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Chemotactic Signaling in Filamentous Cells of *Escherichia coli*

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Video techniques were used to record chemotactic responses of filamentous cells of *Escherichia coli* stimulated iontophoretically with aspartate. Long, nonseptate cells were produced from polyhook strains either by introducing a cell division mutation or by growth in the presence of cephalexin. Markers indicating rotation of flagellar motors were attached with anti-hook antibodies. Aspartate was applied by iontophoretic ejection from a micropipette, and the effects on the direction of rotation of the markers were measured. Motors near the pipette responded, whereas those sufficiently far away did not, even when the pipette was near the cell surface. The response of a given motor decreased as the pipette was moved away, but it did so less steeply when the pipette remained near the cell surface than when it was moved out into the external medium. This shows that there is an internal signal, but its range is short, only a few micrometers. These experiments rule out signaling by changes in membrane potential, by simple release or binding of a small molecule, or by diffusion of the receptor-attractant complex. A likely candidate for the signal is a protein or ligand that is activated by the receptor and inactivated as it diffuses through the cytoplasm. The range of the signal was found to be substantially longer in a *cheZ* mutant, suggesting that the product of the *cheZ* gene contributes to this inactivation.

Escherichia coli is propelled by about six flagellar filaments that emerge at random points on the surface of the cell. Each filament is powered by a rotary motor at its base (9, 11, 60). When the motors turn counterclockwise (CCW), the filaments work together in a bundle that drives the cell steadily forward—the cell runs; when the motors turn clockwise (CW), the bundle flies apart, and the motion is highly erratic—the cell tumbles (36, 38). Runs and tumbles occur in an alternating sequence, each run constituting a step in a three-dimensional random walk (12). When the cell swims in a spatial gradient of a chemical attractant, runs up the gradient are extended; this imposes a bias on the random walk that carries the cell in a favorable direction (12, 37).

Within a few tenths of a second after the addition of a large amount of attractant, the flagella spin exclusively CCW, but the bias (the fraction of time spent spinning CCW) eventually returns to its prestimulus level: the system adapts to the attractant (14, 36, 37, 63). The response and adaptation to a number of attractants, including aspartate, are mediated by proteins that span the cytoplasmic membrane, called transducers or methyl-accepting chemotaxis proteins (for reviews, see references 17, 28, 48, and 62). The transducer for aspartate, which also contains the receptor binding site, is the product of the *tar* gene (named for taxis to aspartate and certain repellents). Adaptation to an increase in the concentration of aspartate results from carboxymethylation of a set of glutamyl residues located on a cytoplasmic domain of this molecule. This is catalyzed by a methyltransferase, the product of the *cheR* gene. Adaptation to a decrease in the concentration of aspartate results from demethylation of these sites, catalyzed by a methylesterase, the product of the *cheB* gene. Evidently, binding of aspartate produces a signal that changes the rotational bias of the flagellar motors, and methylation shuts off this signal. Strains with deletions of the *cheR* or the *cheB* gene (or both) respond to aspartate, but they do not adapt (26, 51, 67); their biases can be set at will by the addition of suitable concentrations of attractants or repellents (or both) (30). The response to the sudden addition of aspartate is markedly delayed in strains carrying mutations in the *cheZ* gene (16, 57), but the reasons for this have not been determined. However, there is evidence that both the *cheY* and *cheZ* gene products interact with other components of the chemotaxis system (50).

The nature of the signal that couples the transducers to the flagella is not known. However, it is clear that signaling does not require metabolism of the attractant (1, 42), protein synthesis (5, 7), or growth (2). Signals that have been proposed include membrane potential (64), free calcium ion (44), cyclic GMP (15), and transmembrane ion fluxes (34, 64). Several studies have shown that membrane potentials are not likely to be involved in signaling in *E. coli* (39, 43, 61), although this mechanism remains viable for some longer bacteria, e.g., spirochetes (24, 25).

To learn more about the properties of the signal that couples the transducers to the flagella, and in particular its range, we combined techniques for iontophoretic stimulation (16, 57) with those for attaching markers to the flagellar motors of long filamentous cells (31). Markers (ordinary polyhook cells fixed with glutaraldehyde) were attached with anti-hook antibodies to the polyhooks of filamentous cells, so that the direction of rotation of their flagellar motors could be followed by phase-contrast microscopy. The filamentous cells have a single cytoplasmic space (31); any signal linking the receptors to the flagella should be free to travel internally from one end of the cell to the other. Iontophoretic ejection of a charged attractant, such as aspartate, from a micropipette produces a localized increase in concentration. We applied stimuli of this kind at various points in the vicinity of filamentous cells and measured the responses of their marked flagella.

Our results show that an internal signal exists, but that it has a short range. The range in a mutant deleted for *cheR* and *cheB* was about 2 μm, whereas the range in a *cheZ* mutant was longer, about 6 μm. These results suggest that signaling is mediated by a substance generated at the transducers and destroyed in the cytoplasm, a substance that reaches the flagellar motors by diffusion.

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TABLE 1. Behavioral properties of strains used in this study

Cells	Strain	Genotype	Marker yield	Prestimulus bias[a]	Sensitivity to aspartate
Normal	AW405	Wild type		CW ≃ CCW	High
	HB9	*fleE hag*		CCW	High
	RP2867	Δ*cheRB*		CW to CW ≃ CCW	Low
	RP5007	*cheZ*		CW	High
Filamentous[b]	HB9	*flaE hag*	High	CCW[c]	ND[d]
	HB203	*flaE hag ftsQ*	Low	CCW[e]	High
	HB238	*flaE hag* Δ*cheRB*	High	CCW	Low[f]
	HB241	*flaE* Δ*cheRB*	High	CW ≃ CCW	Low[g]
	HB254	*flaE hag cheZ*	High	CW ≃ CCW	High

[a] CW ≃ CCW, Roughly 50% CCW; CCW, more than 75% CCW; CW, less than 25% CCW.
[b] Strain HB203 by growth at 42°C; other strains by growth in the presence of cephalexin.
[c] Nearly 100% CCW.
[d] Not determined.
[e] Sometimes CCW, sometimes CW, depending on the preparation.
[f] Somewhat lower than strain RP2867.
[g] Somewhat higher than strain RP2867.

