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The effect of phosphate on the motility of *Rhodobacter sphaeroides*

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1. SUMMARY

When *Rhodobacter sphaeroides* was resuspended in sodium phosphate buffer directly after growth on complete medium there was an almost complete loss of motility. The addition of 1 mM sodium phosphate to *R. sphaeroides* suspended in Hepes buffer also caused an immediate reduction in the mean swimming speed of 35%; an effect that was maintained for more than 60 min. This loss of motility was prevented by the inclusion of succinate in the resuspension medium. Addition of phosphate in the absence of succinate also increased the membrane potential by up to 35% and decreased the rate of respiration by 25%. These effects were reversed in the presence of succinate, phosphate addition decreased the membrane potential by up to 7% and increased the rate of respiration by 23%. These data show that the effect of phosphate buffer on *R. sphaeroides* is highly complex and its inhibition of motility is probably an indirect effect. The use of phosphate buffer in many physiological experiments may have unexpected side effects.

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2. INTRODUCTION

Rhodobacter sphaeroides is a purple non-sulphur photosynthetic bacterium that swims by the rotation of a single sub-polar flagellum [1]. It lacks the methyl accepting chemotaxis proteins found in the enteric bacteria and has consequently been studied as a model system of receptor independent chemotaxis [2–7]. Many chemoattractants, such as the organic acids and potassium cause an increase in the mean swimming speed and a decrease in the stopping frequency [4,8]. Further analysis of these effects suggests some compounds have two different effects on the flagellar motor of *R. sphaeroides*. First, there is a true chemotactic response, and secondly, some compounds cause an additional excitation of motility [6]. Neither of these responses appears to involve changes in the steady state membrane potential or rate of respiratory electron transport [7].

While studying the effect of various compounds on the motility of *R. sphaeroides* it was found that cells resuspended in sodium phosphate buffer showed a dramatic loss of motility. No repellents have been identified in *R. sphaeroides* and previous studies have found that only inhibitors such as vanadate and CCCP have a major inhibitory effect on swimming. We therefore investigated the effect of phosphate more fully to see whether it

has a direct influence on the flagellar motor or an indirect influence on cellular metabolism. The latter is important because *R. sphaeroides* is often resuspended in phosphate buffers before measurement of properties such as membrane transport and energisation.

3. MATERIALS AND METHODS

3.1. Materials

N-2-Hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (Hepes), was obtained from Sigma Chemical Co. All other reagents were of analytical grade.

3.2. Bacteria and growth media

Rhodobacter sphaeroides WS8 (wild-type) was from W.R. Sistrom. Cultures were grown anaerobically at 25 ° C under tungsten filament illumination on medium A of W.R. Sistrom, which contained 20 mM phosphate and 10 mM succinate as previously described [9]. Late log phase cells were harvested by centrifugation under air at a density of approximately 10^9 cells ml^{-1}, washed and resuspended in 10 mM sodium Hepes, pH 7.2, which had been sparged with N_2 for at least 45 min. Cells were then allowed to equilibrate for a further 45 min before being used in experiments. This yielded cell suspensions with an oxygen concentration of approximately 0.1 mM. Cells grown under these conditions possessed both photosynthetic and respiratory electron transport chains allowing measurement of both photosynthetic and respiratory electron transport on identical cells.

3.3. Computer tracking

Cells were sealed in optically flat microslides, illuminated at 1000 μmol photons m^{-2} s^{-1} in a Nikon Optiphot microscope and the mean cell speeds determined by computer tracking as previously described [10].

3.4. Respiratory electron transport

The consumption of O_2 by 3 ml of cells was measured at 25 ° C with a Clarke-type oxygen electrode (Rank Brothers, Bottisham U.K.). Illumination was kept at approximately 10–20 μmol photons m^{-2} s^{-1} as previously described [7].

3.5. Measurement of membrane potential

The membrane potential of cells was estimated by monitoring the change in absorbance at 523 nm with respect to 510 nm. This difference is caused by the electrochromic bandshift of carotenoid pigments which are present in the cytoplasmic membrane of photosynthetically grown cells [11]. The absorbance was measured with a DW2000 dual wavelength spectrophotometer (SLM-Aminco, U.S.A.) as described previously [9]. The absorbance shift has been shown to be related to the membrane potential by comparison with the ion-distribution method [12]. The bandshift was measured both in cells kept in total darkness and in cells under high side illumination.

4. RESULTS

Cells of *R. sphaeroides* suspended in growth medium, which had succinate (10 mM) as carbon source and sodium phosphate (20 mM) as buffer, were highly motile. However when these cells were centrifuged and resuspended in sodium phosphate buffer alone there was an almost complete loss of motility. Centrifugation may cause shock to the cells, therefore to eliminate this possibility sodium phosphate was added to cells that had previously been centrifuged, resuspended in Hepes buffer

Table 1

The effect of phosphate on the motility of *R. sphaeroides* WS8

All samples are the average of at least six replicates. The control cells treated with 1.0 and 10.0 mM sodium phosphate were significantly different ($P > 99.9\%$) from the untreated cells. The succinate concentration was 1 mM.

Phosphate (mM)	Change in the swimming speed of cells (%) after phosphate addition	
	control	plus succinate
0.1	−10	27
1.0	−35	−5
10.0	−37	−5

and allowed to recover their motility (Table 1). Phosphate as low as 1 mM reduced the mean swimming speed of the population by around 35%. All of the components of the growth medium were examined to see if any compound protected against the loss of motility. In the presence of the carbon source succinate there was no significant change in the speed at which the cells swam after phosphate addition, in fact 0.1 mM caused an increase. Potassium is known to increase the swimming speed of *R. sphaeroides* [8], and indeed potassium phosphate had little effect on the swimming speed of *R. sphaeroides*, presumably because the potassium antagonised the effect of phosphate (data not shown).

