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Salt taxis in *Escherichia coli* bacteria and its lack in mutants

(chemotaxis/osmotaxis/chemoreceptors/methyl-accepting chemotaxis-protein mutants)

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ABSTRACT ***Escherichia coli* is attracted to a variety of salts. This attraction is highly reduced in mutants missing a known transducer, the methyl-accepting chemotaxis protein I; there is a smaller role for another transducer, the methyl-accepting chemotaxis protein II. We discuss the relation of salt taxis to osmotaxis.**

All organisms require salt. The search for salt is universal: those organisms that can move are attracted to it, and those that cannot move grow toward it. There is an optimum concentration of salt; too little and too much are both avoided (for reviews see refs. 1–5).

Already 100 years ago Pfeffer discovered that bacteria are attracted to inorganic salts (6). Now we report attraction to a large variety of salts in *Escherichia coli*, a bacterium we use here to explore the biochemical and genetic mechanisms. Although the threshold for salt taxis is high, this is a powerful attraction, in some cases nearly as strong as for the best *E. coli* attractants known, the amino acids L-aspartate and L-serine. The optimum concentration for salt taxis, between 10 mM and 100 mM, is quite similar to the optimum for salt taste in animals (3).

An unanswered question in biology is how salts are sensed. We have studied salt taxis in various sensory mutants of *E. coli*, and here we report that taxis toward salts requires a protein already known to be the receptor for L-serine, the methyl-accepting chemotaxis protein I (MCP I) (7, 8). In addition, there is a smaller role for MCP II, the L-aspartate receptor (7, 8). Just how these proteins act to detect salts remains to be discovered.

MATERIALS AND METHODS

Bacteria were grown at 35°C with shaking in tryptone broth (1% Difco tryptone and 0.5% NaCl) to an optical density at 590 nm of 0.55–0.65. (Cells grown in Vogel–Bonner minimal lactate medium were sometimes used, and these gave similar results.) Then the cells were centrifuged at 6,000 × *g* for 3 min, the supernatant fraction was discarded, the pellet was resuspended, and chemotaxis medium (1 mM potassium phosphate, pH 7.0/0.1 mM potassium EDTA) was added. L-Methionine (1 mM) was included in the chemotaxis medium because growth in tryptone broth (which contains methionine) tends to repress its synthesis, and it is known that methionine is required for chemotaxis (9). This was followed by two more such washes in chemotaxis medium, and finally the bacteria were resuspended in chemotaxis medium at an optical density at 590 nm of 0.01 (about 7×10^6 bacteria per ml). Such procedures have been described in detail (10).

Bacteria in a pond containing this chemotaxis medium were tested for the ability to be attracted into a capillary containing the chemotaxis medium plus various concentrations of salt. (Stock solutions of each 1 M salt had been adjusted to pH 7.0.) This was done for 30 min at 30°C; then the capillary contents were plated, and colonies were counted the next day—all as described for the capillary assay (10). The experimental points were carried out in triplicate; sizes of errors are indicated in the figure legends.

The strains of *E. coli* K-12 used here are chemotactically wild-type AW405 (11); *tsr* mutants AW518 (11), AW641 (12), AW642 (12), and RP5882 (13); *tar* mutants AW539 (11), AW631 (12), and AW633 (12); *trg* mutants AW701 (14) and AW703 (14); *tap* mutant RP3525 (15); and phosphotransferase enzyme I mutant AW509, also called X17 (16, 17).

RESULTS

Salt Taxis in Chemotactically Wild-Type Bacteria. *E. coli* that is wild type for chemotaxis was attracted into a capillary containing inorganic salts, for example NaCl (Fig. 1). The threshold is 0.1–1 mM, roughly 10^4-fold higher than the thresholds for the amino acids L-aspartate or L-serine. There is an optimum attraction when the capillary contains 100 mM salt; since the salt diffuses out of the capillary, the optimum concentration must actually be somewhat less than 100 mM. The magnitude of this optimum response approaches that for aspartate and serine, but the concentration of the optimum is 10–100 times above that for the amino acids. Higher concentrations of salts, for example 400 mM, are less attractive; indeed, they are known to be repellents (18). Lower concentrations are also less attractive, and in fact pure water is avoided (data not shown here, but see figure 1 in ref. 19).

