Role of Methionine in Bacterial Chemotaxis

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The effect of methionine starvation on the motility and behavior of normal and nonchemotactic mutants of Salmonella typhimurium was investigated. Methionine starvation eliminates tumbling in the wild type, but fails to do so in an uncoordinated (frequent tumbling) mutant. In the mutant, methionine starvation significantly extends the period of smooth motility that follows a sharp temporal increase of attractant. These results suggest that methionine metabolism is not tightly coupled to the generation of tumbles, but rather is necessary for the return of some tumble-regulating parameter to a steady-state level.

Bacterial chemotaxis provides a convenient system for studying the processes of sensory reception and simple behavior. The migrational behavior of bacteria can be measured by the capillary assay of Adler (1), the population migration apparatus of Dahlquist et al. (7), and the tracking apparatus of Berg (5). Macnab and Koshland (9) have shown that chemical gradients are sensed by a temporal comparison of concentrations. Hazelbauer and Adler (8) demonstrated that periplasmic proteins are apparently the receptors that bind specific attractants. The biochemical processes that transmit information from the receptors to the flagella are still obscure.

A possible clue to these processes has come from the observation of Adler and Dahl (2) that a methionine auxotroph of Escherichia coli was nonchemotactic toward all attractants tested when starved for methionine. The effect of methionine starvation does not result from lack of protein synthesis because starvation for other amino acids such as threonine or leucine had no effect on chemotaxis. Moreover, methionine starvation does not lead to loss of motility but rather to an alteration in the type of motility displayed.

In this paper we describe studies on the effect of methionine starvation on normal and chemotactically defective strains of Salmonella typhimurium in an attempt to gain further insight into the nature of this phenomenon.

A preliminary account of this work was presented at the 57th Annual Meeting of the Federation of American Societies for Experimental Biology, April 1973.

MATERIALS AND METHODS

Bacteria. All strains used in this study are derivatives of S. typhimurium, strain LT2, and are described in Table 1.

Media. Minimal citrate (VBC) medium as described by Vogel and Bonner (12) was supplemented with glycerol (1% wt/vol) and any required amino acids (20 μg/ml). Liquid nutrient broth consisted of nutrient broth (0.8% wt/vol; Difco) and sodium chloride (0.5% wt/vol). Nutrient agar plates were agar (1.5%; Difco) in liquid nutrient broth. Tryptone semisolid plates were agar (0.25% wt/vol; Difco), tryptone (1% wt/vol; Difco), and sodium chloride (0.5% wt/vol).

Growth, harvesting, and washing of bacterial cultures. All experiments described in this paper were done on cultures grown aerobically in minimal medium at 30°C to mid-log phase (a density of about 5 × 10⁸ bacteria/ml). They were then centrifuged at 3,000 × g for 15 min at 4°C and resuspended in the appropriate medium. The bacteria were washed one additional time before use in an experiment.

Microscopy and photography. Bacteria were observed in dark field. One-half drop of culture, 5 × 10⁶ cells/ml, was placed in a "chamber" constructed by bridging one cover slip over two others on a glass slide.

Motility tracks were photographed using a stroboscopic light source as has been described previously (9).

RESULTS

Effect of methionine starvation on the chemotactic response of Salmonella. The effect of methionine starvation is shown in Fig. 1, which demonstrates that Salmonella, like E. coli, require methionine for chemotaxis. In a methionine auxotroph, the chemotactic response is constant when the methionine concentration in the medium is varied over the range 10⁻³ to 10⁻⁶ M. Below 10⁻⁶ M the response drops sharply, approaching zero at 10⁻⁸ M. The curve is qualitatively very similar to that obtained by Armstrong (3) for E. coli.

