bands corresponded to ^{35}S-labeled polypeptides synthesized by cloned genes expressed in UV-irradiated cells programmed with appropriate λ transducing phage (26) or in minicells containing appropriate plasmids (19). We used null mutations in *tsr* and *tar*, as well as a mutation in *flaI*, to construct a set of isogenic strains of *E. coli* that would be expected to lack completely the polypeptides produced from one or both of the transducer genes. These strains provided a means of identifying the polypeptides produced by *tsr* and *tar* as specific bands or spots on one- and two-dimensional gel patterns of total membrane proteins of intact, unperturbed cells.

Figure 1B shows an autoradiogram of the one-dimensional gel pattern of ^{35}S-labeled membrane proteins from strains lacking one or both transducer gene products. In the MCP region, as indicated by the aligned fluorogram in Fig. 1A,

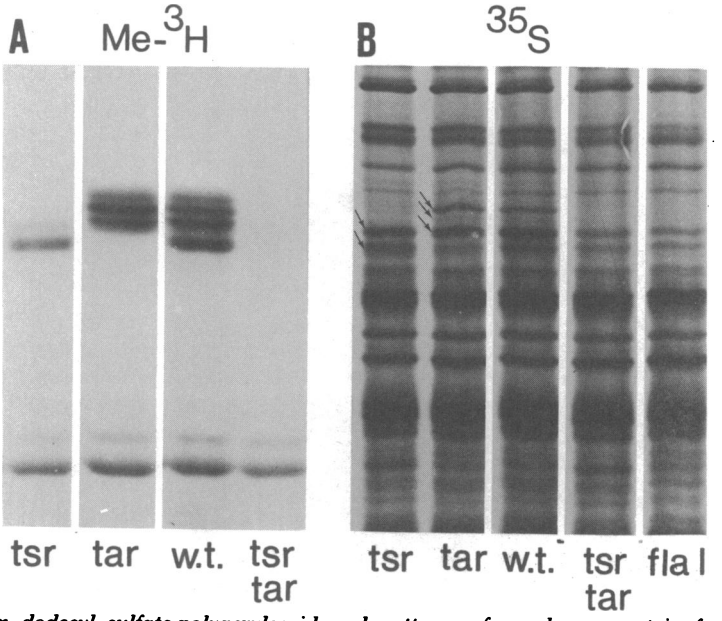

FIG. 1. *Sodium dodecyl sulfate-polyacrylamide gel patterns of membrane protein from wild-type and transducer mutant strains. (A) Fluorogram of methyl-^{3}H-labeled material. The wild-type (w.t.)strain used was HB233; the tsr (MCP I$^-$), tar (MCP II$^-$), and tsr tar mutant strains used were HB237, HB238, and HB243, respectively. MCPs were located in the range from 55,000 to 65,000 apparent molecular weight. The methylated band at the bottom of the figure, which is not related to chemotaxis (12), is at approximately 45,000 apparent molecular weight. (B) Autoradiogram of ^{35}S-labeled material. Strains as in (A). The flaI mutant used was HB261. The tsr-dependent bands (MCP I) and the tar-dependent bands (MCP II) are indicated by arrows in the tar and tsr tracks, respectively. Shrinkage of gels during processing for fluorography made it difficult to align autoradiograms and fluorograms precisely. Fluorograms of ^{35}S-labeled membrane protein exhibit relatively poor resolution of closely contiguous bands, but show that the prominent tsr product band indicated by the top arrow in the tar track corresponds to the uppermost methyl-^{3}H-labeled MCP I band. Thus, the patterns in (A) and (B) (both 9% polyacrylamide) are aligned in approximately that relationship.*

a number of ^{35}S-labeled bands could be correlated with *tsr* and *tar* activities. The arrows pointing to the *tar* track (Fig. 1B) indicate bands correlated with *tsr* (MCP I) activity (i.e., bands present in *tar* strains but missing in strains lacking *tsr* activity). Bands correlated with *tar* activity are indicated by arrows in the *tsr* track. It appears that the band in the midst of the MCP area (marked with an arrow in both the *tsr* track and the *tar* track) was the result of comigration of at least three polypeptides, one produced by *tsr*, one produced by *tar*, and one independent of *flaI* activity (neither *tsr* nor *tar* is expressed in an *flaI* strain [26]). Thus, the products of *tsr* and *tar*, which were present in normal amounts among the membrane proteins of normal, untreated cells and were visualized by radioactive label incorporated during synthesis of the polypeptides, both appeared as more than one band in a sodium dodecyl sulfate gel pattern.

