CHEMOTACTICALLY INDUCED INCREASE IN THE MEMBRANE POTENTIAL OF SPHEROPLASTS OF *RHODOPSEUDOMONAS SPHAEROIDES*

J. P. ARMITAGE and M. C. W. EVANS

Department of Botany and Microbiology, University College London, Gower Street, London WC1E 6BT, England

Received 31 January 1980

1. Introduction

When *Rhodopseudomonas sphaeroides* is stimulated chemotactically there is an increase in membrane potential as measured by the electrochromic absorbance shift of the membrane-bound carotenoids [1].

Further investigation of the nature and role of the membrane potential change in the control of the direction of flagella rotation requires the use of specific ion- and potential-sensitive dyes and well-characterised chemotaxis mutants.

Unfortunately most of these dyes will not pass the barrier of the outer membrane of Gram-negative bacteria and without an indicator of a normal chemotactic response, e.g., direction of flagella rotation, it is difficult to interpret the nature of any response which these dyes may show.

Here we provide evidence that spheroplasts of Gram-negative photosynthetic bacteria respond in a similar manner to whole cells when stimulated chemotactically, showing an increase in membrane potential. The results suggest that removal of the outer membrane may not affect the early sequence of the chemotactic signalling process, which occurs at the inner, cytoplasmic membrane.

2. Materials and methods

2.1. *Organism*

Rhodopseudomonas sphaeroides wild-type 2.4.1 was used throughout this study.

2.2. *Growth conditions and experimental media*

Cells were grown anaerobically, in the light, in batch culture to late exponential phase, as in [2]. For experimentation whole cells, lysed spheroplasts and chromatophores were suspended in 5 mM oxygen-free potassium phosphate buffer (pH 7.0) and whole spheroplasts in the same buffer containing 10% sucrose.

Compounds shown to elicit a chemotactic response in *Rps. sphaeroides* [3] were dissolved at 0.5 M in the same buffer and the pH readjusted if necessary. All cell suspensions were kept anaerobic by bubbling with oxygen-free nitrogen.

2.3. *Preparation of spheroplasts*

Late exponential phase batch-grown cells were resuspended after centrifugation in 25 mM Tris–HCl buffer (pH 7.0) containing 20% (w/v) sucrose and 25 μg/ml lysozyme. After incubating at 0°C for 5 min Na-EDTA was added to give 10^{-3} M final conc. After ~2 h incubation at room temperature spheroplast formation was almost complete, as measured by microscopic examination. The first osmotic shock was then given by carefully adding an equal volume of 5 mM potassium phosphate buffer (pH 7.0) dropwise to the stirred spheroplast suspension at 0°C. After stirring for 30 min at 0°C the spheroplasts were pelleted at 12 000 × *g* at 4°C for 20 min. Resuspension of the pellet in 5 mM potassium phosphate buffer (pH 7.0) and 10% sucrose gave the whole spheroplast suspension. For the lysed spheroplast preparation the final shock was completed by gently resuspending the pellet in 5 mM potassium phosphate buffer (pH 7.0) containing 10 μg/ml RNase and 5 μg/ml DNase and stirring for 30 min at 0°C. After centrifuging under the same conditions as above the lysed spheroplasts were suspended for use in 5 mM oxygen-free potassium phosphate buffer.

2.4. *Preparation of chromatophores*

Whole cells, grown as above, were harvested and resuspended in 10 mM Tris–HCl (pH 7.0) containing 30% sucrose and 100 μg/ml lysozyme and incubated at 0°C for 10 min. The cell suspension was then diluted 1:3 with 10 mM Tris–HCl and sonicated at 0°C until the turbidity of the suspension fell.

Whole cells and large fragments were removed by centrifugation at 10 000 × *g* at 4°C and the chromatophores then pelleted by centrifugation for 1.5 h at 130 000 × *g* at 4°C. Chromatophores were resuspended in 5 mM oxygen-free phosphate buffer (pH 7.0).

