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Chemotaxis in *Escherichia coli* Proceeds Efficiently from Different Initial Tumble Frequencies

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The relationships between the level of tumbling, tumble frequency, and chemotactic ability were tested by constructing two *Escherichia coli* strains with the same signaling apparatus but with different adapted levels of tumbling, above and below the level of wild-type *E. coli*. This was achieved by introducing two different aspartate receptor genes into *E. coli*: a wild-type (wt-tar_s) and a mutant (m-tar_s) *Salmonella typhimurium* receptor gene. These cells were compared with each other and with wild-type *E. coli* (containing the wild-type *E. coli* aspartate receptor gene, wt-tar_e). It was found that in spite of the differences in the adapted levels of tumbling, the three strains had essentially equal response times and chemotactic ability toward aspartate. This shows that the absolute level of the tumbling can be varied without impairing chemotaxis if the signal processing system is normal. It also appears that a largely smooth-swimming mutant may undergo chemotaxis by increasing tumbling frequency in negative gradients.

Escherichia coli and *Salmonella typhimurium* execute a random walk by alternating between two modes of swimming, smooth swimming and tumbling (2). These bacteria swim by means of rotating helical flagella, about six per cell (1). When the flagella rotate counterclockwise (CCW), each bacterium swims in a smooth, directed manner. The smooth-swimming runs are interrupted when the flagella rotate clockwise (CW), and the bacterium tumbles (16). Tumbles serve to reorient the bacterium so it will swim in a different direction. In the presence of certain gradients, the random walk becomes biased. When the bacterium swims up gradients of chemical attractants, such as aspartic acid or serine, tumbles are less frequent and the runs thus become longer (3, 12). When swimming down steep gradients, the bacterium tumbles more frequently and runs are shorter (12). Gradients thus affect the probability of transitions between the tumbling and smooth-swimming states. The gradients are sensed through specific, homologous transmembrane receptors (reviewed in references 18 and 19). Specifically biasing the random walk constitutes the basis of chemotactic motility. The balance between the smooth-swimming and tumbling states has been regarded as a critical parameter in determining the ability of bacteria to carry out chemotaxis efficiently.

Another critical feature of the sensing mechanism is its temporal nature (12). Spatial gradients are detected by using a short-term memory. As the bacterium swims (~20 μm/s), experiences of the recent past are compared with those of the present. The gradient is thus measured over a length several times the length of the bacterium. For aspartate, the memory of the recent past concentration is stored on the aspartate receptors (about 1,000 receptors per cell [7]) as the level of covalent modification (10, 18). The aspartate receptor (Tar) is reversibly methylated on four specific glutamic acid residues on the cytoplasmic portion of the receptor (22). The number of methyl groups is proportional to receptor occupancy. Receptors sensing an increase in the concentration of aspartate send a signal that suppresses tumbling, and, simultaneously, the receptor becomes more highly methylated. Conversely, a decrease in the aspartate concentration increases the tumble frequency and causes receptor demethylation. As a first approximation, the time constant of the memory is the time over which the bacterium averages inputs and is related to how rapidly the levels of methylation change in response to these inputs.

Recent studies suggest that receptor methylation plays more than one role in chemotaxis. The level of receptor methylation sets the adapted tumble frequency of the cell (20, 21). Also, the time dependence of the methylation reaction is involved in temporal comparisons in the small-stimulus limit (10, 15, 18). How does the receptor carry out both of these functions simultaneously? If one could alter the initial adapted tumble frequency (which we call the zero-point level) without changing the signaling system, behavioral studies on the different strains should provide insight into the multiple functions of the receptor.

To approach this problem, we introduced various forms of the *S. typhimurium* aspartate receptor gene (tar_s), which were essentially identical in function but which had different zero points and levels of tumbling, into a common *E. coli* strain. Two receptors were studied in detail, the wild-type receptor (wt-tar_s) and a mutant in which the sequence near the third site of methylation was altered: Ala-312–Thr-313 was changed to Thr-312–Ala-313. The bacteria constructed in this way had normal signaling systems for chemotactic stimuli, and, therefore, their responses to various chemical and behavioral stimuli could be used to study the significance of the adapted levels of tumbling to the chemotactic mechanism.

MATERIALS AND METHODS

Materials. Colicin E-1, L-aspartic acid, L-serine, L-threonine, L-leucine, L-histidine, L-methionine, and thiamine were obtained from Sigma Chemical Co. (St. Louis, Mo.). Agar and nutrient broth components were obtained from Difco Laboratories (Detroit, Mich.). L-[*methyl*-^{3}H]methionine (85 Ci/mmol) was obtained from Amersham Corp. (Arlington Heights, Ill.).

