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Chemotactic Transducer Proteins of *Escherichia coli* Exhibit Homology with Methyl-Accepting Proteins from Distantly Related Bacteria

DAWN M. NOWLIN,[1] DAVID O. NETTLETON,[2] GEORGE W. ORDAL,[2] AND GERALD L. HAZELBAUER[1]*

Biochemistry/Biophysics Program, Washington State University, Pullman, Washington 99164-4660,[1] and School of Basic Medical Sciences and Department of Biochemistry, University of Illinois, Urbana, Illinois 61801[2]

Received 4 February 1985/Accepted 15 April 1985

Transducers are transmembrane, methyl-accepting proteins central to the chemotactic systems of the enteric bacteria *Escherichia coli* and *Salmonella typhimurium*. Methyl-accepting proteins have been reported in a number of species in addition to these enteric bacteria. Those species include *Bacillus subtilis* and *Spirochaeta aurantia*, representatives of groups that diverged from ancestral enteric bacteria and from each other very early in bacterial evolution. An antiserum that reacts with all transducers of *E. coli* precipitated specifically methyl-accepting proteins from *B. subtilis* and *S. aurantia*, indicating that these proteins share antigenic determinants with transducers of *E. coli*. In addition, analysis of tryptic peptides by high-pressure liquid chromatography revealed similarities in the regions of methyl-accepting sites for proteins from all three species. These observations imply that structural features have been preserved in the three species from transducers contained in a common ancestor of eubacteria. It is thus reasonable to predict that other flagellated, chemotactic bacteria will be found to contain methyl-accepting proteins homologous to transducers of enteric bacteria.

Intensive study of chemotaxis in the enteric bacteria *Escherichia coli* and *Salmonella typhimurium* has led to the identification of many molecular components and biochemical reactions involved in the sensory systems of those organisms (4, 13, 20, 28). However, there are many species of flagellated, motile bacteria besides *E. coli* and *S. typhimurium*, and all of them appear to be capable of chemotaxis (22, 26). Investigations of tactic mechanisms in some other bacteria have revealed many parallels to and some differences from the well-characterized system of the enteric bacteria. These observations raise the possibility that sensory systems from different bacteria consist of components that are not only analogous in function but also homologous in structure. In this study, we investigated the possibility of homology among methyl-accepting chemotaxis proteins from distantly related species.

The most extensively characterized components of the enteric sensory system are transducer proteins, which are transmembrane, methyl-accepting proteins central to both the excitation and adaptation phases of chemotactic behavior (13, 28). Transducers serve as chemoreceptors by binding attractants (14, 31) or complexes of a binding protein and attractant (19, 23). Adaptation to tactic stimuli is correlated with changes in the extent of carboxyl methylation of specific glutamyl residues in the transducer that recognized the attractant (13, 28). The amino acid sequences of the four *E. coli* transducers, deduced from the corresponding gene sequences, suggest that the proteins consist of a periplasmic, amino-terminal domain and a cytoplasmic, carboxyl-terminal domain connected by a short, membrane-spanning sequence (2, 3, 21, 24). All four transducers, Tsr, Tar, Tap, and Trg, exhibit substantial homology in their carboxyl-terminal, methyl-accepting domains (2, 3, 21, 24). The amino-terminal, attractant recognition domains of Tsr, Tar, and Tap are less similar (21), and the amino-terminal domain of Trg appears unrelated to the others (2). Thus, it seems that evolution of the various *E. coli* transducers involved conservation of the methyl-accepting domain and divergence of the ligand recognition domain.

Methyl-accepting chemotaxis proteins have been observed in a number of nonenteric species (7, 15, 16, 25, 27, 30). The level of methylation changes during adaptation to chemical stimuli, and these proteins thus appear to be analogous in function to transducers of enteric bacteria. Methyl-accepting proteins of *Caulobacter crescentus*, a close relative of enteric bacteria, are antigenically related to enteric transducers (11) and thus must contain homologous structural regions. Other species in which methyl-accepting chemotaxis proteins have been characterized are *Bacillus subtilis* (1, 10, 30) and *Spirochaeta aurantia* (16), eubacteria representative of groups that diverged from ancestral enteric bacteria and from each other very early in bacterial evolution (9, 29). In this report we show that the methyl-accepting proteins of both of these species exhibit homology with the transducer proteins of *E. coli*.