MATERIALS AND METHODS

Strains. All strains were derivatives of *E. coli* K-12. AW405 (wild type; 6) was the gift of J. Adler. RP2867 (deleted for *cheR* and *cheB*; 49), RP5007 (*cheZ293*; 46), and RP5099 (Tn*10* insertion near *eda*) were gifts of J. S. Parkinson. HB9 (*flaE hag*) and HB203 (*flaE hag ftsQ*, chemotactic at 42°C) were described previously (31). The latter strain was reported as *flaE hag*, but we found that it swarms at 30°C when infected with hybrid bacteriophage carrying the wild-type *hag* gene (λfla1Δ4; 58), suggesting either that it is *hag* and not *flaE* or that the *flaE* phenotype is suppressed by the mutation(s) allowing chemotaxis at 42°C. HB238 (*cheR cheB flaE hag*), HB241 (*cheR cheB flaE*), and HB254 (*cheZ flaE hag*) were constructed from HB9 by P1 transduction with *eda*. A tetracycline-resistant *eda*$^-$ clone was picked after infection of HB9 with P1 phage grown on RP5099, and *eda*$^+$ transductants were selected after infection with P1 grown on RP2867 or RP5007. The strains are listed in Table 1.

Reagents and buffers. All solutions were prepared from reagent-grade chemicals and glass-distilled water. Reagents were obtained as described previously (16, 31). Motility medium contained 90 mM NaCl, 10 mM KCl, 10 mM Tris-chloride (pH 7.0), 10 mM sodium lactate, 0.1 mM tetraethylpentamine (54), and 0.001 mM L-methionine.

Preparation of marked filamentous cells. Strain HB203 was grown in tryptone broth (Difco Laboratories) at 30°C until the early exponential phase and then shifted to 42°C for an additional 1 or 2 h. Chloramphenicol (100 μg/ml) was added to prevent formation of septa on cooling; this concentration was maintained throughout the course of an experiment. Strains HB9, HB238, HB241, and HB254 were grown on tryptone broth at 35°C until the early exponential phase, cephalexin (50 μl/ml) was added, and growth was continued at 35°C for 1 or 2 h. This concentration of cephalexin, or chloramphenicol (100 μg/ml), was maintained throughout the course of an experiment. Markers were prepared from cells of strain HB9 and attached to the filamentous cells, as described previously (31), except that open cover slips were used instead of flow chambers, and filamentous cells attached to cover slips were rinsed with motility medium before the addition of anti-hook antibody. The final preparation was covered with a drop of motility medium of volume about 0.2 ml.

Data acquisition. The cells were viewed by phase contrast through a 40× water immersion objective (Zeiss) mounted on a Nikon S-Ke microscope. The experiments were done at room temperature (22°C). Iontophoretic pipettes were filled with motility medium containing 0.01 mM thorium chloride and either L-aspartate or nickel chloride as described previously (57). Pipette resistances were 15 to 60 MΩ. A current-injection circuit (21) was used to pass current (−1 to −200 nA for aspartate and +3 to +50 nA for nickel) through the pipettes via AgCl-coated Ag wires. Pipettes were positioned with a micromanipulator (Narishige MO-103).

The motion of the markers was recorded on videotape with a vidicon camera (Ikegami ITC-47) and a VHS recorder (Panasonic NV 8950) and displayed on a 9-in. (ca. 23-cm) monitor (Hitachi VM-910U). The overall magnification at the monitor was about 4,000×. Calibration of the micromanipulator was checked by comparing recordings of the tip of the pipette as it was moved from position to position against the recording of an objective micrometer. The micromanipulator was used, in turn, to verify the magnification for each experiment; a recording was made of the tip of the pipette as it was displaced 10 μm. A digital time display was included in the recording that pulsed from black to white whenever the pipette current was turned on or off. Digital records of the direction of rotation of the markers were obtained from the video recordings, as described previously (31).

Concentration changes near filamentous cells. In searching for an internal chemotactic signal by comparing responses to step stimuli applied near the cell body or far away, it is essential to know that the concentration of attractant near the cell is not perturbed strongly by the presence of the cell, in particular that it is not elevated by reflection of molecules from the cell body. Naively, one would expect the molecules to be absorbed rather than reflected, but to be absolutely certain, we determined the magnitude of the effect of reflection by appeal to an electrical analog, as described in Appendix A. Perturbations due to reflection were found to be quite small.