The transducer gene products can be better separated from other membrane proteins in two-dimensional gels in which separation by isoelectric focusing is followed by electrophoretic separation in the presence of sodium dodecyl sulfate (20). Representative gels are shown in Fig. 2; this figure illustrates that *methyl*-^{3}H-labeled MCP I and MCP II were missing in a *tsr* mutant

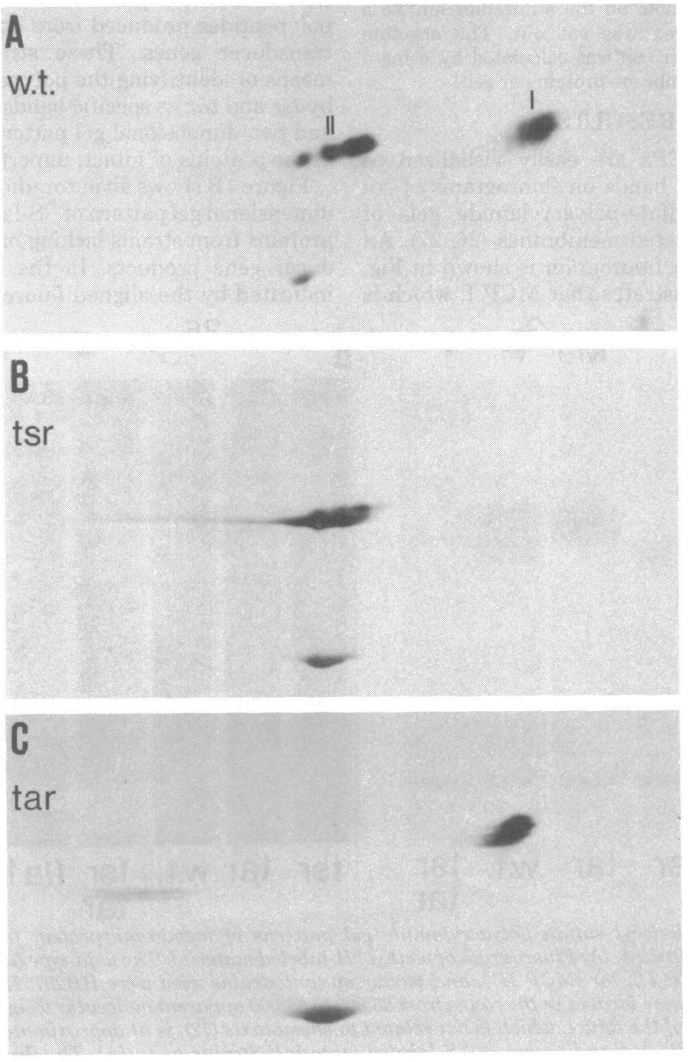

FIG. 2. *Two-dimensional gel patterns of membrane proteins from wild-type (w.t.) and transducer mutant strains. Methyl-^{3}H-labeled material (A through C) or ^{35}S-labeled material (D through F) was submitted to isoelectric focusing (basic to acidic, left to right) and then sodium dodecyl sulfate gel electrophoresis (vertical dimension). The polyacrylamide contents of the gels were 10% (B and C) and 12.5% (A, D, E, and F). MCP I (I) focused at a pI of 5.3 to 5.4, and MCP II (II) focused at a pI of 5.8 to 5.9. Reference spots are circled in (D) through (F). Bacterial strains as in Fig. 1.*

and a *tar* mutant, respectively (Fig. 2A through C), and also shows the ^{35}S-labeled spots corresponding to MCP I and II (Fig. 2D and E). The appearance of these ^{35}S-labeled spots was dependent on a functional *flaI* gene, as well as the respective structural genes (Fig. 2D through F). Previously, *methyl*-^{3}H-labeled transducer MCPs have been visualized on two-dimensional gels as streaks of radioactivity rather than distinct spots (11, 19, 26). Our experience is that in gels in which the range of ampholytes is more acidic than usual (20), the proteins are ofter resolved into distinct spots. In the gels used in these studies, MCP I and MCP II migrated at apparent pI's of approximately 5.35 and 5.85, respectively. Commonly used mixtures of ampholytes (20) produce a rather steep gradient in that pH range. The steep gradient probably accounts for previously published patterns of MCPs (11, 19, 26), in which streaks of MCP I and MCP II appear close to each other toward the acidic end of the pH gradient.

