Glycerol and Ethylene Glycol: Members of a New Class of Repellents of Escherichia coli Chemotaxis

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By using the chemical-in-plug method, we found that glycerol and ethylene glycol caused negative chemotaxis in wild-type cells of Escherichia coli; the threshold concentration was about $10^{-3}$ M for both chemicals. As with other known repellents, the addition of glycerol or ethylene glycol induced a brief tumble response in wild-type cells but not in generally nonchemotactic mutants. Experiments with mutants defective in various methyl-accepting chemotaxis proteins (MCPs) revealed that the presence of any one of three kinds of MCPs (MCP I, MCP II, or MCP III) was necessary to give a tumble response to these repellents. Consistently, it was found that the methylation-demethylation system of MCPs was involved in the adaptation of the cells to these repellents. The effect of glycerol or ethylene glycol was not enhanced by lowering the pH of the medium, and glycerol did not alter the membrane potential of the cells. All of these results suggest that glycerol and ethylene glycol are members of a new class of repellents which produce a tumble response in the cells by perturbing the MCPs in the membrane.

Bacteria can sense various chemicals in their environment and respond by changing their swimming patterns. An increase in the attractant concentration causes counterclockwise rotation of the flagella, which results in smooth swimming of the cell, whereas a decrease in the attractant concentration or an increase in the repellent concentration causes clockwise rotation of the flagella, which results in tumble swimming of the cell. By a combination of these swimming patterns, bacteria can migrate toward a favorable environment or away from an unfavorable environment (1, 13).

It has been established that chemical stimuli are first detected by specific chemoreceptors on the cell surface and then transmitted to integral membrane proteins called methyl-accepting chemotaxis proteins (MCPs) (26). For example, maltose-binding proteins located in the periplasmic space are the chemoreceptors for maltose, and the ligand-bound maltose-binding proteins directly and specifically interact with one of the MCPs, MCP II, for the sensory transduction (11, 21).

For the processing of attractant information, Escherichia coli cells have three kinds of MCPs (12, 25). MCP I is a product of the tss gene and is used mainly for L-serine sensing. MCP II is a product of the tar gene and is used mainly for L-aspartate and maltose sensing. MCP III is a product of the trg gene and is used for D-ribose and D-galactose sensing. Recent studies have shown that in addition to functioning as sensory transducers, MCP I and MCP II function as chemoreceptors for L-serine and L-aspartate, respectively (7, 28). Methylation of MCPs by the methylation-demethylation system specific to MCPs results in sensory adaptation to most attractants (26).

Chemosensory transduction of repellents in E. coli seems to be more complicated than that of attractants. The repellents studied so far are classified into two groups. One consists of repellents such as L-leucine and Ni$^{2+}$, which are processed by a specific MCP, e.g., MCP I for L-leucine and MCP II for Ni$^{2+}$ (25). The other group consists of repellents such as weak acids and uncouplers, which act by perturbing the physiological state of the cell and are not usually mediated by MCPs (15, 22), although a part of the information concerning weak-acid repellents is processed by MCP I (10, 20). When the MCPs are involved in the information processing, the methylation-demethylation system of MCPs is involved in the adaptation to the repellents.

Glycerol is a good carbon source for E. coli. However, it has been shown that glycerol is not a chemoattractant (2). Accidentally, we found that glycerol is a repellent for E. coli cells. Since ethylene glycol, which is analogous to glycerol, has already been reported to be a repellent for E. coli (27), we compared the repellent action of these chemicals with that of known repellents. In this paper, we show that glycerol and ethyl-
ene glycol are members of a new class of repellents which cause a tumble response in *E. coli* cells, probably by perturbing any one of the MCPs in the membrane.

**MATERIALS AND METHODS**

**Bacterial strains and growth conditions.** All of the bacterial strains used in this study were *E. coli* K-12 derivatives. Strains AW405 (wild type), AW457 (cheA), AW518 (tsr-l), AW655 (tsr-12), AW539 (tar-l), AW656 (tar), AW701 (tsr-1), AW569 (tsr-l tar-1), AW659 (tsr-12 tar), AW657 (tsr-12 trg-l), AW658 (tar trg-1), and AW660 (tsr-12 trg trg-1) were obtained from J. Adler of the University of Wisconsin (12). Strains RP3238 (cheW), RP4873 (cheY), RP4080 (cheR217), and RP5698 (Δtsr) were obtained from J. S. Parkinson of the University of Utah (4, 18, 19). Strain RP5698 lacks most of the *tsr* gene (J. S. Parkinson, personal communication). Strain MS5209 (tar-52Δ1) was obtained from M. Simon of the University of California (3). Strain TH403 (tsr-l:TsΔ) was obtained from S. Harayama of the University of Tokyo (5). Strain AS-1 (cheA) has been described previously (8).