MATERIALS AND METHODS

Immunoprecipitation of radiolabeled methyl-accepting proteins. *methyl*-^{3}H-labeled membranes from *B. subtilis* OI1085 (30) or *methyl*-^{3}H-labeled *S. aurantia* M1 cells (16) were suspended in 20 mM Tris hydrochloride (pH 6.8)–5 mM $MgCl_2$–1 mM each of the protease inhibitors paratoluene sulfonyl fluoride, paratoluene sulfonic acid, and phenylmethylsulfonyl fluoride, and the cells were broken by sonication. Sodium cholate was added to a final concentration of 15%, and the mixture was incubated at room temperature for 15 min. After centrifugation for 15 min at 8,000 × *g* in an Eppendorf model 5412 centrifuge, 100 μl of anti-Trg serum (8) was added to solubilized material from 9×10^9 *S. aurantia* cells or from 33 μg of *B. subtilis* membrane protein. Subsequent incubations, washings, and analysis by sodium dodecyl sulfate-polyacrylamide gel electrophoresis with a

* Corresponding author.

separation gel with less bisacrylamide and a lower pH than usual were as previously described (8). For competition experiments, *methyl*-^{3}H-labeled material was mixed with unlabeled, detergent-solubilized extract of HB233, an *E. coli* strain which is wild type for chemotaxis, or of HB244, an *E. coli* strain in which none of the four transducers is synthesized (12). The extracts were from 6.25×10^8 or 1.25×10^9 *E. coli* cells. Our standard immunoprecipitation (8) was from an extract of 1.25×10^8 *E. coli* cells.

Analysis of tryptic peptides by HPLC. *methyl*-^{3}H-labeled samples for high-pressure liquid chromatography (HPLC) were reduced and alkylated (17) and electrophoresed on sodium dodecyl sulfate-polyacrylamide gels. The regions of the dried gels that contained *methyl*-^{3}H-labeled protein were located by fluorography. Appropriate pieces of the dried gels were excised and then rehydrated in 0.2 M *N*-ethylmorpholine acetate buffer (pH 7.5), and the protein in the rehydrated gel was digested with diphenylcarbamylchloride-treated trypsin as described previously (17). The chromatographic samples were from 1.25×10^9 cells of *E. coli* HB586, 1.5×10^{10} cells of *S. aurantia* M1, or 0.8 mg of *B. subtilis* membrane protein. Reverse-phase HPLC was done with a Beckman model 160 liquid chromatograph equipped with a Brownlee Aquapore RP-300 10-μm column (4.6 mm by 25 cm) equilibrated with 35 mM $NaPO_4$ buffer (pH 2.2) and maintained at 30°C. Peptides were eluted at a flow rate of 1 ml/min with a gradient of 0 to 100% acetonitrile. Fractions (0.5 ml) were collected, scintillation fluid was added, and samples were counted.

Analysis of tryptic peptides from *S. aurantia* proteins was difficult because adequate amounts of *methyl*-^{3}H-labeled material could be obtained only by loading in gel lanes quantities of cellular material that resulted in deformed patterns on the gels, prohibiting analysis of single *methyl*-^{3}H-labeled bands. Yields of total labeled protein from *S. aurantia* were poor, and there was the distinct possibility of incomplete tryptic digestion.

RESULTS

Immunoprecipitation of methyl-accepting proteins. An antiserum raised in a rabbit injected with purified Trg protein immunoprecipitates Tsr and Tar as well as Trg (8), indicating that the antibodies in the serum recognize structural features common to carboxyl-terminal domains of all transducers in *E. coli*. We reasoned that this antiserum would be an appropriate probe for conserved structure in methyl-accepting proteins of bacteria distantly related to *E. coli*.