Effect of cations. Chloride salts of the various alkali metals were tested as attractants (Fig. 1). Each was best at 100 mM, and this order of attractiveness was found: LiCl > NaCl > KCl > RbCl > CsCl. This is the same as the sequence of alkali metals in increasing atomic number: Li < Na < K < Rb < Cs.

NH_4Cl was an even better attractant than LiCl (Fig. 1); indeed NH_4^+ salts were the best inorganic attractants found for *E. coli* so far. Taxis toward NH_4Cl is the only inorganic-ion attraction that had been described for *E. coli* (11).

The various monovalent-cation nitrate salts were found to be attractants too, and these data are entirely similar to those presented for the chlorides. The peak concentrations were all at 100 mM in this decreasing order: NH_4NO_3, 42,500 bacteria in the capillary; $LiNO_3$, 41,000; $NaNO_3$, 25,200; KNO_3, 13,500; $RbNO_3$, 10,500; and $CsNO_3$, 9500 (data for other concentrations not shown).

Several divalent cations were also found to be attractants (Fig. 2). This sequence of effectiveness was determined: $MgCl_2 > SrCl_2 > BaCl_2 > CaCl_2$. The peak concentrations for divalent cations were 10 mM, not 100 mM as found for monovalent cations. We found that $CoCl_2$ and $NiCl_2$ failed to attract *E. coli* (data not shown), and indeed Co^{2+} and Ni^{2+}, as well as Mn^{2+} and Zn^{2+}, had previously been shown to be repellents sensed by MCP II (7, 20, 21).

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Abbreviation: MCP I, II, III, and IV, methyl-accepting chemotaxis proteins I, II, III, and IV.

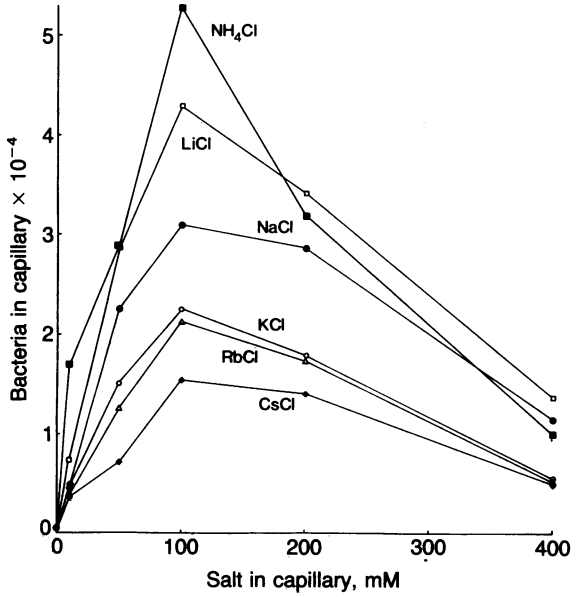

FIG. 1. Chemotaxis toward monovalent-cation chloride salts by chemotactically wild-type *E. coli* (strain AW405). For 30 min at 30°C, bacteria were attracted into capillaries, each containing a salt. This was all done together in a single experiment. In the absence of salt in the capillary, typically 600 ± 200 bacteria accumulated in the capillary; this value has not been subtracted from any of the data presented in this paper. All points were carried out in triplicate; the smallest error encountered was ±3%, the largest error was ±16%, and the average was ±6%.

Organic cations were attractants too. Choline·HCl, L-arginine·HCl, and L-lysine·HCl, all at pH 7, gave accumulations in the capillaries of 19,400, 16,000, and 11,100 at their peak concentrations of 100 mM, 50 mM, and 50 mM cation, respectively (data for other concentrations not shown). Previously we showed that these two amino acids fail to attract *E. coli* (11), but that was done with 10 mM potassium phosphate rather than at the 1 mM concentration used here (see below).