Cells were grown at 35°C with shaking in T broth consisting of 1% tryptone (Difco Laboratories, Detroit, Mich.), 0.5% NaCl, and 10 mM sodium lactate. In the case of strain RP4080, the cells were grown in T-broth supplemented with 0.5% glycerol. At the late log phase of growth (absorbance at 590 nm = 0.7), the cells were harvested.

**Motility medium.** Cells were washed and suspended in a motility medium consisting of 10 mM potassium phosphate buffer (pH 7.0), 0.1 mM potassium EDTA, and 10 mM sodium lactate. The pH of the motility medium usually was 7.0; when necessary, the pH was changed to the final pH, as indicated, with HCl.

**Chemicals.** [3H]triphenylmethylphosphonium bromide ([3H]TPMP') (6 Ci/mmol) was the generous gift of R. M. Macnab of Yale University. Optically pure glycerol was a product of Wako Pure Chemical Industries (Osaka, Japan). Ethylene glycol and other dihydric alcohols were purchased from Tokyo Kasei Co. (Tokyo, Japan).

**Chemical-in-plug method for negative-chemotaxis assay.** The chemical-in-plug method of Tso and Adler (27) was used for the negative-chemotaxis assay. Cells at the late log phase were harvested by centrifugation at 12,000 × *g* for 10 min at room temperature and washed once with the motility medium. The cells were suspended without any addition of the medium to make a dense cell suspension. About 10⁶ cells were then mixed with 20 μl of motility medium containing 0.3% agar (Difco) at 50°C. This cell-agar mixture was immediately poured into a petri dish. After about 4 min of incubation at room temperature, the agar plugs which contained various test chemicals were planted with toothpicks into the cell-agar suspension. Plates were incubated for 30 min at room temperature, and the size of the clearing zone around the plug was measured.

**Measurement of tumble response in free-swimming cells.** The tumble response in free-swimming cells was analyzed by measuring the changes in the fraction of smooth-swimming cells. Cells at the late log phase were harvested by filtration through a membrane filter (pore size, 0.45 μm; Sartorius-Membrane Filter GmbH, Göttingen, Germany), washed three times with 5 ml of the motility medium each time, and resuspended in the same medium to a concentration of about 5 × 10⁷ cells per ml. In a test tube, cells were mixed with a repellent, and a drop of the cell suspension was immediately placed on a glass slide. Swimming cells were observed at 25°C with a dark-field microscope and recorded on videotape. Swimming tracks of these cells were obtained from the video scenes by a photographic method with an exposure time of 1 s, as described previously (17).

To obtain the smooth-swimming fraction, that is, the fraction of smooth-swimming cells in total cells at a given time point, only the swimming tracks having no sharp bend were counted as smooth-swimming tracks. Without any stimuli, the smooth-swimming fraction was about 0.8 for wild-type cells and about 0.9 for cheR mutant cells. In the figures, the smooth-swimming fraction obtained without any repellents is normalized to 100% and expressed as the percentage of control cells.

In the case of the cheR mutant, experiments were carried out at 20°C, since the repellent action on the mutant cells was found to be more clear at low temperatures (Y. Imae, T. Mizuno, and K. Maeda, manuscript in preparation).

**Rotation mode of tethered cells.** Cells in growth medium were sucked and blown out vigorously about 200 times through a Pasteur pipette to shear their flagella. After 10-fold dilution in the motility medium, a drop of the cell suspension was put on a glass slide and mixed with a drop of antiflagellar antiserum (24). The tethered cells thus obtained were washed extensively with the motility medium. Repellents or attractants were added to the tethered cells at 25°C, and the changes in the rotation mode of the cells were observed under a dark-field microscope (14) and recorded on videotape. The rotation mode of the cells was analyzed by a playback in slow motion.

