Proc. Natl. Acad. Sci. USA
Vol. 85, pp. 9451–9455, December 1988
Biochemistry

Osmotaxis in *Escherichia coli*

(bacteria/chemotaxis/behavior)

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Contributed by Julius Adler, September 13, 1988

ABSTRACT **The escape of motile organisms from high concentrations of chemicals was studied in *Escherichia coli*. We have found all chemicals tested to be osmorepellents. It was shown in both a spatial assay and a temporal assay that the known sensory receptors for chemotaxis are not used for osmotaxis, so a different sensory mechanism appears to be employed. According to the temporal assay, the mechanism between sensory receptors and flagella is also not used for tumbling response (at least in solutions above 0.4 osmolar).**

Water is important to life, so all organisms keep in check the concentration of cellular water. For plants and animals on land, dehydration is an obvious threat; finding a source of water is therefore of great concern. For aquatic organisms, the search is for a place to reside that has the most favorable salinity. Perhaps, of all that organisms seek, an ideal osmolarity might well be the most primitive goal. The movement of organisms in search of an environment of optimal water content is called osmotaxis.

In the case of bacteria, osmotaxis was discovered a hundred years ago by Massart (1, 2). In *Escherichia coli*, it was briefly indicated by Tso and Adler (ref. 3, pp. 573–574).

We have shown that osmotaxis can be divided into three ranges: the cells are repelled by pure water, they are attracted to an optimal concentration of osmotic agent, and they are repelled by a high concentration (4). Here we explore the repulsion of *E. coli* by high osmolarity. With the use of mutants, we show that known sensory receptors for chemotaxis are not used in osmotaxis and that the mechanism intermediate between receptors and flagella appears not to be required either. Part of this work has been presented previously (4–6).

MATERIALS AND METHODS

Bacterial Strains. The strains used are all derivatives of *E. coli* K-12. (See Table 2 for a list.)

Spatial Assay Method for Studying Response to Osmotic Agent. Bacteria were grown by shaking them at 35°C overnight in tryptone broth (1% Bacto tryptone/0.5% NaCl) to provide a 3-week supply stored at 20°C. For each day's use, 0.1 ml of these cells was inoculated into 10 ml of tryptone broth and grown by shaking at 35°C to an optical density of 0.6–0.8 at 590 nm. Five milliliters was centrifuged for 3 min at room temperature and the supernatant was discarded; the pellet was resuspended in 5 ml of motility medium (10 mM potassium phosphate, pH 7.0/0.1 mM potassium EDTA), and then the cells were centrifuged again and the supernatant was discarded.

This final pellet of bacteria was resuspended in 20 ml of motility medium in 0.3% agar at 50°C, and this mixture was immediately poured into a plastic Petri dish (30°C) that held the 2% agar plugs containing motility medium and osmorepellent. All was done according to the previously described "chemical-in-plug" method (3). (Note that a gradient forms outside the plug, and the bacteria respond to concentrations much lower than in the plug.) Plates were incubated for 30 min at 30°C and they were then examined and photographed.

Temporal Assay Method for Studying Response to Osmotic Agent. Cells were grown overnight in tryptone broth as above. One-week supplies of bacteria in minimal medium were prepared by inoculating 0.1 ml of these cells into 10 ml of Vogel–Bonner salts (7) containing 25 mM sodium lactate as a carbon and energy source and 1 mM required amino acids and then shaking the flask overnight at 35°C. Daily cultures were made by growing 0.1 ml of this in 10 ml of minimal medium to an optical density of 0.45–0.70 at 590 nm. Cells were centrifuged and washed as above. The final pellet was resuspended in motility medium at an optical density of 0.2 at 590 nm.

Osmolarities tested ranged from 24 (motility medium alone) to 800 mOsM (motility medium plus solute). At 30°C, 5 μl of culture was mixed with 5 μl of test solution on the center of a coverslip by delivering and withdrawing three times with a 20-μl pipette. The coverslip with this droplet was placed upside down between two supporting coverslips on a microscope slide. Cells were then observed and videotaped for several minutes at the slide–droplet interphase.