Bacterial strains and plasmids. RP437 and RP4372 were obtained from J. S. Parkinson (Department of Biology,

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University of Utah, Salt Lake City). RP437 (F⁻ *thr-1 leuB6 his-4 metF159 eda-50 rpsL136 thi-1 ara-14 mtl-1 xyl-5 fhuA31 tsx-78*) is an *E. coli* K-12 derivative and is the wild type for chemotaxis. RP4372 is a derivative of RP437 that has a nonpolar deletion mutation spanning the aspartate and dipeptide receptor genes (17) and a mutation in the serine receptor gene (Δ*tar-5201* and *tsr-1*, respectively), and does not respond to either aspartic acid or serine. The *E. coli* strains K797 and RS1071 were obtained from Barbara J. Bachmann of the *E. coli* Genetic Stock Center (Department of Biology, Yale University, New Haven, Conn.). Both RS1071 (*zah-281*::Tn*10*) and K797 (*phoR79*::Tn*10*) contain the Tn*10* near the *lac* region and cotransduce by P1 bacteriophage, 80 and 10 to 15%, respectively.

RP4372 containing F′ was used in infections by M13 phage. The M13mp8-derived bacteriophages DK601, DK602, and TK603 containing the *S. typhimurium* tar_s have been described previously (23). DK601 carries wt-tar_s. DK602 and TK603 have been altered by site-specific mutagenesis so that Gln-309 has been converted to Glu in DK602, and TK603 is a derivative of DK602 in which Thr-313 has been interchanged with Ala-312. The tar_s allele in TK603 is referred to hereafter as m-tar_s, and the receptor protein product is referred to as m-Tar_s.

Strain construction. *E. coli* strains of RP4372 containing tar_s were constructed by using the gene replacement technique of Blum et al. (5). Briefly, this technique uses colicin E1 resistance to select for integration by homologous recombination of recombinant M13 phage DNA into the chromosome. The M13mp8-derived phages were used to infect F′-containing RP4372 at a high multiplicity of infection (10^2 to 10^4). Since RP4372 does not have amber suppressors, as an extrachromosomal element the M13mp8 phages could neither replicate nor support the growth of bacterial colonies on colicin E1-containing petri plates. tar_s-containing M13mp8 can confer colicin E1 resistance if the phage DNA integrates into the chromosome by homologous recombination. The only region in the tar_s-containing phage DNA that is homologous with the *E. coli* chromosome is a part of the *lac* operon. Upon recombination, tar_s is thus expected to be incorporated in the *lac* region of *E. coli*. Cells (5×10^4, 5×10^5, or 5×10^6), the M13mp8-derived phages DK601, DK602, or TK603 (5×10^8 PFU), and 240 U of colicin E1 were mixed together in 3 ml of top agar, poured onto 10-cm LB plates, and incubated at 30°C. The colicin E1-resistant colonies were assayed for enhanced chemotaxis toward aspartate, but not toward serine, by using semisolid agar swarm plates (0.4% agar). Linkage of the aspartate chemotaxis phenotype to the *lac* region of *E. coli* was determined by P1 transduction by using K797 and RS1071, which contain Tn*10* near the *lac* operon.

Behavioral assays. For behavioral assays, cells were grown in Vogel-Bonner Citrate (VBC) medium (24) supplemented with 1% glycerol (vol/vol); 50 μg/ml each of histidine, leucine, methionine, and threonine; and 10 μg/ml of thiamine.

Agar swarm plates were used to characterize the chemotactic ability of RP4372, RP437, and the tar_s-containing derivatives of RP4372 toward aspartate and serine. Swarm plates (0.3% agar, 30 ml in 10-cm plates) were composed of supplemented VBC medium and also contained either aspartate or serine when needed. After inoculation with a freshly grown culture, swarm diameters were measured as a function of time at 30°C. When possible, the swarm measurements were carried out in a warm room (30°C); otherwise, the cells were grown at 30°C in an incubator and measured at room temperature. Using the warm room resulted in more precise measurements.

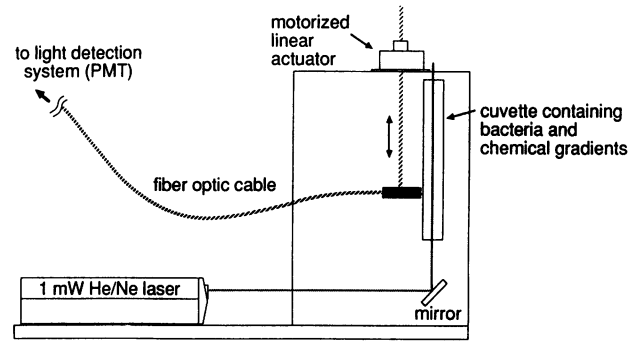

FIG. 1. Schematic diagram of the light-scattering apparatus. The beam of a helium-neon laser is reflected by a mirror up the vertical axis of the sample cell. The sample cell contains the bacteria and the chemical gradients. The amount of scattered light, proportional to the concentration of bacteria, is detected by a photomultiplier tube coupled to an optical fiber bundle. The distribution of bacteria in the sample cell is determined by scanning the end of the bundle near the sample cell with a stepping-motor drive. Repeatedly scanning the sample reveals changes in the distribution as a function of time.