**Measurement of membrane potential of cells.** The membrane potential of the cells, that is, the electrical potential difference between the inside and outside of the cells, was estimated from the distribution of a radioactive lipophilic cation ([3H]TPMP'). Cells were washed and suspended in HEL medium (10 mM HEPES [N-2-hydroxyethylpiperazine-N'-2-ethanesulfonate] buffer [pH 7.0], 0.1 mM EDTA, 10 mM sodium lactate) to a concentration of 10⁶ cells per ml. With shaking at 30°C, cells (1 ml) were mixed with 2 μM tetrathenylboron, and then [3H]TPMP' (390 μCi/2 μmol per ml) was added to a final concentration of 10 μM. After incubation for 15 min, glycerol was added to a final concentration of 1 M. As a control, the same volume of distilled water was added to another tube. At intervals, a 50-μl sample was removed, and the amount of [3H]TPMP' accumulated in the cells was measured by filtration and measured previously (23).

**RESULTS**

Negative chemotaxis to glycerol and ethylene glycol. The chemical-in-plug method was applied to demonstrate that glycerol and ethylene glycol are repellents for *E. coli*. Wild-type cells showed negative chemotaxis to glycerol and ethylene
Tumble response induced by glycerol and ethylene glycol. When glycerol or ethylene glycol was added to the wild-type cells, a transient tumble response was induced (Fig. 2). As with other typical repellents, the adaptation to these chemicals occurred very quickly, and even after the addition of a very high concentration (e.g., 1 M) of glycerol or ethylene glycol, the recovery time was only 1 to 2 min at 25°C.

The dose-response relation was investigated by using an adaptation-deficient mutant, the cheR217 mutant, since it showed the tumble response to these chemicals for more than 30 min. About 0.4 M glycerol or ethylene glycol was necessary to cause an incessant tumbling in almost all of the mutant cells (Fig. 3). The addition of 1,5-pentanediol at up to 0.4 M induced only a slight tumble response in the cheR mutant. This result eliminates the possibility that a sudden increase in the osmotic pressure or glycol as well as to well-known repellents such as acetate and L-leucine (Fig. 1). The threshold concentration of glycerol necessary to induce negative chemotaxis in the wild type was estimated to be about 10^{-3} M. The threshold value of ethylene glycol was also about 10^{-3} M, which is consistent with the value reported by Tso and Adler (27).

Although the threshold values of glycerol and ethylene glycol for negative chemotaxis were similar, the sizes of the clearing zones induced by these repellents were different; glycerol produced a larger clearing zone than ethylene glycol. The clearing zone induced by high concentrations of glycerol was composed of two types of zones (Fig. 1). The inner zone was completely clear and identical to the zone induced by ethylene glycol or other repellents, but the outer zone was slightly turbid, indicating that some cells were still left behind in this zone. The reason for the formation of this outer zone by glycerol is unknown, but we sometimes observed that acetate produced a similar outer zone, although it was much weaker.

FIG. 1. Negative chemotaxis to glycerol and ethylene glycol in wild-type strain AW405. The chemical-in-plug method was used as described in the text. About 10^9 cells were distributed equally in the semisolid plate. After plugs containing various concentrations of glycerol, ethylene glycol, or other repellents were implanted, the plate was incubated at 25°C for 30 min. Plugs 1-3 contained no addition, 30 mM L-leucine, and 17 mM acetate, respectively. Plugs 4-8 contained glycerol (a) or ethylene glycol (b) at 10^{-4}, 10^{-3}, 10^{-2}, 10^{-1}, and 1 M, respectively.

FIG. 2. Transient tumble response in strain AW405 induced by glycerol and ethylene glycol. At the time point indicated by the arrow, glycerol or ethylene glycol (EG) was added to wild-type cells in motility medium to a final concentration of 1 M. Changes in the smooth-swimming fraction were followed at 25°C. The fraction without any repellent was normalized to 100%.
the viscosity of the medium by the addition of these chemicals is a cause of the tumble response in the mutant.

Glycerolialdehyde, which is a possible contaminant in glycerol, did not induce any tumble response in the cheR mutant at up to 0.02 M (data not shown).

The action of glycerol and ethylene glycol was completely overcome by the addition of attractants (Table 1). The generally nonchemotactic mutants, AW457 (cheA), RP3238 (cheW), and RP4873 (cheY), showed no response to glycerol or ethylene glycol. These results suggest that the chemosensory transducing system is involved in the information processing for these repellents.

The addition of a high concentration of glycerol or ethylene glycol caused a slight decrease in the swimming speed in both the wild type and the generally nonchemotactic mutants, probably due to some increase in the viscosity of the medium. At 1 M glycerol or 1 M ethylene glycol, the swimming speed was about 80% of the normal speed, and this speed was not restored by the addition of any attractants.