By use of the procedure of Sager *et al.* (8) behavior was analyzed with equipment from the Motion Analysis Corporation. Videotapes of cells were digitized at 15 frames per sec, and then average angular speeds in degrees per frame and average linear speeds in μm/sec were calculated. Each point in Fig. 2 was determined by averaging the results (from several experiments) for a 10-sec segment every 30 sec over a 3-min period after addition of the stimulus. Segments with fewer than 500 cell-frames of data were not included. Each point in the figure represents at least 10^4 cell-frames.

RESULTS

Osmotaxis in Chemotactically Wild-Type Bacteria

Spatial Assays. Chemotactically wild-type *E. coli* cleared the zone near a plug that contained high concentrations of solute and they formed a ring outside the cleared area, as shown by use of the chemical-in-plug method (3) (Fig. 1). (This is a spatial assay—i.e., there is a gradient emanating from the plug.) For example, *E. coli* is repelled from a plug containing 1 M or higher concentration of ribitol (Fig. 1*A*). This chemical does not serve for either positive or negative chemotaxis in *E. coli* (3, 9).

Even chemoattractive solutes repel at high concentrations, as shown in Fig. 1*B*. Here, bacteria formed an attractant ring to low concentrations of D-ribose (double arrow), but they were repelled from the plug containing 1 M or more concentrated D-ribose (arrow).

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Abbreviations: OsM, osmolar; MCP, methyl-accepting chemotaxis protein.