In addition to using the capillary assay, we also observed the effect of added salt solution under the microscope by use of a television screen; and then we analyzed those videotape

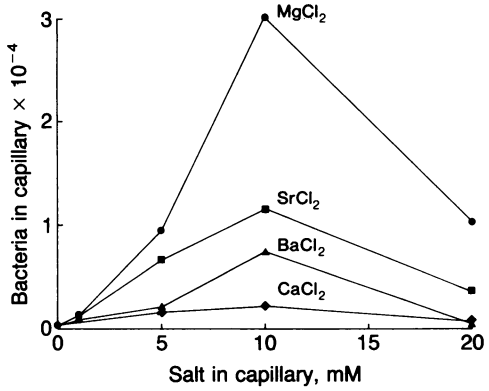

FIG. 2. Chemotaxis toward divalent-cation chloride salts by chemotactically wild-type *E. coli* (strain AW405). For 30 min at 30°C, bacteria were attracted into capillaries, each containing a salt. All points were carried out in triplicate; the smallest error encountered was ±6%, the largest error was ±25%, and the average was ±9%. This was all done together in a single experiment, where 100 mM NaCl as a control attracted 33,000 bacteria into the capillary. (Chelation of such high concentrations of divalent ion by the 0.1 mM EDTA would reduce the concentration of free divalent ion by only a little.)

data by the motion-analysis assay (22). Because *E. coli* are very smooth in our medium, the cells were first made tumblier by adapting to the repellent L-leucine (30 mM). (Initially the bacteria become very tumbly in response to repellent; then they adapt, and for unknown reasons they adapt to a long-lasting, rather tumbly state, which was used here at 5 min after exposure to the leucine.) Addition of 20, 40, or 80 mM LiCl (plus 30 mM leucine to keep its concentration constant) caused *E. coli* to run smoothly with suppression of angular speed (the equivalent of tumbling) for 30, 50, or >70 sec (data not shown). Dilution of 80 mM LiCl or 10 mM $MgCl_2$ to 20 mM and 2 mM, respectively, caused tumbling (data not shown). These are the results we would have expected from the data obtained by capillary assays.

Effect of anions. To study the effect of varying the anion, we measured chemotaxis to various sodium halides (Fig. 3). Cl^- is best, while F^-, Br^-, and I^- are roughly equal. There is no dependence on atomic number of the halides: F < Cl < Br < I.

A similar experiment was carried out with the various potassium halides, and similar results were obtained: at the peak concentration (100 mM), Cl^- was the best (23,000 bacteria in the capillary); then the sequence was Br^- (14,000 bacteria) > F^- (10,200 bacteria) > I^- (8000 bacteria) (data for other concentrations not shown).

Sodium and potassium salts of polyvalent inorganic anions, the phosphates and sulfates, were attractants too. The peak concentration in each case was at 100 mM of monovalent cation for sodium phosphate (pH 7), potassium phosphate (pH 7), sodium sulfate, and potassium sulfate; these gave respectively accumulations of 19,000, 16,700, 19,000, and 13,500 bacteria in the capillaries (data for other concentrations not shown). Thus, sodium or potassium phosphates or sulfates are about as effective as halides other than chlorides.

Salts of organic anions were also tested. The peak concentrations of sodium and cesium L-malate were at 100 mM of monovalent cation and gave accumulations of 25,500 and 5200 bacteria, respectively (data for other concentrations not shown). Previously we reported that L-malate was an attractant (11), but now it appears that this is due in part to its positive charge since the sodium salt was more effective than the cesium salt, as with the chlorides and the nitrates.

Bacteria can detect both cations and anions. A comparison of various cations of a common anion shows differences in response (Fig. 1). For example, NH_4Cl is a better attractant than CsCl. Thus, bacteria sense each of the cations in varying degrees of strength. Similarly, a comparison of the various

