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The Chemokinetic and Chemotactic Behavior of *Rhodobacter sphaeroides*: Two Independent Responses

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Rhodobacter sphaeroides **exhibits two behavioral responses when exposed to some compounds: (i) a chemotactic response that results in accumulation and (ii) a sustained increase in swimming speed. This latter chemokinetic response occurs without any apparent long-term change in the size of the electrochemical proton gradient. The results presented here show that the chemokinetic response is separate from the chemotactic response, although some compounds can induce both responses. Compounds that caused only chemokinesis induced a sustained increase in the rate of flagellar rotation, but chemoeffectors which were also chemotactic caused an additional short-term change in both the stopping frequency and the duration of stops and runs. The response to a change in chemoattractant concentration was a transient increase in the stopping frequency when the concentration was reduced, with adaptation taking between 10 and 60 s. There was also a decrease in the stopping frequency when the concentration was increased, but adaptation took up to 60 min. The nature and duration of both the chemotactic and chemokinetic responses were concentration dependent. Weak organic acids elicited the strongest chemokinetic responses, and although many also caused chemotaxis, there were conditions under which chemokinesis occurred in the absence of chemotaxis. The transportable succinate analog malonate caused chemokinesis but not chemotaxis, as did acetate when added to a mutant able to transport but not grow on acetate. Chemokinesis also occurred after incubation with arsenate, conditions under which chemotaxis was lost, indicating that phosphorylation at some level may have a role in chemotaxis. Aspartate was the only chemoattractant amino acid to cause chemokinesis. Glutamate caused chemotaxis but not chemokinesis. These data suggest that (i) chemotaxis and chemokinesis are separate responses, (ii) metabolism is required for chemotaxis but not chemokinesis, (iii) a reduction in chemoattractant concentration may cause the major chemotactic signal, and (iv) a specific transport pathway(s) may be involved in chemokinetic signalling in *R. sphaeroides*.**

Rhodobacter sphaeroides has a single subpolar flagellum which it rotates unidirectionally. The rotation of the flagellum stops periodically for intervals of between a few seconds and many minutes. Brownian motion reorientates the cell during stopped periods, enabling changes in swimming direction (2). Although *R. sphaeroides* lacks methyl-accepting chemotaxis proteins (MCPs) and the phosphotransferase system-dependent chemotaxis systems found in enteric bacteria (3, 20, 28), it is able to respond chemotactically to a wide range of attractants (21). All compounds shown to be chemoattractants for *R. sphaeroides* are metabolites, and at least limited metabolism seems to be required for the chemotactic response (21, 25). Chemotaxis in enteric bacteria and many other species, on the other hand, relies on the extracytoplasmic detection of chemoeffectors by specific MCPs which signal changes in occupancy to the flagellar motor via specific phosphorylated cytoplasmic proteins (6, 18, 30). The relationship between the metabolism-based chemotactic system of *R. sphaeroides* and the MCP-based system of enteric bacteria has yet to be identified.

A second response is shown by *R. sphaeroides*. On addition of some chemoattractants, there is also a sustained increase in swimming speed (26). This increase in speed has been shown to be independent of any long-term changes in the proton motive force (Δp) or changes in rates of electron transport (9, 22). *Escherichia coli* has been shown to swim faster when grown under rich conditions than when grown under poor conditions (17), possibly reflecting a difference in Δp under the different conditions. Transient fluctuations in the speed have also been reported (10), but sustained chemokinesis in response to the addition of chemoeffectors has not been observed. Behavioral models suggest that in the absence of adaptation, chemokinesis would result in the spreading of a population of bacteria rather than accumulation (27); therefore, the role of chemokinesis in the behavior of *R. sphaeroides* and its interaction with the chemotactic pathway is very interesting, particularly as the chemotactic pathway in this organism is different from that characterized in many other species of bacteria. This study was devised to investigate the nature of the chemokinetic response and its relationship to MCP-independent chemotaxis.

