The behavioural response of anaerobic *Rhodobacter sphaeroides* to temporal stimuli

Helen L. Packer, David E. Gauden and Judith P. Armitage

Author for correspondence: Judy Armitage. Tel: +44 1865 275299. Fax: +44 1865 275297.
e-mail: armitage@bioch.ox.ac.uk

Microbiology Unit,
Department of
Biochemistry, University of
Oxford, South Parks Road,
Oxford OX1 3QU, UK

The behavioural response of *Rhodobacter sphaeroides* to temporal changes in the concentration of chemoeffectors, and to stimuli affecting electron transport, was analysed using tethered cells. Populations of photosynthetically grown tethered cells of *R. sphaeroides* showed a transient response, a stop followed by adaptation, to a stepwise reduction in the concentration of chemoattractants (such as organic acids or sugars) and terminal electron acceptors. A step-down response was also measured in free swimming cells to a reduction in light intensity. As this response appears to apply to all effectors this suggests that there is a sensory pathway in anaerobically grown *R. sphaeroides* which responds primarily to a reduction in a stimulus. *R. sphaeroides* therefore responds when moving down a concentration gradient. This is the inverse of the classical *Escherichia coli*–*Salmonella typhimurium* model of bacterial sensory behaviour in which bacteria respond primarily when there is an increase in an attractant concentration, i.e. when moving up a gradient. *R. sphaeroides* does show a chemokinetic response to an increase in concentration of a limited number of compounds but this response is sustained and accompanied by an increase in the rate of flagellar rotation and therefore not simply equivalent to the transient increase in smooth swimming measured in *E. coli* on addition of an attractant.

Keywords: *Rhodobacter sphaeroides*, chemotaxis, aerotaxis, electron transport, phototaxis

INTRODUCTION

Bacteria are able to sense and respond to a wide range of effectors, including nutrients, light, temperature and pH, and have developed complex signalling systems to detect changes in the presence of these effectors and elicit adaptive motile responses. The sophisticated signalling systems of prokaryotes, as well as being significant molecular pathways in their own right, are also a model for determining the molecular basis of global cellular signalling processes as, in common with all such systems, the signal must be detected, processed (amplified and integrated) and an output produced. The intracellular signalling machinery of the enteric bacteria has been elucidated in some detail (Armitage, 1992; Stock *et al.*, 1989; Taylor *et al.*, 1988). In these organisms a limited range of stimuli is detected through transmembrane methyl-accepting chemotaxis proteins (MCPs) and the signal transduced via CheW to a two-component sensor/regulator system. This sequence of cytoplasmic phospho-

proteins (CheA, CheB, CheY) is involved in processing the sensory signal, transmitting this signal to the flagellar motor and controlling adaptation. However, in some bacterial species transmembrane MCPs have not been identified and different sensory machinery appears to be in operation (Armitage, 1992). One such organism is *Rhodobacter sphaeroides*.

R. sphaeroides has a single flagellum which either rotates unidirectionally to propel the cell forward or stops rotating, during which time Brownian forces re-orientate the cell (Armitage & Macnab, 1987). *R. sphaeroides* is able to respond chemotactically to a wide range of attractants such as amino acids, sugars and organic acids. Until recently MCPs were believed to be absent from *R. sphaeroides* and sensing was thought to be conducted entirely through a metabolism-linked pathway. However, evidence has now been found for the existence of MCP homologues in this species but they may be primarily cytoplasmic rather than transmembrane (Ward *et al.*, 1995b). Downstream of the *mcpA* homologue a large chemotaxis operon has been identified containing genes homologous to the enteric *cheA*, *cheR*, *cheW* and two

Abbreviation: MCP, methyl-accepting chemotaxis protein.

genes encoding homologues of the single enteric *cheY* (Ward *et al.*, 1995a). In addition to showing a chemotactic response, *R. sphaeroides* shows a second response, chemokinesis, to an increase in the concentration of some compounds such as the organic acids and K^+ ions. The addition of these compounds results in an increase in the rate of flagellar rotation which is independent of any change in the electrochemical proton gradient (Brown *et al.*, 1993; Poole *et al.*, 1990). The increase in rotation rate is sustained and may continue for several hours, but a decrease in the stopping frequency which accompanies chemokinesis does adapt, albeit over tens of minutes rather than the seconds to few minutes seen in chemotaxis (Packer & Armitage, 1994). The role of chemokinesis in cell sensing and motility is unknown but it has now been reported in several other bacterial species.