2.5. *Malate dehydrogenase activity*

The intactness of the spheroplasts was measured using malate dehydrogenase (EC 11.1.37) activity as an indicator of cell membrane breakage as in [4]. By comparison with chromatophore lysates and measurement of activity of physically broken spheroplasts only 14% of the malate dehydrogenase activity was found to have been lost from the spheroplasts during preparation whereas 87% of the activity was found in the supernatant of chromatophore preparations. This was taken to indicate that the majority of spheroplasts prepared this way still contained a large percentage of their soluble cytoplasmic contents.

Examination by phase-contrast microscopy supported this view, the spheroplast preparation being made up mainly of large opaque spheres with very few transparent spheroplasts.

2.6. *Measurement of carotenoid bandshift*

The difference in turbidity of suspensions of whole

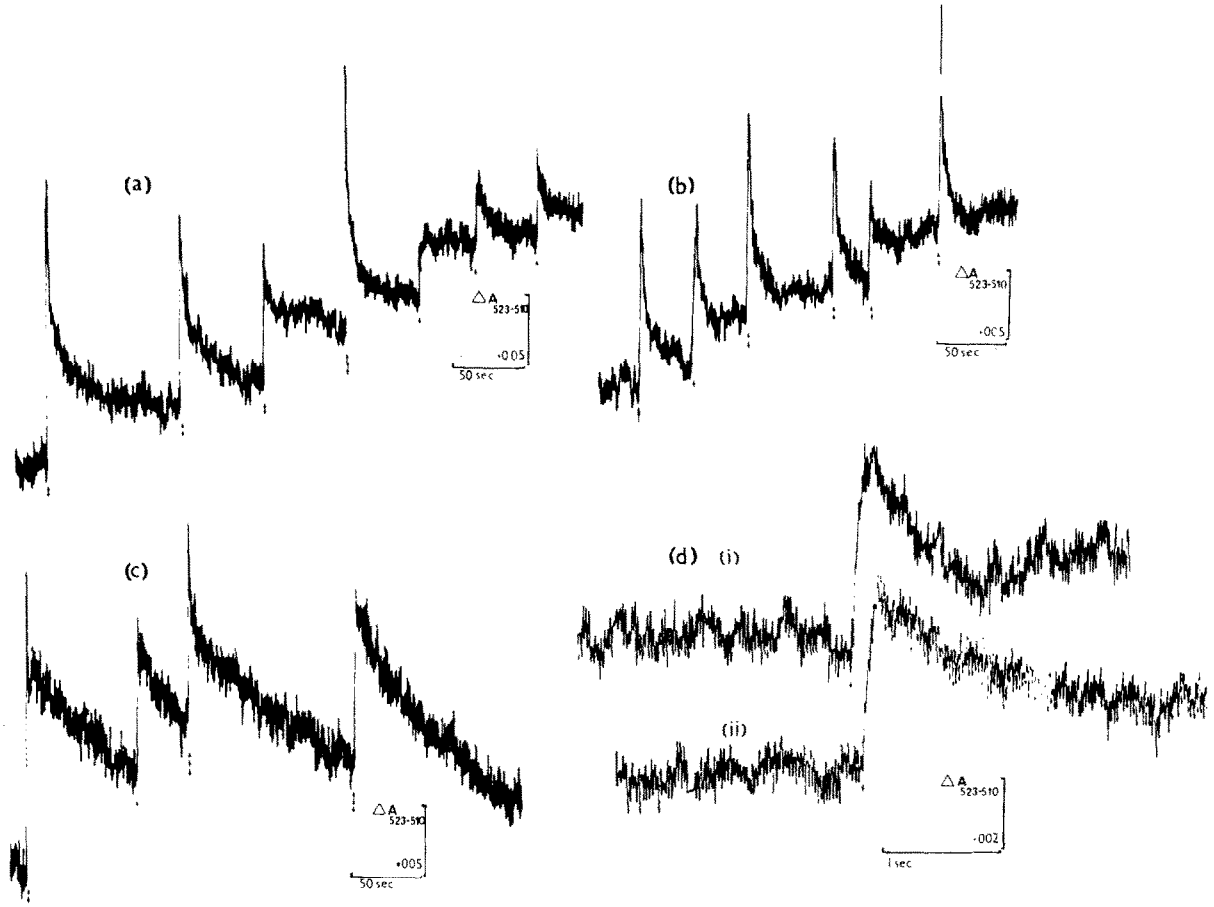

Fig.1. Increase in membrane potential on addition of chemotactic agents to whole cells of *Rps. sphaeroides*. Repeated additions of: (a) L-aspartate; (b) D-galactose; (c) L-cysteine (↑ 0.8 mM; ↑↑ 1.2 mM; ↑↑↑ 1.6 mM). (d) Kinetics of the membrane potential change on addition of 1.6 mM (i) L-aspartate and (ii) L-cysteine.

