Interrelationship between Ca\(^{2+}\) and a methionine-requiring step in *Halobacterium halobium* taxis

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Received 21 September 1981
Accepted 23 September 1981

1. INTRODUCTION

*Halobacterium halobium* possesses both phototaxis [1] and chemotaxis towards (or away from) attractants and repellants [2,3]. According to our data, the positive phototaxis towards green light which is mediated by the bacteriorhodopsin H\(^+\) pump [1,4,5] is governed by \(\Delta \mu \text{H}^+\)-sensing [6]. Blue light evokes negative phototaxis of *H. halobium* cells, and it was suggested that an as yet uncharacterized retinal-requiring pigment P\(_{370}\) [1,4,5] acts by causing a depolarization of the membrane [4,5]. However, we have recently found that blue light does not change the energy level at intensities that repel bacteria and suggested that P\(_{370}\) is a specific photoreceptor [6].

Sensory information from individual bacterial receptors is passed to methyl-accepting chemotaxis proteins (MCP) [7]; their methylation causes adaptation to attractant [8,9]. Recently a discovery of an MCP protein in *H. halobium* was reported [10]. It was shown that the addition of attractants increased, while the addition of a repellent decreased, \(-\text{CH}_3\) incorporation into a protein fraction. L-Ethionine, a non-metabolizing analogue of methionine, blocks methylation and was found to inhibit positive chemotaxis, but not phototaxis, in *H. halobium* [2]. A methyl-requiring process was also reported to be non-essential in the sensing of uncouplers by *Bacillus subtilis* [11]. We consider the sensing of uncouplers to be mediated by \(\Delta \mu \text{H}^+\) reception and thus to be closely related to *H. halobium* positive phototaxis. We therefore attempted to re-investigate the methionine requirement in *H. halobium* phototaxis.

In this paper we report that both positive and negative phototaxis in *H. halobium* is dependent upon methylation, and that a next step in the information processing requires Ca\(^{2+}\).

2. MATERIALS AND METHODS

2.1. Bacteria

*H. halobium* R\(_1\)M\(_1\) was kindly provided by Dr. D. Oesterhelt. Bacteria were cultivated for 72 h at 37°C with limited aeration in a complex Oxoid peptone medium [12]. Bacteria were harvested by centrifugation, washed and resuspended to approx. 2 \(\times 10^9\) cells/ml in the basal salts portion of the growth medium containing Ca\(^{2+}\) as contaminants of the salts (4.3 M NaCl, 27 mM KCl, 80 mM MgSO\(_4\), 14 mM sodium citrate pH 7.0) or in the Ca\(^{2+}\) starvation medium (4.3 M NaCl, 27 mM KCl, 2 mM MOPS, 10\(^{-5}\) M EGTA, pH 7.0). Incubation during 24 h at 37°C under illumination, was found to stimulate motility.

2.2. Reagents

All chemicals (reagent grade) were dissolved in Ca\(^{2+}\) starvation medium. Ionophore A23187 was
dissolved in ethanol and added at a 1:200 v/v ratio. The final concentration of ethanol (0.5%) had no influence on bacterial behaviour.

2.3. Observations

A drop of bacterial suspension (approx. 2 • 10⁻⁹ cells/ml) was placed between a slide and coverslip and sealed with vaseline oil to prevent evaporation. A 10-min incubation in the dark was allowed to ensure anaerobiosis that increases the sensitivity of the response to green light. Cells were observed in phase contrast (Reichert-Univar microscope). The slides were placed on a Biotherm (Reichert) stage at 37°C. Light stimuli provided with an incident light source passed through a blue or a yellow filter.

3. RESULTS

Bipolarly flagellated H. halobium move with a speed of approx. 4 µm/s reversing once about every 17 s. A decrease in yellow green light (λ > 500 nm) or an increase in blue light intensity causes cells to reverse. A quantitative estimate of taxis may be obtained by recording the fraction of cells that reverse to a given stimuli in a given time [6].

Green or blue light stimuli were selected that caused an approximate 50% response of H. halobium cells. Then 2 mM L-ethionine was added and the sensitivity to the same stimuli was recorded at different time intervals (Fig. 1). In accordance with the findings of Schimz and Hildebrand [2], a 2–3 h incubation with ethionine did not decrease the phototaxis sensitivity. However, a longer incubation caused a gradual loss of phototaxis sensitivity. However, a longer incubation caused a gradual loss of phototaxis that completely disappeared in 6 h. The ATP level hardly changed after a 6 h incubation with ethionine (measurements were done with luciferine-luciferase; data not shown). A prolonged incubation of cells with methionine had no effect on phototaxis sensitivity (Fig. 1).