**Sensory transducing pathway for glycerol and ethylene glycol.** For the sensory transduction of most chemotacticants and of typical repellents such as L-leucine and Ni²⁺, the presence of a specific MCP is necessary. To clarify which MCP is required for the sensory transduction of glycerol and ethylene glycol, various mutants having defects in MCPs were analyzed.

All of the mutants having a defect in one of the MCPs showed a tumble response to glycerol and ethylene glycol that was similar to that of the wild type. The mutants tested were AW518 (tsr-l), AW655 (tsr-12), RP5698 (Δtsr), AW539 (tar-l), MS5209 (tar-SΔA1), AW701 (trg-1), and TH403 (trg-1::Tn5). RP5698, MS5209, and TH403 are the null mutants in MCP I, MCP II, and MCP III, respectively. Furthermore, the deletion in mutant MS5209 has been reported to start from the tar gene and extend into an adjacent gene, tap, whose product has recently been identified as an MCP-like protein (3, 29). All of these results indicate that none of the MCPs, including the product of the tap gene, is specifically involved in the sensory transduction of these repellents.

The mutants having double defects in MCP I and MCP II, in MCP I and MCP III, and in MCP II and MCP III also showed a tumble response to glycerol and ethylene glycol (Fig. 4a). The
Thus, elimination of all three MCPs at the same time, however, resulted in a drastic decrease in the ability to respond to glycerol and ethylene glycol: the tsr tar trg triple mutant did not show any detectable tumble response to these repellents (Fig. 4b). This result suggests that the presence of one kind of MCP in the cell is required for the sensory transduction of these repellents.

In addition to the free-swimming cells, tethered cells were used for the analysis of the repellent action of glycerol and ethylene glycol. Tethered cells of the wild type without any stimuli showed frequent changes in the direction of rotation, and the addition of glycerol or ethylene glycol caused a transient fixation of rotation in the clockwise mode. Figure 5a shows a typical result of this type of response to glycerol in a tar mutant. Adaptation to glycerol occurred after 1 to 2 min at 25°C. These results are consistent with the previous results obtained with the free-swimming cells. When 1 M glycerol or 1 M ethylene glycol was added to tethered cells of the wild type, about 80% of the cells showed an increase in clockwise rotation (Table 2). Tethered cells of a tsr tar double mutant showed only counterclockwise rotation in the absence of any stimuli. However, the addition of glycerol or ethylene glycol caused an increase in the clockwise mode of rotation (Fig. 5b). This kind of response was found in about 60% of the cells (Table 2). In the case of the tsr tar trg triple mutant, tethered cells showed exclusively counterclockwise rotation. The addition of glycerol or ethylene glycol caused no response in most cells, and only about 20% of the cells showed an increase in the clockwise rotation (Table 2). Thus, these results confirmed that for the normal response to glycerol or ethylene glycol to occur, the presence of at least one kind of MCP is necessary.

When the mutants having a single defect in MCPs were tested by the chemical-in-plug method, all of them, including the null mutants, showed negative chemotaxis for glycerol and ethylene glycol. The tar tap double mutant also showed a similar result. However, the tsr tar double mutant and the tsr tar trg triple mutant showed no detectable negative chemotaxis for these repellents.

Chemosensory adaptation to glycerol and ethylene glycol. As described above, an adaptation-deficient mutant (the cheR217 mutant) showed a tumble response to glycerol and ethylene glycol but did not show any adaptation to these repellents. Since the mutant has a defect in the methylation system of MCPs (4, 19), these results indicate that the methylation-demethylation system of MCPs plays an important role in the adaptation to these repellents. The require-

![Figure 5](https://example.com/figure5.png)

**FIG. 5.** Changes in the rotation mode of tethered cells by the addition of glycerol. At the time point indicated by the arrow, glycerol was added to tethered cells to a final concentration of 1 M, and the rotation mode of the cells was measured at 25°C. (a) Strain AW656 (tar); (b) strain AW659 (tsr tar). Abbreviations: CCW, counterclockwise rotation; CW, clockwise rotation.

### TABLE 2. Effect of glycerol and ethylene glycol on tethered cells of a wild-type strain and of MCP mutants

<table>
<thead>
<tr>
<th>Strain</th>
<th>% of cells responding to:</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Glycerol (1 M)</td>
</tr>
<tr>
<td>AW 405 (wild type)</td>
<td>80</td>
</tr>
<tr>
<td>AW 659 (tsr tar)</td>
<td>67</td>
</tr>
<tr>
<td>AW 660 (tsr tar trg)</td>
<td>22</td>
</tr>
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</table>

* Tethered cells were challenged with 1 M glycerol or 1 M ethylene glycol at 25°C, and the cells which showed a clear increase in the frequency of clockwise rotation were counted as responding cells. More than 40 tethered cells were tested with each repellent.
ment of MCPs for the sensory transduction of these repellents supports this conclusion.