The tethering assay was performed as described previously (25). The total response time of a cell to a saturating aspartate stimulus was defined as the period of time after the tethered bacterium first senses the increase in aspartate concentration (the cell becomes 100% CCW) until the first CCW to CW rotational transition of the cell (the onset of the adapted state).

The measurement of the redistribution of bacteria by light scattering in gradients of aspartic acid was carried out as described previously (8, 25). A schematic diagram of the present apparatus used to measure redistributions is shown in Fig. 1. The bacteria and chemical gradients were introduced into the observation cuvette from a series of reservoirs and mixing chambers by using peristaltic pumps (25). The concentration of cells used in the experiments was 5×10^6 cells per ml unless otherwise noted. The beam from a 1-mW helium-neon laser (Spectraphysics, Mountain View, Calif.) was directed up the vertical axis of the cuvette (1-cm^2 cross section, 10 cm tall). The amount of scattered light was proportional to the concentration of bacteria and was measured as a function of position in the cuvette by using a stepping-motor-driven fiberoptic bundle coupled to a photomultiplier tube. Repeated scanning revealed the kinetics of redistribution. The bacteria were studied in distributed exponential gradients, with plateau concentrations of 50 μM aspartate and a 1/e distance of 2 cm, and in 0 to 50 μM step gradients (0 to 50 μM over ~5 mm just following its preparation).

Swimming behavior was characterized by video microscopy. Bacteria were grown to mid-logarithmic phase in minimal media with shaking at 30°C. Portions were diluted into fresh VBC (100-fold), and 5 μl of this diluted bacterial suspension was placed between a microscope slide and cover glass which were separated by 50 μm. The swimming behavior of the bacteria was observed by using a video camera (Dage-MTI model 67M Newvicon) attached to a microscope (Zeiss model IM35) at a magnification of ×1500 and was recorded on videotape (Sony VO5800H video recorder, 3/4″ tape) for subsequent analysis. The time was recorded on the videotape (Panasonic WJ810 time/date generator) and provided a temporal resolution of 0.02 s (60 Hz).

TABLE 1. Average run and tumble times of wild-type and tar_s-containing bacteria

Strain (terminology) (relevant genotype)	No. of bacteria tracked	No. of:		Mean duration (seconds) ± one SD	
		Runs	Tumbles	Run	Tumble
RP437 (wt-tar_e cells) (wild type for chemotaxis)	7	93	88	1.51 ± 1.31	0.23 ± 0.19
RP4372 *lac*::DK601 (wt-tar_s cells) (*lac*::DK601$tar_e^-tap^-tsr^-$)	12	88	95	1.94 ± 1.64	0.73 ± 0.64
RP4372 *lac*::DK602[a] (*lac*::DK602$tar_e^-tap^-tsr^-$)	6	37	40	2.13 ± 1.73	0.67 ± 0.69
RP4372 *lac*::TK603 (m-tar_s cells)[b] (*lac*::TK603$tar_e^-tap^-tsr^-$)	4	4	0[c]	6.9 to 34.5[d]	

[a] The swimming behavior of RP4372 *lac*::DK602 was nearly identical to that of the wt-tar_s (DK601-containing) cells. DK602 is an M13 plasmid with tar_s that has Gln-309 changed to Glu-309. Gln-309 is the third site of methylation, which is deamidated prior to methylation. In esterase-containing cells, the extent of deamidation at this site is over 90% (D. Sanders and D.E.K., unpublished observations and reference 25) and thus one expects that the cells containing either the wt-tar_s gene or the Gln-309 → Glu-309 mutant tar_s gene to behave similarly.

[b] In TK603, Ala-312 and Thr-313 were interchanged to become Thr-312 and Ala-313 in the mutant.

[c] Under the conditions of observation, no tumbles of the m-tar_s cells were observed. More bacteria than the number tracked and reported here were observed.

[d] The minimum and maximum run times of the four bacteria tracked. In this case, a run was defined as the time that the bacterium was in the field of view.

Videotape analysis was carried out manually. The run → tumble and tumble → run transitions were pinpointed by repeated slow-motion playback of the videotape forward and backward through the swimming traces. Tumbles were scored only when a significant and abrupt change in the swimming direction was detected (~30°). The duration of the tumble could be assessed by a distinct swimming pattern, distinguishable as a continuous end-over-end motion of the bacteria, which ceased at the end of the tumble. The average length of the runs and tumbles was determined from swimming traces of wild-type and tar_s-containing bacteria.

