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Potassium chemotaxis in *Rhodobacter sphaeroides*

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The addition of potassium to *Rhodobacter sphaeroides* in the absence of magnesium increased the membrane potential, as measured by the carotenoid bandshift. In the presence of magnesium, potassium decreased the membrane potential. Potassium stimulated the mean population swimming speed by 33% and 48% in the presence or absence of magnesium, respectively. Chemotactic responses of *R. sphaeroides* to potassium were also unaffected by the presence or absence of magnesium. These results suggest that the membrane potential and by inference the ΔpH and $\Delta\mu_H+$ are not involved in chemotactic signalling towards potassium.

Bacterial chemotaxis; Motility; Membrane potential; ΔpH

1. INTRODUCTION

Rhodobacter sphaeroides is a purple non-sulphur bacterium that shows strong chemotactic responses to several sugars, organic acids, and ions such as ammonium and potassium [1,2]. In enteric bacteria transmembrane methyl accepting chemotaxis proteins are the receptors that detect chemical gradients [3]; these are absent in *R. sphaeroides* [4]. Instead there appears to be an obligate requirement for transport and metabolism of chemoattractants [1,2]. One response of this organism to the addition of chemoattractants is a prolonged change in swimming with an increase in run length and decrease in stopping frequency [5].

Potassium is a very strong chemoattractant for *R. sphaeroides* [1,5]. It has been shown to have a central role in regulating the intracellular pH and in determining the membrane potential [6]. The membrane potential was shown to be decreased by the addition of potassium [6]; however, we have found that potassium can actually increase the membrane potential as measured by the carotenoid bandshift. Therefore we have investigated the cause of this difference and what effect it has on the chemotactic and motility responses of *R. sphaeroides*. This is particularly important since the membrane potential and/or ΔpH has been suggested to be important in receptor-independent taxis [7,8].

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2. MATERIALS AND METHODS

2.1. *Bacteria and growth media*

R. sphaeroides WS8 (wild type) was from W.R. Sistrom. Cultures were grown on medium A of W.R. Sistrom under tungsten filament illumination and anaerobic conditions at 25°C as previously described [9]. Cells were harvested by centrifugation in late log phase at a density of approximately 10^9 cells/ml, washed and resuspended in 10 mM sodium Hepes, pH 7.2, sparged with N_2 for at least 45 min.

2.2. *Carotenoid bandshift*

The membrane potential of cells was estimated by monitoring the absorbance change caused by the electrochromic bandshift of carotenoid pigments in the cytoplasmic membrane [10]. The absorbance was measured at 523–510 nm using a DW2000 dual wavelength spectrophotometer (SLM-Aminco, USA) as described previously [9]. The total signal was determined by the addition of carbonyl cyanide *m*-chlorophenylhydrazone (10 μM).

2.3. *Motility measurement*

Cells were sealed in optically flat microslides and illuminated at 1000 μmol photons m^{-2} s^{-1} in a Nikon Optiphot microscope; cell speeds were determined by computer tracking as previously described [11].

2.4. *Chemotaxis assay*

Chemotactic responses were assayed in blind wells where two adjoining chambers are separated by an 8 μm pore size polycarbonate membrane. A solution of chemoattractant was placed in the top chamber and a suspension of bacteria in the bottom chamber. The chemotactic movement of bacteria into the top attractant containing chamber was determined by Coulter counting as described previously [1].

3. RESULTS AND DISCUSSION

The effect of potassium on the $A_{523-510}$ of *R. sphaeroides* is shown in fig.1. In the presence of 1 mM $MgCl_2$ the $A_{523-510}$ dropped by up to 15%. The effect