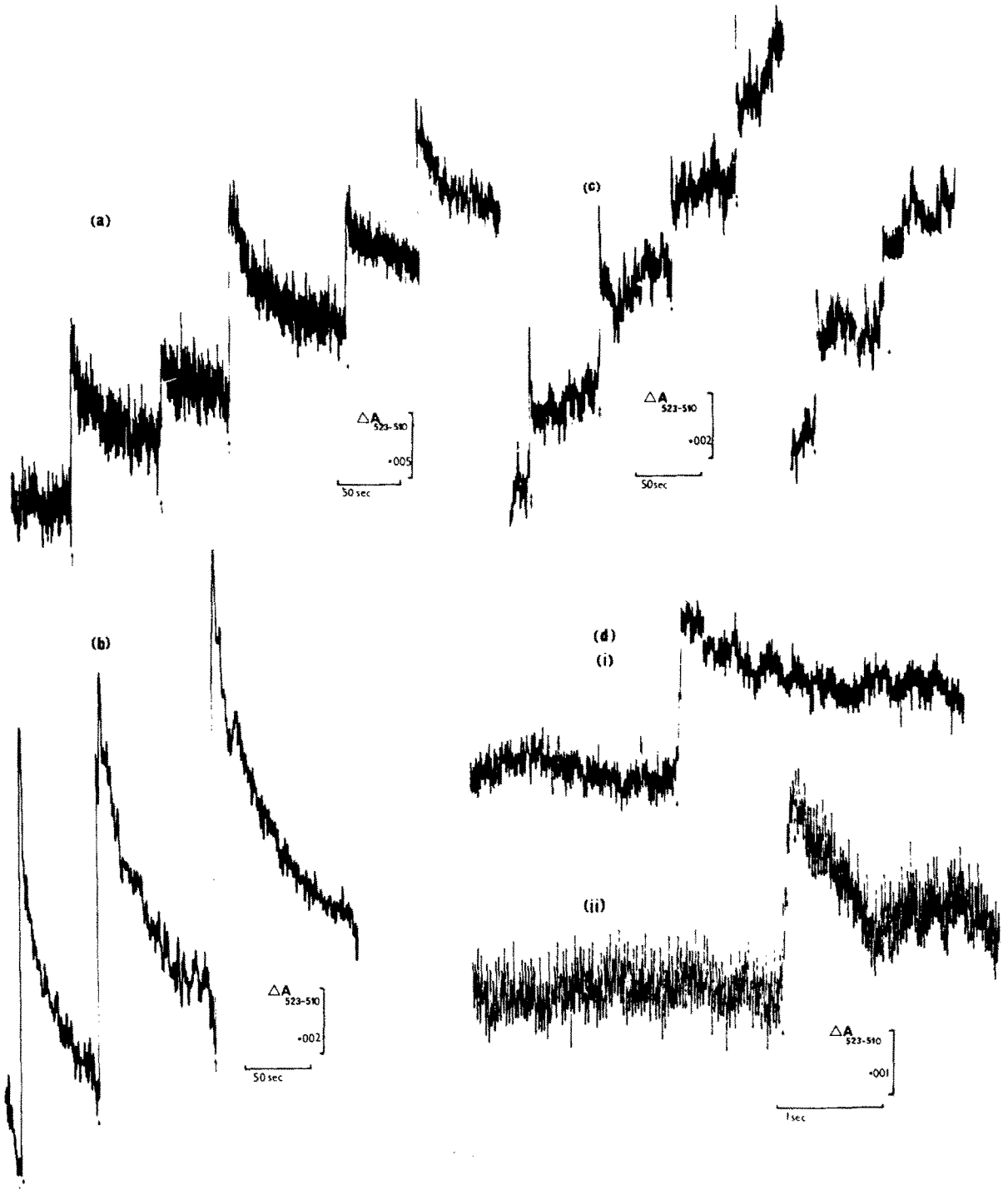

Fig.2. Increase in membrane potential on addition of chemotactic agents to spheroplasts of *Rps. sphaeroides*. Repeated additions of: (a) D-glucose, (b) L-cysteine; (c) D-galactose (↑ 0.8 mM; ↑ 1.2 mM). (d) Kinetics of the membrane potential change on addition of 0.8 mM (i) D-glucose and (ii) L-cysteine.

cells, spheroplasts, lysed spheroplasts and chromatophores was considerable. All samples were therefore equilibrated on the basis of their carotenoid absorbance using an Aminco DW2 spectrophotometer in dual wavelength scanning mode.

Characteristic responses were measured as in [1] the carotenoid $A_{510}-A_{523}$ as an indicator of membrane potential changes. Response times were measured using the spectrophotometer in kinetic mode and a Nicolet 1020A digital oscilloscope.

Isolated chromatophores may not be able to maintain a membrane potential, therefore in experiments with chromatophores a potential was induced across the membranes by illumination with actinic light at an intensity which induced half the maximum carotenoid shift for the preparation being used.

Actinic light induction of an increased membrane potential in spheroplasts, lysed spheroplasts and whole cells did not alter the observed response to chemotactic agents.

3. Results and discussion

We have shown [1] that *Rps. sphaeroides* responds to chemotactic stimuli, either positive or negative, by an increase in membrane potential as measured by the carotenoid bandshift. Fig.1 shows the typical response of whole cells to positive and negative chemotactic compounds and to illumination with actinic light. The responses of all 3 stimulants were sensitive to compounds which directly affect the membrane potential, e.g., *m*-chlorocarbonyl cyanide phenyl hydrazone (CCCP). The response time of the apparatus (~200 μs) and the mixing time of the system ($t_{1/2}$ ~50 ms) meant that only the slower phase of the carotenoid bandshift was seen [5], which for actinic light had a rise time of $t_{1/2}$ ~55 ms and for chemotactic responses $t_{1/2}$ ~75–100 ms followed by a decay $t_{1/2}$ ~300 ms.