Determination of the levels of receptor methylation. Unstimulated and aspartate-stimulated levels of receptor methylation were determined in the wild-type cells (RP437), the receptorless mutant (RP4372), and the tar_s-containing cells (wt-tar_s and m-tar_s) by the methanol diffusion assay of Chelsky et al. (6). Cells were grown to mid-logarithmic phase at 30°C in VBC (optical density at 650 nm = 0.8); they were then centrifuged and suspended in fresh VBC with 300 μCi of L-[*methyl*-^{3}H]methionine and 50 μg of chloramphenicol per ml for 30 min. At that time, the samples (1 ml) were divided and one-half of the samples received aspartate stimulation (1 mM). All samples were incubated an additional 5 min. The cells were spun down, suspended in sodium dodecyl sulfate sample buffer, subjected to acrylamide gel electrophoresis, and analyzed as described previously (6).

RESULTS

Swimming behavior of strains with different set points. Observation of the swimming behavior in the microscope revealed differences between wild-type *E. coli* (wt-tar_e cells) and the tar_s cells. The data are summarized in Table 1. Most striking was the absence of tumbling (under steady-state conditions) in cells containing m-tar_s. The mean smooth-swimming intervals for wt-tar_e cells and wt-tar_s cells were similar, but the mean tumble durations differed significantly.

The data of Table 1 could be used to obtain tumble frequencies and tumble levels. The tumble frequency is a measure of the rate at which a cell leaves the smooth-swimming state and enters the tumbling state and is thus related inversely to the duration of the smooth-swimming interval. According to this definition, the tumble frequency depends only on the length of the smooth-swimming interval (which begins at the end of a tumble) and does not take into account the duration of a tumble. The term "tumble level" is used to denote the fraction of time that a cell is in the tumbling state and, by definition, depends on the duration of the smooth-swimming and tumbling intervals. From the data in Table 1, it was clear that the tumble level of wt-tar_s cells was larger than that of wt-tar_e cells but that the tumble frequencies of the two strains were similar. The tumble frequencies of wt-tar_s and wt-tar_e cells were similar, since the average run intervals were similar, but the tumble level of the wt-tar_s cells was larger because the tumbles lasted longer. The long tumbles probably decrease the efficiency of chemotaxis, since the tumble, once generated, should be terminated so that a new run can be started. This inefficiency may be compensated for in the wt-tar_s cells by slightly longer run lengths. m-tar_s cells had a much lower tumble frequency than wt-tar_e cells, since the average run length of a m-tar_s cell was much longer. The m-tar_s cells also presumably had a lower level of tumbling, although this was not determined directly, since tumble durations could not be measured in these cells. However, it is plausible that the tumbles of the m-tar_s cells are not any longer than the tumbles of the wt-tar_s cells. If the tumble intervals of these two strains are assumed to be equal, a significantly lower tumble level still results for the m-tar_s cells.

Rotational biases of tethered cells. The percentage of the time that the tar_s cells were rotating CW differed significantly from wild-type *E. coli*. Wild-type *E. coli* cells rotated CW 37 ± 4% (± the standard error of the mean of 11 cells), wt-tar_s cells rotated CW 56 ± 5% (9 cells), and m-tar_s cells rotated CW 18 ± 2% (9 cells). The percentage of CW rotation in all three strains was independent of the aspartate concentration. The m-tar_s cells spent significantly less time in the CW state than the wt-tar_s cells did. The inversion of Ala-312 and Thr-313 in Tar_s lowers the CW/CCW ratio and is consistent with a lower resting level of methylation in m-tar_s cells, although no significant difference in the resting level of methylation has been detected (23). The swimming behavior and the tethering data are in qualitative agreement. Cells with wt-tar_s spend the most time tumbling and the most time rotating CW, wild-type *E. coli* has intermediate levels, and m-tar_s cells tumble the least and spend the least time rotating CW.

