METHYLATION OF MEMBRANE PROTEINS IS INVOLVED IN CHEMOSENSORY AND PHOTOSENSORY BEHAVIOR OF *HALOBACTERIUM HALOBIUM*

Angelika SCHIMZ

Institut für Neurobiologie der Kernforschungsanlage Jülich GmbH, Postfach 1913, 5170 Jülich, FRG

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1. Introduction

Halobacterium halobium responds to changes in light intensity [1,2] and to chemical gradients in the surrounding medium [3]. Light detected by photosystem 370 acts as a repellent and light detected by photosystem 565 acts as an attractant [2,4]. Chemical attractants are glucose and several amino acids, whereas phenol acts as a repellent [3]. Reversible covalent modifications of proteins play an important role in sensory transduction. Methylation and demethylation of protein carboxyl groups have been linked to the control of chemosensory responses in *Escherichia coli* [5]. Light-dependent reversible phosphorylation of proteins was found in *Halobacterium* [6]. This paper describes the influence of attractant light of 565 nm wavelength and of the chemoattractant glucose on the methylation of membrane proteins in *H. halobium*. The transferred methyl group is derived from methionine.

2. Materials and methods

Halobacterium halobium R_1, a mutant lacking gas vacuoles, was used for experiments. The cells were grown, as in [3], until they responded to light and chemical stimuli. Bacteria were collected by centrifugation at 13 000 × *g* for 15 min, washed, and resuspended in peptone-free medium (~5 × 10^8 cells/ml) and kept at 40°C. The cells were incubated for 60 min with 30 μg puromycin/ml (Boehringer, Mannheim) [7]. Radioactive methionine (Amersham Buchler, Braunschweig) was then added to 20 ml cultures: 40 μCi L-[1-^{14}C]methionine (60 mCi/mmol) or 200 μCi of L-[*methyl*-^{3}H]methionine (75 Ci/mmol), or 40 μCi of L-[*methyl*-^{14}C]methionine (60.2 mCi/mmol), respectively. After 60 min, the ^{3}H-labelled cultures were subjected to stimulation for 20 min at 25°C.

2.1. *Stimulation*

D-Glucose was added to 2 mM final conc. For the light stimulus a 100 W tungsten lamp connected with an interference filter, 565 nm, half-width 5.9 nm (Schott, Mainz) was used. Cells were continuously illuminated with an intensity of 4.8 × 10^{14} $h\nu/cm^2s$. Methylation was stopped with formaldehyde (final conc. 2.5%). To allow correction for variable sample loss during the isolation procedure of the membranes, aliquots from a normalization culture labelled with L-[*methyl*-^{14}C]methionine were added to each of the ^{3}H-labelled samples [5]. Thus, each sample originally contained an identical amount of ^{14}C-labelled proteins, and variable sample loss should be reflected in the ^{14}C-values. Methylation of proteins was expressed by the ratio of ^{3}H/^{14}C found in each gel section. Cell membranes were isolated by the procedure in [8]. The membrane fractions were resuspended in 0.5 ml 20 mM sodium–potassium phosphate buffer at pH 6.8, and 50 μl aliquots were washed with 400 μl cold acetone. Electrophoresis of the membrane proteins was performed on SDS–acrylamide gels after [9]. The gels were cut into 1.5 mm slices, and each slice was solubilized in 4 ml toluene-based scintillation cocktail containing 10% NCS solubilizer (Amersham/Searle, USA). Radioactivity was measured in a liquid scintillation spectrometer (Betaszint BF 5000, Berthold, Wildbad).

3. Results and discussion

3.1. *Inhibition of protein biosynthesis*

To study methyl transfer from methionine to

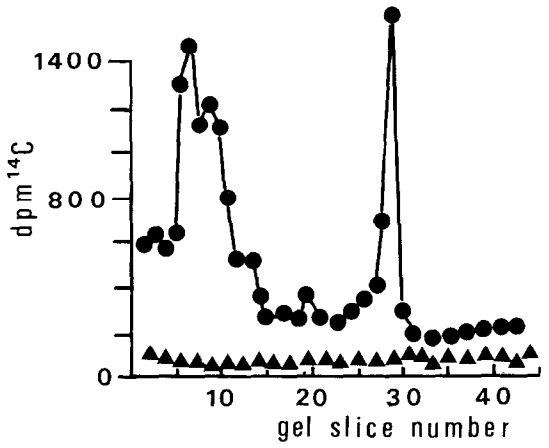

Fig.1. Inhibition of protein biosynthesis. Halobacteria were incubated with L-[1-^{14}C]methionine for 1 h. Isolated membranes were subjected to SDS–acrylamide electrophoresis, and radioactivity of membrane proteins was determined in 1.5 mm gel sections. (▲) Sample incubated with 30 μg puromycin/ml for 1 h prior to the addition of methionine; (●) sample without puromycin treatment.