MATERIALS AND METHODS

Growth media and conditions. *R. sphaeroides* WS8 (wild type; obtained from W. Sistrom) was grown as previously described in 200-ml bottles at 25°C under continuous illumination (1) with either 5 mM succinate or, when specifically mentioned, fructose as a carbon source. L-Alanine (25 mM), sometimes supplemented with 1 g of Casamino Acids per liter, was used as the sole carbon and nitrogen source in some experiments. A fluoroacetate-resistant mutant (*R. sphaeroides* WS8 FA4), obtained following chemical mutagenesis (12b), was grown under identical conditions. Cells were harvested in late log phase and resuspended in N_2-sparged 10 mM sodium–*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) buffer (pH 7.2). Chloramphenicol (50 $\mu g\ ml^{-1}$) was normally added to prevent protein synthesis and growth. The resus-

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pended cells were incubated under illumination for 1 h before any experiment was started to ensure anaerobic conditions. All chemokinesis experiments were carried out with 10^9 bacteria ml^{-1} and in bright light to maintain a high Δp.

To reduce the intracellular concentration of ATP, cultures were resuspended in HEPES buffer as described above but without chloramphenicol and with the addition of 1 mM sodium arsenate. This concentration was chosen for the majority of experiments, as preliminary studies indicated that there was no difference in behavior between populations incubated in 1 or 10 mM arsenate, either for 1 h or overnight. Previous experiments showed that the intracellular ATP levels were below 50 μM under these conditions. Controls lacked arsenate. Behavior was examined after incubation for 1 h anaerobically in the light. Under these conditions, *R. sphaeroides* stopped growing but remained motile, probably because a high Δp was maintained in the light.

Computerized motion analysis of free-swimming cells. Samples were drawn into optically flat microslides (Camlab Ltd., Cambridge, England), which were then sealed with Vaseline. These were illuminated at 1,200 μM photons m^{-2} s^{-1} on a Nikon Optiphot microscope. Cell tracks were determined by motion analysis on an upgraded system similar to the one previously described (Seescan plc, Cambridge, England) (24). This system enabled detailed analysis of individual cell tracks, providing data on the run speed (the transient velocity between stops; expressed as micrometers per second), stopping frequency, duration of stops (seconds), duration of runs (seconds), and length of the run (micrometers) for each cell. Individual free-swimming cells were monitored for about 2 s, which is the average time cells swam into the plane of focus, and the mean values of at least 100 cells were then determined for each sample of three replicates. The effects of chemoeffectors on the motile behavior of the populations were determined within 10 s of their addition to the cell samples.

Analysis of tethered cells. Ten microliters of *R. sphaeroides* in HEPES buffer was incubated anaerobically under illumination with an equal volume of antiflagellar antibody (diluted 20-fold in 10 mM HEPES) for 20 min on a coverslip in a humidity chamber. After incubation, the coverslip was placed onto a miniature flow cell (5). The cells tethered by their flagella were then observed as either HEPES buffer or buffer containing 1 mM attractant was pumped through the chamber. Video recordings of the cells were made continuously and then analyzed by using rotation analysis software described previously (24).

Chemotaxis measurements. Chemotaxis was assayed by the plug plate method as previously described (13). This method measures the accumulation of bacteria around a chemical source. Motile cells were suspended in cooled 0.25% (wt/vol) soft agar in HEPES buffer with chloramphenicol (50 μg ml^{-1}) and poured into petri plates. Plugs of 2% (wt/vol) solid agar containing the possible chemoeffector or HEPES (control) were inserted into the soft agar plates. These were then incubated for 2 h anaerobically in the light before being examined for areas of accumulation.