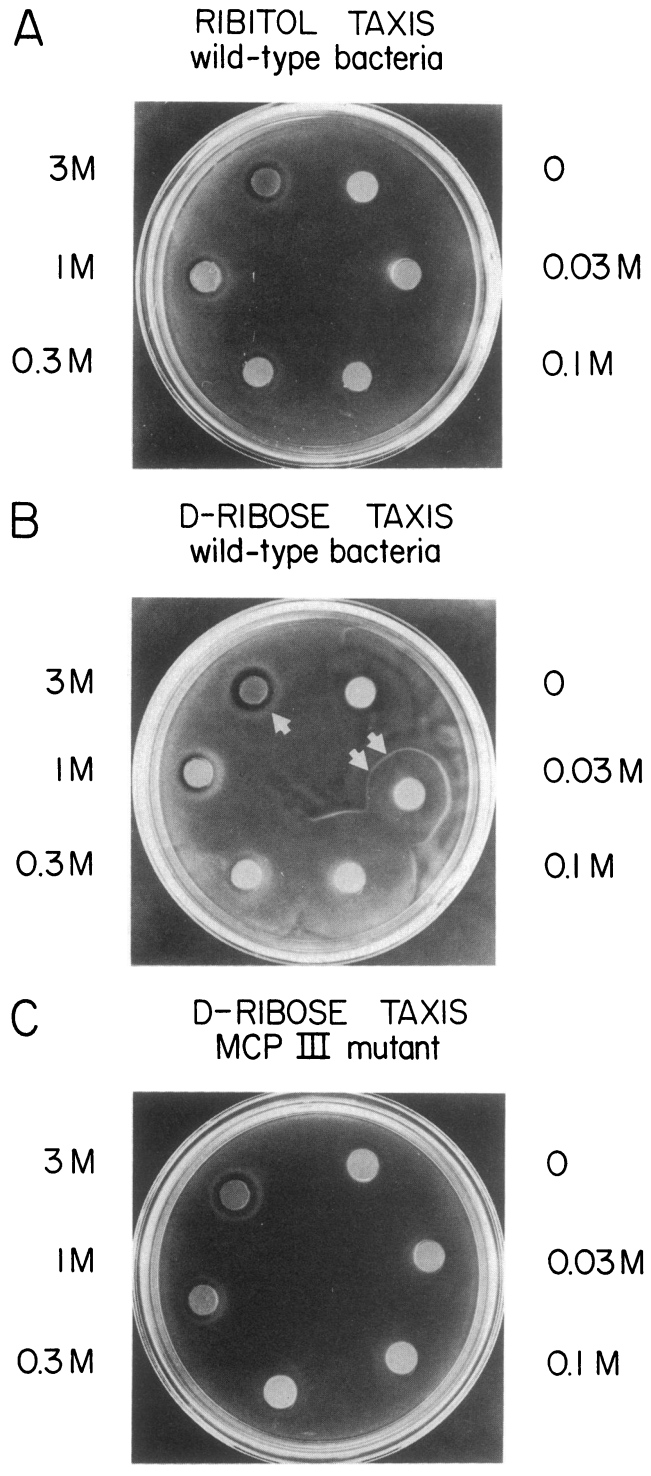

FIG. 1. Osmotaxis in chemotactically wild-type and mutant *E. coli*. The chemical-in-plug method was used. (*A*) A chemotactically wild-type strain (AW405) was repelled by 1 M and 3 M ribitol plugs. (*B*) Wild type (AW405) was tested with D-ribose. The inner ring outside a zone of clearance (see 1 M and 3 M, single arrow at 3 M) is believed to be due to osmorepulsion, while the outer ring (best apparent at 0.03 M and 0.1 M, double arrow at 0.03 M) is believed to be due to chemoattraction. (*C*) *trg* mutant (AW701), known to be missing attraction to D-ribose, was tested with D-ribose. Only the inner ring (see 1 M and 3 M), believed to be due to osmorepulsion, was observed. The *trg* mutant is deficient in methyl-accepting chemotaxis protein (MCP) III.

At high concentrations, chemorepellents may be osmorepellents in addition. For example, Co^{2+}, a known chemorepellent (3), causes two rings, an outer ring probably due to negative chemotaxis and an inner ring probably due to osmotaxis (data not shown). Two rings were reported also for the repellent glycerol (see figure 1*A* in ref. 10). Bacterial chemorepulsion and osmorepulsion appear to be separate behaviors: (*i*) high concentration of repellent generates two repulsion rings in the chemical-in-plug assay; (*ii*) chemorepulsion was defined as having a threshold below 0.1 M in the plug assays (3), while the threshold for osmorepulsion is higher; and (*iii*) all chemorepulsion studied so far relies on MCP I or MCP II, so that mutants missing these fail to carry out chemorepulsion, but osmorepulsion does not require MCPs (see below).

Using this chemical-in-plug method, we have found that most chemicals at high concentrations repel chemotactically wild-type cells (Table 1). These chemicals include ones that are attractive, repulsive, or neutral when tested at lower concentrations (see legend of Table 1).

Table 1. Negative taxis by chemotactically wild-type *E. coli*

Chemical*	Negative taxis 0.03 OsM	0.1 OsM	0.3 OsM	1 OsM	3 OsM
Sugars and related compounds					
D-Galactose	−	−	−	+	+
D-Gluconate	−	−	−	+	+
D-Glucose	−	−	−	+	+
Glycerol†	+	+	+	+	+
Lactose	−	−	−	+	Δ
Maltose‡	−	−	−	−	−
D-Mannitol	−	−	−	+	Δ
D-Mannose	−	−	−	+	+
1,5-Pentanediol†	−	−	−	+	+
Ribitol§	−	−	−	+	+
D-Ribose§	−	−	−	+	+
D-Sorbitol	−	−	−	+	+
Sucrose	−	−	−	+	+
Amino acids					
L-Aspartate‡	−	−	−	−	−
L-Serine‡	−	−	−	−	−
Other organic compounds					
Acetate	+	+	+	+	+
Benzoate	+	+	+	+	+
Inorganic compounds					
$Co(NO_3)_2$	+	+	+	+	+
$NiCl_2$	+	+	+	+	+
KCl‡	−	−	−	−	−
NaCl‡	−	−	−	+	+
$NaNO_3$	−	−	−	+	+
Na_2SO_4	−	−	+	+	Δ

The chemical-in-plug method was used to test negative taxis in chemotactically wild-type strain AW405. The column headings refer to osmolarity in the plug. (Note that M approximates OsM for uncharged chemicals; for a salt such as NaCl 1 M ≈ 2 OsM; for a salt such as Na_2SO_4 1 M ≈ 3 OsM.) A + means that a zone of clearance around the plug was clearly observed by eye (as in Fig. 1); this zone was larger than 1 mm in width and it was surrounded by a ring of bacterial accumulation immediately outside the zone of clearance. A − means that no zone of clearance (or one less than 1 mm in width) was observed. A Δ means that the chemical to be tested was not soluble at this concentration in agar.

*The following chemicals are attractants for which the threshold is between 10^{-8} and 10^{-5} M: L-aspartate, D-galactose, D-glucose, lactose, maltose, D-mannitol, D-mannose, D-ribose, L-serine, and D-sorbitol; the following are chemorepellents: acetate, benzoate, glycerol, Co^{2+}, and Ni^{2+}; and the following are neither attractants nor chemorepellents: 1,5-pentanediol, D-gluconate, ribitol, and sucrose (refs. 3, 9–11; unpublished data).