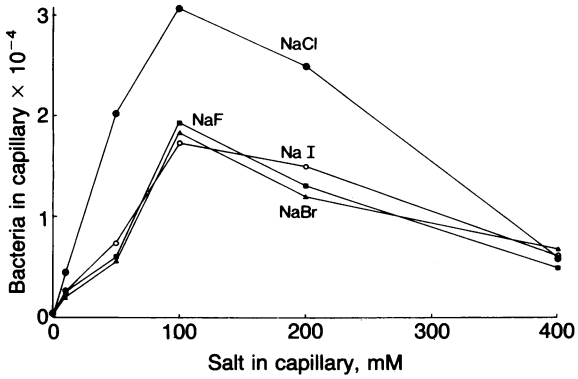

FIG. 3. Chemotaxis toward sodium halide salts by chemotactically wild-type *E. coli* (strain AW405). For 30 min at 30°C, bacteria were attracted into capillaries, each containing a salt. All points were carried out in triplicate; the smallest error encountered was ±4%, the largest error was ±25%, and the average was ±12%. The data for NaBr were obtained in a separate experiment and then normalized by comparison with NaCl present in both experiments.

anions of a common cation also shows differences, though much smaller than for cations (Fig. 3). For example, NaCl is a better attractant than NaBr. In general, Cl^- is preferred over other anions.

Another way to study this is to place the same anion in the bacterial suspension and the capillary, but to have one cation in the bacterial suspension and a different cation in the capillary; or to place the same cation in the bacterial suspension and the capillary but one anion in the bacterial suspension and a different anion in the capillary. For example, bacteria are attracted into a capillary containing 50 mM NH_4Cl when the bacterial suspension contains 50 mM NaCl, but not *vice versa*; and bacteria are attracted into a capillary containing 50 mM NaCl when the bacterial suspension contains 50 mM NaI, but not *vice versa* (data not shown). So also this method tells that bacteria can discriminate both among cations and among anions.

Inhibition of salt taxis by other salts. If two attractants are sensed by the same receptor, then placing one in both the capillary and the bacterial suspension (at an equal, saturating concentration) will inhibit taxis toward another present only in the capillary, while if the two are sensed by different receptors, there will be little or no inhibition; this is called a "competition experiment" (16, 23).

Such experiments were done with 10 mM or 50 mM NaCl in both the capillary and bacterial suspension, and in the capillary there was 100 mM of KCl, NH_4Cl, $LiNO_3$, KF, potassium phosphate, Na_2SO_4, choline·HCl, L-lysine·HCl, or L-arginine·HCl. There was always severe inhibition: 50–90% inhibition by 10 mM NaCl and 80–99% inhibition by 50 mM NaCl. There was no inhibition of motility as judged by microscopic observation. Those results indicate that NaCl and all of these chemicals are sensed by the same receptor.

When both the capillary and bacterial suspension contained NaCl (10 mM or 50 mM), attraction to $MgCl_2$ in the capillary (10 mM) was inhibited by 92% and 97%, respectively. In the reciprocal experiment, when both the capillary and bacterial suspension contained $MgCl_2$ (5 or 10 mM), attraction to NaCl in the capillary (100 mM) was inhibited by 98% and 99%, respectively. These results indicate that NaCl and $MgCl_2$ are sensed by the same receptor.

In contrast, NaCl in both the capillary and bacterial suspension (10 mM or 50 mM) inhibited attraction to 10 mM L-aspartate only slightly (7% and 12%, respectively). NaCl inhibited attraction to 10 mM L-serine somewhat more strongly (26% and 35%, respectively); this may suggest a stronger interaction of salts and serine at the MCP I level.

Sucrose at 50 mM in both the capillary and bacterial suspension inhibited taxis toward 100 mM NaCl in the capillary only slightly (3%). This helps to show that osmotaxis (exemplified by sucrose taxis) and salt taxis are different phenomena (see review of osmotaxis in *Discussion*).

In past work we had used 10 mM potassium phosphate (pH 7.0; plus 0.1 mM EDTA) for the chemotaxis medium (10), while in this report we use just 1 mM potassium phosphate (pH 7.0; plus 0.1 mM EDTA). That change from 10 mM to 1 mM made possible this discovery of salt taxis. (A further reduction to 0.1 mM did not help.) Potassium phosphate at 10 mM (or any other salt at 10 mM) competes with salt taxis severely (see competition by NaCl above).

