# MEMBRANE POTENTIAL CHANGES DURING CHEMOTAXIS OF *RHODOPSEUDOMONAS SPHAEROIDES*

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Received 6 April 1979

## 1. Introduction

Many motile species of bacteria when confronted with a stimulus gradient, e.g., light, chemical, respond by moving towards (positive taxis) or away from (negative taxis) the source of the stimulus. Flagellate bacteria achieve this by controlling the direction of rotation of their flagella, anticlockwise rotation results in smooth swimming and occasional clockwise rotation results in tumbling in peritrichously flagellate bacteria or reversal in polar flagellate bacteria, in both cases this often results in a change of direction when smooth swimming is resumed (reviewed in [1,2]). It appears that when presented with a spatial gradient of either a positive or negative stimulus bacteria respond by longer periods of anticlockwise flagella rotation, therefore smooth swimming, resulting in net movement up a positive gradient or down a negative gradient. A sudden increase in a temporal gradient has been reported to give a slightly different result, if the stimulus is a repellent, a sudden increase in concentration results in rapid tumbling or reversal, whereas an attractant again produces a prolonged period of smooth swimming. It is thought that a sudden change of gradient, with the possible exception of a temporal repellent, always results in a period of tumbling suppression.

When the stimulus is chemical, the gradient is sensed by the binding of the chemotactic compound to specific receptors, usually bound to the inner membrane. The information about the number of receptors bound, and therefore the gradient, is transmitted to the flagella by a little understood process which seems to involve methylation of specific chemotaxis proteins (methyl-accepting chemotaxis proteins) in the cell envelope. These may be involved in both the transfer and interpretation of the signals, possibly by controlling specific ion gates [2].

Earlier studies have indicated that a change in the membrane potential may be part of the signal [3], suggesting a sensory system similar to the binding and transmission of signals in higher organisms. All previous work, however, used techniques which were slow, indirect and destructive to measure any changes in potential.

The membrane-bound carotenoids of photosynthetic bacteria are well characterised, natural indicators of the potential difference across the membrane, changes in membrane potential resulting in an electrochromic absorption shift [4]. We have recently shown that the photosynthetic bacterium *Rhodopseudomonas sphaeroides* responds chemotactically to gradients of some compounds even when grown phototrophically [5]. Using the carotenoid absorption shift of *Rps. sphaeroides* we have now investigated changes in membrane potential of living cells in physiological conditions when exposed to a temporal chemotactic stimulus.

## 2. Materials and methods

### 2.1. *Organism*

*Rhodopseudomonas sphaeroides* wild-type 2.4.1 was used throughout this study. We have already shown that this strain exhibits both positive and negative chemotaxis [5].

2 2 *Growth conditions and experimental media*

Cells were grown anaerobically in the light in batch culture to late exponential phase, as described previously, but without acetate in the medium [5] After centrifugation the cells were resuspended in 5 mM oxygen-free potassium phosphate buffer (pH 7 0) to $10^9$ cell/ml

Compounds to be tested for their ability to elicit a change in membrane potential were dissolved at 0 5 M in the same buffer and the pH readjusted if necessary All solutions were kept anaerobic by bubbling with oxygen-free nitrogen

2 3 *Measurement of carotenoid shift*

Spectrophotometer measurements of the carotenoid absorption were carried out using an Aminco DW2 spectrophotometer in dual wavelength mode, using the difference $A_{510}-A_{523}$, which gave the maximum response in this species

Measurement of response times was made using the spectrophotometer in kinetic mode and a Nicolet 1020A digital oscilloscope The overall response time of the instrument system was <200 μs

Chemotactic solutions, usually 10 μl, were added to 3 ml cell suspension at 30°C in an anaerobic top stirred cell The mixing time of the system, examined by dye addition, was <100 ms ($t_{1/2}$ ~50 ms) The response of the experimental system was therefore limited by the mixing time

## 3 Results and discussion

The light or chemically-induced shift in the absorption spectrum of the carotenoids of some bacteria was shown [4] to be linearly related to a change in membrane potential, and to be sensitive to compounds which directly affect the membrane potential, e g , *m*-chlorocarbonylcyanide phenyl hydrazone (CCCP), valinomycin

Figure 1 shows that our system responded in a similar manner, illumination with actinic light resulted in a large shift in carotenoid adsorption, presumably caused by the pumping of protons across the membrane during photosynthesis The response time of the instrumentation meant that only the slow phase of the carotenoid shift was resolved but this had similar kinetic properties to those previously described ($t_{1/2}$ ~55 ms)