†See also ref. 10.

‡Failure to observe osmotaxis in the plug assay is most likely due to strong attraction in chemotaxis. NaCl osmotaxis was weak (KCl and NaCl chemotaxis, unpublished data of Y. Qi and J.A.).

§See Fig. 1.

We tested the possibility that osmotaxis is but oxygen taxis in disguise. An experiment was done in a glove-box; all material used was put under vacuum and flushed with nitrogen, and an anaerobically utilizable energy source, 1 mM D-glucose, was provided. Repulsion rings in response to the chemical in the plug (1 M D-glucose, 1 M D-sorbitol, or 1 M sucrose) were still observed under these anaerobic conditions (data not shown). A further argument against oxygen taxis is that inorganic compounds and aerobically nonmetabolizable organic compounds (e.g., ribitol, sucrose, and 1,5-pentanediol), which should not create local oxygen gradients, nonetheless induce repulsion rings (Table 1). We conclude that "osmotaxis rings" are not due to oxygen taxis.

Temporal Assays. Bacterial behavior upon osmotic challenge was observed directly under a microscope. (This is a temporal assay of behavior: the chemical is added and quickly mixed in to produce a sudden change in osmolarity everywhere in the drop.) Wild-type *E. coli* cells, which alternate between running and tumbling in control buffer, invariably tumbled for a few minutes when challenged with solutions higher than 0.1 OsM before returning gradually to normal behavior. Chemicals tested included KCl, NaCl, sucrose, and ribitol. For each of these chemicals the duration and strength of tumbling increased with osmolarity. When confronted with solutions of 0.6 OsM or higher, bacteria first became nonmotile for 1 or 2 min and then tumbled incessantly for up to hours. Some chemicals are better osmorepellents than others; KCl induced more tumbling than NaCl (data not shown).

Tumbling was studied quantitatively by use of a computerized motion analysis system (8) (Fig. 2). At concentrations greater than 100 mOsM, angular speed increased and linear speed decreased; this indicates that tumbling increased with increasing osmolarity. At concentrations between the first point and 100 mOsM, there was no indication of increased tumbling but a suggestion of attraction instead. We previously showed that *E. coli* are attracted to an optimum osmolarity of ribitol or sucrose in that range (4). Part of the attraction here, however, results from chemotaxis towards KCl (Y. Qi and J.A., unpublished data).

Osmotaxis in Chemotaxis Mutants

Spatial Assays. We used the chemical-in-plug method to find out if various chemotaxis mutants can perform osmotaxis. First, mutants missing each one of the transducers required for chemotaxis were tested for osmotaxis: *tsr*, missing MCP I, a protein needed for taxis toward L-serine and away from acetate and benzoate (12, 13); *tar*, missing MCP II, a protein needed for taxis toward L-aspartate and maltose and away from Co^{2+} and Ni^{2+} (12, 13); *trg*, missing MCP III, a protein needed for D-galactose and D-ribose taxis (14–16); *tap*, missing MCP IV, a protein needed for taxis toward dipeptides (17); and *ptsI*, missing enzyme I of the phosphotransferase system, which is needed for taxis toward various sugars such as D-mannose and D-sorbitol (18).

Each mutant was tested for osmotaxis away from D-glucose, ribitol, D-ribose, and $NaNO_3$ (each in a different plug), and also away from chemicals sensed specifically by the particular transducer defective in that mutant. Each of the mutants could perform osmotaxis away from each of these chemicals (Table 2, Osmotaxis in plug assay). The *trg* mutant failed to form the outer, ribose-chemotaxis, ring, as expected, but it could still form the inner, osmotaxis, ring (Fig. 1*C*). The *tar* mutant did not form the Co^{2+} outer ring, believed to be due to negative chemotaxis, but it did form the inner ring, thought to be due to osmotaxis (data not shown). The wild type failed to be repelled osmotactically by L-aspartate, maltose, L-serine, and KCl (see Table 1); apparently the positive chemotaxes to these attractants are so strong that they obscure osmotaxis, but once chemotaxes are removed the demonstration of osmotaxis becomes clear (Table 2).