This effect is specific for methionine starvation because chemotaxis was not inhibited by
TABLE 1. Genotypes and sources of bacterial strains

<table>
<thead>
<tr>
<th>Strain no.</th>
<th>Description</th>
<th>Source/reference</th>
</tr>
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<tbody>
<tr>
<td>ST2</td>
<td>metE</td>
<td>Diethylsulfate mutagenesis of ST1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>SL4041</td>
<td>trpA&lt;sup&gt;+&lt;/sup&gt; hisC527&lt;sup&gt;(amber)&lt;/sup&gt; che&lt;sup&gt;(amber)&lt;/sup&gt;</td>
<td>P. V. Vary and B. A. D. Stocker (11)</td>
</tr>
<tr>
<td>ST4</td>
<td>trpA&lt;sup&gt;+&lt;/sup&gt; hisC527&lt;sup&gt;(amber)&lt;/sup&gt; che&lt;sup&gt;(amber)&lt;/sup&gt; met</td>
<td>Diethylsulfate mutagenesis of strain SL4041</td>
</tr>
<tr>
<td>ST4&lt;sup&gt;1&lt;/sup&gt;</td>
<td>trpA&lt;sup&gt;+&lt;/sup&gt; met His&lt;sup&gt;-&lt;/sup&gt; Che&lt;sup&gt;-&lt;/sup&gt; &lt;sup&gt;a&lt;/sup&gt;</td>
<td>Selected as a spontaneous Che&lt;sup&gt;-&lt;/sup&gt; revertant of strains ST4&lt;sup&gt;4&lt;/sup&gt;</td>
</tr>
<tr>
<td>ST4&lt;sup&gt;2&lt;/sup&gt;</td>
<td>trpA&lt;sup&gt;+&lt;/sup&gt; hisC527&lt;sup&gt;(amber)&lt;/sup&gt; met</td>
<td>Selected as a spontaneous Che&lt;sup&gt;-&lt;/sup&gt; revertant of strains ST4&lt;sup&gt;4&lt;/sup&gt;</td>
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<sup>a</sup> ST1 is a wild-type S. typhimurium LT2 having good motility and chemotaxis. It was formerly referred to as LT2-S2 (9).

This strain is probably hisC527<sup>(amber)</sup> and che<sup>(amber)</sup> containing an amber suppressor. The evidence for this is that the histidine requirement was lost concomitant with reversion to the Che<sup>-</sup> phenotype. Also, the motility of this mutant, i.e., tumbling frequency, is intermediate between that of the wild type and strain SL4041, indicating a leakiness characteristic of suppressed amber mutations.

<sup>c</sup> Che<sup>-</sup> revertants of ST4 were isolated as follows. A drop from a full grown nutrient broth culture of ST4 was placed on a tryptone semisolid plate and incubated at 30°C in a wet incubator. After 24 to 48 h, asymmetric rings indicative of chemotactic swarming bacteria formed. Samples were taken from the leading edge of the swarms, and single colonies were isolated from them. These were checked for amino acid growth requirements and the ability to form symmetrical rings in tryptone semisolid medium.

starvation for histidine of tryptophan in a his<sup>-</sup>, trp<sup>-</sup>, met<sup>-</sup> triple auxotroph. Adler and Dahl (2) found no effect during starvation of E. coli for threonine or leucine, and Armstrong (3) reported no effect during arginine, histidine, proline, shikimic acid, or tryptophan starvation.

Chemotaxis is blocked regardless of how methionine starvation is imposed. Starvation in a metE mutant (blocked in the conversion of homocysteine to methionine) and a metA mutant (blocked in the conversion of homoserine to O-succinylhomoserine) blocked chemotaxis equally well. Growth of wild-type cells in the presence of methionine will repress all of the enzymes in the methionine biosynthetic pathway (10). Chemotaxis is blocked in repressed cells if methionine is absent from the medium. These results show that the methionine effect does not result from accumulation of a methionine precursor.

Effect of methionine starvation on motility.

Methionine-starved cultures of S. typhimurium are motile, but there is a striking difference between their motility and that of nonstarved cultures. Normal motility consists of "runs" in which bacteria swim in a generally straight line for a few seconds. This period of coordinated motility, the run, is then interrupted by a brief period of apparent rapid turning, which is called a "tumble." The tumble usually lasts less than 1 s. When coordination resumes, the bacteria swim off again in a new direction that is random with respect to the former direction. Figure 2A shows this normal motility pattern in the presence of methionine.