Methyl-accepting proteins have been identified as components of the respective sensory systems for both *B. subtilis* (1, 10, 30), a gram-positive organism, and *S. aurantia* (16), a spirochete. These proteins appear on sodium dodecyl sulfate-polyacrylamide gels as a group of bands with apparent molecular sizes in the range of 60,000 to 70,000 daltons. We radiolabeled methyl-accepting chemotaxis proteins of *B. subtilis* (30) and *S. aurantia* (16) in vivo with [*methyl*-^{3}H]methionine in the presence of an inhibitor of protein synthesis. The anti-Trg serum immunoprecipitated these *methyl*-^{3}H-labeled proteins from detergent-solubilized samples of both species (Fig. 1A and B, lanes 1 and 2), whereas no precipitation was observed with serum obtained from the rabbit before immunization (data not shown). The presence of an extract of wild-type *E. coli* containing an excess of unlabeled transducers greatly reduced the amount of radiolabeled protein from *B. subtilis* or *S. aurantia* in the immunoprecipitates (Fig. 1A and B, lanes 3 and 4), whereas substantial radiolabeled protein from either species was precipitated in the presence of an equivalent amount of extract from an *E. coli* mutant (12) that lacked all four transducers (Fig. 1A and B, lanes 5). These competition experiments indicate that precipitation reflects the interaction of antibodies specific for *E. coli* transducers with methyl-accepting proteins of *B. subtilis* and *S. aurantia*. Formation of precipitating immune complexes indicates that more than one and probably several antigenic determinants on the methyl-accepting proteins are recognized by antibodies in the serum, thus creating the cross-linked precipitate. The anti-Trg serum precipitated every band of methyl-accepting chemotaxis protein from both species, although precipitation was inefficient for the fastest migrating band in the pattern from *S. aurantia* (Fig. 1). Thus, each form contained antigenic sites sufficient for precipitation. As in *E. coli*, the multiple bands from these species are likely to reflect both the presence of more than one kind of transducer, each with a slightly different electrophoretic migration, and multiple electrophoretic forms of each transducer resulting from different extents of covalent modification (4, 13, 20, 28). Precipitation of *B. subtilis* or *S. aurantia* proteins by anti-Trg serum is only about 10% as efficient as precipitation of Tsr or Tar proteins of *E. coli* (8), indicating that interspecies homology is less extensive than the 60% amino acid identity between the carboxyl-terminal domains of Trg and Tsr or Tar (2).

Analysis of methylated tryptic peptides. Anti-Trg serum reacts equally well with the various forms of single transducers from *E. coli* that differ in the extent of methylation (8). Therefore, the principal antigenic determinants that have been recognized are unlikely to be methyl-accepting residues themselves. However, because methylation is a common feature of the proteins recognized, regions containing methyl-accepting glutamyl residues are likely candidates for homology between methyl-accepting proteins from different species. For the transducers of *E. coli*, the tryptic peptides containing the methyl-accepting sites of each protein are very similar (2, 21). These long (23- or 25-residue) peptides of Tsr, Tar, and Trg elute from a reversed-phase HPLC column at almost the same concentration of acetonitrile, after most other peptides derived from those proteins (17, 18). The modified peptides appear as a series of *methyl*-^{3}H-labeled peaks corresponding to differing extents of modification (Fig. 2A; 17, 18). We analyzed *methyl*-^{3}H-labeled tryptic peptides in the polypeptides present in the fastest migrating band of the methyl-accepting chemotaxis proteins of *B. subtilis* by HPLC and observed that the major species of methyl-labeled peptides eluted at positions similar to those for the transducers of *E. coli* (Fig. 2). This indicates that the amino acid composition and the positions of trypsin cleavage in the region surrounding some methyl-accepting sites are similar in the proteins from *E. coli* and *B. subtilis*. Tryptic digestion of the full complement of methyl-accepting chemotaxis protein bands from *B. subtilis* (all bands in Fig. 1A, lane 1) generated more *methyl*-^{3}H-labeled peptides than shown in Fig. 2B (data not shown), but the additional peaks were clustered near the peaks evident in Fig. 2B and thus may represent peptides of similar properties. The similarities in the chemical nature and size of some peptides containing methyl-accepting sites of the *E. coli* and *B. subtilis* proteins were consistent with the observation that methyl transferase from *E. coli* and *B. subtilis* catalyze methylation in vitro of methyl-accepting proteins from the other species (5).