RESULTS

Chemotaxis and chemokinesis. Different metabolites were tested for the ability to cause an increase in the mean run speed of free-swimming populations of *R. sphaeroides* (Table 1). Similar increases in mean run speed were obtained whether the bacteria had been grown on succinate or fructose as the carbon source. All of the metabolizable compounds which caused chemokinesis elicited a chemotactic response in the plug plate assay. Most organic acids produced strong chemokinetic responses, whereas sugars produced little or no response, even when they were the sole carbon source for growth (Table 1). Fructose consistently caused a slight increase in mean run speed when free-swimming populations were examined, but it did not cause a measurable change in the rotation rate of the cell bodies of tethered cells. The slight increase in swimming speed may reflect a real change in flagellar rotation rate which was damped by the high load on the rotating cell body of a tethered cell compared with a free-swimming cell.

TABLE 1. Increase in the mean run speed of *R. sphaeroides* immediately after the addition of chemoeffectors

Chemoeffector (1 mM)	Chemokinesis (increase in run speed)		Chemotaxis[a]
	μm s^{-1}[b] (mean ± SE)	%[c]	
Acetate	6.2 ± 1.2	36.8	+
Propionate	6.9 ± 2.4	41.4	+
Pyruvate	6.0 ± 1.1	35.9	+
Malonate	5.6 ± 1.2	38.5	−
Glucose	1.0 ± 1.8	6.0	+
Fructose	1.3 ± 0.4	7.5	+

[a] Tested on plug plates (see Materials and Methods). +, positive response; −, no accumulation.

[b] The mean run speeds for glucose, fructose, and fumarate are not significantly different from the control value.

[c] The mean run speed of the control was 11.2 ± 0.3 μm s^{-1}, and the results are given as the increase over the control value.

Figure 1 shows the change in mean run speed (the speed between stops) of a population of cells when a chemokinetic effector was added. Figure 1A shows the response of an unstarved population, and Fig. 1B shows the response of a starved population. The starved population had, on average, a slower mean run speed than the unstarved population; however, both types of cell population responded to the chemoeffector, indicating that the chemokinetic response was not simply a deenergized population becoming more energetic in the presence of a metabolite.

The idea that a change in metabolic state is not required for chemokinesis was supported by the response of *R. sphaeroides* to the nonmetabolizable analogue of sodium succinate, sodium malonate. When tested on plug plates, sodium malonate did not induce a chemotactic response at any concentration; however, the addition of concentrations of 1 mM and above to free-swimming cells caused a sustained chemokinetic response (Table 1). There was no measurable response to concentrations below 1 mM. In addition, the fluoroacetate-resistant mutant, FA4, transported acetate well but grew poorly if given acetate as a carbon source and showed only a weak chemotactic response toward acetate (23a). The addition of 1 mM acetate increased the mean run speed from 14.8 to 23.8 μm s^{-1}, comparable to the increase measured with wild-type cells.

The motile behavior of *R. sphaeroides* was also measured after short and prolonged incubation in sodium arsenate. A concentration of 10 mM arsenate was initially chosen, as it has been shown to deplete the intracellular ATP concentration in *E. coli* within 10 min and to prevent chemotaxis (15, 16). However, 1 mM arsenate was found to have the same effect on the behavior of *R. sphaeroides*, and this concentration was therefore used for the majority of the experiments to reduce the possibility of secondary effects. Sodium arsenate had no effect on the general swimming behavior of *R. sphaeroides* at either concentration when measured after incubation for ei-

