The role of methylation in the taxis of *Halobacterium halobium* to light and chemo-effectors

S.I. Bibikov, V.A. Baryshev and A.N. Glagolev

Laboratory of Molecular Biology and Bioorganic Chemistry, Moscow State University, Moscow 117234, USSR

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In *Halobacterium halobium* tactic responses towards light and chemoeffectors are accompanied by changes in the methylation level of methyl-accepting chemotaxis proteins (MCP). Taxis towards green light absorbed by the bacteriorhodopsin proton pump appears to be governed by $\Delta\tilde{\mu}_H$+-sensing. The addition of CCCP, an uncoupler, prevented the increase of MCP methylation in response to green light illumination, but had no effect on CH_3-incorporation followed by the addition of the attractants glucose, leucine and histidine. Similarly, CCCP did not change MCP demethylation in response to blue light illumination, a repelling stimuli.

The sensitivity to an uncoupler of methylation linked to $\Delta\tilde{\mu}_H$+-mediated green light taxis is to be expected, while the independence of demethylation caused by the blue light of CCCP is an indication that in the latter case a specific photoreceptor governs phototaxis. Informed processing from the blue light receptor to MCP does not involve a change in the membrane potential.

Halobacterium halobium *photobehavior* $\Delta\tilde{\mu}_H$+*-sensing* *Protein methylation* *Blue-light taxis*

1. INTRODUCTION

Both the chemotactic and phototactic responses of an archaebacterium, *Halobacterium halobium*, are mediated by methylation of methyl-accepting chemotaxis proteins (MCP) [1,2], first discovered to be involved in the chemotaxis of *Escherichia coli* [3]. Attractants cause MCP methylation that represents adaptation to them, while adaptation to repellents is followed by MCP demethylation [4].

Halobacterium halobium possesses two phototactic responses: a positive reaction towards green light absorbed by bacteriorhodopsin; and a negative reaction towards blue light mediated by an as yet unidentified pigment P370 [5,6]. In [7,8], taxis towards green light was governed by $\Delta\tilde{\mu}_H$+-sensing, while blue-light taxis seemed to be independent of changes in the membrane potential. In *E. coli* $\Delta\tilde{\mu}_H$+-sensing was found to be independent of MCP methylation [9–11]. It therefore seemed important to study the relationship between methylation and $\Delta\tilde{\mu}_H$+-sensing in *H. halobium*, since the sum of indirect evidence pointed to the active role of MCP in $\Delta\tilde{\mu}_H$+-sensing in this species [1,2,7,8,12].

Here, we report that $\Delta\tilde{\mu}_H$+-sensing in *H. halobium* specifically involves MCP methylation; besides, we found that a change in the level of methylation following the addition of chemoattractants or a blue-light stimulus can be observed in cells uncoupled by the addition of CCCP.

Abbreviations: CCCP, *m*-chlorocarbonylcyanidephenylhydrasone; MCP, methyl-accepting chemotaxis protein; P370, blue-light taxis receptor; SDS, sodium dodecyl sulfate

2. MATERIALS AND METHODS

Halobacterium halobium R_1M_1 lacking bacterioruberin and gas vacuoles was kindly provided by Dr D. Oesterhelt. Cells were grown for 96 h on a complex medium containing peptone (Oxoid) [13], as in [14]. Stationary-state cells containing bacteriorhodopsin were harvested by centrifugation, washed and resuspended in the basal salts portion of the growth medium.

CCCP and SDS were from Sigma; puromycin

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was from Serva; L-[*methyl*-^{3}H]methionine (0.5 Ci/mmol) and L-[*methyl*-^{14}C]methionine (60 mCi/mmol) were from the Institute of Isotopes of the Hungarian Academy of Sciences. Other chemicals used were of reagent grade.

2.1. *CH_3-labelling procedure*

Cells (10^9/ml) were incubated in a basal salts medium containing puromycin (30 mg/ml) for 60 min to inhibit protein synthesis. The sample was then divided into two portions. Experiments were performed with the first portion, incubated with 20 μM L-[*methyl*-^{3}H]methionine for 60 min to label the cytoplasmic *S*-adenosylmethionine. The second portion, used as a control, was incubated for 60 min with 40 μM L-[*methyl*-^{14}C]methionine and then kept in the dark. Methylation was halted by the addition of 2.5% (final conc.) formaldehyde to a 5 ml aliquot of the cell suspension. Aliquots were sampled simultaneously from both portions of the bacterial suspension and mixed together. The result of electrophoresis was presented as the dpm ^{3}H/dpm ^{14}C ratio, making it possible to control stimuli-independent changes in methylation levels according to [3].

2.2. *Preparation of total protein*

Cells suspended in a basal salts medium were mixed with cold acetone (1:9, v/v) [15]. Proteins were collected by centrifugation, washed in cold acetone and dried overnight.