Analysis of tryptic peptides from methyl-accepting chemotaxis proteins of *S. aurantia* was technically difficult because of the low cellular content of these proteins and was

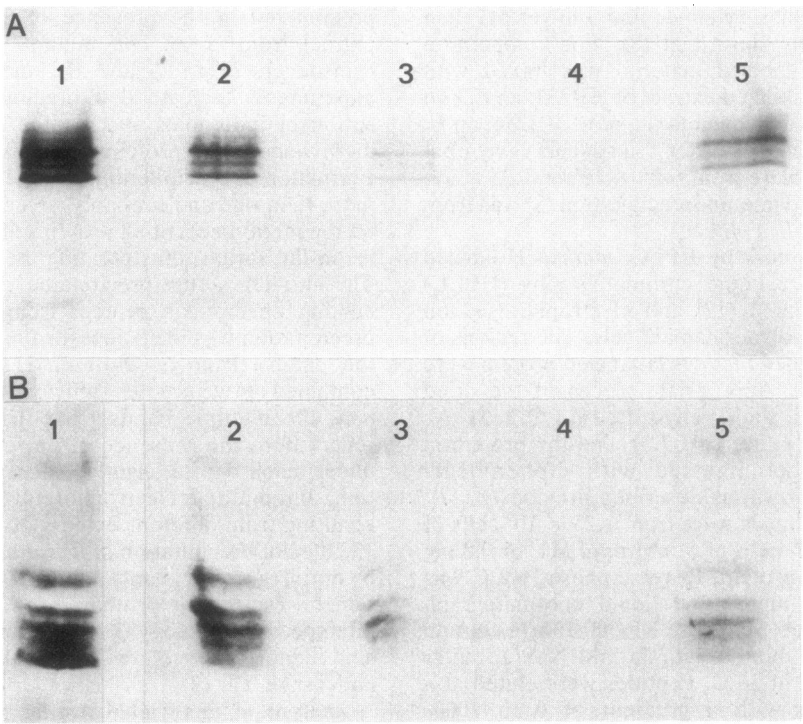

FIG. 1. Immunoprecipitation of methyl-accepting chemotaxis proteins of *B. subtilis* and *S. aurantia* by an antiserum raised to the Trg transducer of *E. coli*. The figures are fluorographs of the region of the gel containing the methyl-accepting chemotaxis proteins. The methyl-labeled bands from *S. aurantia* migrated with apparent molecular sizes between 55,000 and 65,000 daltons, in the same range observed for the transducers of *E. coli*, whereas the methyl-labeled bands from *B. subtilis* appear to be approximately 10,000 daltons larger. (A) Methyl-accepting proteins of *B. subtilis*. Lanes: 1, *methyl*-^{3}H-labeled protein (5.1 μg of membrane protein in sample); 2, immunoprecipitate of *methyl*-^{3}H-labeled protein; 3, immunoprecipitate of *methyl*-^{3}H-labeled protein in the presence of an unlabeled *E. coli* extract that contained transducer molecules in a fivefold excess relative to the antiserum; 4, immunoprecipitate as in lane 3 except that a tenfold excess of unlabeled *E. coli* extract was present; 5, immunoprecipitate as in lane 4 except that the *E. coli* extract was from a mutant that lacked all four transducers. (B) Methyl-accepting proteins of *S. aurantia*. Lanes: 1, *methyl*-^{3}H-labeled protein (1.7×10^8 cells in sample); 2, immunoprecipitate of *methyl*-^{3}H-labeled proteins; 3 to 5, immunoprecipitates of *methyl*-^{3}H-labeled proteins in the presence of unlabeled *E. coli* extracts as described for panel A, lanes 3 to 5.

limited to samples containing the complete spectrum of *methyl*-^{3}H-labeled bands visible in Fig. 1B (see above). The pattern shown in Fig. 2C includes the major species that were routinely observed. The radioactivity in fraction 12 is at the position of free methanol that could be produced by demethylation occurring during processing of the sample. A peak at that position is sometimes observed in patterns of *E. coli* protein. Variation was observed in the relative sizes of peaks and in the number of minor peaks surrounding the major ones (Fig. 2C). In any case, the peptides eluting in fractions 100 to 125 (Fig. 2C), which were always detected, could be *S. aurantia* homologs to the major *methyl*-^{3}H-labeled peptides from *E. coli* and *B. subtilis* (Fig. 2A and B).