The absence of methionine shown in Fig. 2B causes the trajectories of the bacteria to be smoother, showing much less frequent tumbling. Although the bacteria do not swim in precisely straight lines, vigorously motile individual bacteria were not observed to display a bona fide tumble for up to 1 min of continuous observation. The same impression is gained from looking at a field of bacteria (20 to 50 in view at any one time). Any bacterium that is seen not to be swimming in a smooth coordinated manner is invariably either nonmotile or poorly motile. Thus, in normally motile cells, tumbling is completely suppressed by methionine starvation.

Effect of methionine starvation on the re-

![Graph of L-Methionine Molarity vs. Bacteria Accumulated (× 10<sup>9</sup>)](http://jb.asm.org/) FIG. 1. Methionine dependence of chemotaxis in a methionine auxotroph. A culture of strain ST2 was washed in methionine-free VBC-glycerol, and samples (0.03 ml) of the washed suspension were then diluted into tubes containing 3.0 ml of VBC-glycerol and L-methionine at the concentration indicated on the abscissa. The tubes were incubated at 30°C with shaking for 15 min before assaying for chemotaxis. Chemotaxis was measured at 30°C for 30 min by the Pfeffer capillary assay described by Adler (1) using 1 mM L-serine as an attractant.
In the absence of methionine, it seemed of interest to see whether methionine starvation would alter the motility pattern of an uncoordinated mutant. This mutant, strain SL4041, is non-chemotactic as judged both by the capillary assay and by the fact that it does not swim on tryptone semisolid media. The motility pattern is one of constant tumbling or uncoordination, with no smooth coordinated swimming. Five methionine auxotrophs were independently derived from strain SL4041, and their motility was examined both in the presence and absence of methionine. In all five cases the continuous tumbling was unaffected by methionine starvation. Figure 3A and B show this for one of the auxotrophs, ST4.

The possibility that the auxotrophs were leaky and contained enough methionine to support tumbling was considered; however, the probability that this alternative is correct for all five mutants is small. In addition, two Che+ revertants of strain ST4, ST41 and ST42, were isolated. When either strain is starved for methionine, tumbling is eliminated. Because strains ST41 and ST42 contain the same methionine biosynthetic mutation as the parent, ST4, it is unlikely that ST4 is a leaky auxotroph.

The observation that ST4 continues to tumble in the absence of methionine suggests one of two possibilities. The first is that strain SL4041 contains a mutation that allows tumbling to occur in the absence of methionine, and the second is that the abnormal motility pattern of strain SL4041 is not the result of continuous tumbling, but rather the result of a defective flagellar apparatus. In the first alternative, the mutation is assumed to effect the regulation of tumbling, i.e., the flagella are capable of coordinating properly, but they are constantly receiving signals that prevent smooth swimming. In the second alternative, the flagella are receiving the proper signals, but they are unable to coordinate their flagella properly, regardless of which signals are being received.

To choose between these alternatives, strain ST4 was subjected to a positive temporal gradient of serine. Normally, chemotactic bacteria subjected to such a temporal increase in attractant concentration respond by suppressing tumbling for several minutes. We call this a

![Figure 2](http://jb.asm.org/)

**Fig. 2. Effect of methionine starvation on the motility of strain ST41.** Bacteria were washed and then diluted to a cell density of about $2 \times 10^8$ cells/ml in VBC-glycerol containing $10^{-5}$ M L-histidine, $10^{-3}$ M L-tryptophan, and $10^{-2}$ M L-methionine, (A), or the same without methionine (B). Strain ST41 was used here instead of ST2 or ST42 because ST41 has a relatively higher rate of spontaneous tumbling, and the difference between the presence and absence of methionine is more noticeable during the 5-s exposure period. In the presence of methionine (A), the motility tracks consist of relatively smooth, gently curved segments in which the bacterial soma is oriented along the direction of the track interrupted by "tumbles" where the bacterial soma shows random orientation. In the absence of methionine (B), tumbling is virtually absent and the motility tracks are nearly all smooth.