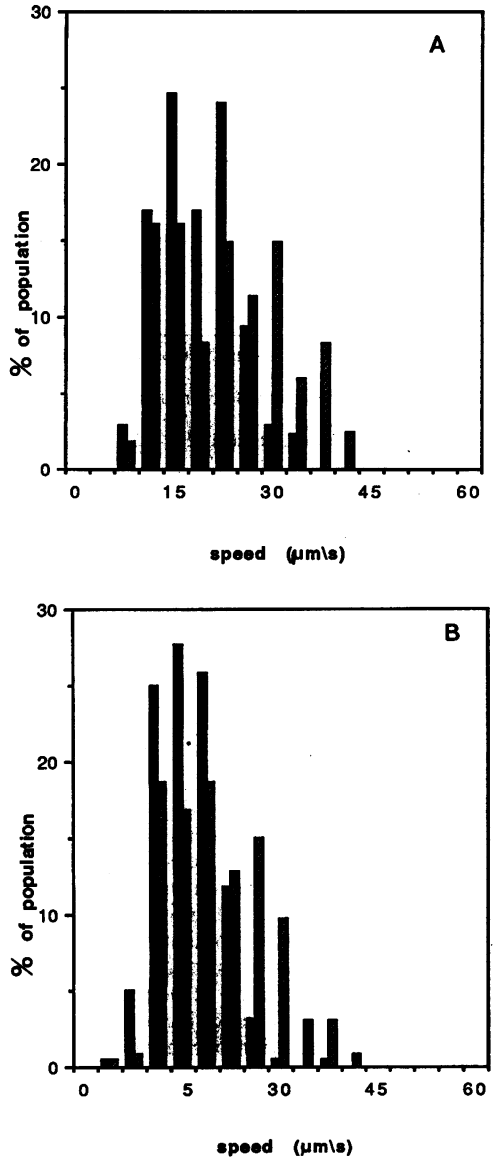

FIG. 1. Histograms of the change in run speed of populations of *R. sphaeroides* immediately after the addition of 1 mM propionate. (A) Effect on a fully energized population; (B) effect on a starved population. ■, before addition of 1 mM propionate; ▩, after addition of 1 mM propionate.

ther 1 h or overnight. The chemokinetic response measured after incubation with arsenate was not significantly different from the control response; however, chemotaxis was lost, whether measured by the population response in plug plates or as the step-down response of individual tethered cells (Table 2). This finding suggests that some form of phosphorylation-dependent metabolism is required for chemotaxis but not for chemokinesis.

Alanine supplemented with Casamino Acids was used as the sole carbon and nitrogen source for growth, under which growth conditions amino acids have been shown to become strong chemoattractants (25). The ability of amino acids to cause chemokinesis under these growth conditions was tested. Only one amino acid, aspartate, gave an increase in the mean swimming speed of the cells even though all the amino acids

TABLE 2. Effects of sodium arsenate on chemotaxis and chemokinesis in *R. sphaeroides*[a]

Chemoeffector (1 mM)	Chemokinesis (mean run speed [µm/s^{-1}] ± SE; n = 5)		Chemotaxis	
	Control	+ Arsenate	Control	+ Arsenate
Control	16.8 ± 1.6	16.1 ± 1.7	−	−
Acetate	20.3 ± 2.0	19.5 ± 1.4	+	−
Propionate	21.5 ± 2.9	21.5 ± 1.1	+	−
Pyruvate	20.2 ± 0.5	19.0 ± 1.5	+	−

[a] Cells were resuspended in 10 mM HEPES (pH 7.2) plus 1 mM arsenate and incubated for 1 h before measurements were taken. There was no significant difference between the data after 1 h in 1 mM arsenate and 15 h in 1 mM arsenate or 5 h in 10 mM arsenate (data not shown). All chemokinetic compounds were added as the sodium salt.

tested caused chemotactic responses. The increase in mean swimming speed was about 20%, from 14.1 (±0.9) to 16.9 µm/s^{-1}. Surprisingly, glutamate, which is structurally related to aspartate, did not cause a measurable chemokinetic response.

These data strongly suggest that the chemotactic and chemokinetic responses are independent and that the increase in translational velocity is probably independent of metabolism.

Changes in the pattern of free-swimming cell motility. The tracks of free-swimming cells were analyzed in detail, after addition of different concentrations of effectors, to determine the nature of the behavioral changes. Different responses were seen, dependent on the compound added and its concentration. Table 3 shows the effects of different concentrations of acetate on cell motility. The lowest concentration which produced a measurable effect on motile behavior was 0.01 mM. After the addition of 0.01 mM acetate, the mean run speed and the run duration increased, almost doubling the mean run length. In addition to the change in mean run speed, the stopping frequency of the population decreased; however, the mean stop duration remained unchanged. An increase in the concentration to 10 mM, however, caused the mean run speed to decrease, with a corresponding decrease in run length. In addition, the run duration also decreased, and the stopping frequency and stop duration increased (Table 3). Microscopic observation confirmed that in the presence of higher concentrations of chemokinetic effector, the percentage of motile cells in a population decreased. Except for longer stopped periods, the cells appeared normal and resumed normal swimming immediately after the concentration of acetate was reduced by dilution.