RESULTS

General properties of filamentous cells. We began with strains carrying *flaE hag* mutations to produce filamentous cells with polyhooks, but without flagellar filaments. Our hope was that these cells would be nonmotile, but would

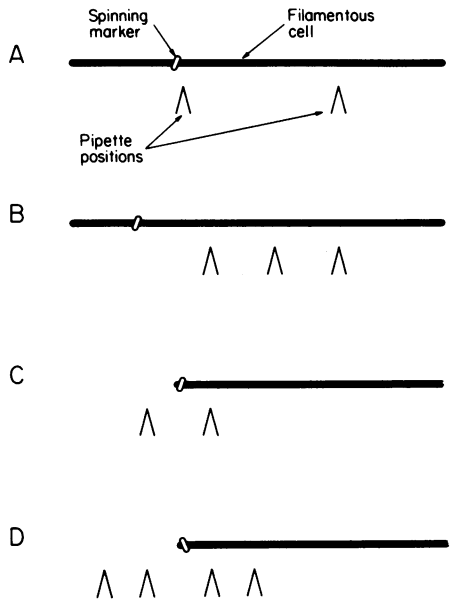

FIG. 1. Schematic illustration of different modes of iontophoretic stimulation of filamentous cells carrying flagellar markers. (A) Pulse stimulation with the pipette close to the cell surface either near the marker or far away. (B) Large step stimulation with the pipette close to the cell surface at different distances from the marker. (C) Small step stimulation with the pipette close to the cell surface or far away at about the same distance from the marker. (D) As C, but at more than one distance from the marker. Scale: the marker in A is 20 μm from the left end of the cell.

have appendages to which glutaraldehyde-fixed polyhook markers could readily be attached. We introduced a temperature-sensitive mutation in septation, *ftsQ* (8), and produced filaments by growing the cells at 42°C. Although we used these cells for measurements of impulse responses (see below), their rotational biases proved to be highly variable, and the yield of spinning markers was low. Therefore, we produced filaments by growing *flaE hag* cells at 35°C in the presence of the β-lactam antibiotic cephalexin (27, 55). Compared with cells of normal size (Table 1), these filamentous cells tended to have higher CCW biases and lower sensitivites to aspartate (as judged by the size of the stimulus required to produce a response). Relatively late in the course of this work we found that cells that were *flaE* rather than *flaE hag* had normal biases and sensitivities. Markers could be attached to these cells, even though some of their polyhooks presumably carried flagellar filament stubs (59). Experiments were done on both *flaE hag cheRB* and *flaE cheRB* cells (Table 1); similar results were obtained in either case (see below).

Impulse responses and transition times of strains wild type for chemotaxis. The peak concentration for a diffusive wave of an attractant or repellent generated by a short iontophoretic pulse is inversely proportional to r^3, where r is the distance from the tip of the pipette (10). Thus, only receptors close to the pipette are strongly affected. Impulse responses (16) obtained when the tip of the pipette was near the surface of the cell, but either close to a marker or far away, were compared (Fig. 1A, Fig. 2A). The positive lobe of the impulse response obtained when the pipette was close to a marker was about 50% wider than normal, and the negative lobe appeared relatively shallow and prolonged (cf. Fig. 2B). The impulse response obtained when the pipette was 27 μm or farther from the marker was barely discernible. Responses of intermediate size could be obtained when the pipette was closer to the marker than this, but similar responses were observed when the pipette was moved out from the cell surface (data not shown); evidently, these responses arose primarily from changes in concentration of aspartate near the marker in the external medium.

We also measured transition times (the time between the onset of a large stimulus and the first reversal) for adaptation to step stimuli delivered iontophoretically (57). When an iontophoretic pipette is switched on, the concentration of attractant or repellent a distance r away approaches its steady-state value in a time of order $t = r^2/6D$, where D is the diffusion coefficient of the chemical (10); for $r = 10$ μm and $D = 10^{-6}$ cm^2/s, this time is relatively short, 0.2 s. The steady-state concentration is inversely proportional to r (not r^3), so receptors are strongly affected over a longer range. The transition time decreased as the pipette was moved along the surface of a filamentous cell (strain HB203) 15 to 35

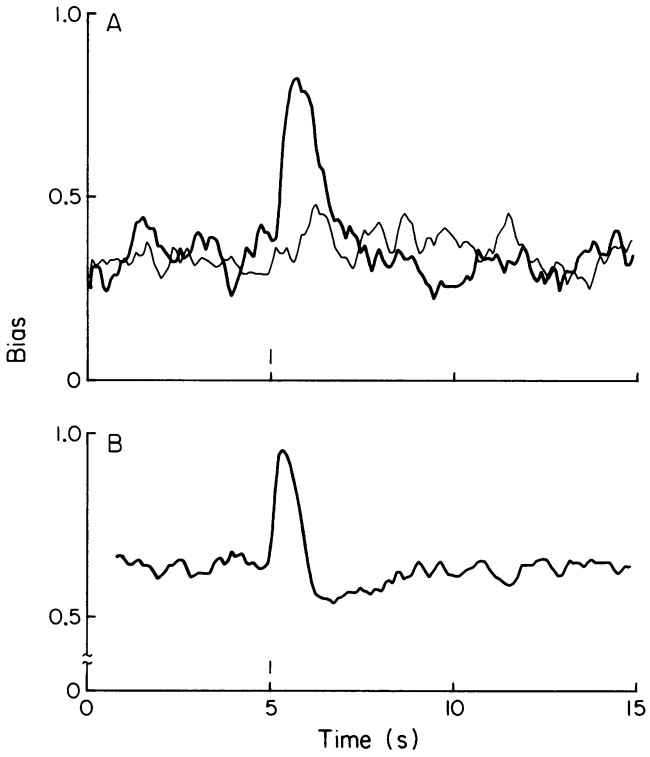

FIG. 2. Responses of wild-type cells for chemotaxis to brief pulses of L-aspartate (A) when the tip of the pipette was near the surface of a filamentous cell (strain HB203 *flaE hag ftsQ*) either near the marker (thick curve) or far away (thin curve) and (B) when the tip of the pipette was near a normal tethered cell (strain AW405). The stimuli were given at 5 s (vertical lines). All data were smoothed so that each point of a curve represents the average bias in a 0.1-s interval centered at that point. (A) In all cases the pipette was within 4 μm of the cell surface. Pipettes containing 1 mM L-aspartate or 3.3 mM α-methylaspartate were placed near (less than 5 μm in four cases, 15 μm in one case) or far (27 to 40 μm) from a marker and pulses 20 to 62 ms long and −50 or −100 nA in amplitude were delivered. Pulses of the same duration and amplitude were used at either position on a given cell. Responses obtained when the pipette was near the marker (81 stimuli) or far away (93 stimuli) were obtained by averaging the data from five cells. (B) The impulse response for strain AW405 was obtained by averaging data from 17 cells and 378 stimuli.