**Response to a steep temporal gradient.** A more severe test of tumble suppression by methionine starvation was performed by subjecting *Salmonella* to a sharp temporal decrease in attractant concentration. Normally such a stimulus dramatically increases the frequency of tumbling for up to 10 to 15 s after the stimulus is applied (9). Under conditions that normally elicit a tumbling response for unstarved bacteria, methionine-starved bacteria showed no change in behavior. The bacteria continued to swim in a smoothly coordinated manner. Tumbling is apparently blocked during methionine starvation so that even a severe stimulus cannot reinstate it.

**An uncoordinated mutant.** Because normal bacteria exhibit only smooth motility in the absence of methionine, it seemed of interest to see whether methionine starvation would alter the motility pattern of an uncoordinated mutant. This mutant, strain SL4041, is non-chemotactic as judged both by the capillary assay and by the fact that it does not swim on tryptone semisolid media. The motility pattern is one of constant tumbling or uncoordination, with no smooth coordinated swimming. Five methionine auxotrophs were independently derived from strain SL4041, and their motility was examined both in the presence and absence of methionine. In all five cases the continuous tumbling was unaffected by methionine starvation. Figure 3A and B show this for one of the auxotrophs, ST4.

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To choose between these alternatives, strain ST4 was subjected to a positive temporal gradient of serine.Normally, chemotactic bacteria subjected to such a temporal increase in attractant concentration respond by suppressing tumbling for several minutes. We call this a
"smooth response." The result with strain ST4 is shown in Fig. 3C. Inhibition of tumbling was observed in 90% of the bacteria. These bacteria swam in a coordinated manner with a linear velocity typical of normal strains. This period of smooth motility died away in a few minutes, giving rise to the tumbling pattern characteristic of this mutant strain. These observations prove that the flagella of strain ST4 (and therefore strain SL4041) are capable of normal coordinated motility for a prolonged period of time, and hence the defect is in the tumble regulation and not in the flagellar apparatus.

**Effect of methionine starvation on duration of the smooth response of the uncoordinated mutant.** The influence of methionine starvation on the smooth response of strain ST4 was investigated, and the results are shown in Table 2. For an aspartate gradient in the absence of methionine, the response lasted up to 10 min, whereas in the presence of methionine the response lasted only 1.5 min. A similar effect

**Table 2. Effect of amino acid starvation on duration of the smooth response**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Starvation conditions</th>
<th>Response duration* (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ST42 (che+, control)</td>
<td>- Histidine</td>
<td>2.3 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>- Tryptophan</td>
<td>2.5 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>- Methionine</td>
<td></td>
</tr>
<tr>
<td>ST4 (uncoordinated)</td>
<td>- Histidine</td>
<td>1.2 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>- Tryptophan</td>
<td>1.5 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>- Methionine</td>
<td>10 ± 2.0</td>
</tr>
</tbody>
</table>

*Stimulus is a temporal gradient of 0 → 1 mM L-aspartate generated in the apparatus described by Macnab and Koshland (9).

*No response listed because methionine-starved che+ strains always swim in a smooth coordinated manner.

omitted. The motility tracks of ST4 under these conditions is similar to photograph (A), indicating that methionine starvation does not eliminate tumbling in this mutant. (C) The effect of a positive temporal gradient of attractant on the motility tracks of ST4. Bacteria were washed and resuspended in VBC-glycerol containing all three amino acids as in (A). To create a temporal gradient, 0.5 ml of bacteria suspension was rapidly pipetted into 0.5 ml of resuspension medium containing 2 mM L-serine in a small test tube. A vortex mixer was used to assure rapid mixing. Immediately after mixing, a drop of the solution was taken for photomicrography. The photograph seen here was taken approximately 30 s after the mixing. The motility tracks indicate smooth coordinated motility.
was observed with a serine gradient. This result suggests that methionine starvation might in fact improve the chemotactic response of the mutant when swimming in a spatial gradient, i.e., in the capillary assay. However, strain ST4 failed to respond in the capillary assay in either the presence or absence of methionine. If improvement occurred, the effect on the uncoordinated bacteria was not sufficient to allow chemotaxis. The positive results in the temporal gradient apparatus therefore were undoubtedly caused by the much steeper gradient per unit time in that apparatus.