Response times to aspartate stimuli. Even though the swimming behaviors and the CW/CCW rotation ratios of the strains were different, the response times of the three strains, each of which had normal signaling systems but different aspartate receptors, were found to be very similar. Figure 2 depicts the responses measured in the tethering assay for cells with the wild-type *E. coli* receptor (wt-Tar_e),

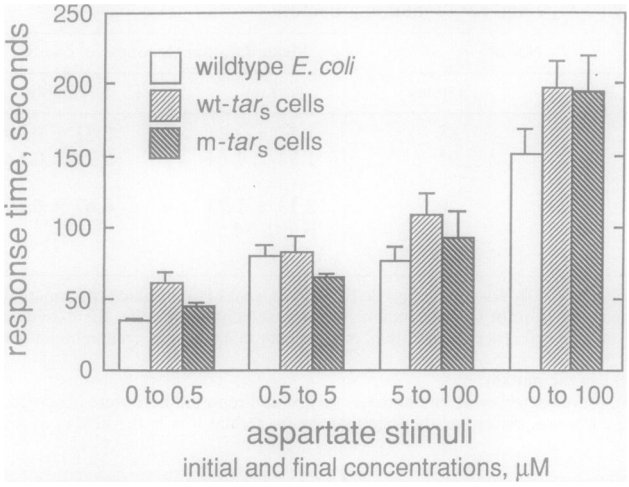

FIG. 2. The response times of wild-type *E. coli* and tar_s-containing cells to aspartate stimuli were measured by the tethering assay. The response time to a positive stimulus is the period of time between the first detection of the stimulus (100% CCW rotation) and the onset of the adapted state (the first CCW → CW transition). Response times were measured for a series of aspartate stimuli (0 to 0.5 μM, 0.5 to 5 μM, 5 to 100 μM, and 0 to 100 μM). Error bars are one standard deviation.

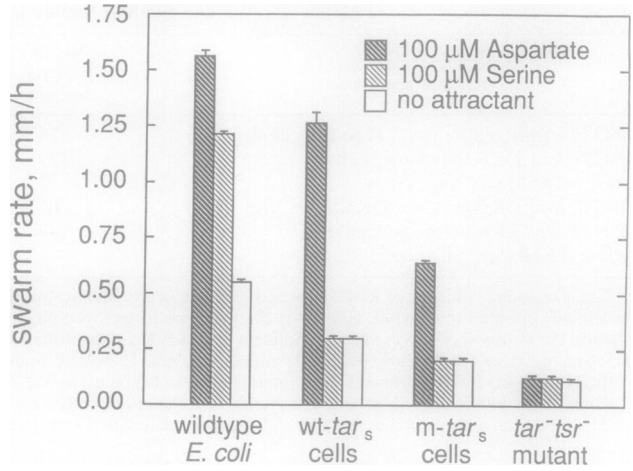

FIG. 3. The swarm rates (mm/h) of wild-type *E. coli* (RP437), an *E. coli tar tsr* mutant lacking aspartate and serine receptor function (RP4372), and two modified strains of RP4372 that contained tar_s were compared. The tar_s-containing RP4372 cells carried an M13mp8 phage that had a wild-type tar_s (wt-tar_s) or an altered tar_s (m-tar_s, Thr-313 and Ala-312 interchanged) insert. Swarm rates were measured in a warm room (30°C) in the presence of either 100 μM aspartate, 100 μM serine, or in the absence of any added attractant. Rates were measured in triplicate. Error bars are one standard deviation.

the wild-type *S. typhimurium* receptor (wt-Tar_s), or the mutant receptor (m-Tar_s), in response to a variety of aspartate stimuli (0 to 0.5 μM, 0.5 to 5 μM, 5 to 100 μM, and 0 to 100 μM). They are essentially indistinguishable. Even though the strains have the wild-type level, above the wild-type level, and below the wild-type level, respectively, of CW rotation before stimulation, they return to those levels in essentially the same period of time after stimulation. This is consistent with the observation that the overall rates of methylation of wt-Tar_s and m-Tar_s are the same (23). Furthermore, it is consistent with the notion that the amount of the Tar protein participating in the response is similar in all three strains, since it has been shown previously that the response time to an aspartate stimulus depends on the concentration of the receptor in the cell (13).

Methylation levels. Total levels of receptor methylation were determined in order to obtain an independent estimate of Tar in the membrane. In the absence of aspartate, methylation levels were close to their expected values. Since wt-Tar_e was present in an *E. coli* strain (RP437) that had a serine receptor and the Tar_s receptors were added back to a strain in which the serine receptor was deleted (RP4372), the total level of methylation in the absence of aspartate was lower in the tar_s cells than in wild-type *E. coli*. The effect of the serine receptor was taken into account by comparing the difference between unstimulated and aspartate-stimulated levels of methylation. It was assumed that the aspartate stimulus did not affect the level of methylation on the serine receptor. The difference in the level of methylation for aspartate-stimulated cells minus unstimulated cells was similar, and the variation in the differences was smaller than the experimental errors in the absolute values. Thus, the differences in the methylation were not so precisely determined as to require a correction for the effects of receptor interaction, where it has been determined that small increases in methylation on the serine receptor occurred as a result of a positive aspartate stimulus mediated through Tar (14). The total level of methylation was consistent with the response times, indicating that Tar was present in similar amounts in each of the cells.