Effect of bacterial concentration. Here we use *E. coli* at an optical density at 590 nm of 0.01 (about 7×10^6 bacteria per ml). In earlier work, *E. coli* was used at 10 times this concentration (10). We now find that this higher concentration of bacteria reduces NaCl taxis by more than 20-fold. That results from inhibition by materials given off by the bacteria (probably salts), since a supernatant fraction from bacteria at this higher concentration (7×10^7 bacteria per ml) inhibited NaCl taxis. Lowering the concentration of bacteria further, to half that used in the present report, did not stimulate salt taxis significantly. Thus, about 7×10^6 bacteria per ml is optimal for this study.

Salt Taxis in Receptor Mutants. Mutants missing each of various known chemotaxis transducers were tested for taxis to LiCl (Fig. 4 *Left*) and $MgCl_2$ (Fig. 4 *Right*). This includes *tsr* mutants missing MCP I—the receptor for the attractant L-serine and for certain repellents (7, 8); *tar* mutants missing MCP II—the receptor for the attractant L-aspartate, for the attractant maltose by way of its binding protein, and for certain other repellents (7, 8); *trg* mutants missing MCP III—the receptor for the attractants D-galactose and D-ribose by way of their binding proteins (14, 24); a *tap* mutant missing MCP IV—the receptor for dipeptides by way of a binding protein (15); and an enzyme I mutant defective in the phosphotransferase system, which is needed for taxis toward certain sugars (17).

The only mutants seriously disturbed in salt taxis were MCP I mutants: AW518 (Fig. 4) as well as AW641, AW642, and the MCP I deletion RP5882 (data not shown). There was a 95% loss of taxis toward LiCl (Fig. 4 *Left*) and $MgCl_2$ (Fig.

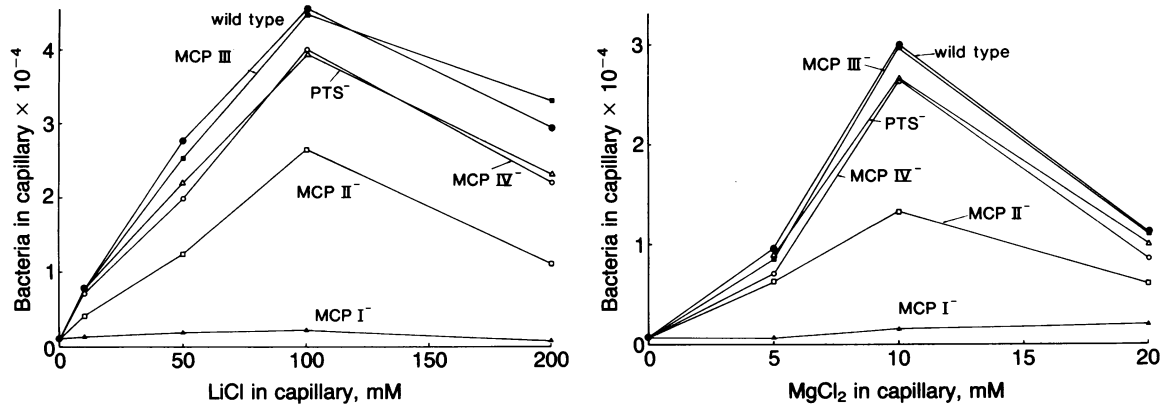

FIG. 4. Chemotaxis toward LiCl (*Left*) and $MgCl_2$ (*Right*) in receptor mutants. For 30 min at 30°C, bacteria were attracted into capillaries, each containing a salt. ●, Wild type (AW405); ▲, *tsr* or MCP I^- (AW518); □, *tar* or MCP II^- (AW539); ■, *trg* or MCP III^- (AW701; similar results were obtained with AW703); ○, *tap* or MCP IV^- (RP3525); △, phosphotransferase enzyme I^- (AW509). All points were carried out in triplicate; the smallest error encountered was ±5%, the largest error was ±22%, and the average was ±7%. To compare *Left* and *Right*, we used NaCl with wild-type bacteria in both; 100 mM NaCl attracted 35,000 wild-type bacteria into the capillary in the *Left* experiment and 33,500 in the *Right* experiment. In a similar experiment, taxis toward 1 mM L-serine was found to be 52,000, 1000, 53,500, 54,400, 42,000, and 45,000 bacteria in the capillary in 30 min for the same strains (wild type, *tsr*, *tar*, *trg*, *tap*, and enzyme I^-, respectively). Taxis toward 1 mM L-aspartate was found to be 76,000, 70,500, 2000, 74,900, 65,500, and 66,600 bacteria in the capillary in 30 min for the same strains, respectively.