The addition of 1 mM malonate to a population of cells caused an increase in the swimming speed of the population comparable to the response seen when acetate was added. However, the effect on the pattern of cell motility was different (Table 4). Although there was a change in the mean swimming speed, and therefore run length, the other parameters were identical to those of the control; i.e., there was no change in stopping frequency. When the concentration was increased to 10 mM, malonate did not cause a significant increase in the percentage of stopped cells, or decrease in swimming speed, unlike the response seen after the addition of high concentrations of metabolites. The addition of malonate therefore simply caused an increase in the rate of flagellar rotation. The addition of malonate at concentrations below 1 mM did not elicit a change in motile behavior.

Changes in the pattern of behavior of tethered cells. Tethered cells were used to determine the responses of individual

TABLE 3. Effects of increasing concentrations of sodium acetate on the motile behavior of *R. sphaeroides*[a]

Concn (mM)	Stop duration (s)	Stopping frequency (Hz)	Run duration (s)	Run length (μm)	Run speed (μm s^{-1})
0	0.4 ± 0.1	1.0 ± 0.1	0.5 ± 0.1	6.3 ± 0.7	11.5 ± 0.4
0.01	0.4 ± 0.0	0.6 ± 0.1	0.8 ± 0.1	12.3 ± 2.2	14.4 ± 1.6
0.1	0.5 ± 0.1	0.6 ± 0.1	0.7 ± 0.1	13.8 ± 1.5	16.8 ± 0.5
1.0	0.5 ± 0.1	0.6 ± 0.1	0.7 ± 0.1	16.8 ± 1.9	19.9 ± 1.1
10	0.5 ± 0.1	0.8 ± 0.1	0.6 ± 0.1	11.9 ± 1.8	17.9 ± 1.4
50	0.6 ± 0.1	0.9 ± 0.1	0.5 ± 0.2	8.6 ± 2.4	14.2 ± 1.2

[a] The mean values and standard errors of the means are shown for three repeats measured immediately after the addition of the sodium acetate. Data were collected over about 3 min for about 100 cells, each tracked for up to 2 s.

cells to a chemoeffector. Cell body rotation was measured initially with anaerobic HEPES buffer flowing through the chamber. This was then changed to HEPES containing a chemoeffector, and the change in rotational behavior was measured immediately. Figure 2 shows the changes in rotation rate with time of a typical cell. On addition of 1 mM pyruvate, the stopping frequency of the cell changed from 0.37 to 0.02 stops s^{-1}, the mean run duration increased (2.31 to 38.27 s), and the mean run speed as measured by rotation rate increased (3.98 to 6.5 Hz). This trend was observed for all of the measured cells. Tethered cells therefore behaved similarly to the free-swimming bacteria, with the increase in swimming speed reflecting the long-term change in rotation rate (the chemokinetic response) and the additional change in stopping frequency (the chemotactic response). Removal of the pyruvate caused most of the tethered cells to stop rotating for between 10 and 30 s. Rotation then resumed at the prestimulus rate and stopping frequency.

The step-down response to the removal of an attractant was lost if the buffer also contained 1 mM arsenate (data not shown). Figure 2B shows responses to the addition and removal of 1 mM malonate. There was an significant increase in rotation rate when malonate was added, but no change in stopping frequency. When malonate was removed, the rate of rotation returned to the prestimulus level, but there was again no change in the stopping pattern of the cells. These data support the idea that the change in stopping frequency is the chemotactic response and is independent of the chemokinetic response.