The chemotactic response is fairly well understood in the enteric bacteria. When *Escherichia coli* is swimming up a spatial gradient of an attractant, the cell responds by reducing the probability of tumbling, therefore smooth swimming is increased in comparison to behaviour in an isotropic environment (Berg & Brown, 1972; Berg & Tedesco, 1975; Block *et al.*, 1983; Dahlquist *et al.*, 1976). When the cell swims down the concentration gradient the probability of tumbling is the same as it is in an isotropic solution. The duration of the sensory response depends upon the size of the stimulus. When small stepwise increases in attractant concentration are delivered to tethered *E. coli*, the cells rapidly respond by dramatically increasing the counter-clockwise bias of flagellar rotation and then adapt within 4 s (Segall *et al.*, 1982). When a large stepwise concentration change is made which would saturate the receptors, the cells again show the rapid response but then recover slowly over several minutes. When an attractant is rapidly removed, the cells respond by spinning clockwise but this change in bias is slight when compared to that for the addition of attractant, and the adaptation times are considerably shorter, suggesting that the dominant chemotactic behavioural response in enteric bacteria is to an increase in chemoeffector concentration rather than a decrease.

Although it has long been known that *R. sphaeroides* shows chemotaxis, the mechanism involved at the level of the flagellar motor has not been investigated. In this study the sensory response of *R. sphaeroides* was investigated to characterize the behaviour of individual cells when faced with a temporal change in the concentration of a stimulus and to compare this to the *E. coli*-*Salmonella typhimurium* model of sensory behaviour.

METHODS

Growth media and conditions. *R. sphaeroides* WS8 (wild-type, obtained from W. Siström) and WS8N (a spontaneous nalidixic-acid-resistant mutant) were grown as previously described with either 5 mM sodium succinate or fructose as the carbon source (Armitage *et al.*, 1985). Cultures were grown either photoheterotrophically, under continuous illumination at $50 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Harrison *et al.*, 1994), or anaerobically in the dark with 5 mM fructose as the carbon source and 40 mM DMSO as

electron acceptor (Zannoni & Marrs, 1981). All cultures were grown to late exponential phase.

Analysis of responses of tethered cells in the flow chamber.

R. sphaeroides cells (5 μl), harvested and resuspended in 10 mM sodium HEPES buffer (pH 7.2) with $50 \mu\text{g}$ chloramphenicol ml^{-1} , were incubated on a cover slip in a humidity chamber with an equal volume of *R. sphaeroides* anti-flagellar antibody. After 20 min incubation, the coverslip was loaded onto a flow chamber (Berg & Block, 1984) and after at least a further 15 min equilibration in the flow chamber, the cells were observed under phase contrast microscopy at $1000\times$ magnification. Buffers containing different concentrations of chemoeffectors, oxygen or other electron acceptors were passed through the chamber and the behaviour of tethered cells was analysed. The rotational behaviour of individual tethered cells was measured using the motion analysis system previously described (Poole *et al.*, 1988). The rotational rate of each cell was recorded every 0.06 s. The probability of each of 10 individual cells being stopped (bias) was calculated for each point in time, the mean stop probability was then calculated for the 10 cells and smoothed over 1.8 s intervals. This was plotted as stop probability against time. For experiments looking at the effects of chemoeffectors, the merged data came from 10 cells in the same field. Cells grown on DMSO showed reduced motility and these data therefore come from 10 cells from different fields, but from the same sample and treated identically.

Analysis of the response of cells to changes in light intensity.