μm from a marker (Fig. 1B). Near the lower end of this range, similar transition times were obtained when the pipette was moved out from the cell surface (data not shown). A similar distance dependence was observed with the repellent Ni^{2+} (with strain HB9). These experiments show that *E. coli* does not have a long-range chemotactic signaling system.

Changes in rotational biases of strains defective in adaptation. Accurate determination of the chemotactic impulse response requires the use of as many as 100 repetitive stimuli (16), and preparations of marked filamentous cells were too fragile to make work on this scale very practical. Therefore, we used an alternative approach: measurements of changes in bias generated by small step stimuli with mutants defective in adaptation (e.g., *cheR cheB* mutants, which fail to adapt at all, or *cheZ* mutants, which adapt slowly; 16). We established, first, that there is an internal chemotactic signal, and then we determined its range.

A small step change in the concentration of aspartate applied near the marker of a *cheRB* filamentous cell (*flaE* or *flaE hag*) generated a step change in bias that persisted for the longest step tested (60 s), whereas the bias of a *cheZ* filamentous cell returned about one-fourth of the way to its prestimulus level in a similar period of time. Shifts in bias to small step changes in concentration applied either on the cell or off the cell at about the same distance from a marker at the end of the cell were compared (Fig. 1C). Examples of the response are shown in Fig. 3, and the results are summarized in Table 2. In every case, responses were larger with the pipette on the cell than off the cell, by as much as 100% (Table 2). This was true even when the pipette was farther from the marker when on the cell than when off the cell (cell no. 1, 4, 5, and 8 to 10). As discussed in Appendix A, given equal pipette-to-marker distances, reflection of aspartate from the cell body would raise the concentration of aspartate at the marker when the pipette was on the cell relative to the value expected when the pipette was off the cell by no more than 2%. This effect is more than offset by the difference in displacement of the pipettes, which, through the inverse dependence of steady-state concentration on distance, gives rise to larger changes in concentration. In any event, since aspartate is taken up and metabolized under chemotaxis conditions (42), the cells probably lower rather than raise the concentration of asparate at the marker. Why, then, should the response be larger with the pipette on the cell than with the pipette off the cell? When the pipette is on the cell, more receptors in regions away from the marker bind attractant than when the pipette is off the cell. Evidently, these extra receptors contribute to the change in bias of the flagellar motor at the end of the cell. Therefore, *E. coli* must have a short-range signaling system.

Accurate determination of the range of this system proved more difficult. The ideal experiment (Fig. 1D) would be one in which comparisons were made of responses with the pipette on or off the cell for a series of different pipette-to-marker distances. In practice, this could not always be done because of variations in response from stimulus to stimulus, shifts in prestimulus bias, and episodes in which markers stopped spinning or fell off. Therefore, we combined data from all cells in which stimuli were given at more than one position on the cell body.

To interpret the effect of small concentration changes on responses, we measured the dose-response curves of *cheRB* and *cheZ* filamentous cells. One type of measurement was made by placing the pipette near a marker on a filamentous cell and recording the responses to currents of different amplitude. From earlier studies of transition times of tethered wild-type cells (Segall, unpublished data), we know that the change in concentration varies linearly with current, particularly for currents less than 20 nA. The other type of measurement was made on markers at the end of a filamentous cell by fixing the amplitude of the stimulus current and moving the pipette to different points off the end of the cell. The *cheRB* filamentous cells had a dose-response curve with a clear threshold (Fig. 4A). This probably accounts for the relatively low sensitivity of *cheRB* strains to attractant stimuli (Table 1; 16). The *cheZ* filamentous cells, on the other hand, had a dose-response curve that was reasonably linear (Fig. 4B). These cells were more sensitive to attractant stimuli than were *cheRB* cells (Table 1).

The decay of the internal signal with distance was measured by comparing the sizes of the responses produced to identical step stimuli when the tip of the pipette was placed near the surface of a cell at different distances from the marker (as in Fig. 1B and D). The stimuli were small enough that the response was not saturated at the point of closest approach. The responses of *cheRB* filamentous cells decayed very rapidly with distance (Fig. 5A). When the effects