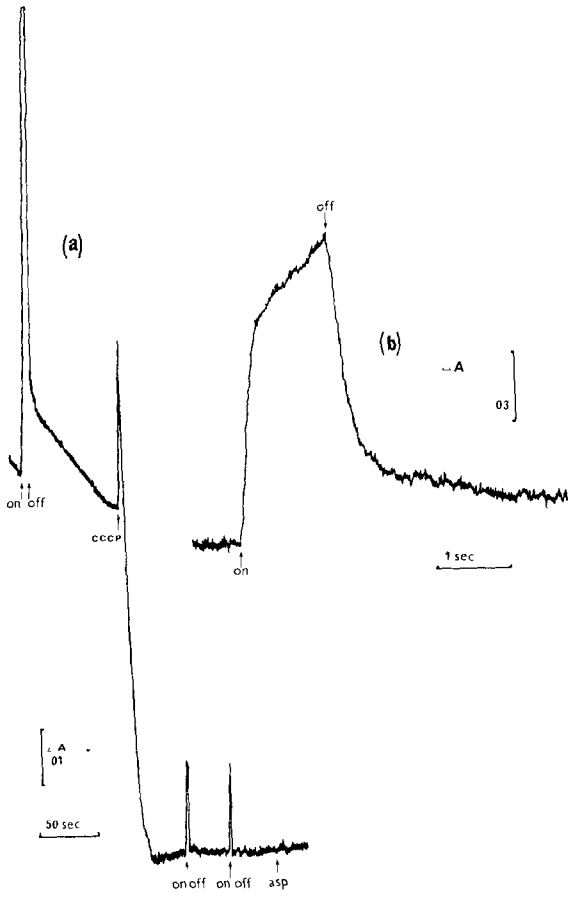


Fig 1 Light-induced membrane potential change of *Rps sphaeroides* (a) Increase in membrane potential as measured by carotenoid absorption shift ($A_{510}-A_{523}$) on illumination with actinic light (780 nm) before and after addition of $10^{-5}$ M CCCP L-aspartate (1 6 mM) was added (↑ asp) after reduction of the membrane potential (b) Kinetics of the light induced membrane potential change (detailed in section 2)

Addition of uncoupling concentrations of CCCP caused a rapid fall in membrane potential and, as previously described, the slow phase of the light induced carotenoid shift was considerably reduced

To investigate the effect of chemotactic agents on the membrane potential of *Rps sphaeroides* we have used compounds which we had shown to be chemotactic, and which were simple and non-toxic We used both sugars and amino acids as they have been shown in other Gram-negative systems, e g , *Escherichia coli*, to possibly use different types of binding proteins [2]

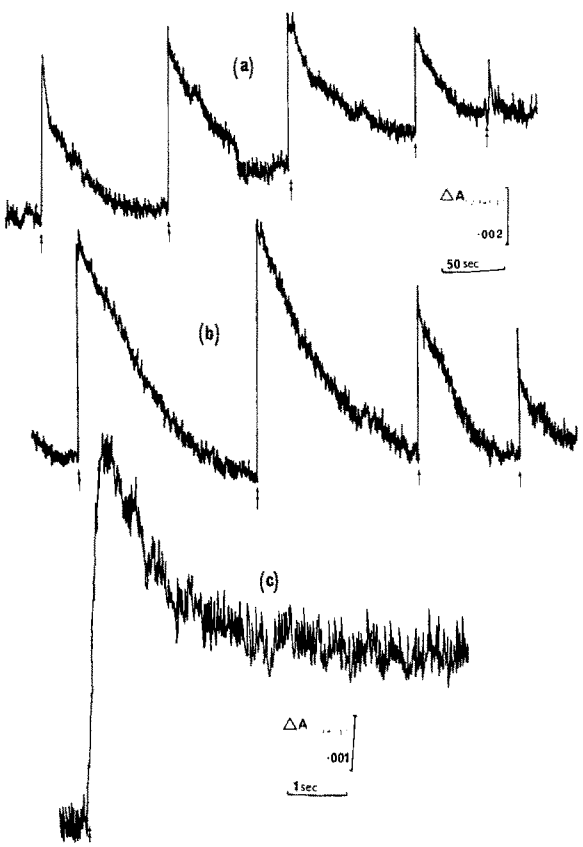


Fig.2. Increase in membrane potential on addition of chemoattractants. Repeated additions of (a) L-alanine and (b) D-galactose (↑ 1.6 mM; ↑ 0.8 mM). (c) Kinetics of the membrane potential change on addition of 1.6 mM L-alanine.