Cells were grown anaerobically under high light intensity ($120 \mu\text{mol m}^{-2} \text{s}^{-1}$) (Harrison *et al.*, 1994). Growth at this light intensity reduced the quantity of bacteriochlorophyll in the culture by fourfold compared to cells grown at $50 \mu\text{mol m}^{-2} \text{s}^{-1}$. For free swimming cells, samples taken directly from the growing culture were drawn by capillary action into 0.05 mm diameter optically flat microslides (Camlabs) after the addition of $50 \mu\text{g}$ chloramphenicol ml^{-1} . The microslides were sealed at the ends with Vaseline and left in the light (12 V halogen light source, with phase contrast optics) under the microscope for 15 min before experimentation. The cells were video-recorded for 5 min at a light intensity of $240 \mu\text{mol m}^{-2} \text{s}^{-1}$ and then the light intensity was reduced rapidly, by the addition of neutral density filters, to $13 \mu\text{mol m}^{-2} \text{s}^{-1}$ and the response recorded. After 5 min the light level was returned to the original level.

The behaviour of cells was analysed manually from the video recordings as the motion analysis system was unable to detect the cells during the period of light level change. For manual measurement the video was advanced frame by frame and the cell paths plotted and measured.

RESULTS

Background behaviour of tethered cells

Wild-type tethered cells of *R. sphaeroides* either spin counter-clockwise, which is equivalent to a run in a free swimming cell, or cease rotation, which is equivalent to a stop in a free swimming cell. The rotational behaviour of 50 photosynthetically grown cells taken from 10 different populations was pooled, analysed and the mean was calculated. The behaviour of populations of cells was measured independently. Cells were tethered in the flow chamber and their rotational behaviour was analysed for 10 min while N_2 -sparged 10 mM HEPES buffer, pH 7.2, was passed through the flow chamber. Tethered cells of *R. sphaeroides* under constant non-stimulated environ-

Fig. 1. The response of *R. sphaeroides* to a stepwise increase and decrease in the concentration of the chemoattractant sodium acetate. (a) Response of rotation rate of a single tethered cell to the addition and removal of 1 mM sodium acetate (arrows). The rotation rate increased and the stopping frequency decreased after the step-up in sodium acetate and the removal of acetate resulted in a transient stop followed by adaptation. (b) Stopping probabilities of 10 cells from the same field of view. The addition of 1 mM sodium acetate caused a sustained decrease in stopping frequency of the population and removal resulted in all the cells transiently stopping.

mental conditions had a mean stopping frequency of 0.22 ± 0.17 and thus stopped on average just less than once every 5 s. The behaviour of individual cells was variable with some cells only stopping once during the measurement period of 600 s (e.g. after a 596.4 s run duration), whereas other cells stopped frequently. The mean stop duration was 1.04 ± 3.18 s (min = 0.06 s, max = 102.5 s) and the mean run duration was 2.53 ± 13.3 s (min = 0.06 s, max = 596.4 s). The rotation rates of the cells were also variable with the mean rotation rate at $4.28 \pm 1.3 \mu\text{m s}^{-1}$ (min = $3.5 \mu\text{m s}^{-1}$, max = $11 \mu\text{m s}^{-1}$). Cells which did not rotate during the experiment were not measured as it was not possible to determine whether the lack of rotation was physiological or an artefact of tethering. In populations of free swimming cells some non-motile cells were, however, usually observed.

Response to changes in concentration of chemoeffectors

The response of populations of tethered cells to a range of chemoeffectors was investigated. Fig. 1(a) shows the response of a single tethered cell to changes in the concentration of the chemokinetic chemoeffector, sodium acetate. Initially HEPES buffer, pH 7.2, alone was passed over the cells. This was then changed for buffer containing 1 mM sodium acetate and then returned to buffer alone. The single cells showed an increase in rotation rate to about 7.5 Hz and a reduction in stopping frequency. On removal of the sodium acetate the cells stopped for about 50 s before starting to rotate again and returned to a pre-stimulus stopping frequency and rotation rate within 3 min. Fig. 1(b) shows the merged data from 10 cells from the same field of view and shows the mean response to the addition and removal of 1 mM sodium acetate. The unstimulated cell populations had a background stop probability of 0.22. When sodium acetate was added the population stop probability was reduced, dropping to 0.05. Sodium acetate is a chemokinetic effector for *R. sphaeroides* and the response seen on its addition is a chemokinetic response (Packer & Armitage, 1994). In this experiment the stop probability was reduced for at least 100 s after which adaptation began. If extrapolated it would take about 10 min for complete adaptation to occur, a much longer period than would allow integration of this response into chemotaxis. We have previously shown in free swimming cells that this reduction in stopping frequency could be sustained for up to 60 min (Packer & Armitage, 1994).