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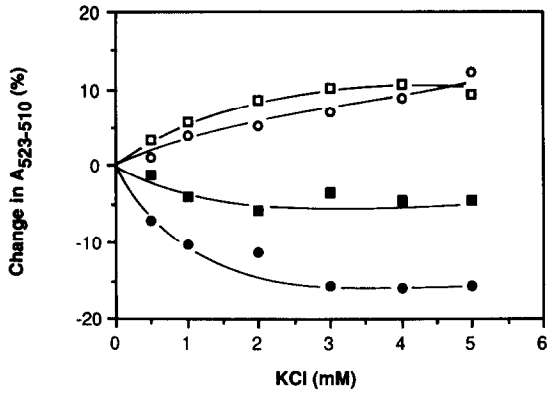

Fig.1. The effect of KCl on the carotenoid bandshift ($A_{523-510}$), which is a measure of membrane potential. Cells were incubated in the dark (○,●), or the light (□, ■). Minus magnesium open symbols, plus magnesium (1 mM) closed symbols. The data are expressed as a percentage of the total signal.

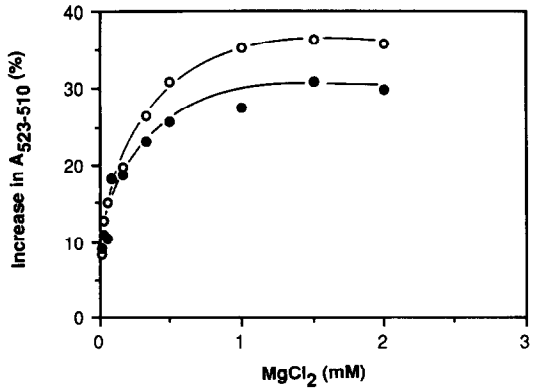

Fig.2. The effect of $MgCl_2$ on the carotenoid bandshift ($A_{523-510}$) in the dark. (○) Minus KCl, (●) plus KCl (1 mM). The data are expressed as a percentage of the total signal.

was the same whether cells were in the light or dark. When magnesium was omitted the $A_{523-510}$ increased after potassium addition, again the effect was the same in the light or dark. The drop in $A_{523-510}$ in the presence of magnesium agrees with the results of Abee et al. [6] who included $MgCl_2$ in their assays. Magnesium, as well as an intact outer membrane, is required for high rates of potassium uptake in *R. sphaeroides* [6,12]. Both the increase in membrane potential after potassium addition, and the decrease when magnesium is present have been observed in chromatophores [13]. In that study membrane potential increased following potassium addition in the absence of valinomycin, under which condition potassium does not move across the membrane. However, in the presence of valinomycin, where potassium movement did occur, the membrane potential dropped [13]. Consequently, in whole cells, it is likely that it is the uptake of potassium that leads to a decline in the membrane potential.

Magnesium alone increased the measured $A_{523-510}$ by up to 35%, but in the presence of 1 mM KCl the increase was slightly smaller (fig.2). While it is possible that such increases were due to light scattering effects, it is unlikely that these contributed significantly to the increase because the dual wavelength technique minimises their effects [13,14]. Furthermore it has been demonstrated that the increase in the amplitude of the bandshift signal following addition of magnesium [13] could be explained in terms of changes in the membrane surface potential and, therefore, in the overall membrane potential [15].

Potassium can either increase or decrease the $A_{523-510}$, depending on whether magnesium is present. The effects of potassium on motility and chemotaxis were therefore investigated. In the absence of magnesium, potassium stimulated the mean swimming speed of *R. sphaeroides* from 20.2 μm/s to 29.9 μm/s (fig.3A). Inclusion of $MgCl_2$ (1 mM) in the buffer did not significantly change this effect, with the mean swimming speed increasing from 19.5 μm/s to 26 μm/s (fig.3B). There was also a strong chemotactic response towards potassium, which was greatest at 1 mM, in the presence or absence of magnesium (fig.4). Magnesium alone did not cause a chemotactic response (data not shown).