DISCUSSION

The three species considered here include representatives of the two major groups into which bacteria are traditionally divided, gram negative and gram positive, as well as a representative spirochete, motile bacteria with no external flagella but with flagellalike filaments intercalated between an inner and outer membrane (6). Homology among methyl-accepting chemotaxis proteins of these divergent species implies that structural features have been preserved from proteins contained in a common ancestor. Such an ancestral cell would have existed at a very early time in the course of evolution. Analysis of phylogenetic relationships by using sequences of rRNA (9, 29) has led to the identification of a minimum of eight major groups of eubacteria, each about equally related. It appears that these groups originated and diverged 3.5 to 3.8 billion years ago (29). The species considered in these studies are representatives of three of the major lines of eubacterial descent. Thus, our findings imply that homologous regions of transducers in contemporary bacteria have been conserved from a protein present in a common eubacterial ancestor extant more than 3.5 billion years ago. It is an intriguing possibility that the protein from which contemporary transducers are descended was itself a transducer functioning as part of a sensory system in the earliest eubacterial cell.

Because homology has been observed among transducer proteins from species that are thought to be related only through the ancestor common to all eubacteria, it seems reasonable to expect homology to extend to transducers in other species related through the same common ancestor, that is, all eubacteria. We believe that it is very likely that the chemotactic systems of most, if not all, flagellated eubacteria will be found to include methyl-accepting transducer proteins and that these transducers will exhibit homology with transducers that have already been characterized. Thus we expect, as current studies reveal, that at least some, if not most, aspects of chemotactic mechanisms are common to all flagellated eubacteria.

The existence of analogous or even homologous methyl-accepting proteins in species outside the eubacterial king-