Time course of the response. The increase in mean run speed of a population of *R. sphaeroides* in the presence of a chemoeffector was usually sustained for many hours. Analysis of both tethered and free-swimming cells was carried out to determine the time course of the other changes in cell motility. The increase in mean run speed of a population was maintained for over 180 min, whereas analysis of the cell tracks showed that the initial increase in run duration, caused by the decrease in stopping frequency, returned to the prestimulus level earlier but still took many tens of minutes. Similar long adaptation times were seen with tethered cells in the flow chamber (unpublished data). Figure 2 shows a tethered cell for which an increase in pyruvate concentration caused an increase in rotation rate and a decrease in stopping frequency. In this experiment, the pyruvate was removed after about 10 min, resulting in a step-down response, but no adaptation to the increase in pyruvate had occurred during this time. Once the cell population had returned to the prestimulus run speed, the repeated addition of the chemoeffector caused a percentage increase in speed comparable to the original response. Therefore, the increase in the rate of flagellar rotation was apparently sustained for as long as the compound was present at the increased concentration.

The time taken to return to a prestimulus stopping frequency after the addition of an attractant was still longer than that for enteric bacteria. In contrast, when tethered cells were subjected to a decrease in the concentration of attractant, there was a brief period when the cells stopped, equivalent to a tumble in other species, and when rotation resumed after a few seconds, it was at the prestimulus stopping frequency and rotation rate. A step-down in chemoattractant concentration, therefore, produced a rapid transient response, the stop, followed by rapid adaptation and a return to prestimulus behavior. The time course for returning to a prestimulus run speed after the addition of a chemoeffector was variable and appeared to depend on the type of stimulus and its strength.

DISCUSSION

The identification of compounds which elicited an accumulation response but not an increase in mean swimming speed, and vice versa, has allowed us to start identifying the changes in motile behavior that characterizes the two responses. Chemokinesis is characterized by an increase in mean swimming speed, sustained over a long period of time. The data from malonate addition suggest that after reaching a maximum speed at about 1 mM, any further increase in concentration has no additional effect on behavior. In contrast, the addition of an effector which also causes chemotaxis causes an additional change in stopping frequency. At concentrations below 10 mM, the stopping frequency is suppressed, but if the concentration

TABLE 4. Effects of increasing concentrations of sodium malonate on the motile behavior of *R. sphaeroides*[a]

Concn (mM)	Stop duration (s)	Stopping frequency (Hz)	Run duration (s)	Run length (μm)	Run speed (μm s^{-1})
0	0.4 ± 0.1	0.14 ± 0.1	0.4 ± 0.1	15.6 ± 3.9	17.5 ± 3.4
1	0.4 ± 0.1	0.16 ± 0.1	0.4 ± 0.1	17.4 ± 2.8	25.0 ± 3.4
10	0.4 ± 0.1	0.16 ± 0.1	0.3 ± 0.1	16.7 ± 2.7	19.9 ± 2.1

[a] The mean values and standard errors of the means are shown for five repeats measured immediately after addition of sodium malonate.