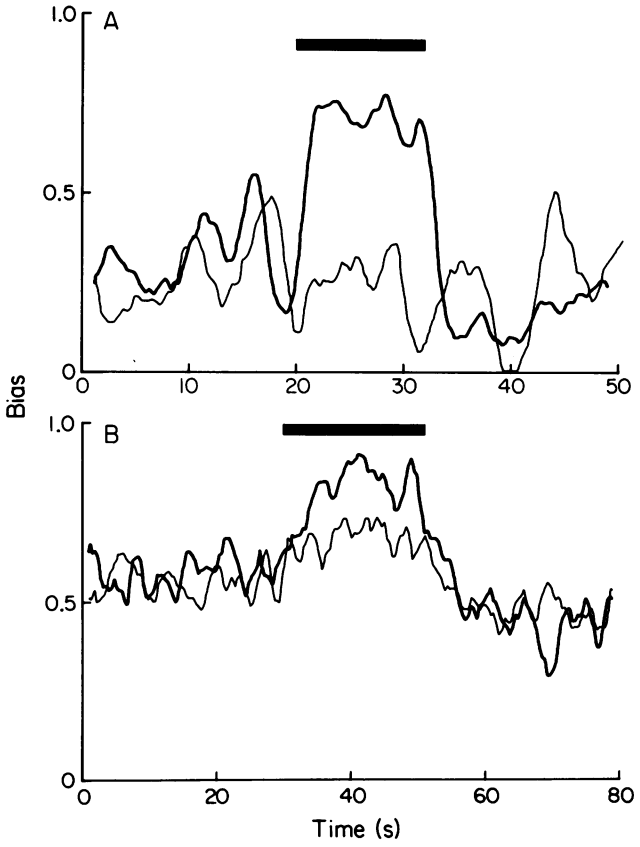

FIG. 3. Change in rotational bias of motors at the ends of filamentous cells to small step changes in concentration of aspartate applied on the cells (thick curves) or off the cells (thin curves) for a *cheRB* cell (A) and a *cheZ* cell (B). All data were smoothed, so that each point on a curve represents the average bias in a 2-s interval centered at that point. (A) Cell no. 5 of Table 2. The current was on from 20 to 32 s (bar); 12 and 8 responses were averaged to generate the thick and thin curves, respectively. (B) Cell no. 9 of Table 2, with the pipette at the positions closer to the marker. The current was on from 30 to 51 s (bar); 16 responses were averaged to generate each curve.

of the nonlinear dose-response curve were included, the response was found to decay approximately as expected for a space constant of 2 μm (thick curve; see below). The response of *cheZ* filamentous cells decayed more slowly with distance (Fig. 5B), roughly as expected for a space constant of 6 μm (thick curve; see below).

DISCUSSION

Our principal findings from iontophoretic stimulation of filamentous cells (Fig. 1) are as follows. (i) *E. coli* does not have a long-range chemotactic signaling system. Pulse stimuli applied within a few micrometers of the cell surface about 30 μm away from a flagellar motor did not affect its rotational bias (Fig. 2). (ii) *E. coli* does have a short-range chemotactic signaling system. Step stimuli applied within about 10 μm of a flagellar motor at the end of a cell were more effective when applied near the cell surface than off the end of the cell (Fig. 3, Table 2). (iii) The range of this signal is substantially smaller with *cheRB* cells than with *cheZ* cells (Fig. 5). These findings are consistent with our earlier observations that correlations in bias fluctuations of motors on the same cell have a range of at most a few micrometers (31). They suggest that these fluctuations are, in fact, due to variations in strength of a chemotactic signal.

Nature of the chemotactic signal. Our results rule out signaling by changes in membrane potential. Such changes have a long range, which can be estimated from the specific resistances of the cell membrane and cytoplasm (4). The specific resistance of the cytoplasmic membrane of *E. coli* is at least 2,000 Ωcm^2 (23). The specific resistance of the cytoplasm of *E. coli* is not known, but that for the squid giant axon is about 60 Ωcm (4). The space constant (the distance over which changes in membrane potential decrease by 1/*e*) for a cell 0.5 μm in diameter having these specific resistances is 205 μm. If the specific resistance of the cytoplasm were actually 10 times larger, the space constant would still be 65 μm, much greater than the range that we have observed for the chemotaxis signal. This does not contradict the observation that changes in membrane potential affect the flagellar motors of *E. coli* (33, 43) or rule out the possibility that such changes are used for signaling in other species, such as *Spirochaeta aurantia* (24, 25) or *Spirillum volutans* (35). However, chemotactic responses of *E. coli* to aspartate are not mediated by any such long-range signaling system. Chemotactic responses of *E. coli* to serine are normal under valinomycin-induced voltage clamp, indicating that these responses also are not mediated by changes in membrane potential (39).

Simple release or binding of a small molecule (such as Ca^{2+}) when an attractant is bound to a receptor also is

TABLE 2. Changes in rotational bias of motors at the ends of filamentous cells in response to step stimuli of aspartate applied on or off the cell

Cell no.[a]	Concn in pipette (mM)	Step amplitude (nA)	Step duration (s)	Pipette position[b]	Distance to marker (μm)	No. of trials	Response[c]		Difference on − off[d] (%)
							Mean	SE	
1	2	−50	60	On	4.5	2	0.45	0.05	18
				Off	3.2	1	0.37		
2	2	−50	60	On	2.4	3	0.33	0.05	6
				Off	2.7	3	0.31	0.08	
3	2	−30	60	On	2.8	6	0.33	0.05	24
				Off	3.1	4	0.25	0.07	
		−30	60	On	4.8	3	0.13	0.08	46
				Off	4.8	1	0.07		
4	0.2	−49	12	On	6.2	3	0.84	0.04	33
				Off	5.5	2	0.56	0.09	
		−47	12	On	6.2	5	0.83	0.02	64
				Off	5.5	5	0.30	0.04	
5	0.2	−20	12	On	6.3	7	0.56	0.05	91
				Off	5.9	5	0.05	0.06	
		−19	12	On	6.3	5	0.43	0.09	100
				Off	5.9	3	−0.09	0.02	
6	0.1	−50	40	On	8.3	3	0.80	0.03	38
				Off	9.5	3	0.50	0.12	
			40	On	16.5	2	0.56	0.09	48
				Off	18.4	2	0.29	0.11	
7	0.2	−13	60	On	9.4	5	0.29	0.06	17
				Off	9.7	5	0.24	0.03	
8	0.2	−17	12	On	6.7	4	0.12	0.02	33
				Off	6.5	4	0.08	0.03	
		−15	12	On	6.7	3	0.11	0.01	100
				Off	6.5	3	0.00	0.06	
9	0.2	−8	21	On	5.4	16	0.33	0.05	52
				Off	5.1	16	0.16	0.02	
				On	9.5	10	0.20	0.03	55
				Off	11.2	15	0.09	0.03	
10	0.2	−10	12	On	6.9	7	0.48	0.06	20
				Off	5.3	7	0.40	0,08	

[a] Cells 1 to 5 were *cheRB* (1 to 3 were strain HB238, 4 and 5 were strain HB241) >27 μm long with markers <1 μm from the end spinning >8 Hz. Cells 6 to 10 were *cheZ* (strain HB254) >18 μm long with markers <3 μm from the end spinning >4 Hz.