In the case of the wild type, glycerol or ethylene glycol induced a tumble response for about 2 min. However, the response in the tsr tar double mutant continued for much longer (Fig. 4a). Tethered cells of the double mutant also showed a longer response to these repellents; the response continued for more than 5 min, and a frequent clockwise rotation was observed even 8 min after the addition of glycerol (Fig. 5b). Thus, in this mutant, the adaptation to these repellents occurs quite slowly. However, the addition of D-ribose (10 mM) or the dilution of glycerol quickly restored the rotation mode to the original state, namely, counterclockwise rotation (data not shown). These results indicate that these repellents did not cause irreversible alteration in the sensory transducing system of the mutant. In the case of the tethered cells of the tsr tar trg triple mutant, the percentage of responding cells was small, but the time course of the response in the responding cells was similar to that of the tsr tar double mutant shown in Fig. 5b. Of course, in the triple mutant, the addition of D-ribose did not restore the rotation mode.

Effect of the pH of the medium on the repellent action of glycerol and ethylene glycol. Changes in the pH of the medium did not alter the repellent action of glycerol and ethylene glycol in the wild-type cells, whereas the repellent action of acetate was strongly affected by the pH (Fig. 6). The mutants defective in the cheA, cheW, or cheY gene showed no response to 1 M glycerol or 1 M ethylene glycol even at pH 5.5, although it has been reported that acetate induces a drastic tumbling in these generally nonchemotactic mutants at low pH (10). Furthermore, glycerol and ethylene glycol caused a clear tumble response in the tsr tar double mutant, as described above (Fig. 4a), whereas acetate did not induce any tumbling in the mutant even at pH 5.5 (10). These results indicate that the repellent action of glycerol and ethylene glycol is different from that of acetate, which is known to act at low pH by altering the physiological state of the cells (10).

Effect of glycerol on the membrane potential of the cells. The membrane potential of the cells was measured by using a lipophilic cation, \([\text{^3H}]\text{TPMP}^+\). For this purpose, we used a mutant, AS-1, which is permeable to \([\text{^3H}]\text{TPMP}^+\) without EDTA treatment and is chemotactically like the wild type (8). The membrane potential of the cells was about −130 mV, and the addition of 1 M glycerol did not cause any significant changes in the membrane potential. The earliest time point of the measurement was 30 s after the addition of glycerol. Under the conditions used, the cells showed drastic tumbling for about 2 min.

Similar results were obtained by using a wild-type strain, AW405, although in this case EDTA treatment was necessary to make the cells permeable to \([\text{^3H}]\text{TPMP}^+\). Thus, glycerol is not an uncoupler-type repellent.

Repellent action of other monohydric and dihydric alcohols. As reported by Ts'o and Adler (27), various alcohols were repellents to E. coli. Dose-response curves to various alcohols were obtained by using a cheR mutant (Fig. 7). The monohydric alcohols methanol and ethanol induced a weak tumbling at 0.4 M (Fig. 7a), whereas other long-chain monohydric alcohols caused an irreversible inhibition of motility at 0.4 M (data not shown). Most dihydric alcohols caused a drastic tumbling at 0.4 M (Fig. 7b and 7c).
c). 1,2-Propanediol caused a drastic tumbling at 0.2 M, but 1,3-propanediol caused only a slight tumbling at 0.2 M. With most dihydric alcohols, a concentration higher than 0.5 M caused a severe deterioration of motility (data not shown).

DISCUSSION

Wild-type cells of *E. coli* showed negative chemotaxis to glycerol and ethylene glycol. These chemicals caused transient increases in the tumbling frequency of the free-swimming cells and in the frequency of clockwise rotation of the tethered cells. The generally nonchemotactic mutants showed no response to these chemicals. Thus, glycerol and ethylene glycol are repellents, information about which is processed by the chemosensory transducing system.