**DISCUSSION**

The methionine effect, originally discovered by Adler and Dahl (2) in *E. coli*, has now been shown to occur also in *Salmonella*. Our studies of this effect indicate that tumbling is not merely reduced but completely eliminated in a methionine-starved culture. Moreover, methionine-starved *Salmonella* fail to tumble when stimulated by a steep temporal decrease in attractant concentration, an experiment that normally causes violent tumbling in unstarved *Salmonella*. The loss of tumbling explains at the behavioral level why methionine starvation causes loss of chemotaxis. As demonstrated by Berg and Brown (6) and Macnab and Koshland (9), when bacteria experience an increase in attractant concentration, e.g., by swimming up a gradient of attractant, tumbling is suppressed and the bacteria swim longer than usual in the up-gradient direction. If the bacteria find themselves swimming down the gradient, the frequency of tumbling is increased only slightly relative to normal. As a result, chemotactic migration occurs essentially from a selective suppression of spontaneous tumbling. Because methionine starvation removes this capacity to tumble, selectivity of direction is also eliminated.

Although our results show that a supply of methionine is necessary for tumbling in normal strains, the experiments with ST4 indicate that mutants can be isolated that continue to tumble in the absence of methionine. This puts severe restrictions on the possible role of methionine in relation to tumble regulation. Any mechanism in which methionine or a methionine derived metabolite is a substrate for a tumble generating reaction seems eliminated. A hypothetical example of such a mechanism would involve S-adenosyl-methionine donating a methyl group to a protein in the basal region of the flagella that would perturb the orientation of the flagella and result in a tumble. Methionine starvation would deplete the supply of S-adenosyl-methionine and turn off the tumble-generating reaction. If bacteria operated by this mechanism, a mutant that tumbled in the absence of methionine could not occur. Strain ST4, an uncoordinated mutant, does just that, eliminating this hypothesis.

An alternative is that methionine, or its metabolite, has some less direct, perhaps regulatory role in the control of tumbling. The data of Table 2 suggest that this regulatory role is to create a biochemical environment that turns off the smooth response. In the presence of methionine, strain ST4 showed a smooth response of only 1.5 min, whereas in the absence of methionine, the response duration is increased sixfold to a value of roughly 10 min. Thus, the presence of methionine in the cell is necessary for rapid return to an environment that allows tumbling from an environment that causes smooth swimming.

At present little is known about how tumbles are regulated at the molecular level. Presumably there exists within the cell some parameter, e.g., metabolite concentration, ion concentration, or membrane potential, that serves to integrate information from chemoreceptors and modify motility. When a stimulus is encountered, the value of this parameter changes but must return to its steady-state level soon after the stimulus has ended. Methionine may serve to assume that the tumble-regulatory parameter returns to its steady-state value.

The observation on the che+ and mutant strains agree with this hypothesis. In che+ bacteria, this regulatory parameter is apparently delicately balanced so that only sporadic tumbling occurs. A slight perturbation in its steady-state level caused by methionine starvation would thus effectively eliminate tumbling. Strain ST4, however, has a much higher frequency of spontaneous tumbling. In this case methionine starvation could decrease the probability of tumbling without completely eliminating it.

The validity of our interpretation rests ultimately on the nature of the mutation in the uncoordinated mutant. We have implicitly assumed that strain ST4 is qualitatively similar to the wild type, differing only in the quantitative aspects of gradient sensing or tumble regulation. The temporal gradient results support this view (Table 2).

In recent publications, Armstrong (3, 4) presented evidence that in *E. coli* the methionine effect reflects a need for S-adenosyl-methionine in chemotaxis. Experiments in our laboratory with *Salmonella* concur with this conclusion.
Thus, the methionine requirement may reflect a need for $S$-adenosyl-methionine in turning off the smooth response.

ACKNOWLEDGMENTS

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LITERATURE CITED