[b] On or off the cells, as illustrated in Fig. 1C.

[c] Change in rotational bias: the mean value during the step less the mean value for 20 to 30 s before and after the step. The steps were spaced about 1 min apart.

[d] Change in bias on, less change in bias off, divided by change in bias on, times 100.

unlikely as a signaling mechanism. With such a mechanism, the signal would simply diffuse away from the point of release; the initial change in bias of nearby markers would decline, and the bias of distant markers would increase. This was not observed when *cheRB* filamentous cells were given step stimuli lasting 30 s; the biases of markers about 4 μm from the pipette shifted rapidly and remained high for this period, whereas the biases of markers 7 μm away remained low. Since a molecule of diffusion coefficient D can diffuse in one dimension a mean-square distance x^2 in time $t = x^2/2D$, a signal molecule of this kind would remain localized within 3 μm for 30 s only if its diffusion coefficient were smaller than about $D = 1.5 \times 10^{-9}$ cm^2/s. This is several hundred times smaller than the diffusion coefficient of, say, Ca^{2+} in axoplasm (29).

To account for the kind of decay shown in Fig. 5 (or found for the bias correlation ratio described earlier; 31) the signal must be inactivated as it moves away from its point of origin, either by conversion to some inert form or by transport out of the cell. The smaller the signal's diffusion coefficient and the higher its rate of inactivation, the shorter the range. A model for diffusion with decay is discussed in Appendix B.

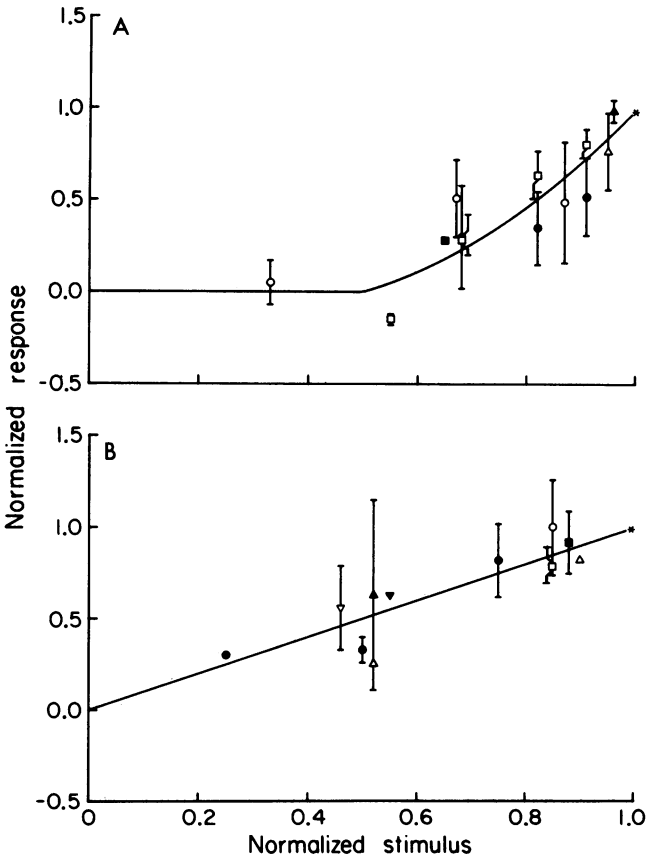

FIG. 4. Dose-response curves of *cheRB* filamentous cells (A) and *cheZ* filamentous cells (B). For each cell, the change in bias (Table 2, footnote *c*) generated by a given current or at a given position was averaged and scaled to the mean response to the largest stimulus given. The stimulus amplitudes were scaled to the largest stimulus amplitude. The error bars are the standard errors in each normalized response (including the uncertainty due to the standard error in the mean response to the largest stimulus). The different symbols identify different cells (six in A, one strain HB238 and five strain HB241; eight in B, strain HB254). The curves were drawn by eye.