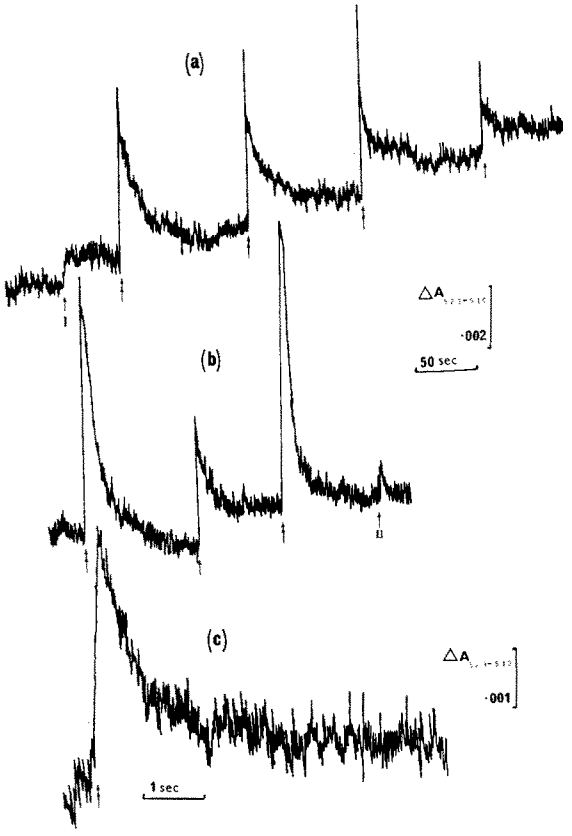


Fig.3. Increase in membrane potential on addition of chemorepellents. Repeated additions of (a) L-leucine and (b) acetate (↑ 1.6 mM; ↑ 0.8 mM) with controls: buffer (↑ I) (10 μl); (↑ II), indole (1.6 mM). (c) Kinetics of potential change on addition of 1.6 mM L-leucine.

Figures 2 and 3 show that there was an increase of membrane potential, as shown by the absorption change due to the carotenoid shift, when either a positive or negative chemotactic compound was added to the cells. The direction of the shift was the same for both positive and negative compounds but the size and extent of the response depended on the compound and the amount added.

The system used here had a faster response time than those previously used but the results in general resemble those in [3]. Kinetic studies of the response times when either attractants or repellents were added showed a 3-phase response (fig.2c,3c). There was an initial fast rise in membrane potential ($t_{½}$ ~75–100 ms) followed by a fast decay in potential ($t_{½}$ ~300 ms) and then a slower decay phase returning to a steady membrane potential over 1.5–2 min, dependent on the size of the initial response and the compound used, probably caused by uptake and metabolism of the test compound. The rise time of the membrane potential change is only a little slower than the response time of the experimental system. We cannot exclude the possibility that more rapid transient changes occur.

Control experiments (fig.3), involving the addition of a non-chemotactic compound, indole or buffer alone, resulted in no absorption change. There was also no significant change in carotenoid adsorption if a chemotactic compound was added to the cells after the release of the membrane potential by CCCP (fig.1).

Repeated rapid addition of an attractant in an attempt to saturate the receptor sites, and thus remove the 'gradient' from the sensory system resulted in a reduction of the size of the carotenoid shift (fig 2a,b) This reduction occurred when a concentration of attractant of the same order of magnitude as that already established as being near to saturation had been reached, about $10^{-3}$ M for L-alanine However the membrane potential increase was not completely abolished, perhaps reflecting uptake and metabolism of the compounds

The results presented here show that the addition of a chemotactic compound to *Rps sphaeroides* caused an increase in membrane potential as indicated by the shift in carotenoid absorbance, in agreement with the fluorescence work [4] with *E coli* The inability of another group to find a change in membrane potential using Gram-positive bacteria [6] suggests that either this part of the chemotactic signal is lacking in Gram-positive bacteria or occurs as a very fast transient, outside the range of their slow measuring system There is some evidence that the flagella motor of Gram-positive and -negative bacteria is not identical, and this early potential increase may involve the driving motor Unfortunately there are no motility or chemotaxis mutants of *Rps sphaeroides* It is therefore not possible to establish at which stage of the chemotaxis sensing system the increase in membrane potential occurs We cannot exclude transients, occurring faster than the response time of our system

Previous results have indicated that an increase in membrane potential may suppress tumbling during phototaxis [7]. We suggest that the increase in membrane potential reported here, which occurs when either an attractant or repellent is added to *Rps sphaeroides* may be involved in the brief early period of prolonged smooth swimming when the chemoreceptors are bound This is almost certainly not the signal identifying the type of chemotactic stimulus and an alternative signalling system must exist to separate a positive from a negative chemotactic compound

We conclude that when *Rps sphaeroides* encounters either a temporal or spatial gradient or either an attractant or repellent there is an increase in membrane potential causing a brief period of smooth swimming It is probably only then that the type of chemotactic agent is distinguished and a second signalling system involving tumble generation activated

## References

[1] MacNab, R B (1978) CRC Crit Rev Biochem 5, 291–341
[2] Goy, M F and Springer, M S (1978) in Receptors and Recognition B (Hazelbauer, G L ed) vol 5, pp 1–34, Chapman and Hall, London
[3] Szmelcman, S and Adler, J (1976) Proc Natl Acad Sci USA 73, 4387–4391
[4] Jackson, J B and Crofts, A R (1971) Eur J Biochem 18, 120–130
[5] Armitage, J P , Keighley, P and Evans, M C W (1979) FEMS Microbiol Lett 6/3, in press
[6] Miller, J B and Koshland, D E , jr (1977) Proc Natl Acad Sci USA 74, 4752–4756
[7] Harayama, S and Imo, T (1977) J Bacteriol 131, 34–41