On favorable two-dimensional gels, *methyl*-^{3}H-labeled MCPs were resolved into a series of distinct spots that lay along a diagonal from a position of higher apparent molecular weight and more acidic pI toward a position of lower apparent molecular weight and more alkaline pI. MCP II often appears to consist of four partially overlapping spots arranged along a diagonal (Fig. 2A) (8). Carboxyl methylation of a glutamyl residue eliminates a negative charge and thus should cause a slight alkaline shift of the pI of an MCP. We have argued elsewhere (8) that a diagonal of spots would occur if an MCP were multiply methylated and that each additional

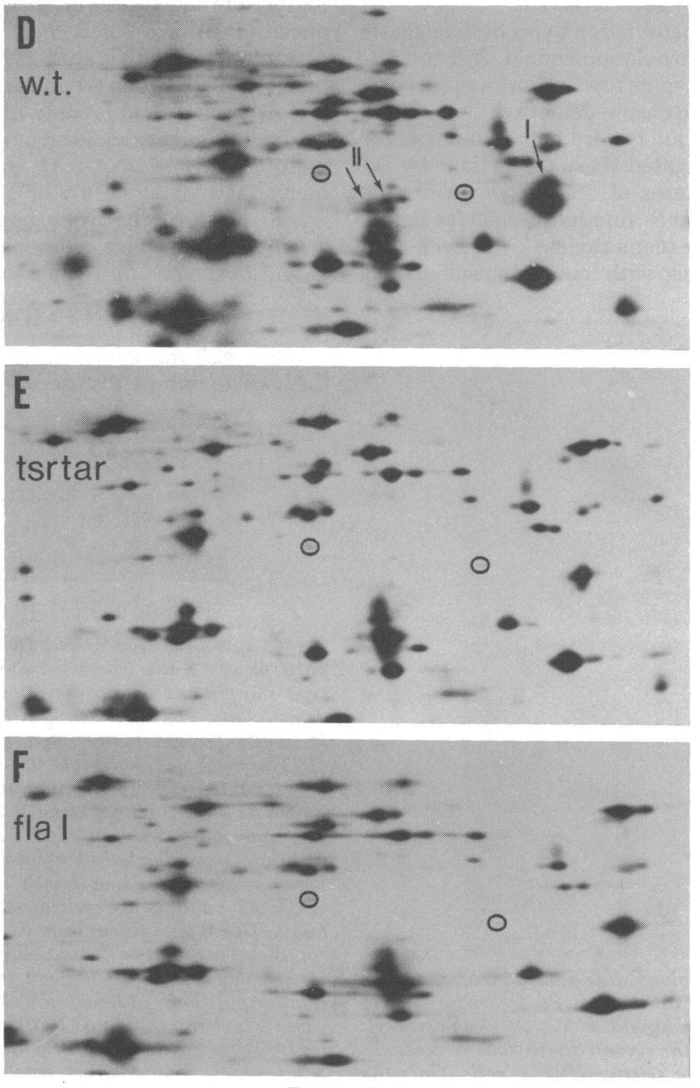

FIG. 2.—D-F

methyl group would result in slightly faster migration in sodium dodecyl sulfate-gel electrophoresis, probably as the result of increased binding of the detergent (5, 6). Thus, we used the observation of a diagonal pattern to support the hypothesis that MCPs are multiply methylated.

However, the pattern is more complicated than a single diagonal. On gels with particularly good resolution, MCP II consisted of six spots. This pattern was observed with ^{35}S-labeled (Fig. 2D) and *methyl*-^{3}H-labeled (Fig. 3B) material. Smearing or overexposure of the spots produced a pattern like that in Fig. 2A (a diagonal of apparently four overlapping spots). In two-dimensional gels with good resolution, *methyl*-^{3}H-labeled MCP I was resolved into a large number of spots (Fig. 3A). We have not yet obtained a gel containing ^{35}S-labeled protein in which the MCP I spots have been resolved.