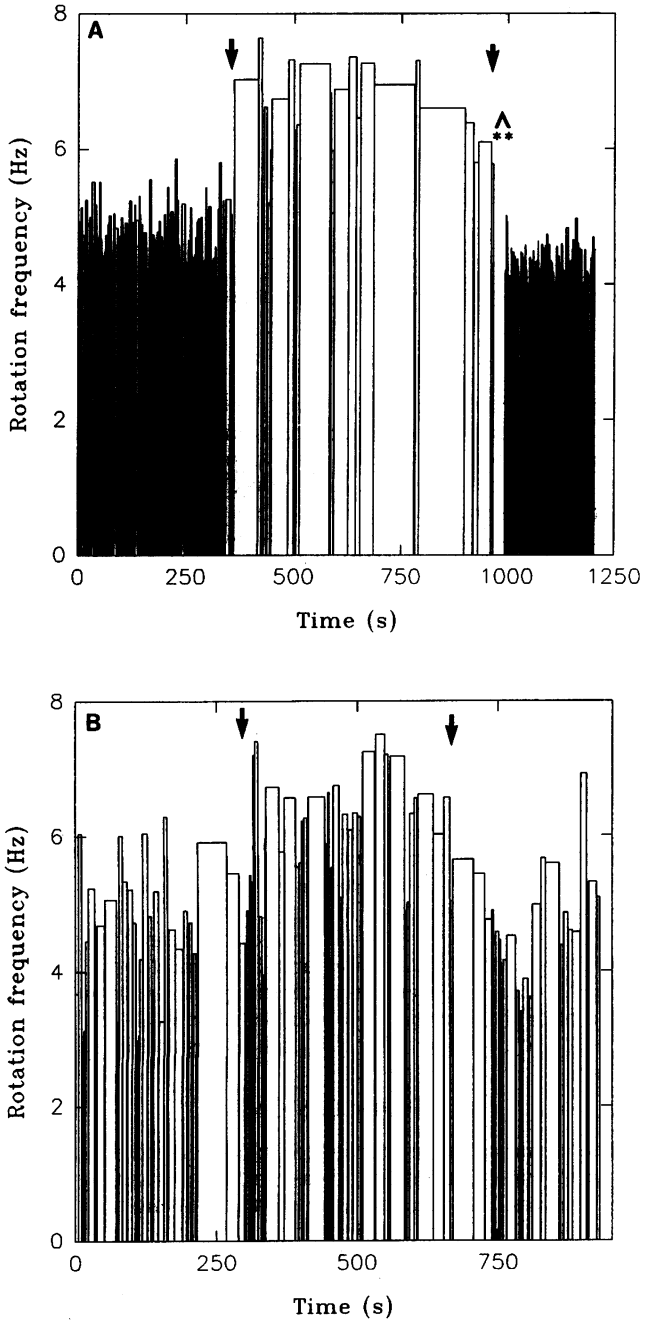

FIG. 2. Behavior of a typical tethered cell in a flow chamber after addition and removal of 1 mM pyruvate (A) and 1 mM malonate (B). HEPES buffer was pumped through the chamber; the chemoeffector introduced at the first arrow, and buffer alone was reintroduced at the second arrow. The ** shows the prolonged stop after the removal of pyruvate.

is taken higher, the stopping frequency increases until at 50 mM, although the cells appear normal, the increase in stop length makes a percentage of the population appear nonmotile. It is possible that the increase in stop duration seen with high concentrations of chemoeffectors is the result of a second chemotactic signal overriding the chemokinetic signal, but this will require further characterization of the chemotactic response.

Examination of the behavior of tethered cells to changes in attractant concentration showed that an increase in an attractant caused a decrease in stopping which, while adapting faster than the chemokinetic response, was still sustained over tens of minutes. A step-down in attractant concentration, however, caused a stop response for about 10 to 30 s followed by a return to prestimulus behavior, i.e., a fast adaptive response. The step-down response seen with tethered cells is similar to the increased tumbling seen when enteric bacteria move away from an attractant and shows adaptation on a time course that would allow accumulation. Analysis of this form of behavior in previous models (27) shows that an increase in direction changing (in this case as the result of stops) when moving down a gradient leads to efficient chemotactic accumulation, as long as adaptation is part of the response. The long-term decrease in stopping, however, when the attractant was increased would not allow gradient sensing or lead to accumulation according to current models (27). It is possible that gradient sensing in *R. sphaeroides* relies only on sensing a reduction in attractant concentration. This might be particularly efficient for the stop-swim motility pattern of *R. sphaeroides*.

Chemokinetic responses were induced by some metabolites, in particular the weak organic acids and some transportable nonmetabolites. The data presented here suggest that metabolism is not directly involved in chemokinesis but transport is essential; transportable nonmetabolites such as malonate caused chemokinesis; acetate caused chemokinesis in a fluoroacetate-resistant mutant, and cells still exhibited chemokinesis when incubated in concentrations of arsenate that inhibited chemotaxis. Although most chemokinetic effectors are organic acids, the effect is probably not the result of a change in intracellular pH, as benzoate did not cause a chemokinetic effect, although it did get into the cells; in addition, incubation of cells at extracellular pHs between 6 and 9 had no major effect on either the mean run speed of cells or the chemokinetic response (unpublished data from this laboratory).