When the acetate was removed from the buffer, all of the cells in the population stopped. The stops were never completely synchronized between cells, suggesting variation in the signalling time from cell to cell, and resulting in a calculated stop probability of 0.95 (Fig. 1b). The response time of the cell population was 64 s. The adaptation time, i.e. the time for all the cells to return to pre-stimulus behaviour, was 196 s. The cells therefore responded to the addition of a chemokinetic effector by a long-term reduction in their stop probability and responded to the rapid removal of the attractant by a transient stop and adaptation over several minutes. A second round of addition and removal of a chemoeffector had the same effect as the initial addition and removal.

The increase in concentration of an attractant which does not cause chemokinesis had no effect on either the stopping behaviour or rotation rate of the cell populations. The response of tethered cells grown with fructose as the carbon source to the stepwise addition and removal of fructose is shown in Fig. 2(a) and (b). Initially the unstimulated cell populations had stop probabilities of 0.19 and 0.35 (Fig. 2a, b). The addition of either 1 mM or 5 mM fructose did not cause any significant change in the behaviour of the cell populations, with mean stop probabilities of 0.15 and 0.35, respectively. When fructose was removed from the buffer, there was a transient increase in stopping probability. The response to the removal of 1 mM fructose was less sustained than the

Fig. 2. The response of two populations of 10 cells to the stepwise addition and removal of fructose. The cells were grown using fructose as the sole carbon source. (a) shows no response to 1 mM fructose, added at the first arrow but a transient stop followed by adaptation following removal of the fructose at the second arrow. (b) shows the same experiment as (a) but 5 mM fructose was added at the first arrow. A transient step-down response occurred when the attractant was removed from the buffer. The cells were all from the same field of view in the microscope.

response to the removal of 5 mM fructose. Removal of 1 mM fructose resulted in a response time of 41 s; the stop probability rose to 0.4 and adapted over 29 s. The response time to the removal of 5 mM fructose was 76 s; the mean stop probability increased to 0.88 with an adaptation time of 71 s. The cells therefore only showed a behavioural response to the removal of fructose and did not respond to its addition.

To determine whether the step-down response was a common response to all chemoeffectors in photo-synthetically grown *R. sphaeroides*, a range of chemoeffectors was investigated. The non-chemokinetic chemotactic effectors fructose and glucose only caused a behavioural response when the concentration was de-

Fig. 3. The response of a population of 10 anaerobically grown tethered cells to a change in oxygen concentration. Initially the cells were in N_2 -sparged HEPES buffer, pH 7.2; this was changed at the arrow to air-saturated buffer and changed back to N_2 -saturated buffer at the second arrow. A transient step-down response occurred when the concentration of oxygen was increased. The cells were from the same population.

creased, not when it was increased. The chemokinetic chemoeffectors succinate, pyruvate, propionate, lactate and malate all produced the same response as acetate with a sustained decrease in stopping frequency on their addition but a transient step-down response on their removal.

Response to changes in light intensity

The response of populations of free swimming cells of *R. sphaeroides* to a step-down in light intensity was analysed. Initially the population was swimming at $29.3 \pm 6.8 \mu\text{m s}^{-1}$. When the light level was decreased from 240 to $13 \mu\text{mol m}^{-2} \text{s}^{-1}$, the whole cell population stopped swimming for $2.8 \pm 0.65 \text{ s}$ and then resumed swimming at a lower swimming speed of $14.5 \pm 6.2 \mu\text{m s}^{-1}$. When the light level was returned to the original level, the cell populations did not show any change in stopping frequency but simply increased their swimming speed back to pre-stimulus levels.