Swarm rates. Measurements of the swarm rates are displayed in Fig. 3. The swarm rates of all three strains are enhanced in an aspartate gradient relative to no attractant. The *E. coli* wild-type strain has the serine receptor, so it responds to serine, whereas the *Salmonella* receptor was introduced into the *tar tsr* strain, so it does not respond. The similarity of the wt-Tar_e and wt-Tar_s responses on swarm plates containing 100 μM aspartate indicate that the absence of the serine and dipeptide receptors has little effect on the aspartate response at this concentration of aspartate. In the swarm plates, cells with m-Tar_s receptors respond to the aspartate gradient less well than cells with either the wt-Tar_s or wt-Tar_e receptors.

When the aspartate gradient was varied (Fig. 4), there was a slight difference in rates, but, in general, the m-tar_s cell was somewhat less efficient. At low and moderate aspartate concentrations (up to 100 μM aspartate), the wild type swarmed better than either tar_s construct, but at higher concentrations of aspartate (500 and 1,000 μM), the swarm rates of the tar_s-containing cells equaled or bettered the wild-type rate. Since the swarm plate assay requires the growth of a bacterial colony to create an aspartate gradient, other gradients will form along with the intended gradient, e.g., pH, oxygen, etc. Signals transmitted through the aspartate receptor undoubtedly played an important role at low aspartate concentrations. To see this, compare the swarm rates of bacteria in the absence of an added attractant (Fig. 3). tar^-tsr^- bacteria (also tap^-) had the smallest swarm rate; the introduction of either wt-tar_s or m-tar_s increased the swarm rate significantly, and in wild-type cells, which contained both *tar* and *tsr* (and *tap*), the swarm rate was larger yet. Although the swarm assay proved that the tar_s-containing cells possessed significant chemotactic ability, the gradient conditions of the swarm assay were not defined, especially at low aspartate concentrations where other gradients competed and influenced cell behavior.

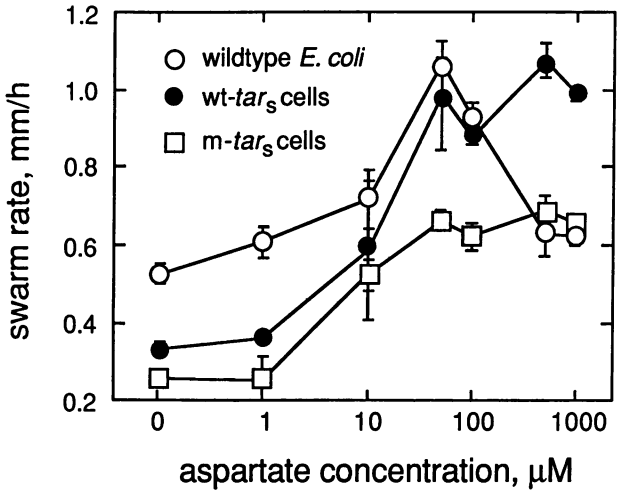

FIG. 4. Swarm rates as a function of aspartate concentration for wild-type *E. coli* (○), cells with wt-tar_s (●), and cells with m-tar_s (□). Cells were grown in a 30°C incubator, except for brief measurements at room temperature. Error bars are the standard error of the mean of three measurements.

Defined gradient chemotaxis. A more controlled test of the chemotactic ability of the tar_s cells toward aspartate was used. The ability of cells to move up exponential gradients of aspartic acid was monitored by light scattering. In Fig. 5, the distribution of bacteria is plotted as functions of the position in the cuvette. The approximate concentration profile is indicated at the bottom of Fig. 5 (50 μM plateau; the 1/e distance = 2 cm). The number of cells at the top of the gradient was estimated by the area under the peak. The peak areas are plotted as a function of time in Fig. 6. The rates of movement up the gradient were approximately constant for the first 50 min of the assay and were obtained from a linear regression analysis of the data (Fig. 6). The average of four

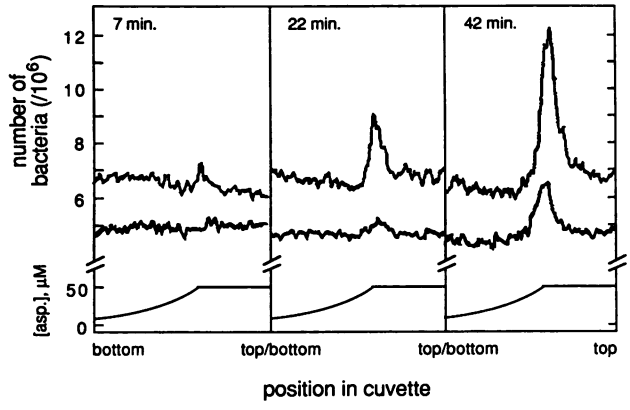

FIG. 5. The top two traces in each of the three panels show the redistribution of bacteria in an exponential gradient of aspartic acid. The traces were obtained at 7 min (left), 22 min (middle), and 42 min (right). The tar_s cells, containing wt-tar_s (middle traces) and m-tar_s (top traces), accumulated at the top of the exponential gradient, which is represented in the bottom traces of each panel. The abscissa in each panel is the distance from the bottom to the top of the region scanned in the cuvette, 6 cm. The aspartic acid decreased exponentially toward the bottom of the cuvette (the left end of each panel) with a 1/e distance of 2 cm, from a 50 μM plateau at the top of the cuvette (the right end of each panel).