There was no recovery in the motility of cells to which phosphate had been added over 60 min (Fig. 1). The swimming speed of control cells eventually declined over this time, as is usual for cells that have been sealed in flat microslides.

In an attempt to see whether phosphate used as a resuspension buffer had other marked effects on general cellular physiology, we examined its effect on the membrane potential and rate of oxygen uptake. When succinate was omitted, sodium phosphate caused a significant increase in the membrane potential of cells in high light (Fig. 2). Once again succinate antagonised the effect such that addition of phosphate to cells in the presence of 1 mM succinate caused a small drop in the membrane potential (Fig. 2). Similar effects on the membrane potential were observed if the measurements were made on cells in total darkness.

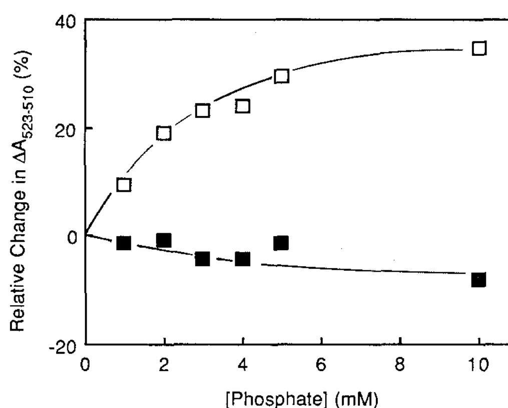

Fig. 2. The effect of phosphate on the membrane potential of *R. sphaeroides*, under high light conditions, as measured by the carotenoid bandshift. (□) Cells in Hepes buffer; (■) cells in Hepes buffer plus 1 mM succinate.

In the absence of succinate the rate of oxygen consumption dropped from 9.9 nmol O_2 min^{-1} (mg protein)$^{-1}$ $\pm$ 2.2 to 7.4 nmol O_2 min^{-1} (mg protein)$^{-1}$ $\pm$ 1.3 after the addition of sodium phosphate (1 mM), a decrease of 25%. In the presence of succinate (1 mM) the addition of sodium phosphate (1 mM) increased the oxygen consumption increased from 43.5 nmol O_2 min^{-1} (mg protein)$^{-1}$) $\pm$ 3.34 to 53.4 nmol O_2 min^{-1} (mg protein)$^{-1}$ $\pm$ 2.3, an increase of 23%. The much higher rate of oxygen consumption in the presence of succinate is almost certainly due to the provision of an oxidisable carbon source.

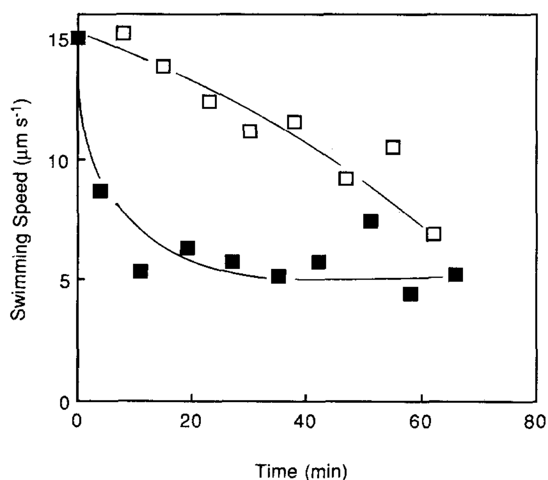

Fig. 1. The effect of phosphate addition on the swimming speed of *R. sphaeroides*. (□) Control cells; (■) cells plus 1 mM sodium phosphate.

5. DISCUSSION

It is clear that phosphate ions alone had a highly inhibitory effect on the motility of *R. sphaeroides* (Table 1, Fig. 1). This occurred even though cells had been grown on a succinate minimal medium containing 20 mM phosphate as a buffer. Cells grown under these conditions did not show active accumulation of phosphate, although this did occur if the phosphate concentra-

tion was limiting during growth i.e. below 0.1 mM (data not shown). The effects of phosphate must therefore be a result of either passive entry or exchange of the ion. The inhibition of motility by phosphate could be completely removed by the addition of the carbon source succinate. This suggests that the effect is complex because it is altered by the metabolism of a carbon and electron source. This is supported by the observation that phosphate also increased the membrane potential while decreasing the rate of respiration (Fig. 2). An increase in the membrane potential is known to inhibit the rate of respiration in photosynthetic bacteria, perhaps explaining the observed decrease in respiration [13]. Again in the presence of succinate the effects of phosphate were reversed; the membrane potential fell and the respiration rate increased. Overall this suggests that the inhibition caused by the addition of phosphate ions is an indirect effect on cells and probably not caused by direct inhibition of the flagellar motor. In the absence of added succinate, phosphate alone led to an increase in the membrane potential suggesting an electrogenic accumulation or exchange of phosphate (Fig. 2). However, as stated above, active accumulation was not observed under these conditions. It may be possible that the change in membrane potential is caused by the simultaneous operation of a cation-phosphate^{-2} symporter [14] and a cation efflux system, by an alteration in the rate of electron transport-linked proton efflux and ion transport [15]. Alternatively phosphate is known to be involved in antiport systems [16], so the increase in membrane potential might be caused by the electrogenic exchange of differentially charged internal and external phosphate ions in a futile cycle.

While the precise reason for the effect of phosphate is unknown, it is clear that the use of such a highly biologically active ion in resuspension buffers may lead to complex side effects, and it should be used cautiously as a buffer in transport and physiology studies. In addition it should be noted that motility is a sensitive and rapid indicator of inhibitory effects on whole cells.

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