Response to changes in concentration of an electron acceptor

The effect of a change in O_2 concentration on photo-heterotrophically grown cells was investigated. Tethered cells were incubated under normal light intensity in the flow chamber with N_2 -saturated HEPES buffer containing 1 mM succinate. The background mean stop probability was 0.02 (Fig. 3). When the oxygen concentration in the buffer was increased by switching to air-saturated buffer, the cells responded transiently by stopping (stop probability of 0.87). The response time of the cell population was 39 s and the adaptation time was

Fig. 4. The response of a population of 10 cells grown anaerobically in the dark to a stepwise change in the concentration of the terminal electron acceptor DMSO is shown. The experiment was conducted under low illumination. Initially the cells were in N_2 -saturated HEPES buffer, pH 7.2, containing 20 mM DMSO. (a) DMSO was removed at the arrow. (b) DMSO was replaced at the arrow. A transient response occurred on the removal of DMSO. The same cells were subjected to the step-up and step-down in DMSO, but because of the reduced swimming seen in DMSO grown populations, the cells were from several fields and the data merged.

68 s. The stop probability of the cell population did not completely return to the pre-stimulus level of 0.02 but remained at 0.2. When aerobic buffer was changed back to anaerobic buffer, the cell population did not respond and the mean stop probability remained at 0.23.

Cells grown anaerobically in the dark with DMSO as a terminal electron acceptor were tethered and incubated in a buffer containing 20 mM DMSO and 2 mM fructose, and the rotational rate of the cells was measured under low light ($34 \mu\text{mol m}^{-2} \text{s}^{-1}$). The cell population had a mean stop probability of 0.24 (Fig. 4). When the buffer was changed to DMSO-free buffer, the cells briefly stopped rotating and the stop probability increased to 0.94 (Fig. 4a). The response time was brief, 23 s, and the

adaptation time was 30 s. There was no response when the concentration of DMSO was increased back to 20 mM and the mean stop probability after the change was 0.2 (Fig. 4b).

DISCUSSION

R. sphaeroides has previously been shown to respond chemotactically to a wide range of chemoeffectors, all of which are metabolites, with the accumulative response dependent upon at least partial metabolism (Poole *et al.*, 1993). Analysis of the behaviour of individual cells of anaerobic, photosynthetically grown *R. sphaeroides* to changes in the concentration of chemoeffectors has identified adaptive responses. Tethered cells responded to a step-down in the concentration of a chemoeffector, such as an organic acid or sugar, by stopping flagellar rotation and then returning to pre-stimulus behaviour over a few minutes. We have previously shown that the presence of arsenate inhibits the chemotactic response in *R. sphaeroides* and analysis of tethered cells in the presence of arsenate showed that the transient stop to a step-down in chemoeffector concentration was no longer present, indicating that the transient increase in stopping is part of the chemosensory system (Packer & Armitage, 1994) and suggesting that periodic stopping of motor rotation in *R. sphaeroides* is the equivalent of tumbling in enteric bacteria. However, in addition to this, a stepwise increase in the concentration of a compound which was both a chemo-attractant and a chemokinetic effector produced a long-term but adaptive decrease in the stop probability.

It appears, therefore, that the motile behaviour of *R. sphaeroides* is as follows: (i) unstimulated swimming is punctuated by stops for random direction changing; (ii) when an increase in the concentration of a non-chemokinetic metabolite is sensed, e.g. when a cell moves up a gradient, there is no change in behaviour and the cell continues swimming normally; (iii) if there is a decrease in concentration of the metabolite, e.g. when a cell swims down a gradient, the cell stops transiently. Mathematical modelling of this pattern (L. Kruglyak, personal communication) using simulation based on that used for *E. coli* (Schnitzer *et al.*, 1990) predicted accumulation of cells at regions of high effector concentration. The behaviour of a population of chemokinetically stimulated cells was also mathematically simulated and led to an accumulation of cells only if direction-dependent changes in stopping frequency (i.e. chemotaxis) also occurred; however, this model gave weaker accumulation than a solely chemotactic effector. The decrease in stop probability on the addition of a chemokinetic effector is not the same as the response of *E. coli* to attractant as, in *R. sphaeroides*, this response is large, sustained over tens of minutes and accompanied by an increase in rate of rotation of the flagellar motor. However, as the change of stop probability does slowly adapt this may add to a chemotactic response.