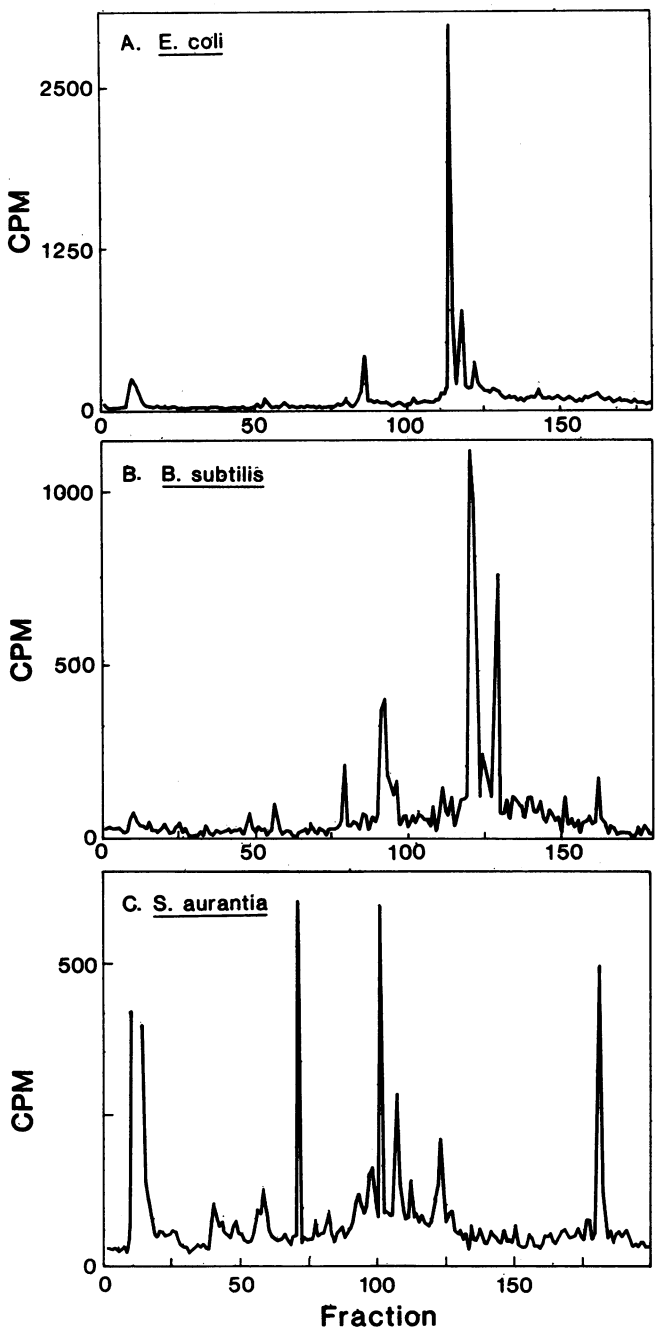

FIG. 2. Tryptic peptide maps of *methyl*-^{3}H-labeled, methyl-accepting chemotaxis proteins of *E. coli*, *B. subtilis*, and *S. aurantia*. (A) Tryptic peptide map of *E. coli* Trg methyl-accepting chemotaxis protein. Trg protein was produced in HB586, a *tsr tar tap* mutant that contains pTH57, a pBR322-*trg* hybrid plasmid (8). The sample was a mixture of all the electrophoretically resolved forms of Trg (17). In such a mixture a few forms of the two methyl-accepting tryptic peptides dominate the pattern. (B) Tryptic peptide map of *B. subtilis* methyl-accepting protein. The sample corresponded to the lower, most intense band seen on fluorograms (see Fig. 1A, lane 1). (C) Tryptic peptide map of *S. aurantia* methyl-accepting proteins. The sample corresponded to all the *methyl*-^{3}H-labeled bands of apparent molecular sizes between 55,000 and 65,000 daltons (see Fig. 1B, lane 1).

dom (9) is an intriguing possibility. Investigations of this possibility are in progress.

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LITERATURE CITED

1. **Ahlgren, J. A., and G. W. Ordal.** 1983. Methyl esterification of glutamic acid residues of methyl-accepting chemotaxis proteins in *Bacillus subtilis*. Biochem. J. **213**:759–763.
2. **Bollinger, J., C. Park, S. Harayama, and G. L. Hazelbauer.** 1984. Structure of the Trg protein: homologies with and differences from other sensory transducers of *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **81**:3287–3291.
3. **Boyd, A., K. Kendall, and M. I. Simon.** 1983. Structure of the serine chemoreceptor in *Escherichia coli*. Nature (London) **301**:623–626.
4. **Boyd, A., and M. I. Simon.** 1982. Bacterial chemotaxis. Annu. Rev. Physiol. **44**:501–517.
5. **Burgess-Cassler, A., and G. W. Ordal.** 1982. Functional homology of *Bacillus subtilis* methyltransferase II and *Escherichia coli* cheR protein. J. Biol. Chem. **257**:12835–12838.
6. **Canale-Parola, E.** 1978. Motility and chemotaxis of spirochetes. Annu. Rev. Microbiol. **32**:69–99.
7. **Craven, R. C., and T. C. Montie.** 1983. Chemotaxis of *Pseudomonas aeruginosa*: involvement of methylation. J. Bacteriol. **154**:780–786.
8. **Engström, P., D. Nowlin, J. Bollinger, N. Magnuson, and G. L. Hazelbauer.** 1983. Limited homology between Trg and other transducer proteins of *Escherichia coli*. J. Bacteriol. **156**:1268–1274.
9. **Fox, G. E., E. Stakebrandt, R. B. Hespell, J. Gibson, J. Moniloff, T. A. Dyer, R. S. Wolfe, W. E. Balch, R. S. Tanner, L. J. Magrum, L. B. Zablen, R. Blakemore, R. Gupta, L. Bonen, B. J. Lewis, D. A. Stahl, K. R. Luehrsen, K. N. Chen, and C. R. Woese.** 1980. The phylogeny of prokaryotes. Science **209**:457–463.
10. **Goldman, D. J., S. W. Worobec, R. V. Hecker, R. B. Siegel, and G. W. Ordal.** 1982. Chemotaxis in *Bacillus subtilis*: effects of attractants on the level of methylation of methyl-accepting chemotaxis proteins and the role of demethylation in the adaptation process. Biochemistry **21**:915–920.
11. **Gomes, S. L., and L. Shapiro.** 1984. Differential expression and positioning of chemotaxis methylation proteins in *Caulobacter*. J. Mol. Biol. **178**:551–568.
12. **Hazelbauer, G. L., P. Engström, and S. Harayama.** 1981. Methyl-accepting chemotaxis protein III and transducer gene *trg*. J. Bacteriol. **145**:43–49.
13. **Hazelbauer, G. L., and S. Harayama.** 1983. Sensory transduction in bacterial chemotaxis. Int. Rev. Cytol. **81**:33–70.
14. **Hedblom, M. L., and J. Adler.** 1980. Genetic and biochemical properties of *Escherichia coli* mutants with defects in serine chemotaxis. J. Bacteriol. **144**:1048–1060.
15. **Hirota, N.** 1984. Chemotaxis in thermophilic bacterium PS-3. J. Biochem. **96**:645–650.
16. **Kathariou, S., and E. P. Greenberg.** 1983. Chemoattractants elicit methylation of specific polypeptides in *Spirochaeta aurantia*. J. Bacteriol. **156**:95–100.
17. **Kehry, M. R., and F. W. Dahlquist.** 1982. The methyl-accepting chemotaxis proteins of *Escherichia coli*. Identification of the multiple methylation sites on methyl-accepting chemotaxis protein I. J. Biol. Chem. **257**:10378–10386.
18. **Kehry, M. R., P. Engström, F. W. Dahlquist, and G. L. Hazelbauer.** 1983. Multiple covalent modifications of Trg, a sensory transducer of *Escherichia coli*. J. Biol. Chem.