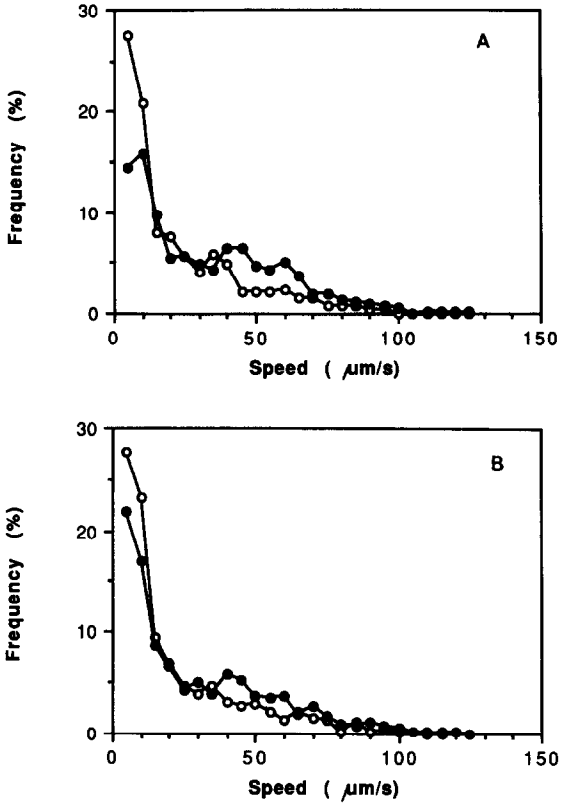

Fig.3. The effect of KCl on the motility of *R. sphaeroides* in the presence and absence of $MgCl_2$. (A) Cells in the absence of $MgCl_2$ (○) control, (●) plus KCl (1 mM). (B) Cells in the presence of $MgCl_2$ (1 mM) (○) control, (●) plus KCl (1 mM).

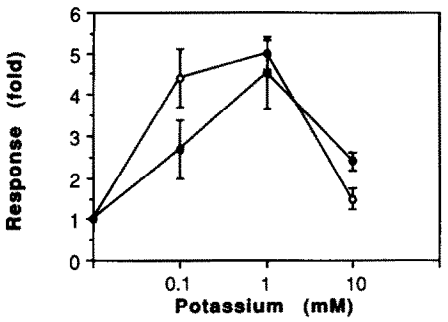

Fig.4. The effect of $MgCl_2$ on chemotaxis towards KCl in *R. sphaeroides*. (○) Control cells, (●) cells plus $MgCl_2$ (1 mM). The response is the fold increase in the cell number swimming into the attractant chamber relative to a buffer control.

Potassium is clearly a crucial ion in the regulation of intracellular pH, membrane potential [6] and possibly intracellular turgor [16]. It also has a profound effect on the motility and chemotaxis of *R. sphaeroides*. The ability of potassium to cause these effects is independent of whether magnesium is present and hence the effect of potassium on the $A_{523-510}$. It is difficult to see how the membrane potential can be directly involved in tactic signalling if it can be increased or decreased upon potassium addition. Furthermore magnesium, which increases the membrane potential, is not an attractant, demonstrating that the ability to increase the membrane potential is not correlated with the ability to cause a tactic response. These data strongly support the contention that the membrane potential is not directly involved in tactic signalling.

The total $\Delta\tilde{\mu}_{H+}$ has been shown to be little changed after potassium addition [6]. This suggests that tactic signalling is not dependent upon changes in the total $\Delta\tilde{\mu}_{H+}$. In the presence of magnesium the ΔpH has been shown to increase in response to the potassium induced decrease in membrane potential [6]. Given this, it is likely that the potassium induced increase in the membrane potential in the absence of magnesium would cause a corresponding drop in ΔpH. If correct, this suggests that the ΔpH and pH_i are not important in tactic signalling, because they also can go up or down depending on whether magnesium is absent or present. Instead potassium may directly affect motor function, an effect possibly mediated by cellular turgor or even an indirect one mediated by an effect of potassium on metabolism. The latter is possible since metabolism of acetate via acetyladenylate is now known to increase the clockwise rotation of the *Escherichia coli* flagellar motor [17]. The flagellar motor of *R. sphaeroides* only rotates clockwise [18], therefore any stimulus that increases the clockwise rotation would increase the mean population swimming speed.

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