Photoheterotrophically grown cells responded to oxygen by transiently stopping. Parallel experiments to measure the membrane potential of cells under similar conditions

revealed that this dropped slightly upon addition of oxygen if the cells were incubated in low light but not if they were in high light (D. E. Gauden & J. P. Armitage, unpublished data). This suggests that in high light, where photosynthetic electron transport is very rapid, there is no significant effect on electron flow when oxygen is present. But at low light, when photosynthetic electron transport will be operating at a reduced rate, electron transport to O_2 causes a transient reduction in photosynthetic electron transport. Anaerobically DMSO-grown *R. sphaeroides* showed chemotaxis to DMSO. Tethered DMSO-grown cells showed an increase in stopping to a step-down removal of DMSO, conditions under which there is a fall in the rate of electron transport (Gauden & Armitage, 1995). Similarly, in photoheterotrophically grown cells, a drop in light intensity results in a decrease in the rate of cyclic electron transport and also a step-down response followed by adaptation. A return to high light did not cause any change in stopping pattern even though electron transport rates increased, although swimming speed did increase, presumably because the electrochemical proton gradient increased. *R. sphaeroides* may therefore sense the rate of electron transport and adapt to a particular rate; a decrease in the rate, even briefly, causing a transient increase in stopping frequency. Whether the primary signal is a change in the size of the electrochemical proton gradient or the redox state of one of the electron transport components is unresolved but recent data suggest it may be a redox sensing mechanism (Gauden & Armitage, 1995).

None of the non-chemokinetic chemoeffectors caused a transient response to an increase in the concentration of an effector. It is, however, possible that there are very short stops or pauses in flagellar rotation in addition to the long stops. Any change in the number of short stops might be missed when the behaviour of tethered cells is followed, as the increased viscous drag on the cell body and the compliance of the hook might mask these and the tracking system cannot detect stops shorter than 60 ms. Alternatively, as the transient stop and adaptation to a decrease in the concentration of effector is the only response which seems to occur for all effectors, photosynthetically grown *R. sphaeroides* may have a chemotactic pathway which only responds to a decrease in stimulus. The longer term reduction in stop probability on the addition of chemokinetic effectors may be under the control of a different chemosensory pathway. The identification of a second *cheY* homologue in *R. sphaeroides* suggests that, unlike *E. coli*, there are at least two sensory pathways controlling the flagellar motor. Preliminary examination of the behaviour of aerobically grown cells indicates that they show a different pattern of behaviour to anaerobically grown cells, suggesting that chemosensing in general in *R. sphaeroides* is much more complex than that shown by *E. coli* and is influenced by the physiological state of the cell.

This study shows that the behavioural mechanism in photosynthetically grown *R. sphaeroides* appears to be the opposite of the 'optimistic' response of enteric species where cells only respond to an increase in attractant

concentration. Photosynthetic *R. sphaeroides* responds 'pessimistically' only showing a response if the attractant concentration decreases.

ACKNOWLEDGEMENTS

The authors would like to thank the Wellcome Trust and the BBSRC for their generous support of this work.