2.3. *Isolation of membrane proteins*

Cell envelopes were isolated according to [16]. Cells were frozen in liquid nitrogen immediately after the addition of formaldehyde and were then slowly thawed at room temperature. The procedure led to the destruction of ~99% cells. Thawed material was sonicated at 60 W (Braunsonic 1510) until the viscosity of the suspension decreased. Membranes were separated by centrifugation at 250 000 × *g* for 45 min.

2.4. *Determination of methylation levels*

The acetone protein powder, or the pelleted membranes, were resuspended in a SDS sample buffer and then layered on a 10–20% gradient gel [17]. Bovine serum albumin, ovalbumin and lysozyme were added as markers. After the gels were run, they were cut into 3 mm slices that were solubilized in 0.5 ml of 2% SDS. Radioactivity was measured in a Mark III (Tracor Europa) liquid scintillation spectrometer.

2.5. *Application of stimuli*

A mixture of 1 mM D-glucose, 1 mM D,L-leucine and 0.5 mM L-histidine was used as a chemoattractant [18]. Green–yellow light (90 W × m^2) was emitted by a 2.5 kW xenon lamp (Osram) supplied with a 540 nm cut-off orange filter and a heat filter. Blue light (340 nm < λ < 420 nm, 2 W × m^2) was emitted by 100 W incandescent lamp.

3. RESULTS

3.1. *Methylation of the total protein*

Illumination of cells with green light caused the methylation of proteins with app. M_r 60 000 (fig.1), as deduced from the position of the markers on the gel. Changes in methylation levels were observed in the same band in response to chemoattractants and blue light (fig.2). Similar changes in methylation were observed in the isolated membrane proteins fraction (not shown), in accordance with [2]. Therefore, MCP seems to be the only noticeably methylated proteins in *H. halobium*. This helps to simplify the process of registering MCP methylation by analyzing the total protein instead of isolating the cytoplasmic membrane fraction.

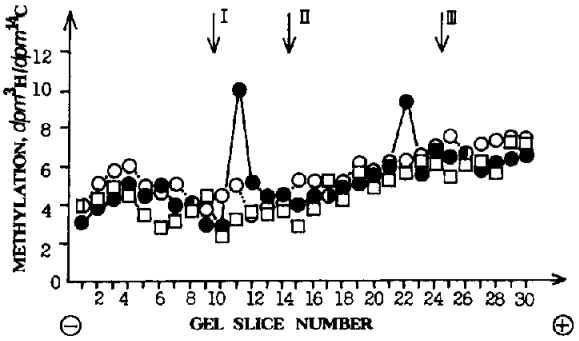

Fig.1. Methylation in response to green light: Light stimuli were applied for 40 min: (□) control; (•) green-light illumination, without CCCP; (○), green-light illumination, cells preincubated with 20 μM CCCP for 20 min. Markers (M_r): (I) bovine serum albumin (M_r 67 000); (II) ovalbumin (M_r 43 000); (III) lysozyme (M_r 14 200).

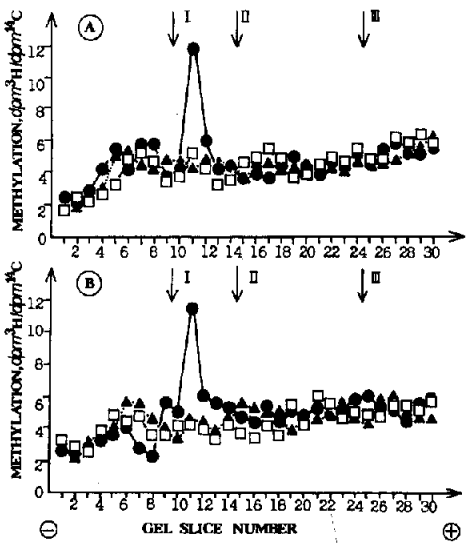

Fig.2. Changes in methylation levels produced by chemoattractants and blue light. Cells were incubated with chemoattractants for 40 min, blue light was then delivered for 20 min: (A) without uncoupler; (B) cells preincubated with 20 μM CCCP for 20 min; (□) control; (•) the addition of chemoattractant mixture; (▲) blue light illumination. Markers as indicated in legend to fig.1.