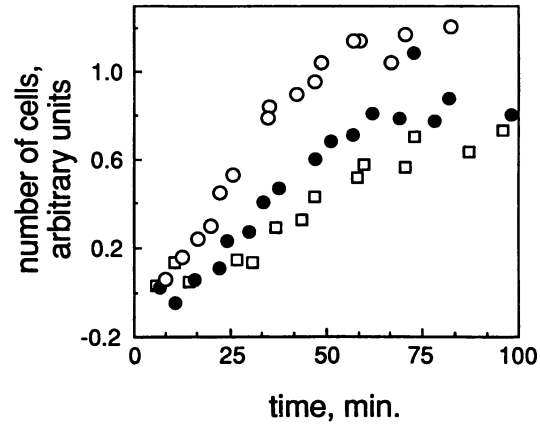

FIG. 6. Measurements of the rate of redistribution of cells in a defined exponential aspartate gradient by light scattering. Exponential gradients were of the form described in Fig. 5. Redistribution of wild-type *E. coli* (□) and *E. coli* cells with wt-tar_s (●) and m-tar_s (○) were measured. Typical data of each strain are plotted.

experiments yielded the rates 1,200 ± 400; 1,300 ± 400; and 2,300 ± 100 cells per s for cells expressing wt-Tar_e, wt-Tar_s, and m-Tar_s, respectively (rate ± standard deviation). These numbers represent the increase per second in the number of cells at the top of the gradient. Not surprisingly, the wt-tar_s and wt-tar_e cells moved up this gradient at an equivalent rate, but it was surprising to find that the m-tar_s cells moved up the gradient at almost two times the rate of wild type. This was apparently not a strong function of the steepness of the gradient. The respective rates of movement of the m-tar_s cells and the wt-tar_s cells across a 0 to 50 μM stepwise increase in the aspartate concentration were 4,800 cells per s and 2,600 cells per s (5×10^6 cells per ml), very similar to the rates of movement in the exponential gradient in relative terms. Movement of wild-type cells in the same gradient had been previously measured to be about 800 cells per s at a concentration of 2×10^6 cells per ml (25). This would be equivalent to 2,000 cells per ml at a bacteria concentration of 5×10^6 cells per ml, assuming that the rate of accumulation is independent of cell concentration.

DISCUSSION

The effect on the chemotactic ability of *E. coli* of varying the fraction of time a cell spends tumbling was studied in three different receptors in an otherwise similar signaling system. The wild-type *E. coli* signaling system was present in all cases. Three different strains had three different steady-state adapted levels of tumbling. The *E. coli* wild-type cell with wt-Tar_e gave the wild-type level. The wild-type *S. typhimurium* aspartate receptor (wt-Tar_s) gave a higher tumbling level in the *E. coli* cell. A mutant *S. typhimurium* receptor (m-Tar_s) in which a small sequence change was made, from Ala-312–Thr-313 to Thr-312–Ala-313, gave a level of tumbling and a tumble frequency (as defined in Results) much lower than the wild type.

When these strains were tested for chemotaxis by response times by using tethering assays, swarm rates in agar gel plates, and migration up a defined gradient, they behaved similarly, showing excellent chemotaxis in all three cases. However, there were some differences. The m-tar_s strain migrated more rapidly in the defined gradient and less well than the other two in the agar swarm plate assay. The low

tumble frequency of the m-*tar*$_s$ cell appears to be a disadvantage in this case, which probably explains its lower swarm rate in the agar and its better-than-wild-type rate in the defined gradients (see below).

The fraction of time a cell is tumbling does not seem to play a major role in the chemotactic ability as long as the rest of the signaling system is intact and the change in tumbling frequency in response to gradients is normal. It should be emphasized that the conclusion in no way conflicts with the finding that esterase-minus mutants, which tumble incessantly, and transferase-minus mutants, which are always smooth swimming, are not capable of chemotaxis. In those cases, both the signaling machinery and the absolute tumbling frequency are altered. In the system described here, only the receptor has been changed to produce a different adapted-state "zero-point" tumbling frequency.