The multiple methylation hypothesis suggests that extensive methylation should shift the distribution of MCP spots toward lower, more basic spots and that extensive demethylation should shift the distribution toward higher, more acidic spots. We investigated these predictions by examining the patterns of ^{35}S-labeled membrane proteins from *cheR* (methyltransferase-defective) and *cheB* (demethylase-defective) mutants. An analysis with one-dimensional gels (Fig. 4) revealed that ^{35}S-labeled bands that corresponded to MCP I and MCP II shifted to positions of higher apparent molecular weight in *cheR* strains and to positions of lower apparent molecular weight in *cheB* strains. On two-dimensional gels the distinctive diagonal patterns of MCP I and MCP II shifted in the predicted manner in absence of methyltransferase and demethylase activities, respectively (Fig. 5). In both one- and two-dimensional patterns of material from *cheR* mutants, new, predominant bands appeared at positions of apparent molecular weight that were above the respective methyl-labeled sets of bands. The new species were probably unmethylated MCP, implying that little completely unmethylated protein existed in unstimulated, wild-type cells. This same conclusion has also been reached from examinations of the patterns of ^{35}S-labeled MCPs produced in UV-irradiated *cheR* and *cheB* strains programmed with appropriate transducing phage λ (4–6). In the two-dimensional pattern it was evident that minimally methylated MCP I exhibited a higher molecular weight than minimally methylated MCP II, whereas the maximally methylated forms of both proteins migrated close to the same apparent molecular weight (Fig. 5). This relationship was also apparent in the one-dimensional patterns (Fig. 4).

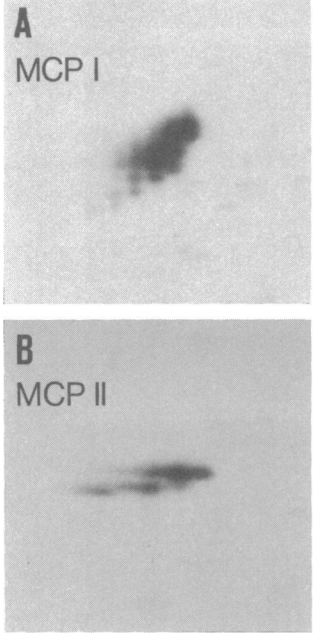

FIG. 3. *Well-resolved two-dimensional gel patterns of methyl-^{3}H-labeled MCPs. Procedures were as described in the legend to Fig. 2; 10% polyacrylamide was used in the second dimension. Membranes were from wild-type (strain HB233) cells. Only the areas around MCP I (A) and MCP II (B) are shown.*

DISCUSSION

Using null mutations in the genes coding for the two major methyl-accepting proteins (*tsr*

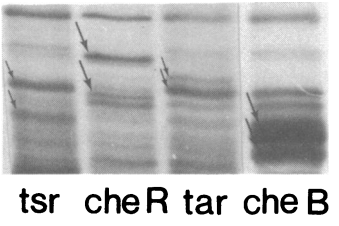

FIG. 4. *Sodium dodecyl sulfate-polyacrylamide gel patterns of ^{35}S-labeled membrane proteins of wild-type and mutant strains defective in methylation or demethylation of MCPs. tsr (MCP I$^-$) and tar (MCP II$^-$) mutants were as described in the legend to Fig. 1B. The cheR and cheB mutants were strains RP4372 and RP4779, respectively. Similar patterns were observed for strains RP4373 (cheR) and RP4788 (cheB). The same major bands of MCP I and MCP II visible in Fig. 1 are indicated by arrows in the tar and tsr lanes, respectively. New bands appearing in cheR and cheB mutants are indicated by arrows in those lanes. This figure differs from Fig. 1B in that the gel contained a slightly different percentage of acrylamide, so that the bands are not in exactly the same relative positions, although the order of bands is the same. The band indicated by the upper arrow in the tsr track and the lower arrow in the tar track is the band which resulted from comigration of at least three polypeptides (see Fig. 1B and text).*