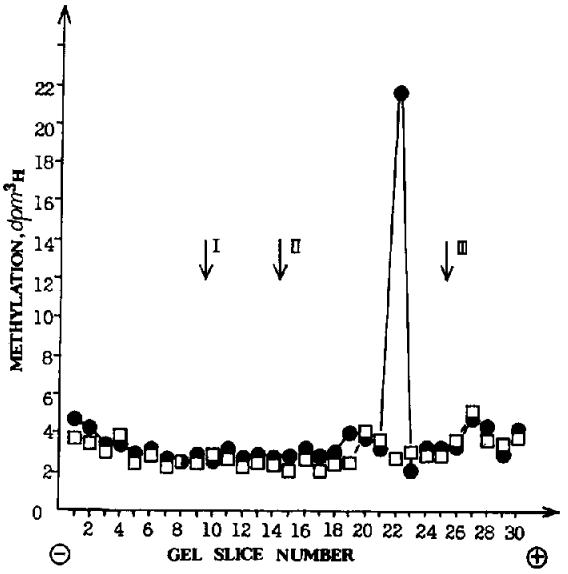

Fig.3. Methylation produced by mixed illumination. Cells were illuminated with blue light for 20 min and then green light was additionally turned on for 40 min: (□) control; (•) illumination. Markers as indicated in fig.1.

3.2. *The low* M_r *methylated protein*

Illumination of cells with green light caused the methylation of an additional polypeptide with an app. M_r 20 000 (fig.1). The amount of label in this band increased markedly if bacteria were illuminated with blue light for a certain time and then green light was additionally turned on, i.e., an attractant stimulus was added to a repellent stimulus (fig.3). Under the same conditions there was no methylation of the heavy MCP band (fig.3).

3.3. *The effect of CCCP on methylation and demethylation*

The uncoupler CCCP caused pronounced de methylation of the 60 000-M_r MCP band when added to cells at 20 μM final conc. (not shown). A repellent is expected to cause demethylation, and the above result is thus consistent with our previous finding that CCCP repelled *H. halobium* in a spatial gradient assay [8]. Green-light illumination no longer caused methylation in either the heavy or the light MCP bands if cells were preincubated with CCCP (fig.1). However, the addition of chemoattractant mixture produced a heavy MCP methylation that was indistinguishable from that without the uncoupler. Likewise, demethylation in response to a blue-light stimulus was also unchanged in the presence of CCCP (fig.2).

4. DISCUSSION

In bacterial taxis, information flows from specific receptors through MCP proteins and numerous *che* (chemotactic) gene products to the flagella (review [19]). The sensing of $\Delta\tilde{\mu}_{H^+}$, suggested to account for taxis away from uncouplers, phototaxis and aerotaxis [20], was found to be independent of MCP in *E. coli* [9–11]. Here, however, we find that in *H. halobium*, $\Delta\tilde{\mu}_{H^+}$-sensing employs an information processing pathway that is analogous to the one found in ordinary chemoreception. This conclusion is based on several lines of evidence that are given below. The addition of an uncoupler that repells *H. halobium* [8] caused MCP demethylation.

The illumination of bacteria by green light, which

is absorbed by the bacteriorhodopsin proton pump, increases the membrane potential and attracts cells via $\Delta\bar{\mu}_{H^+}$-sensing [7,8]; green light produced methylation of MCP [1,2], here. If cells were uncoupled by the addition of a high [CCCP] (20 μM), changes in MCP methylation caused by green light were completely suppressed, while methylation caused by chemoattractants, or demethylation produced by repelling blue-light stimuli, remained intact. This means that $\Delta\bar{\mu}_{H^+}$ is not generally required for MCP methylation; rather, methylation caused by $\Delta\bar{\mu}_{H^+}$-sensing, that must employ a specific MCP protein, is lost with the dissipation of $\Delta\bar{\mu}_{H^+}$. The archaebacterium *H. halobium* thus uses an information pathway for signalling changes in $\Delta\bar{\mu}_{H^+}$ that is quite different from that of *E. coli*.

Blue-light taxis in *H. halobium* is governed by P370, a retinal-dependent system [5,6]. It is believed that blue light absorbed by P370 causes a membrane depolarisation and the subsequent reversal of bacterial flagella [21–23]. However, we were unable to register changes of $\Delta\psi$ upon illuminating cells with blue light [8].

There remained a possibility, though, that the changes in $\Delta\psi$ produced by blue light were very rapid and transient, and thus above the sensitivity of the permeant cation accumulation technique we had employed. It now appears that this possibility may be safely ruled out, since the demethylation of MCP caused by blue light proceeded normally in uncoupled cells. The blue-light receptor thus appears to be a specific photoreceptor of bacterial taxis which probably interacts with an appropriate MCP protein when excited by illumination.

The finding of a low M_r band (20 000) that is methylated upon illumination with green light and is highly methylated when a sequential blue–green stimulus is introduced might be relevant to reports of a low-M_r (19 000) band associated with *B. subtilis* chemotaxis [24]. In *B. subtilis*, however, methylation of the 19 000 M_r protein was not modified by chemoeffector stimuli. The light polypeptide of *H. halobium* might either be the product of MCP degradation, or represent a parallel pathway of information processing. Further research is necessary to clarify this point.

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