258:5050–5055.
19. **Koiwai, O., and H. Hayashi.** 1979. Studies on bacterial chemotaxis. IV. Interaction of maltose receptor with a membrane-bound chemosensing component. J. Biochem. **86**:27–34.
20. **Koshland, D. E., Jr.** 1981. Biochemistry of sensing and adaptation in a simple bacterial system. Annu. Rev. Biochem. **50**:765–782.
21. **Krikos, A., N. Mutoh, A. Boyd, and M. I. Simon.** 1983. Sensory transducers of *E. coli* are composed of discrete structural and functional domains. Cell **33**:615–622.
22. **Pfeffer, W.** 1904. Pflanzenphysiologie, 2nd ed., vol. 2. Wilhelm Engleman, Leipzig, Germany.
23. **Richarme, G.** 1982. Interaction of the maltose-binding protein with membrane vesicles of *Escherichia coli*. J. Bacteriol. **149**:662–667.
24. **Russo, A. F., and D. E. Koshland, Jr.** 1983. Separation of signal transduction and adaptation functions of the aspartate receptor in bacterial sensing. Science **220**:1016–1020.
25. **Schimz, A.** 1981. Methylation of membrane proteins is involved in chemosensory and photosensory behavior of *Halobacterium halobium*. FEBS Lett. **125**:205–207.
26. **Seymour, F. W. K., and R. N. Doetsch.** 1973. Chemotactic responses by motile bacteria. J. Gen. Microbiol. **78**:287–296.
27. **Shaw, P., S. L. Gomes, K. Sweeney, B. Ely, and L. Shapiro.** 1983. Methylation involved in chemotaxis is regulated during *Caulobacter* differentiation. Proc. Natl. Acad. Sci. U.S.A. **80**:5261–5265.
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. **Stackebrandt, E., and C. R. Woese.** 1982. The evolution of prokaryotes, p. 1–30. *In* M. J. Carlile, J. F. Collins, and B. E. B. Moseley (ed.), Molecular and cellular aspects of microbial evolution. Cambridge University Press, Cambridge.
30. **Ullah, A. H. J., and G. W. Ordal.** 1981. In vivo and in vitro chemotactic methylation in *Bacillus subtilis*. Biochem. J. **199**:795–805.
31. **Wang, E. A., and D. E. Koshland, Jr.** 1980. Receptor structure in the bacterial sensing system. Proc. Natl. Acad. Sci. U.S.A. **77**:7157–7161.