Cell Envelopes of Chemotaxis Mutants of *Escherichia coli* Rotate Their Flagella Counterclockwise

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Received 3 December 1984/Accepted 15 January 1985

Flagella rotated exclusively counterclockwise in *Escherichia coli* cell envelopes prepared from wild-type cells, whose flagella rotated both clockwise and counterclockwise, from mutants rotating their flagella counterclockwise only, and even from mutants rotating their flagella primarily clockwise. Some factor needed for clockwise flagellar rotation appeared to be missing or defective in the cell envelopes.

Cells of wild-type *Escherichia coli* or *Salmonella typhimurium* are able to rotate their flagella both counterclockwise and clockwise, to produce smooth swimming and tumbling, respectively (14), but cell envelopes prepared from these wild-type cells rotate their flagella almost exclusively counterclockwise (6).

We have now studied cell envelopes prepared from smooth or tumbling chemotaxis mutants of *E. coli*. Intact smooth mutants rotate their flagella almost entirely counterclockwise (14), and in this report we show that cell envelopes prepared from smooth mutants likewise rotate their flagella counterclockwise (Table 1). Intact tumbling mutants have primarily clockwise-rotating flagella (14), but their cell envelopes have counterclockwise-rotating flagella (Table 1).

A study of cell envelopes of chemotaxis mutants of *S. typhimurium* has been published by Ravid and Eisenbach (20). They also found that *cheB* and *cheZ* tumbling mutants give rise to cell envelopes which rotate their flagella counterclockwise, but two other *S. typhimurium* tumbling mutants, *cheV* MY1 and *cheC* ST120, gave rise to cell envelopes with clockwise-rotating flagella. In contrast, the present study shows that *cheV* tumbling and *cheC* tumbling mutants of *E. coli* produced envelopes with counterclockwise-rotating flagella (Table 1). The reasons for the differences between these *S. typhimurium* and *E. coli* mutants are not known.

Methionine, S-adenosylmethionine, and ATP are known to be required for clockwise rotation of bacterial flagella (1, 2, 5, 13, 21, 22). Eisenbach and Adler previously reported that the counterclockwise rotation of flagella of wild-type cell envelopes could not be changed into clockwise rotation by the addition of L-methionine (0.1 mM), S-adenosylmethionine (0.1 mM), and ATP (1 mM) to the medium used for lysing the cells plus the addition of L-methionine (0.1 mM) to the medium used for washing the cell envelopes (6). Now we report that the same is true for cell envelopes prepared from tumbling bacteria: the envelopes still rotated their flagella exclusively counterclockwise. For the seven strains listed as tumbling mutants in Table 1, entirely counterclockwise rotation was observed for 11, 7, 22, 10, 11, 7, and 16 envelopes, respectively, when they were treated with the three reagents as described above; these data are included in Table 1.

What makes the cell envelopes of wild type and tumbling mutants rotate their flagella exclusively counterclockwise? It is known that counterclockwise rotation can be caused by a low speed of flagellar rotation; switching to clockwise rotation requires a higher level of energy (8). However, a low energy level does not appear to be the cause of failure to observe clockwise flagellar rotation of the cell envelopes: the frequency of rotation of the tethered cell envelopes was as high as that of intact cells, as demonstrated by measurements of the wild-type strain AW405 and the tumbling mutants RP487*cheB*, AW662, and AW672 (data not shown). Neither is the exclusively counterclockwise rotation due to a smaller size of the envelopes. (As envelope size decreases, viscous drag on the motor also decreases, so that the speed of rotation corresponding to an energy level that permits clockwise rotation must increase.) When viewed by phase contrast microscopy, the cell envelopes do appear smaller than intact cells, but this is an artifact; the envelopes are almost the same size as intact cells when viewed by Nomarski differential interference-contrast microscopy, a more appropriate method for judging the size of such small particles (12).

Repellents bring about clockwise rotation of flagella in intact wild-type (14) or *cheR* (a mutant ordinarily having counterclockwise-rotating flagella) (7, 19) cells, but addition of the repellents sodium benzoate (30 mM) or indole (3 mM) did not cause clockwise rotation of the flagella of cell envelopes from the wild-type strain AW405 or the mutant strain RP4080. Similar results were reported for *S. typhimurium* cell envelopes (20). Thus in cell envelopes, the sites of action of repellents are missing or unable to communicate with the flagella.

In previous work (6, 20) rotation of flagella of cell envelopes depended on the addition of an energy source such as L-lactate. In the present study no such requirement was found; apparently the envelopes studied here are not as depleted of endogenous materials as were those studied previously. (Observation by Nomarski microscopy supports this: rotating envelopes have more cytoplasmic matter than do their nonrotating counterparts.) However, that does not appear to be a crucial matter because the cell envelopes prepared from wild-type cells and from tumbling mutants certainly have lost some requirement for clockwise rotation of their flagella, whether or not an added energy source is needed.