REFERENCES

- Armitage, J. P. (1992). Behavioural responses in bacteria. *Annu Rev Physiol* **54**, 683–714.
- Armitage, J. P. & Macnab, R. M. (1987). Unidirectional intermittent rotation of the flagellum of *Rhodobacter sphaeroides*. *J Bacteriol* **169**, 514–518.
- Armitage, J. P., Ingham, C. & Evans, M. C. W. (1985). Role of the proton motive force in phototactic and aerotactic responses of *Rhodospseudomonas sphaeroides*. *J Bacteriol* **163**, 967–972.
- Berg, H. C. & Block, S. M. (1984). A miniature flow cell designed for rapid exchange of media under high-powered objectives. *J Gen Microbiol* **130**, 2915–2920.
- Berg, H. C. & Brown, D. A. (1972). Chemotaxis in *Escherichia coli* analysed by three-dimensional tracking. *Nature* **239**, 500–504. [reprinted with addendum in *Antibiot Chemother* (1974) **19**, 55–78].
- Berg, H. C. & Tedesco, P. M. (1975). Transient response to chemotactic stimuli in *Escherichia coli*. *Proc Natl Acad Sci USA* **72**, 3235–3239.
- Block, S. M., Segall, J. E. & Berg, H. C. (1983). Adaptation kinetics in bacterial chemotaxis. *J Bacteriol* **154**, 312–323.
- Brown, S., Poole, P. S., Jeziorska, W. & Armitage, J. P. (1993). Chemokinesis in *Rhodobacter sphaeroides* is the result of a long term increase in the rate of flagellar rotation. *Biochim Biophys Acta* **1141**, 309–312.
- Dahlquist, F. W., Elwell, R. A. & Lovely, P. S. (1976). Studies of bacterial chemotaxis in defined concentration gradients. A model for chemotaxis toward L-serine. *J Supramol Struct* **4**, 329(289)–342(302).
- Gauden, D. E. & Armitage, J. P. (1995). Electron transport dependent taxis in *Rhodobacter sphaeroides*. *J Bacteriol* **177**, 5853–5859.
- Harrison, D. M., Packer, H. L. & Armitage, J. P. (1994). Swimming speed and chemokinetic response of *Rhodobacter sphaeroides* investigated by natural manipulation of the membrane potential. *FEBS Letts* **348**, 37–40.
- Packer, H. L. & Armitage, J. P. (1994). The chemokinetic and chemotactic behaviour of *Rhodobacter sphaeroides*: two independent responses. *J Bacteriol* **176**, 206–212.
- Poole, P. S., Sinclair, D. R. & Armitage, J. P. (1988). Real time computer tracking of free-swimming and tethered rotating cells. *Anal Biochem* **175**, 52–58.
- Poole, P. S., Brown, S. & Armitage, J. P. (1990). Swimming changes and chemotactic responses in *Rhodobacter sphaeroides* do not involve changes in the steady state membrane potential or respiratory electron transport. *Arch Microbiol* **153**, 614–618.
- Poole, P. S., Smith, M. J. & Armitage, J. P. (1993). Chemotactic signalling in *Rhodobacter sphaeroides* requires metabolism of attractants. *J Bacteriol* **175**, 291–294.
- Schnitzer, M. J., Block, S. M., Berg, H. C. & Purcell, E. M. (1990). Strategies for chemotaxis. In *Biology of the Chemotactic Response*, pp. 15–34. Edited by J. P. Armitage & J. M. Lackie. Cambridge: Cambridge University Press.

- Segall, J. E., Manson, M. D. & Berg, H. C. (1982).** Signal processing times in bacterial chemotaxis. *Nature* **296**, 855–857.
- Stock, J. B., Ninfa, A. J. & Stock, A. M. (1989).** Protein phosphorylation and regulation of adaptive responses in bacteria. *Microbiol Rev* **53**, 450–490.
- Taylor, B. L., Johnson, M. S. & Smith, J. M. (1988).** Signalling pathways in bacterial chemotaxis. *Bot Acta* **101**, 101–104.
- Ward, M. J., Bell, A. W., Hamblin, P. A., Packer, H. L. & Armitage, J. P. (1995a).** Identification of a chemotaxis operon with two *cheY* genes in *Rhodobacter sphaeroides*. *Mol Microbiol* **17**, 357–366.
- Ward, M. J., Harrison, D. A., Ebner, M. & Armitage, J. P. (1995b).** Identification of a methyl-accepting chemotaxis protein in *Rhodobacter sphaeroides*. *Mol Microbiol* **18**, 115–121.
- Zannoni, D. & Marrs, D. L. (1981).** Redox chain and energy transduction in chromatophores from *Rhodospseudomonas capsulata* cells grown anaerobically in the dark on glucose and dimethylsulphoxide. *Biochim Biophys Acta* **637**, 96–106.
-

Received 7 August 1995; revised 12 October 1995; accepted 23 October 1995.