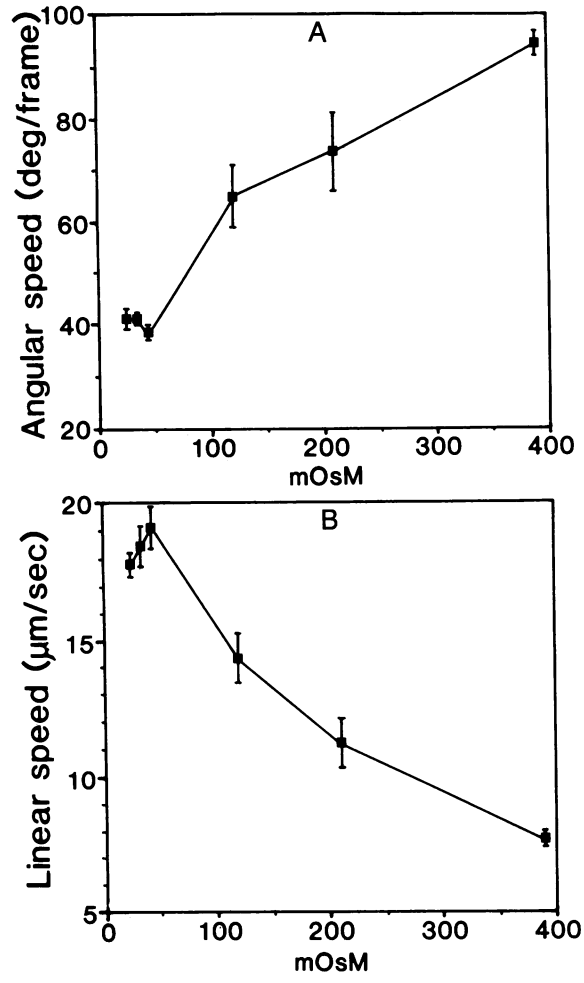

FIG. 2. Behavior of chemotactically wild-type *E. coli* (AW405) in response to osmolarity as measured by motion analysis procedure (8). (*A*) Mean angular speed (±SEM, $n > 30$) of cells in degrees per frame as a function of KCl concentration. (*B*) Mean linear speed in μm/sec (±SEM, $n > 30$) as a function of KCl concentration. The first point, a control at 24 mOsM, represents the osmolarity in the absence of any KCl. Points for 34 and 44 mOsM were collected in a separate experiment and were normalized by comparing their control point to the average first point.

Mutants missing more than one MCP were also tested for osmotaxis. Whenever sufficient tumbling remained, then osmotaxis remained; that was found for *tsr trg* and *tar trg* (Table 2). The loss of both Tsr and Tar, the major MCPs, results in an almost complete loss of tumbling. In this case there was also loss of osmotaxis: This was found for *tsr tar*, *tsr tar trg*, and *tsr tar trg tap*. (But see below under *Temporal Assays*.)

Each of the *che* mutants, known to be lacking a product required in chemotaxis for sending information on transducer occupancy to flagella, was also tested for osmotaxis: These are *cheB* (methylesterase$^-$), *cheR* (methyltransferase$^-$), *cheA*, *cheW*, *cheY*, and *cheZ*. A strain "gutted" of all its chemotaxis machinery was also tested. Finally, we also examined mutants (*flaAII* and *flaBII*) that are motile but defective in chemotaxis owing to flagellar defects leading to a failure in switching between counterclockwise and clockwise rotation. All of these above mutants failed to carry out osmotaxis in this spatial assay (Table 2, Osmotaxis in plug assay), but see below under *Temporal Assays*. Paralyzed (*mot*) and nonflagellated (*fla*) mutants were also unable to perform osmotaxis, as expected for nonmotile cells (Table 2).