Although 20% of tethered intact cells rotate, only about 1% (6) or, in the present case, 0.1 to 0.01% of tethered cell envelopes rotate. This indicates that the vast majority of cell envelopes either have lost something essential for flagellar rotation, have sustained damage to the flagella, or have become so leaky that an adequate proton motive force

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cannot be attained. For reasons not understood, tumbly mutants (other than strain AW662) gave a much lower percentage of rotating envelopes than did smooth mutants or wild-type cells.

Any materials that are needed to achieve rotation of flagella of cell envelopes, both clockwise and counterclockwise rotation, remain to be discovered.

LITERATURE CITED

TABLE 1. Rotation of tethered *E. coli* cells and cell envelopes

<table>
<thead>
<tr>
<th>Chemotaxis genotype</th>
<th>Name</th>
<th>Reference</th>
<th>Cells</th>
<th>Direction of rotation*</th>
<th>No. observed</th>
<th>Cell envelopes</th>
<th>Direction of rotation*</th>
<th>No. observed</th>
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<tbody>
<tr>
<td>Wild type</td>
<td>AW405</td>
<td>4</td>
<td>100% R</td>
<td>100% R</td>
<td>100% CCW</td>
<td>100% CCW</td>
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<tr>
<td>No chemotaxis mutations</td>
<td>RP487</td>
<td>19</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>29</td>
<td></td>
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<tr>
<td>Smooth mutants</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>cheA104</td>
<td>RP487cheA</td>
<td>11, 15</td>
<td>93% CCW, 7% R</td>
<td>27</td>
<td>100% CCW</td>
<td>22</td>
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<td>cheC497</td>
<td>AW405cheC</td>
<td>3</td>
<td>100% CCW</td>
<td>34</td>
<td>100% CCW</td>
<td>20</td>
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<td>cheD193</td>
<td>RP4793</td>
<td>16</td>
<td>90% CCW, 10%</td>
<td>60</td>
<td>100% CCW</td>
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<td>RP4080</td>
<td>1, 7</td>
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<td>35</td>
<td>100% CCW</td>
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<td>100% CCW</td>
<td>36</td>
<td>100% CCW</td>
<td>10</td>
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<td>33</td>
<td>100% CCW</td>
<td>32</td>
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<td>AW660</td>
<td>10</td>
<td>100% CCW</td>
<td>41</td>
<td>100% CCW</td>
<td>19</td>
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<td>Tumbly mutants</td>
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<td>cheB286</td>
<td>RP487cheB</td>
<td>9, 15</td>
<td>2% CCW, 86%</td>
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<td>RP4493</td>
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<td>6% CCW, 86%</td>
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<td>100% CCW</td>
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<tr>
<td>cheE518</td>
<td>AW662c</td>
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<td>89% CW, 11% R</td>
<td>129</td>
<td>100% CCW</td>
<td>59</td>
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<tr>
<td>cheV2640</td>
<td>RP2640</td>
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<td>47</td>
<td>100% CCW</td>
<td>13</td>
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<tr>
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<td>RP487cheZ</td>
<td>9, 15, 18</td>
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<td>46</td>
<td>100% CCW</td>
<td>25</td>
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<td>ΔcheR-cheB</td>
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<td>17</td>
<td>98% CW, 2% R</td>
<td>41</td>
<td>100% CCW</td>
<td>15</td>
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<td></td>
</tr>
<tr>
<td>tsr tar trg tumbley</td>
<td>AW672d</td>
<td>*</td>
<td>89% CW, 11% R</td>
<td>104</td>
<td>100% CCW</td>
<td>36</td>
<td></td>
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</tr>
</tbody>
</table>

* Experimental procedures are the same as described previously (6); the cells were lysed in 10 mM potassium phosphate (pH 7.0)–5 mM MgSO4–0.1 mM EDTA, and all the microscopic observations were in 10 mM pyruvate–10 mM potassium phosphate (pH 7.0)–5 mM MgSO4–0.1 mM EDTA.
* CCW, CW, and R (reversing) indicate that tethered cells or tethered cell envelopes rotate counterclockwise, or clockwise, or both counterclockwise and clockwise. This was determined simply by microscopic observation of samples on a glass slide. Cells or cell envelopes were observed typically for 5 to 10 min; 100% CCW means that during this time all the rotating cells or cell envelopes rotated exclusively counterclockwise. (The direction of rotation of tethered cells is the same as the direction of rotation of their flagella [14]). Only rotating cells or cell envelopes were scored for results.
* Strain AW662 has the cheE518 mutation of AW618 (9) transferred into strain RP487.
* This is a tumbly mutant found in "bumps" at the front of a swarm of strain AW660 on a tryptone swarm plate (C. B. Ball, Ph.D. Thesis, University of Wisconsin-Madison, Madison, 1979).