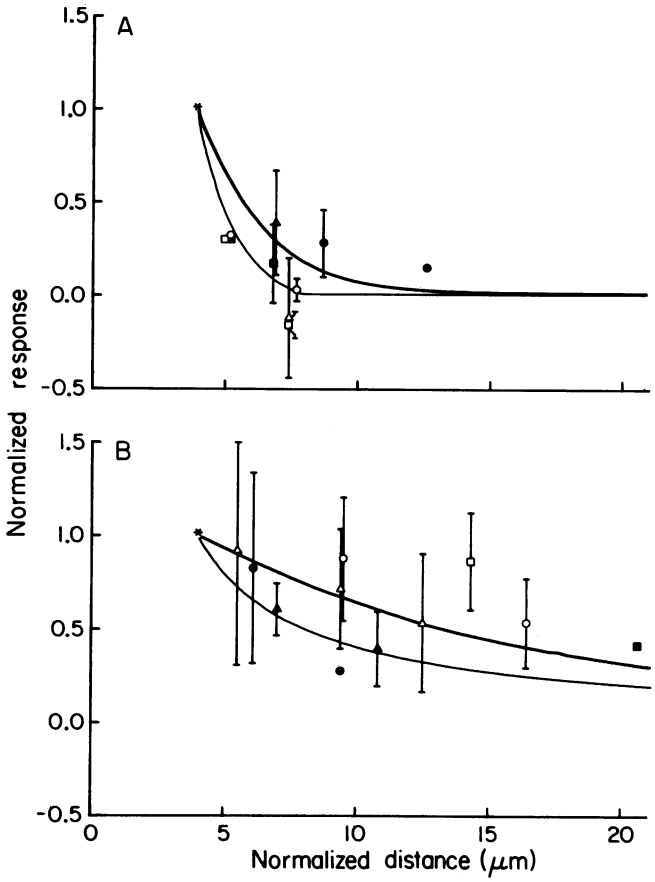

FIG. 5. Distance dependence of the chemotactic signal in *cheRB* filamentous cells (A) and *cheZ* filamentous cells (B). For each cell, the change in bias (Table 2, footnote *c*) generated by a fixed stimulus at a given position was averaged and scaled to the mean response obtained at the position of closest approach. The error bars are the standard errors in each normalized response. Since the position of closest approach varied from cell to cell, the distance for this position was scaled to 4 μm (roughly the mean value), and the other distances for the same cell were scaled by the same factor (so that their ratios remained constant). The thin curves are the predicted dependence of change in bias on distance if the response is determined solely by the concentration of attractant in the external medium at the marker in the manner specified by the dose-response curves of Fig. 4. The thick curves are the predicted dependence for signaling by diffusion with decay, computed as described in Appendix B, for space constants of 2 μm (A) and 6 μm (B).

The decay length of such a signal (the range for an *e*-fold diminution) is the square root of the ratio of the diffusion coefficient and the inactivation rate constant (or of the product of the diffusion coefficient and the signal decay time).

These considerations allow us to rule out signaling by diffusion of the receptor-attractant complex, i.e., a mechanism in which transducers interact directly with the flagellar motors. The decay time of the receptor-aspartate complex can be estimated from its dissociation constant (6 μM for *Salmonella typhimurium*, which has the same sensitivity to aspartate as *E. coli*; 19) and from the on rate constant, which for small ligands is typically in the range 10^7 to 10^8 $M^{-1}s^{-1}$ (18). This gives an off rate constant for aspartate of 60 to 600 $M^{-1}s^{-1}$ or a decay time of the complex of about 20 to 2 ms, respectively. Maximum values of diffusion coefficients of membrane proteins are about 10^{-8} cm^2/s (41), yielding a

decay length of 0.13 μm or less. This is much smaller than the range that we observed with *cheZ* cells. In addition, there is no evidence in *E. coli* that transducers are this closely associated with the flagellar motors (22).

As noted above, it is generally agreed that binding of attractant to transducers changes the level of a signal that affects the rotational bias of the flagellar motors, and that methylation of transducers returns this level to its prestimulus value (17, 28, 48, 56, 62). We favor a hypothesis in which the signal is a molecule that diffuses through the cytoplasm and raises the CW bias of the motor. If inactivation of this molecule were enhanced by the *cheZ* gene product, a possibility suggested by P. Engström (Ph.D. thesis, Uppsala University, Uppsala, Sweden, 1982), then *cheZ* cells would have responses with slower kinetics than wild-type (or *cheRB*) cells, as observed in measurements of response latencies and impulse responses (16, 57; compare the rise and fall times of the *cheZ* and *cheRB* step responses shown in Fig. 3). This also would explain why the signal has a longer range in the *cheZ* cells; the decay time is about 10 times longer in *cheZ* cells than in wild-type cells, so the decay length should be $10^{0.5}$ times as long, which is roughly the factor that we observed. According to this model, *cheZ* mutants have a strong CW bias because they have a higher concentration of signal: the signal shifts the bias CW. Since strains lacking transducers are CCW biased (53), the transducers appear to be involved in the generation of the signal. Furthermore, since addition of attractant shifts the bias CCW, generation of the signal must be depressed when the transducer binds the attractant. Methylation then returns the activity of the signal-generation site to its initial value.

An estimate of the diffusion coefficient of the signal can be made from measurements of signal decay times (3 s in *cheZ* cells, as deduced from measurements of step response kinetics) and decay lengths (about 6 μm in *cheZ* cells; Fig. 5B), which give $D \simeq 10^{-7}$ cm^2/s. An estimate of the size of the signal molecule can be made given the viscosity of the cytoplasm. Estimates of this parameter for *E. coli* range from 5 to more than 10 times the viscosity of water (32, 65; J. E. Tanner, private communication), implying a diffusion coefficient for the signal molecule in water of 5×10^{-7} to 1×10^{-6} cm^2/s. This range of diffusion coefficients corresponds roughly to those for globular proteins in the molecular weight range of 80,000 to 10,000 (18). Unfortunately, this estimate of molecular weight is very sensitive to the size of the diffusion coefficient. There is enough uncertainty in the above analysis, including the measurements of cyotplasmic viscosity, that we cannot rule out the possibility that the signal is a small molecule, perhaps one that binds to larger molecules in the cytoplasm or on the cell membrane and thus undergoes buffered diffusion. If the signal is a protein that is reversibly modified, an obvious candidate would be one or more of the chemotaxis-related gene products for which there is no known function, such as *cheA* (68,000 daltons), *cheW* (15,000 daltons), or *cheY* (11,000 daltons) (47). The suggestion that a complex of the *cheB* and *cheZ* proteins is the signal (45) is unlikely to be correct (J. S. Parkinson, private communication), since *cheRB* deletion strains still respond to attractants. Strains carrying defects in the *cheA*, *cheW*, or *cheY* gene are extremely CCW biased, as would be expected if one of the missing gene products were a signal that decreases the CCW bias (45). Since the *cheY* gene is in the same operon as the *tar*, *tap*, *cheR*, *cheB*, and *cheZ* genes and is expressed at a higher level (20), it is currently the stronget candidate for such a signal. Studies of cell envelope preparations that spin exclusively CCW in the absence of cytoplasmic constituents have led to an analogous hypothesis, in which the *cheY* gene product either generates or enables CW rotation (52).

