Role of Metabolism in the Chemotactic Response of *Rhodobacter sphaeroides* to Ammonia

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Received 7 November 1988/Accepted 23 February 1989

***Rhodobacter sphaeroides* only showed chemotaxis towards ammonia if grown under nitrogen-limited conditions. This chemotactic response was completely inhibited by the addition of methionine sulfoximine. There was no effect of methionine sulfoximine treatment on motility or taxis towards propionate, demonstrating that the effect is specific to ammonia taxis. It is known that methionine sulfoximine inhibits glutamine synthetase and hence blocks ammonia assimilation. Methionine sulfoximine does not inhibit ammonia transport in *R. sphaeroides*; therefore, these results suggest that limited metabolism via a specific pathway is required subsequent to transport to elicit a chemotactic response to ammonia. Bacteria grown on high ammonia show transport but no chemotactic response to ammonia, suggesting that the pathway of assimilation is important in eliciting a chemotactic response.**

Enteric bacteria swim by the counterclockwise rotation of their peritrichous flagellar bundle. Periodically individual flagella rotate clockwise, causing the bundle to fly apart and the cell to tumble (12). Chemical gradients are usually detected by enteric bacteria via cell membrane receptors (methyl-accepting chemotaxis proteins [MCPs]) that span the cytoplasmic membrane (8). The binding of an attractant to an MCP signals the flagellar motors, via changes in phosphorylation of the *che* gene products, to promote smooth swimming. Adaptation to the continued presence of a chemoattractant is mediated by methyl ation of the MCPs at specific glutamate residues (3, 4, 9, 10).

The purple non-sulfur photosynthetic bacterium *Rhodobacter sphaeroides* lacks MCPs and has a single subpolar flagellum that only rotates clockwise, stopping periodically. Direction changing in this species is not caused by counter-rotation of the flagellum but by Brownian motion of the cell when the flagellum stops rotating (2). The principal attractants for *R. sphaeroides* include the weak organic acids, weak bases, ions such as potassium, and light (1, 14). Chemoattractants cause an increase in smooth swimming, but adaptation times appear to be very long, perhaps as a result of the lack of a specific MCP-based sensing system (13). There appears to be an absolute requirement for transport in chemotactic signaling in this species, but it has not been clearly established whether subsequent incorporation or metabolism of the attractant is required (11, 14).

Ammonia is only an attractant for *R. sphaeroides* in cells that have been grown on limiting nitrogen; excess nitrogen appears to repress taxis (11). When grown on limiting nitrogen, *R. sphaeroides* assimilates ammonia via the glutamine synthetase/glutamate synthase pathway, which contrasts with growth on excess nitrogen, for which ammonia is assimilated by glutamate dehydrogenase (5). We had earlier assumed that a single transport system for methylamine and ammonia exists in *R. sphaeroides* and that this system was only present in nitrogen-limited cells (11). However, work by Cordts and Gibson (6) showed that transport of ammonia in *R. sphaeroides* is constitutive, but with a second methylamine-ammonia transport system being derepressed by

growth on limiting nitrogen. It was also shown in that study that methionine sulfoximine does not inhibit ammonia transport, although it is an inhibitor of glutamine synthetase and hence of ammonia assimilation (6). We have therefore looked more closely at the role of transport and incorporation in relation to ammonia taxis in *R. sphaeroides*.

R. sphaeroides WS8 was grown and harvested, and its chemotactic responses were assayed in blind wells in which two adjoining chambers were separated by a polycarbonate membrane (8- μ m pore size). The membrane prevented free mixing of the two chambers but allowed a diffusion gradient of attractant to form and allowed bacteria to swim between the chambers. Bacteria were placed in the bottom chamber, and an attractant solution was placed in the top chamber. The movement of bacteria into the top attractant chamber was determined by Coulter counting and compared with that of controls for which there was only buffer in the attractant chamber (11). The concentration of attractant that caused a peak chemotactic response was always calculated in each experiment, and although it was variable, it was consistent with a certain level of starvation (13). Plug plate assays were also performed in which a slurry of bacteria was suspended in sloppy agar (0.25%) and plugs of attractant (50 mM) were placed into the sloppy agar. Chemotaxis can be measured by the formation of rings of bacteria around the attractant plugs. To obtain nitrogen-limited cells, the low-ammonia medium of Ingham and Armitage was used, while cells grown in excess ammonia were grown on the same medium plus 10 mM ammonia (11). In some experiments, nitrogen-limited cells were obtained by replacing ammonia with filter-sterilized L-glutamine (10 mM).