membrane proteins, the incorporation of the entire molecule must be prevented. Cells were labelled with L-[1-^{14}C]methionine either in the presence or in the absence of puromycin. Puromycin has been reported to inhibit the synthesis of bacterioopsin in *Halobacterium* [7]. In the absence of puromycin cells incorporated methionine into membrane proteins (fig.1) whereas puromycin-incubated cells did not. Thus, puromycin can be regarded as an effective inhibitor of protein biosynthesis in *Halobacterium*.

3.2. *Stimulus-dependent methylation of membrane proteins*

Methyl incorporation into a membrane protein was significantly higher in the presence of glucose or of attractant light than in unstimulated cells (fig.2). The M_r of the methylated proteins was ~60 000 in both stimulated samples. They are different therefore from the proteins which undergo light-dependent phosphorylation [6]. At this time it cannot be decided whether the same protein or different proteins of similar M_r are methylated by light or chemical stimuli. Stimulation of the cells by the repellent phenol lowered the level of methylation in a membrane protein (not shown).

The requirement for methionine in the transduction of chemical stimuli in *H. halobium* was already indicated by the fact that ethionine, an inhibitor of *S*-adenosyl methionine formation, inhibits the response of the bacteria to chemoattractants [3]. Recently, methylated proteins have been found in *Halobacterium* membranes [10]. In *E. coli*, it has been shown that methyl groups from labelled methionine are transferred to membrane proteins until a plateau of methylation is reached. The cells maintain a basal level of methylation in the absence of stimuli. When the cells are stimulated by an attractant the level of methylation rises to a new plateau which is maintained as long as the stimulus is present [11]. The same seems to be true for halobacteria when the cells were stimulated by continuous illumination with attractant light or by addition of glucose. Methylation of membrane proteins seems to be involved also in photosensory and chemosensory behavior of *H. halobium*.

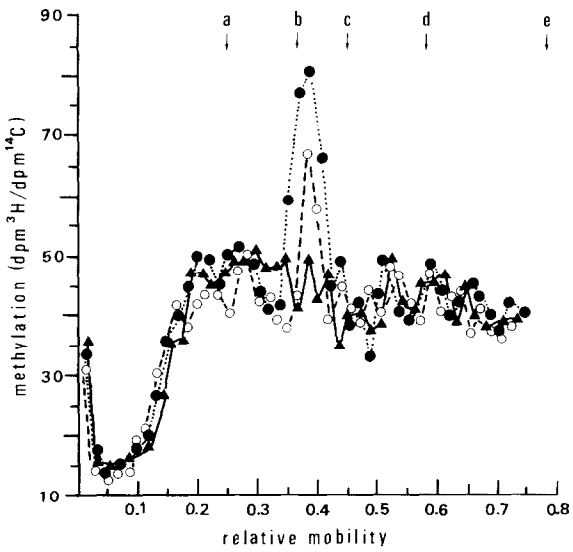

Fig.2. Effect of attractant light and glucose on the methylation of membrane proteins. Cells were labelled with L-[*methyl*-^{3}H]methionine. Aliquots of a ^{14}C-labelled culture were added to all samples prior to the isolation of the membranes to allow correction for variable sample loss. Therefore the ordinate indicates the methylation level as the ratio of ^{3}H/^{14}C. Isolated membranes were subjected to SDS–acrylamide electrophoresis, and radioactivity was determined in 1.5 mm gel sections. (○) Sample stimulated by attractant light (565 nm wavelength); (●) sample stimulated by D-glucose at dim red light; (▲) control without glucose at dim red light. Mobilities are relative to bromphenol blue. The M_r standards used, indicated by arrows, were: (a) phosphorylase *b* (94 000); (b) bovine serum albumin (67 000); (c) ovalbumin (43 000); (d) carbonic anhydrase (30 000); (e) soybean trypsin inhibitor (20 100).

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