Table 2. Tests for osmotaxis in chemotaxis mutants of *E. coli*

Mutant	Osmotaxis in plug assay[a]: D-Glucose, ribitol, D-Ribose, or $NaNO_3$[c]	Osmotaxis in plug assay[a]: Others	Tumbling upon osmolarity increase[b]	Strain	Ref.
Receptor mutants					
tsr (MCP I$^-$)	+	+ for L-serine,[d] acetate, benzoate	+	AW518	19
tar (MCP II$^-$)	+	+ for L-aspartate,[d] maltose, Co^{2+}, Ni^{2+}	+	AW539	11
trg (MCP III$^-$)	+	+ for D-galactose, D-ribose[e]	+	AW701	15
tap (MCP IV$^-$)	+	Not tested[f]	+	RP3525[g]	20
ptsI (enzyme I$^-$)	+	+ for D-mannose, D-sorbitol	+	AW509	18
tsr trg	+[h]		+	AW657	15
tar trg	+[i]		+	AW658	15
tsr tar	–[j]		+	AW569	15
tsr tar trg	–		+	AW660	15
tsr tar trg tap	–		+	CP362	21
Motile but nonchemotactic mutants					
cheB (methylesterase$^-$)	–		+	AW689[g]	[k]
cheR (methyltransferase$^-$)	–		+	AW688[g]	[k]
cheA	–		+	AW690[g]	[l]
cheW	–		+	RP4009[g]	22
cheY	–		+	AW691[g]	[l]
cheZ	–		+	AW692[g]	[l]
flaAII (*cheC*) smooth, *sczA1*	–		+	RP1581[g]	23[m]
flaAII (*cheC*) tumbly, *scyA2*	–		+	RP2634[g]	23[m]
flaBII (*cheV*) smooth, *sczB1*	–		+	RP1592[g]	23[m]
flaBII (*cheV*) tumbly, *scyB10*	–		+	RP2638[g]	23[m]
"Gutted" strain (*tsr tar trg tap cheA cheB cheR cheW cheY cheZ*)	–		+	HCB326	24
Nonmotile mutants					
motA	–		–	M473	25
motB	–		–	M524	26
flaI	–		–	RP3098[g]	27

[a]Plugs contained the chemical named at 3 OsM. A + means a clearly visible ring of bacteria surrounding the vacant region around a plug; a – means no detectable response.
[b]Tumbling was observed microscopically upon challenge by the chemical at 0.4–0.6 OsM. A + means a tumbling response was observed; a – means not.
[c]Each of the four chemicals was used, one at a time, in every case.
[d]The repulsion was weak but definite.
[e]See Fig. 1*C*.
[f]We could not test *tap* mutants with high concentrations of dipeptides because of their low solubility.
[g]Methionine auxotrophs were used with 0.1 mM L-methionine present at all points, since it is required for tumbling (28).
[h]Osmotaxis away from 3 OsM D-ribose, ribitol, D-glucose, or $NaNO_3$ was positive on the same plate that showed a positive chemotactic response to 10 mM Na L-aspartate (which looked like the one for 30 mM D-ribose in Fig. 1*B*).
[i]Osmotaxis away from 3 OsM D-ribose, ribitol, D-glucose, or $NaNO_3$ was positive on the same plate that showed a positive chemotactic response (though weak) to 10 mM L-serine (which looked like the one for 30 mM D-ribose in Fig. 1*B*).
[j]Osmotaxis away from 3 OsM D-ribose, ribitol, D-glucose, or $NaNO_3$ failed, as did positive chemotaxis to 30 mM D-ribose on the same plate (unlike the response for 30 mM D-ribose in Fig. 1*B*).
[k]AW688 and AW689 were made by M. Buechner by phage P1 transduction of Δ*cheR* from RP4944 and Δ*cheB* from RP4953, respectively (strains from J. S. Parkinson, University of Utah), into RP487, selecting for *eda*$^+$, and screening for cotransductants that were smooth or tumbly, respectively.
[l]AW690, AW691, and AW692 were previously called RP487*cheA*, RP487*cheY*, and RP487*cheZ*, respectively (29, 30).
[m]These strains come from J. S. Parkinson. They are similar to those in ref. 23.

Temporal Assays. We also tested osmotic responses of chemotaxis mutants directly under a microscope (Table 2, Tumbling upon osmolarity increase). Each of the transducer mutants responded to osmorepellent. A quadruple mutant defective in all four MCPs, which is entirely smooth-swimming, also tumbled and adapted like the single-MCP mutants.

cheA, cheR, cheW, cheY, flaAII (smooth variety), and *flaBII* (smooth variety) all run with little or no tumbling. These smooth mutants tumbled to addition of KCl with a concentration dependence and adaptation time similar to those of wild type. *flaBII* was least capable of adapting back to smooth behavior.

cheB, cheZ, flaAII (tumbly variety), and *flaBII* (tumbly variety) all are tumbly. We found that these tumbly mutants became even tumblier in high osmolarity—e.g., 0.6 OsM KCl.