In B. subtilis, Ca²⁺ was shown to cause flagellar reversals at a certain step following reception [13] and we confirmed this finding in a study of Phormidium uncinatum and H. halobium behaviour [14]. It is known that Ca²⁺ easily penetrates the cells in the presence of the ionophore A23187; a 50% water solution of the salts medium was applied to insure A23187 penetration in our previous study [14]. We treated H. halobium with this ionophore and found that starting with 10⁻³ M Ca²⁺, cells in the presence of A23187 began oscillatory reversal, with a period of < 3 s (Table 1). Ca²⁺

<table>
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<th>Ethionine CaCl₂ Motility pattern</th>
<th>% reversals without</th>
<th>% reversals with</th>
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<tr>
<td>2 mM (M)</td>
<td>A23187</td>
<td>A23187</td>
</tr>
<tr>
<td>10⁻¹</td>
<td>5–10</td>
<td>motionless</td>
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<tr>
<td>10⁻²</td>
<td>5–10</td>
<td>&gt;90</td>
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<tr>
<td>10⁻³</td>
<td>5–10</td>
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<td>10⁻⁴</td>
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<td>+</td>
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<td>10⁻²</td>
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Fig. 2. Ca\(^{2+}\) dependence of *H. halobium* phototaxis. Cells were Ca\(^{2+}\)-starved for 24 h. Sensitivity was estimated as in Fig. 1, after a 30 min incubation with a given Ca\(^{2+}\) concentration. Ca\(^{2+}\) was added together with 10\(^{-5}\) M EGTA. Free Ca\(^{2+}\) was calculated in accordance with [13]. Stimuli: (©), decrease in green light; (○), increase in blue light. The dashed line represents the sensitivity of photoreactions after 24 h in a medium without Ca\(^{2+}\).

had little effect on *H. halobium* behaviour without the ionophore. However, a prolonged 24-h incubation of cells in the presence of 0.1 M Ca\(^{2+}\) caused all the cells to undergo oscillatory reversal. When 10\(^{-2}\) M Ca\(^{2+}\) + A23187 was added to cells pretreated with ethionine (Table 1), the extremely low reversal rate changed to oscillatory reversals.

A 24-h incubation in a medium without Ca\(^{2+}\) caused a sufficient loss in spontaneous or photoinduced reversals. The requirement of Ca\(^{2+}\) for phototaxis was briefly mentioned previously [4]; it seemed to be interesting to compare the relative dependence of both photoresponses on Ca\(^{2+}\). Cells with reduced phototaxis, after being Ca\(^{2+}\)-starved, were supplied with different concentrations of Ca\(^{2+}\) (no ionophore) (Fig. 2). Green or blue light stimuli that caused a 50% response in control cells were applied. An effective restoration of both blue- and green-light responses was observed upon a gradual increase of the Ca\(^{2+}\) concentration.

4. DISCUSSION

A relationship between chemotaxis and phototaxis in *H. halobium* is evident from the finding that the incubation of cells in the presence of ethionine impairs both chemotaxis [2] and photoreponses (present study). Since a reasonably longer period of incubation with ethionine is necessary to inhibit phototaxis (Fig. 1) than to suppress chemotaxis [2], one may speculate that the information inputs from these two different groups of stimuli feed into different MCP proteins. After a comparatively short period of ethionine treatment, the sensitivity of the negative phototaxis to blue light increased two-fold whereas that of the positive phototaxis remained unchanged. This difference may also reflect the involvement of two different MCP proteins, one collecting information from the P\(_{370}\) receptor and another from the Protomer \(\Delta\mu H^+\)-sensor (protomer) which mediates green light sensing [6]. Since the addition of Ca\(^{2+}\) together with the ionophore A23187 restored the reversing ability of *H. halobium* cells pretreated with ethionine, we conclude that MCP proteins are located at a stage prior to that regulated by Ca\(^{2+}\).

The large concentrations of Ca\(^{2+}\) necessary to cause oscillatory reversals are probably due to a low permeability of the cells to A23187. In a previous study we found that in a hypotonic solution, Ca\(^{2+}\) (+A23187) caused reversals with a \(K_d = 10^{-8}\) M [14].

The fact that it is very difficult to either starve *H. halobium* cells for Ca\(^{2+}\) or to observe oscilla-
tory reversals in the presence of Ca$^{2+}$ (without A23187) indicates that the bacteria maintain Ca$^{2+}$ intracellularly at a level that is essentially independent of its outer concentration. In *B. subtilis* it was found that Ca$^{2+}$, although regulating flagellar reversals, does not actually become redistributed between the cell and the outer medium during taxis [13]. We would suggest that the regulation of flagellar rotation is carried out by redistributing Ca$^{2+}$ between the cytoplasm and binding sites in the cell wall.

The close coincidence of the Ca$^{2+}$ requirements of both photo-reactions (Fig. 2) further indicates that the Ca$^{2+}$-dependent step is the next stage after the methylation process. Perhaps, information from all of the receptors and MCP proteins becomes unified at the level of Ca$^{2+}$. This is illustrated by the tentative scheme given above.

ACKNOWLEDGMENTS

The authors are most grateful to Dr. D. Oesterhelt for providing the R$_{1}$M$_{1}$ *H. halobium* strain and to Calbiochem Co. for a kind gift of A23187. The authors are grateful to Dr. M.Yu. Sherman for helpful discussions.

REFERENCES