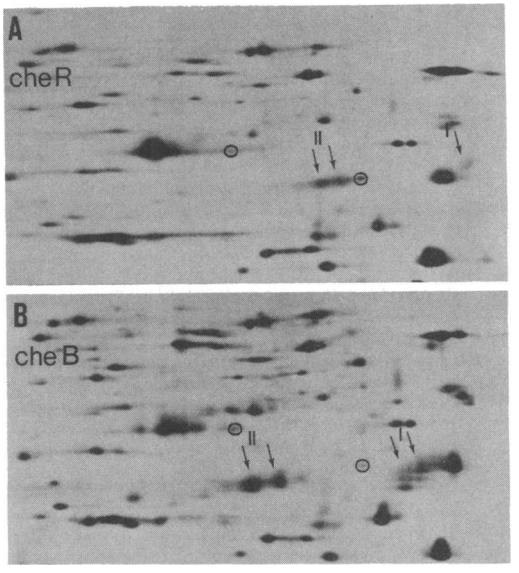

FIG. 5. *Two-dimensional gel patterns of ^{35}S-labeled membrane proteins from mutants defective in methylation or demethylation of MCPs. Fluorograms were produced as described in the legend to Fig. 2. The cheR and cheB mutants were strains RP4373 and RP4779, respectively. Similar patterns were observed for strains RP4372 (cheR) and RP4788 (cheB). MCP I and MCP II are indicated by arrows labeled I and II, respectively. The same reference spots as in Fig. 2D and E on each side of the MCP II spots are circled. See Fig. 2D for the position of MCP II in a wild-type strain.*

and *tar*), we identified the products of these genes among the membrane proteins of *E. coli* visualized on one- and two-dimensional gels. In cytoplasmic membranes no single protein is present as a predominant species. Instead, many polypeptides are present in moderate amounts (2). A visual examination of the two-dimensional gel patterns of ^{35}S-labeled proteins indicates that the products of *tsr* and *tar* are members of that moderate-copy-number group. It would be difficult to make a quantitative determination of the relative abundance of the two products in the membrane on the basis of two-dimensional patterns or on the basis of one-dimensional patterns in which the proteins migrate as multiple bands. Since the lack of methyltransferase or demethylase activity in a strain results in migration of the bulk of each transducer MCP in a single band, one-dimensional patterns of the membrane proteins from *cheR* and *cheB* strains provide a way to compare the intensities of the transducer MCP bands on autoradiograms with the total intensity of membrane proteins in a gel sample. Such comparisons provide estimates that the Tsr and Tar proteins together constitute about 1.5% of the membrane proteins, corresponding to roughly 2.5×10^3 60,000-dalton molecules per cell, with *tsr* protein representing more than one-half of this amount.

MCPs appear as sets of bands on sodium dodecyl sulfate-polyacrylamide gels (26, 27). Investigations of the basis for these unusual patterns have led several investigators, including us, to conclude that MCPs are multiply methylated and that the addition of each methyl group results in a slightly faster migration of the MCP polypeptide chain during electrophoresis in the presence of sodium dodecyl sulfate (4–6, 8). Methylation of any glutamyl residue should have the same effect on the pI of the protein. If each methylation also had the same effect on electrophoresis of the polypeptide in sodium dodecyl sulfate, the MCP polypeptides in a two-dimensional pattern should be arrayed as a single diagonal line of spots progressing in regular steps toward the alkaline side of the gel and down in apparent molecular weight, representing progressively more methylated species. The existence of a more complicated pattern of MCP spots implies either that not all methylations have an equivalent effect on electrophoretic migration or that MCP polypeptides can differ in some way, other than extent of methylation, that affects the positions of spots in two-dimensional patterns. The former possibility would mean that there would be only a single spot of totally unmethylated or totally methylated protein. There are two distinct MCP II spots in the patterns from both *cheR* (methyltransferase) and *cheB* (demethylase) mutants. These results argue against explanations of the complicated patterns based on differences in location or order of methylation of the multiple acceptor sites on MCP polypeptides. The argument is not conclusive in the case of the *cheB* mutants since it is not known whether MCPs are all totally methylated in those strains. The case is stronger for the *cheR* patterns since residual methylation activity has been found to be <5% in a *cheR203* strain and even less in a *cheR202* strain (9). Thus, the two MCP II spots of about equal intensity present in the pattern from a *cheR203* mutant (Fig. 5A) or a *cheR202* mutant (data not shown) are probably not the unmethylated form of MCP II and an equal amount of a singly methylated form. Instead, it seems probable that these spots represent two different, unmethylated forms of the protein.