4 *Right*) as well as a 90–98% loss of taxis toward NH_4Cl, $LiNO_3$, RbCl, CsCl, NaF, NaCl, NaBr, NaI, $NaNO_3$, sodium phosphate (pH 7), Na_2SO_4, KF, KCl, KBr, KI, KNO_3, potassium phosphate (pH 7), $BaCl_2$, $CaCl_2$, choline·HCl, L-lysine·HCl, L-arginine·HCl, and sodium L-malate. (Taxis toward L-aspartate, an MCP II attractant used as a positive control, was normal.)

Mutants missing MCP II (strains AW539, AW631, and AW633) are also disturbed in salt taxis (Fig. 4 *Left* for LiCl and Fig. 4 *Right* for $MgCl_2$ in AW539). There was a 20–50% loss in monovalent-cation salt taxis (NH_4Cl, LiCl, NaCl, KCl, RbCl, CsCl, $LiNO_3$, choline·HCl, L-arginine·HCl, and L-lysine·HCl), and a 60–80% loss in divalent-cation salt taxis ($MgCl_2$, $BaCl_2$, and $CaCl_2$). (Taxis toward L-serine, an MCP I attractant used as a positive control, was normal.) Apparently both MCP I and MCP II play a role in salt taxis, though the role of MCP II is relatively minor. However, MCP III, MCP IV, and the phosphotransferase system appear not to be involved (Fig. 4). In *Salmonella typhimurium* and *E. coli* the receptor for taxis toward divalent cations was reported to be the Mg^{2+}/Ca^{2+}-dependent adenosine triphosphatase (25), but in *E. coli* we found that the mutant AN120 (26) missing this activity was normal for Mg^{2+} taxis (data not shown).

The MCP I mutants fail to show attraction to L-serine or repulsion by MCP I repellents as expected, but they did show a residual taxis (typically around 5–10% of wild type) toward all of the salts tested (and named above). One possibility is that this residual taxis to salts in MCP I mutants is carried out by MCP II; a second possibility is that this represents positive osmotaxis—i.e., the search for an optimum osmolarity (18, 19) (see *Discussion*).

It is known that attractants handled by MCP bring about methylation of the MCP (7, 8), and that this increase in methylation is responsible for adaptation to these attractants (27). A role of MCP in salt taxis was demonstrated directly by the finding that addition of salts to wild-type bacteria (AW405) causes an increased methylation of MCP (Fig. 5). Separation of the several MCPs (7) in the wild-type strain (AW405) shows that it is almost entirely MCP I that is methylated by addition of the salts LiCl (80 mM) or $MgCl_2$ (10 mM) (data not shown). In an MCP II mutant (AW539), the MCP I was methylated upon addition of LiCl or $MgCl_2$; in an MCP I mutant (AW518), LiCl or $MgCl_2$ brought about a slight methylation of MCP (data not shown). Thus, also by this direct measure, salt taxis goes mainly through MCP I but to a minor degree through other MCP.

Most likely salts interact directly with MCP, rather than indirectly by way of a salt-binding protein, since salt taxis survived an osmotic shock procedure (28) known to remove periplasmic proteins that interact with attractants and then bind to MCP (data not shown).