Methionine sulfoximine is known to inhibit the incorporation of ammonia via the glutamine synthetase-dependent pathway, and the effect of methionine sulfoximine at 10 μ g ml⁻¹ on the chemotactic response of ammonia-limited cells was tested (Fig. 1). It can be seen that control cells showed an almost twofold increase in the percentage of cells that swam into the attractant chamber of a taxis well in response to ammonia. When methionine sulfoximine was added, the response was almost totally abolished. There was no change in the speed (Fig. 2) or distribution of cell speed (data not shown) in response to methionine sulfoximine, demonstrat-

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FIG. 1. Effect of methionine sulfoximine addition on chemotaxis to ammonia. ○, Untreated cells; ●, cells plus 10 µg of methionine sulfoximine ml⁻¹. All points are averages of triplicate experiments; bars indicate standard errors of the mean.

ing that methionine sulfoximine is not a general inhibitor of motility. It is possible that methionine sulfoximine affected general chemotactic signaling in *R. sphaeroides* without impairing motility; however, methionine sulfoximine at the same concentration which inhibited ammonia taxis had no effect on the chemotactic response to propionate (Fig. 3). As a further test, the effect of 10 µg of methionine sulfoximine ml⁻¹ on chemotaxis towards propionate, acetate, succinate, or fructose was measured in plug plates, and there were no observable differences in the responses seen on plates with or without methionine sulfoximine. These results show that methionine sulfoximine specifically inhibited the chemotactic response to ammonia.

Ammonia utilization was measured in cells suspended in 10 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) (pH 7.2)–10 mM succinate. NH₄Cl₂ (100 µM) was added at time zero, and samples were taken at 0, 5, and 10 min. Cells were centrifuged for 30 s in a microcentrifuge, and the supernatant ammonia was analyzed by the phenol hypochlorite method (7). Rates of utilization were then calculated by subtraction of the ammonia remaining after 10 min from the time zero sample. Cells grown on high ammonia utilized ammonia at a rate of 11.9 nmol min⁻¹ mg of

FIG. 2. Effect of methionine sulfoximine on motility of *R. sphaeroides*. All points are averages of triplicate experiments; bars indicate standard errors of the mean.

FIG. 3. Effect of methionine sulfoximine addition on chemotaxis to propionate. ○, Untreated cells; ●, cells plus 10 µg of methionine sulfoximine ml⁻¹. All points are averages of triplicate experiments; bars indicate standard errors of the mean.

protein⁻¹ and were able to completely deplete the supplied ammonia. This rate is lower than the value of 66 nmol min⁻¹ mg of protein⁻¹ reported by Cordts and Gibson; however, the value reported here is not the initial rate of uptake, which was approximately two- to threefold higher, but is the utilization rate measured over 10 min. The limit of detection of the phenol hypochlorite assay is 3 to 5 µM; this result therefore indicates that cells had a high affinity for ammonia and suggests the presence of a transport system. However, it is possible that this apparent transport results from the metabolic drag caused by assimilation. The strongest evidence against this comes from the results of Cordts and Gibson, in which a constitutive ammonia transport system was shown to be present in *R. sphaeroides* 2.4.1, in addition to the transport system shown to be repressed by ammonia (6). This second system has been shown to also transport methylamine. These systems are unequivocally active transport systems, because they were demonstrated to be able to form a 100-fold concentration gradient of ammonia (6). Growing strain WS8 on limiting nitrogen, whether with limiting ammonia or glutamine, slightly increased the rate of ammonia utilization (17.9 ± 0.9 µmol min⁻¹ mg of protein⁻¹ [mean ± standard error of the mean] for limiting ammonia and 21.8 ± 5.0 µmol min⁻¹ mg of protein⁻¹ for glutamine). As expected, addition of methionine sulfoximine at 10 µg ml⁻¹ completely inhibited the incorporation, and hence the measured consumption rate, of ammonia. It has already been shown that this inhibition of utilization is not caused by an inhibition of uptake but by inhibition of glutamine synthetase-dependent incorporation (6). Cells treated with methionine sulfoximine still maintained a 100-fold ammonia concentration gradient, indicating continued transport needed to counter the passive efflux from the intracellular ammonia pool of 1 mM.

The results presented here show that some metabolism of ammonia in addition to transport is required for there to be a chemotactic response. Previous attempts to demonstrate chemotaxis in cells grown on high ammonia have been unsuccessful, even though an active transport system is now known to be present under these conditions in *R. sphaeroides* 2.4.1 and probably in strain WS8 (6). This suggests that while transport is necessary for a response it does not per se constitute the chemotactic signal. It also shows that either the pathway of ammonia assimilation or at least the state of the cells under nitrogen limitation is crucial in causing a

chemotactic response to ammonia. Indeed, this suggests that *R. sphaeroides* responds more to the overall metabolic consequences of ammonia assimilation than it does to ammonia itself. *R. sphaeroides* has a very poor ability to adapt to chemotactic stimulation, a result that might suggest that cells would become trapped in a gradient of one compound (13). However, the lack of a response of *R. sphaeroides* to ammonia when grown under nitrogen excess suggests an ability to respond only when limited by the nutrient. An organism such as *R. sphaeroides* would normally be severely nutrient limited in the natural environment and would only benefit by responding to an attractant, whether it be ammonia, propionate, potassium, or light, that limits the growth of the organism. *R. sphaeroides* may avoid being caught in a gradient of one compound because, instead of sensing the chemical itself, it senses the overall metabolic change brought about by the compound. This also obviates the need for a complex and energetically expensive detection and signaling system such as exists in enteric bacteria. Overall, the mechanism of tactic signaling in *R. sphaeroides* appears to be very different from that in enteric bacteria; it requires transport and may respond to the metabolic change brought about by the compound.

We thank the Wellcome Trust for their generous support of this work.

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