We suggest that one reasonable way to interpret the pattern of six MCP II spots is as two diagonal lines of three spots each. Along a diagonal the spots would carry progressively more methyl groups toward the alkaline and lower-apparent-molecular-weight direction, as discussed above. In the simplest case, all polypeptides in spots on the lower diagonal would be separated from polypeptides on the upper diag-

onal by the same feature that causes apparently unmethylated MCP II from *cheR* mutants to produce two spots. The two unmethylated forms would be expected to appear just above and to the right of the right ends of the two diagonals. In fact, the two presumably unmethylated MCP II spots present in Fig. 5A are located at just the expected positions (compare with MCP II pattern in Fig. 2D and 3B). The greater number of MCP I spots and the trouble in resolving ^{35}S-labeled material make interpretation of the MCP I pattern more difficult; however, based on our interpretation of the MCP II pattern, we suggest that the pattern of MCP I spots (Fig. 3A) can be viewed as consisting of three or four diagonal lines, each containing four or five spots.

In summary, we have observed that the polypeptides of MCP I and MCP II are distributed into a variety of forms that can be distinguished on two-dimensional gels. The considerations which we have discussed suggest that there should be at least one other way besides extent of methylation in which MCP polypeptides can differ and that molecules of a given MCP are probably distributed among more than one structural subclass. The chemical basis and functional significance of this subclass division are presently under investigation.