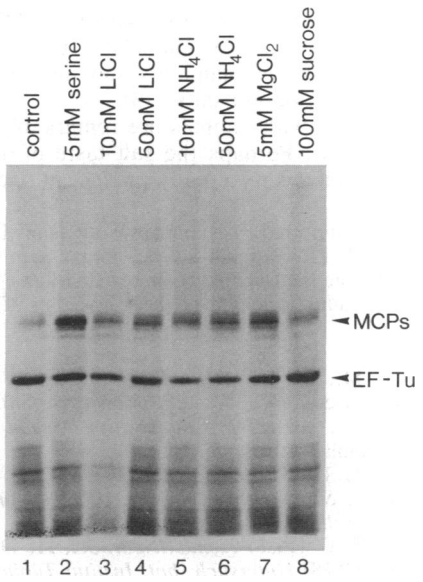

FIG. 5. Methylation of MCP induced by salts. Wild-type (AW405) bacteria were grown in tryptone broth containing 0.5% glycerol and then transferred into 1 mM potassium phosphate, pH 7.0/0.1 mM potassium EDTA/50 μg of chloramphenicol per ml/0.5% glycerol (as energy source). After cells were shaken with L-[*methyl*-^{3}H]methionine and a salt or other stimuli for 30 min at 30°C, they were treated with 5% trichloroacetic acid and then subjected to sodium dodecyl sulfate/9% polyacrylamide gel electrophoresis, as described (7, 27). EF-Tu is the endogenous, methylated elongation-factor Tu, and its density was used to normalize the MCP bands: Lanes 1–8, respectively: methylation of MCP was 100%, 612%, 354%, 341%, 400%, 489%, 345%, and 61%. Salts (or other agents) used are indicated on the top of each lane.

DISCUSSION

In this report we show that *E. coli* bacteria are attracted to a variety of salts, and that this is under control of MCP.

Are the cells attracted to cations or to anions or to both? Clearly the nature of the cation is critical; NH_4^+ and Li^+ are the best monovalent cations, and Mg^{2+} is the best divalent cation. The nature of the anion has a lesser but definite effect; Cl^- is the best halide. Thus, cations and anions can both be sensed, and both play a role in determining the size of the response.

Taxis toward salts is highly reduced in *tsr* (MCP I$^-$) mutants; there is some reduction in *tar* (MCP II$^-$) mutants too. It appears that both MCP I and MCP II participate in salt taxis.

As to the mechanism of salt taxis, it is clear that MCP is involved, but how it is involved remains to be determined. MCP I plays the major role in attraction to salts, but MCP II can also participate. Salts bring about methylation of MCP, so MCP appears to be required for adaptation to salts just as it is required for adaptation to organic attractants (27). Those few salts that repel (see above) are specific for MCP II; they already have been shown to lead to demethylation of MCP, just like organic repellents (7).

The residual taxis to salts in MCP I mutants may be due to positive osmotaxis. Previously we reported that *E. coli* are attracted by the optimum concentration of osmotic agent and that they are repelled by both lower and higher concentrations (18, 19). As examples of this, see the cases for ribitol and sucrose (figure 1 of ref. 19). Inorganic salts of course are also osmotic agents. The size of attraction is roughly the same to ribitol and sucrose in wild-type bacteria and to salts in MCP I mutants, about 1/20th to 1/10th the size of attraction to salts in wild-type bacteria. We previously showed that osmotaxis does not operate via any of the known receptors for chemotaxis, including MCP (18, 19), so it is expected that MCP mutants should still show osmotaxis toward salts even though they have lost "salt taxis."

In higher organisms, one can also speak of a separate salt taxis and osmotaxis (or salt tropism and osmotropism in the case of non-motile organisms). In animals there are specialized receptor cells for tasting salt (3); and in insects (29, 30) and on the tongue of certain mammals (ref. 2, p. 67) there are other receptor cells, involved with thirst, for tasting water.

Like *E. coli*, the nematode *Caenorhabditis elegans* is attracted to a number of salts at concentrations up to 100 mM (31, 32), and it is repelled at higher concentrations (31, 33). This worm also can distinguish cations from anions (31). Mutants of *C. elegans* defective in taxis toward NaCl (other salts are not reported) were isolated and studied; as in *E. coli*, these are pleiotropic mutants—i.e., various nonsalt attractants also fail (34–36).

Dethier has pointed out that all kinds of salts work for the salt taste in more complex animals: "The picture that emerges from studies of mammals and insects is . . . no receptor specific to sodium salts" (3).

Thus, the results with animals are remarkably similar to salt taxis in *E. coli*. Perhaps the salt taste of animals has evolved from mechanisms already present in bacteria.

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