We note, finally, that a signaling system with a short range is quite reasonable for *E. coli*, which is normally less than 3 μm long. It is more important for such a cell to be able to respond quickly (to have a signal with a short decay time) than to signal over a long range (to have a signal with a long decay length). On the other hand, by using a signal that can diffuse a few micrometers before being inactivated, the cell can both couple inputs from receptors of the same kind, and thus improve the precision with which it measures concentrations (13), and integrate inputs from receptors of different kinds (3, 14, 66).

APPENDIX A

Concentration changes near filamentous cells estimated from measurements of electrical potential. The equation determining concentrations during steady-state diffusion (the Laplace equation) is the same as the equation determining electrical potentials in charge-free (electrically neutral) space, e.g., in solution of an electrolyte. With diffusion, the concentration at the tip of the pipette is constant, the concentration far away is zero, and gradients in concentration vanish normal to reflecting boundaries. With electrical currents, the potential at a point electrode is constant, the potential far away is zero, and gradients in potential vanish normal to insulating boundaries. Therefore, in a problem involving steady-state diffusion, one can determine concentrations by arranging suitable electrodes in a solution of an electrolyte, injecting current, and measuring electrical potentials.

A circular glass dish (12.5 cm in diameter, 6.5 cm deep) was filled with 0.1 M NaCl. To simulate a semi-infinite container, Ag-AgCl wire loops were placed near the side and top boundaries of this solution. Current was injected in the body of the solution from a 0.05- to 0.1-cm bead of AgCl at the tip of an insulated Ag wire and collected by the loops. A similar bead electrode was used to measure the potentials. To avoid artifacts due to buildup of ions at the AgCl-water interface, alternating currents (17 Hz) were used. The resistance of the system was constant at about 40,000 Ω for frequencies between 10 and 1,000 Hz. Potentials measured by the sensing electrode (input resistance, 1 MΩ) were recorded with an accuracy of about 1% on the 0.1-V scale of a strip-chart recorder running at 12.5 cm/min. All measurements were made near the bottom surface of the dish, since filamentous cells are attached to an analogous boundary. As expected, the potential varied inversely with the distance from the current-injecting electrode (10). The effect of a nonabsorbing filamentous cell was modeled with a glass rod (0.3-cm diameter). The diameter of the rod provided the scale on which comparisons could be made; for example, when the current-injecting electrode was one diameter from the rod, the situation modeled was that in which the tip of the pipette was one cell diameter from the surface of the filamentous cell. The potential-sensing electrode was placed at various points on the glass rod to measure the potential corresponding to the concentration near the surface of the cell. For each configuration of rod and electrodes, the potential was measured with the rod in place; then the rod was removed without moving the electrodes, and the potential was measured again. This provided an estimate of the relative change in potential due to the presence of the rod or, analogously, the relative change in concentration due to the presence of a reflecting filamentous cell. For the configurations used to determine differences in response for stimuli applied close to the cell body or far away (Table 2), we found that the presence of the cell had less than a 2% effect on the difference in concentration of attractant or repellent expected at the marked flagellar motor.

APPENDIX B

Signaling by diffusion with decay. The model for signaling by diffusion with decay assumes that an internal chemotactic signal is generated by the transducers at a rate that varies with the local external attractant concentration and decays throughout the cell at a

rate proportional to the signal concentration. A filamentous cell is modeled as a linear structure that does not change the concentration of attractant in its neighborhood. The time-independent diffusion equation for this model is

$$Dd^2S/dx^2 - k_dS = k_p(A) \quad (1)$$

where D is the diffusion coefficient of the signal molecule, S is its concentration, x is the position along the filamentous cell, k_d is the first-order rate constant for inactivation of the signal molecule, and k_p is the rate constant for its production, which depends on the attractant concentration, A. Note that both S and A are functions of x. Equation 1 implies that the concentration of signal molecules produced at one point in the cell decays as $\exp(-x/(D/k_d)^{1/2})$, where x is the distance from that point. The decay length is $(D/k_d)^{1/2}$. To calculate the total concentration of S at a particular point in the cell, one sums the contributions from all the points at which S is produced, taking into account the boundary conditions at the ends of the cell; i.e., Green's function for equation 1 is determined, multiplied by k_p, and then integrated over the length of the cell (40).

The problem then reduces to determining the dependence of flagellar bias on S and of k_p on A. The best that can be done at this stage is to use the dose-response curves shown in Fig. 4. Since the dose-response curve for *cheZ* filamentous cells is reasonably linear, this procedure is likely to be valid for these cells. In these experiments, A is much smaller than the dissociation constant of the receptor, so the rate of production of S is proportional to A under the assumption that k_p depends linearly on the fraction of receptor occupied. We assume, as well, that the change in bias depends linearly on S. For the *cheRB* cells, we assume that the threshold occurs in the dependence of k_p on A, not in the dependence of bias on S—the result is about the same if the threshold is assumed to occur in the dependence of bias on S. The thick curves shown in Fig. 5 were computed from the model with these assumptions.

One assumption made in formulating this model is that the rate of production of S is independent of S. This is reasonable if S is a small molecule that is synthesized de novo, but it might not be correct if S is a protein that is cycled between a signaling and an inactivated state. The only alteration to equation 1 would be the addition to k_d of a term dependent on A, so that k_d would no longer be constant. Such an alteration would increase the rate of destruction of S and shorten the signaling space constant and thus strengthen our argument against the membrane receptor serving as the signal.

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