ACKNOWLEDGMENT

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LITERATURE CITED

1. **Adler, J.** 1966. Chemotaxis in bacteria. Science **153**:708–716.
2. **Ames, G. F.-L., and K. Nikaido.** 1976. Two-dimensional gel electrophoresis of membrane proteins. Biochemistry **15**:616–623.
3. **Bonner, W. M., and R. A. Laskey.** 1974. A film detection method for tritium-labeled proteins and nucleic acids in polyacrylamide gels. Eur. J. Biochem. **46**:83–88.
4. **Boyd, A., and M. I. Simon.** 1980. Multiple electrophoretic forms of methyl-accepting chemotaxis proteins generated by stimulus-elicited methylation in *Escherichia coli*. J. Bacteriol. **143**:809–815.
5. **Chelsky, D., and R. W. Dahlquist.** 1980. Structural studies of methyl-accepting chemotaxis proteins of *Escherichia coli*: evidence for multiple methylation sites. Proc. Natl. Acad. Sci. U.S.A. **77**:2434–2438.
6. **De Franco, A. L., and D. E. Koshland, Jr.** 1980. Multiple methylation in the processing of sensory signals during bacterial chemotaxis. Proc. Natl. Acad. Sci. U.S.A. **77**:2429–2433.
7. **De Franco, A. L., J. S. Parkinson, and D. E. Koshland, Jr.** 1979. Functional homology of chemotaxis genes in *Escherichia coli* and *Salmonella typhimurium*. J. Bacteriol. **139**:107–114.
8. **Engström, P., and G. L. Hazelbauer.** 1980. Multiple methylation of methyl-accepting chemotaxis proteins during adaptation of *Escherichia coli* to chemical stimuli. Cell **20**:165–171.
9. **Goy, M. F., M. S. Springer, and J. Adler.** 1978. Failure of sensory adaptation in bacterial mutants that are defective in a protein methylation reaction. Cell **15**: 1231–1240.
10. **Harayama, S., E. T. Palva, and G. L. Hazelbauer.** 1979. Transposon-insertion mutants of *Escherichia coli* K12 defective in a component common to galactose and ribose chemotaxis. Mol. Gen. Genet. **171**:193–203.
11. **Hayashi, H., O. Koiwai, and M. Kozuka.** 1979. Studies on bacterial chemotaxis. II. Effect of *cheB* and *cheZ* mutations on the methylation of methyl-accepting chemotaxis protein of *Escherichia coli*. J. Biochem. (Tokyo) **85**:1213–1223.
12. **Hazelbauer, G. L., and P. Engström.** 1980. Parallel pathways for transduction of chemotactic signals in *Escherichia coli*. Nature (London) **283**:98–100.
13. **Hazelbauer, G. L., and S. Harayama.** 1979. Mutants in transmission of chemotactic signals from two independent receptors of *Escherichia coli*. Cell **16**:617–625.
14. **Hazelbauer, G. L., R. E. Mesibov, and J. Adler.** 1969. *Escherichia coli* mutants defective in chemotaxis toward specific chemicals. Proc. Natl. Acad. Sci. U.S.A. **64**:1300–1307.
15. **Kleene, S. J., M. L. Toews, and J. Adler.** 1977. Isolation of glutamic acid methyl ester from an *Escherichia coli* membrane protein involved in chemotaxis. J. Biol. Chem. **252**:3214–3218.
16. **Kondoh, H., C. B. Ball, and J. Adler.** 1979. Identification of a methyl-accepting chemotaxis protein for the ribose and galactose chemoreceptors of *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **76**:260–264.
17. **Kort, E. N., M. F. Goy, S. H. Larsen, and J. Adler.** 1975. Methylation of a membrane protein involved in bacterial chemotaxis. Proc. Natl. Acad. Sci. U.S.A. **72**: 3939–3943.
18. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
19. **Matsumura, P., M. Silverman, and M. Simon.** 1977. Synthesis of *mot* and *che* gene products of *Escherichia coli* programmed by hybrid ColE1 plasmids in minicells. J. Bacteriol. **132**:996–1002.
20. **O'Farrell, P.** 1975. High resolution two-dimensional electrophoresis of proteins. J. Biol. Chem. **250**:4007–4021.
21. **Parkinson, J. S.** 1978. Complementation analysis and deletion mapping of *Escherichia coli* mutants defective in chemotaxis. J. Bacteriol. **135**:45–53.
22. **Randall, L. L., and S. J. S. Hardy.** 1977. Synthesis of exported proteins by membrane-bound polysomes from *Escherichia coli*. Eur. J. Biochem. **75**:43–53.
23. **Reader, R. W., W.-W. Tso, M. S. Springer, M. F. Goy, and J. Adler.** 1979. Pleiotropic aspartate taxis and serine taxis mutants of *Escherichia coli*. J. Gen. Microbiol. **111**:363–374.
24. **Ridgeway, H. F., M. Silverman, and M. I. Simon.** 1977. Localization of proteins controlling motility and chemotaxis in *Escherichia coli*. J. Bacteriol. **132**:657–665.
25. **Silverman, M., P. Matsumura, R. Draper, S. Edwards, and M. Simon.** 1976. Expression of flagellar genes carried by bacteriophage lambda. Nature (London) **261**:248–250.
26. **Silverman, M., and M. Simon.** 1977. Chemotaxis in *Escherichia coli*: methylation of *che* gene products. Proc. Natl. Acad. Sci. U.S.A. **74**:3317–3321.
27. **Springer, M. S., M. F. Goy, and J. Adler.** 1977. Sensory transduction in *Escherichia coli*: two complementary pathways of information processing that involve methylate proteins. Proc. Natl. Acad. Sci. U.S.A. **74**:3312–3316.
28. **Springer, M. S., M. F. Goy, and J. Adler.** 1979. Protein methylation in behavioral control mechanisms and in signal transduction. Nature (London) **280**:279–284.
29. **Springer, W. R., and D. E. Koshland, Jr.** 1977. Identification of a protein methyltransferase as the *cheR* gene product in the bacterial sensing system. Proc. Natl. Acad. Sci. U.S.A. **74**:533–537.
30. **Stock, J. B., and D. E. Koshland, Jr.** 1978. A protein methylesterase involved in bacterial sensing. Proc. Natl. Acad. Sci. U.S.A. **75**:3659–3663.
31. **Van Der Werf, P., and D. E. Koshland, Jr.** 1977. Identification of a γ-glutamyl methyl ester in bacterial membrane protein involved in chemotaxis. J. Biol. Chem. **252**:2793–2795.