The smooth "gutted" strain lacking all MCPs and *che* products also tumbled and adapted to osmorepellents.

DISCUSSION

This report demonstrates osmotaxis by *E. coli* away from high concentrations of osmotic agents—i.e., away from the loss of cellular water.

Wild-type bacteria and mutants missing any one chemoreceptor such as MCP respond negatively to high osmolarity in

both spatial assays (osmorepellent emanating from a plug) and temporal assays (osmorepellent added with rapid mixing). When two MCPs are missing and only one of these is a major MCP (Tsr or Tar), the bacteria can still run and tumble; then they still show osmotaxis away from high osmolarity in the spatial assay as well as in the temporal assay. However, when both the major MCPs are missing or when in addition one or two other MCPs are missing, the bacteria almost exclusively run; then the cells fail to carry out osmotaxis in the spatial assay but not in the temporal assay. It appears that a certain level of tumbliness is necessary to demonstrate osmotaxis in the spatial assay, but that this is not needed for the temporal assay. *che* mutants, which are primarily smooth or primarily tumbly, failed to form osmotaxis rings around plugs of high osmolarity in the spatial assay, but they did tumble when osmotic agent was added in the temporal assay. Some smooth (*cheR*) or tumbly (*cheB*) mutants are known to fail spatial chemotaxis tests but they can pass temporal tests (31–33).

At this time we are not sure how much of the chemotaxis machinery is required for osmotaxis. On the basis of the spatial assays just described, it would appear that the *che* genes are required for osmotaxis; but on the basis of the temporal assays this appears not to be the case. The two assays are very different. In one case, the bacteria encounter small osmolarity differences as they travel along a gradient emanating from the plug, while in the other case, they are suddenly confronted with a very large change in osmolarity. More research is necessary to answer this question: Where does the osmotaxis stimulus enter the chemotaxis pathway? The evidence does suggest, however, that in temporal assays at high concentrations the osmotactic stimulus bypasses the *che* machinery and affects the switch at the base of the flagellum directly. Elsewhere (4) we have reported the effect of osmotic stimulus on tethered cells and discussed the possible causes of osmotically induced tumbles.

Our results indicate that osmotaxis is independent of the structure of the solutes but depends rather on their concentration. Chemicals known to be attractants, repellents, or neither at low concentrations are shown to be osmorepellents at higher concentrations. Thus the ability of the chemical to attract or repel chemotactically is not related to ability to repel osmotactically. Osmotaxis is not a behavior in response to specific solutes but to any solute. It is therefore most likely a response to changes in concentration of the *solvent*, water.

Underway are further studies of how wild-type and mutant cells respond to optimal and less-than-optimal osmolarity (4). Isolation of osmotaxis mutants should help us to understand the mechanism of osmotaxis.

Responses other than motile ones to osmolarity changes have been reported in bacteria. They include the release of low-molecular-weight compounds from the cytoplasm (34), the regulation of synthesis of OmpF and OmpC porins (35), the production of osmoprotectants in the cytoplasm (36) and membrane-derived oligosaccharide in the periplasm (37), the regulation of K^+ uptake systems (38), and changes in buoyant density (39). While the adjustments to changes in osmolarity have been documented, little is known about the mechanism that actually senses the osmolarity. Any of the above responses could possibly be related to the mechanism for osmotaxis, although we have tested the following mutants for osmotaxis by both spatial and temporal assays and found a normal response: *envZ ompR* (defective in regulation of porin synthesis) (35), and *trkA, -D, -E, -G*, and *-H* and *kdpA, -B, -C, -D*, and *-E* (defective in K^+ uptake, kindly provided by W. Epstein, University of Chicago) (unpublished data).

We have recently discovered a pressure-sensitive ion channel on the surface of *E. coli* (40). This channel or similar entities could serve as an osmolarity sensor.

We are grateful to M. Buechner for technical and experimental advice and to B. Sager for donating motion-analysis expertise. We thank M. Buechner, A. H. Delcour, M. C. Gustin, K. Lewis, B. Martinac, and Y. Saimi for critical reading of the manuscript. This study was supported by National Institutes of Health Grants AI08746 and DK39121, National Science Foundation Grant BNS-8804849, a grant from the Lucille P. Markey Charitable Trust, and a grant from the Graduate School of the University of Wisconsin–Madison.

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