Migration of the mutant with the low zero-point tumble frequency. The tumble frequency of the m-*tar*$_s$ cells in the absence of an aspartate gradient was too low to be measured (see Table 1). From tethering experiments (summarized in Fig. 2) it is clear that the CW/CCW ratio changes transiently with increases in the aspartate concentration, and thus the bacteria still possess the ability to generate tumbles when aspartate concentrations decrease. The tracking studies of Berg and Brown (3) showed that the frequency of tumbling of bacteria moving down an aspartate gradient was essentially the same as that of bacteria in the absence of a gradient. Bacteria moved up the aspartate gradient because the frequency of tumbles decreased when the bacteria were moving up the gradient. Since the m-*tar*$_s$ cells used in our study already have an undetectable tumble frequency in the absence of a gradient, it seems unlikely that this bacterium moves up gradients by further decreasing the frequency of tumbling. We are thus led to the conclusion that the tumble frequency of the m-*tar*$_s$ cell must increase when it swims down an aspartate gradient. This is surprising, in view of the tracking results of Berg and Brown (3). However, Berg and colleagues (4) have shown that tethered cells subjected to a temporal ramp of decreasing aspartate concentration have a lower CCW rotation compared with adapted cells. This is evidence that cells can migrate up favorable gradients by increasing their tumble frequency in a negative gradient. On swarm plates the opposite relationship exists; here wild-type *E. coli* and the wt-*tar*$_s$ cells move at twice the rate of the m-*tar*$_s$ cells (in the presence of 100 μM aspartate). On swarm plates it is not likely to be the shape of the gradient that is the distinguishing factor, since the m-*tar*$_s$ cells migrate more effectively than either wild-type or the wt-*tar*$_s$ cells in the limits of steep and shallow gradients. The answer is more likely to be a result of the nature of the medium through which the bacteria are traveling. In the light-scattering experiments with defined gradients, there are no impediments to the motion of the bacteria, such as the tunnels and channels that exist in the agar. Apparently, the low tumble frequency of the m-*tar*$_s$ cell is something of a disadvantage in this case.

Generation of tumbles. The introduction of mutations into the aspartate receptor has resulted in bacteria that have altered CW/CCW rotation ratios of single motors and consequently, altered tumble/run ratios. Macnab and Han (11) and Ishihara et al. (9) found that motors switch asynchronously, and they have analyzed the relationship between these ratios. Berg and co-workers have suggested that a critical number of flagella are required to generate a tumble (the voting hypothesis [9]). Our data are consistent with this hypothesis. If the flagella switch asynchronously, then the binomial distribution gives probabilities for the different combinations of CW and CCW states. By the voting hypothesis, when the number of flagella rotating CW (n_{cw}) exceeds a critical number of flagella (n_{crit}), the bacterium tumbles. The fraction of time a swimming bacterium spends tumbling (f_{tumble}) is simply the sum of the probabilities of those states, where $n_{cw} > n_{crit}$ and is given by

$$f_{tumble} \approx \sum_{n = n_{crit}}^{n_f} f_{cw}^{n}(1 - f_{cw})^{n_f - n}$$

where n_f is the number of flagella on the bacterium and f_{cw} is the fraction of time a single flagellum rotates in the CW state. Assuming that n_f equaled six, n_{crit} was found to be between three and four, both for wild-type *E. coli* cells and the wt-*tar*$_s$ cells. By using the value of f_{cw} determined for m-*tar*$_s$ cells and n_{crit} equal to either three or four, values for f_{tumble} were calculated to be 0.01 and 0.001, respectively, which is qualitatively consistent with the extremely small tumble frequency of the m-*tar*$_s$ cells. It should be possible to prepare mutated *tar*-containing cells that cover a range of measurable run and tumble intervals and thus determine more precisely the relationship between the CW/CCW ratios and the tumble-run ratios.

Conclusion. These behavioral studies have demonstrated that bacteria with altered zero-point frequencies of tumbling can move up gradients as effectively as bacteria with wild-type tumbling frequencies, provided that the signaling system is intact. The classical *che* mutants also have strong biases toward smooth swimming or tumbling and cannot undergo chemotaxis, but, unlike these *che* mutants, the constructs in the present study have an intact, functioning signaling system. Changes in the tumble frequency are generated appropriately by the bacteria, even though the basal levels of tumbling are significantly different from that of wild-type *E. coli*. The results imply that the short-term temporal comparisons of a wild-type signaling system are adequate to adjust to changes in the initial levels of the tumble frequency. This ability of the excitation-adaptation apparatus is certainly advantageous to the cell, since it allows the bacterium to move up gradients effectively before complete adaptation to other stimuli or in other circumstances in which the tumble frequency deviates significantly from the normal value.

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