All of the repellents extensively studied so far either require the presence of a specific MCP for their sensory transduction or cause some physiological perturbations in the cell. By the use of mutants having various defects in the MCPs, it was revealed that the presence of a specific MCP was not required for the sensory transduction of glycerol and ethylene glycol. However, it was found that the *tsr tar trg* triple mutant showed no response to glycerol or ethylene glycol and that the methylation-demethylation system of MCPs was involved in the adaptation to these repellents. These results indicate that the presence of any one MCP is required for the sensory transduction of glycerol and ethylene glycol. Furthermore, the physiological state of the cell was not detectably perturbed by these repellents. Therefore, we conclude that glycerol and ethylene glycol are members of a new class of repellents for *E. coli*. Since *E. coli* cells can utilize glycerol but not ethylene glycol as a carbon source, glycerol is a metabolizable repellant and ethylene glycol is a nonmetabolizable analog.

There has been no report on repellents which could induce any response in the *tsr tar* double mutant. However, glycerol and ethylene glycol caused a tumble response in this double mutant. This result also indicates that these repellents are different from the repellents extensively studied so far.

The nonspecific requirement of MCPs for the chemosensory transduction of glycerol and ethylene glycol indicates that the chemoreception of these repellents is not mediated by the binding-protein-type receptors which have affinity to a specific MCP. The requirement of quite high concentrations of these chemicals for the induction of the tumble response to the cells supports this idea. Recently, it has been shown that the MCPs, besides functioning as chemosensory transducers, also function as chemoreceptors for many attractants and repellents (7, 28). Therefore, it is quite likely that these repellents have direct or indirect interaction with any one of the MCPs.

The adaptation time to these repellents in the wild type and in many of the MCP mutants was short. However, the tumble response to these repellents in the *tsr tar* double mutant continued for much longer. The tethered cells of the double mutant also showed a longer response. These results may have some relation to the results reported by Hazelbauer and Engström (6), in which the adaptation time of the *tsr tar* double mutant to D-ribose or D-galactose was quite long. The rate of methylation or demethylation of MCP III, which is the only remaining MCP in
this double mutant, might be slow. The slow adaptation of this double mutant is not due to the irreversible alteration of MCP III by these repellents, since the addition of D-ribose or the dilution of glycerol quickly restored the swimming pattern of the mutant cells to the original state at any time.

*E. coli* cells are peritrichously flagellated, have six to eight flagella on a cell, and swim by rotating these flagella as a bundle. Upon tumbling, most flagella on a cell change their rotation mode from counterclockwise to clockwise (16). Actually, under the conditions where most cells of the wild type showed tumbling with glycerol or ethylene glycol, about 80% of the tethered cells showed a drastic increase in the clockwise rotation. In the case of the *tsr tar trg* triple mutant, these repellents did not induce any detectable tumbling. However, when the tethered cells of the triple mutant were challenged with these repellents, about 20% of the cells showed an increase in the clockwise rotation. Since this mutant is not a deletion type, the remaining response in the triple mutant may be due to the presence of the *tap* gene or defective MCP proteins which are still somewhat active in inducing a tumble response by these repellents. Whatever the reason for the remaining response in the mutant, the response found in about 20% of the tethered cells is not due to the difference in the sensitivity of individual cells to these repellents, since none of the free-swimming cells of the triple mutant showed detectable tumbling with these repellents. Rather, the response seems to be related to the nonsynchronous reversal of peritrichous flagella on a cell, which has been seen in *Salmonella typhimurium* (R. M. Macnab, unpublished data [cited in reference 9]). Thus, individual flagella on a peritrichously flagellated cell might show different levels of response to a stimulus.

The addition of glycerol or ethylene glycol did not induce any tumbling in the free-swimming cells of the triple mutant but induced clockwise rotation in about 20% of the tethered cells. This indicates that clockwise rotation in one to two flagella on a cell is not enough to induce tumbling in the cell. In the case of the *tsr tar* double mutant, about 60% of the tethered cells showed clockwise rotation with these repellents, whereas most of the free-swimming cells showed tumbling. Thus, about half of the flagella on a cell should rotate clockwise at the same time to induce tumbling in the cell. It should be noted, therefore, that the swimming pattern of peritrichously flagellated bacteria is not a simple reflection of the rotation mode of the individual flagella.

Specific chemoreceptor proteins have not been found for the repellents studied so far, and most of the repellents are known to be hydrophobic or membrane permeable and to require relatively high concentrations for induction of the tumble response. These facts suggest that the primary target of most repellents is the cell membrane itself, just as in the case of the repellents reported here. If this is the case, then the perturbation of MCPs in the membrane, which is necessary to induce tumbling in the cell, is a secondary effect of the alteration of the membrane. The MCP specificity of the repellents would be explained if each repellent had different effects on the membrane.

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**LITERATURE CITED**