Many metabolic compounds that caused chemokinesis also elicited a chemotactic response; however, most amino acids and sugars, although strong chemoattractants under the right growth conditions, did not cause chemokinesis. Aspartate was an exception, as it induced both chemokinesis and chemotaxis. Glutamate, which is structurally related to aspartate and has a related metabolic fate, did not cause chemokinesis, although it did induce chemotaxis. This finding suggests that the metabolic fate of the chemoeffector may be important in chemotaxis but not in chemokinesis. Arsenate has a general inhibitory effect on all reactions involving phosphorylation, and it is not possible in these experiments to identify whether the loss of chemotaxis is the result of the inhibition of metabolism of the chemoeffector or inhibition of phosphorylation reactions homologous to those involved in sensory transduction in enteric bacteria. We do, however, have preliminary data suggesting that there are *cheA* and *cheY* homologs in *R. sphaeroides* (29).

If metabolism is not necessary for chemokinesis, what causes the signal? The limited number of compounds which elicit chemokinesis suggests that specific transport pathways may have a role in signalling, but unfortunately there are very few data on the transport pathways in members of the family *Rhodospirillaceae*. The signal could be a direct signal related to transport path, or it could be an indirect signal such as a change in ionic flux during transport. Although no change in steady state $\Delta\psi$ was measured during chemokinesis, a change in the ion flux across the membrane would not be measured by this method. However, the size of the response was not dependent on the size of the baseline Δp (12a). The possible connection between transport rates and the chemokinetic

response is currently being investigated, but the dearth of data for *R. sphaeroides* is limiting.

How could the rate of flagellar rotation change in the absence of a change in steady-state Δp? In *E. coli*, resurrection experiments have shown that the rate of rotation of the flagella is related to the number of force-generating units incorporated into the flagellar motor (7) and also suggested that this number might be variable (8, 14). *R. sphaeroides* may somehow modulate the number of force-generating units interacting with its motor, increasing them during chemokinetic stimulation. Certainly the unstimulated speed of individual cells of *R. sphaeroides* fluctuates, even though the measured Δp is maximal. Rapid cell swelling does occur following addition of chemokinetic compounds, and the time courses of the two responses are similar (21a). However, whether a structural change could happen as rapidly as chemokinetic stimulation is, perhaps, doubtful. Alternatively, there could be an intracellular switch controlled by specific transport pathways or rates of transport. For example, fumarate release has been shown to have a role in chemotaxis (but not chemokinesis) in *Halobacterium halobium* and in envelopes of *E. coli* (4, 19), and Ca^{2+} has been implicated in the behavior of *Bacillus subtilis*. Fumarate did not, however, cause any exceptional responses, eliciting chemotaxis and weak chemokinesis under the experimental conditions used here. The identification of the signal may have to await the characterization of the mechanisms involved in transporting the chemoeffectors and the isolation of chemotaxis and chemokinesis mutants to enable the analysis of the separate responses. It is, of course, possible that chemokinesis is a chance by-product of some metabolic process (such as transport) and provides no advantage under natural conditions. Even if this proves to be the case, it does suggest that the rate of flagellar rotation can be altered by more than just the size of Δp.

The data presented here show that motility in *R. sphaeroides* can be controlled by several events: changes in stopping frequency, changes in stop durations, changes in the rate of flagellar rotation, and changes in the duration of runs. While the adaptive change in stopping frequency would result in accumulation in response to a gradient, the chemokinetic response would result in dispersion. The interaction between chemotaxis and chemokinesis appears to be complex, with both able to act independently as well as interactively. Under natural conditions, in the absence of a gradient, chemokinesis would serve to spread the population, but chemotaxis would occur when a gradient was encountered, the cells stopping and reorienting if they move down the gradient.

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