The same stimuli applied to spheroplasts or lysed spheroplasts produced similar responses to those seen in whole cells (fig.2). Spheroplasts in 10% sucrose showed rather larger responses than lysed spheroplasts but the background noise level was higher because of increased scattering artefacts, therefore only the responses of lysed spheroplasts have been shown here.

One exception to the normal responses shown by spheroplasts was that to D-galactose. In whole cell preparations of *Rps. sphaeroides* this was a very good positive chemotactic agent and produced a large carotenoid bandshift. However in spheroplasts the response was much smaller (fig.2) when compared to whole cells or the spheroplast response to, e.g., L-aspartate. This result suggests that in *Rps. sphaeroides*, as in *Escherichia coli*, the galactose binding protein may be a periplasmic binding protein, the majority of which was lost during spheroplast formation [6].

Chromatophores, which because of the method used for isolation can be assumed to be almost exclusively inside-out membrane vesicles [7] showed a large actinic light-induced response (fig.3), but showed no response to the addition of either attractants or repellents, even with a membrane potential maintained at half maximum by illumination with actinic light.

The response of isolated spheroplasts, and the lack of response of inside-out chromatophores, to chemotactic stimulation strongly supports the assumption that the carotenoid shift reported [1] is due to the binding of attractants and repellents to receptor sites on the outside of the cytoplasmic membrane, followed by an increase in membrane potential. These results indicate that the early sequence of events in chemo-

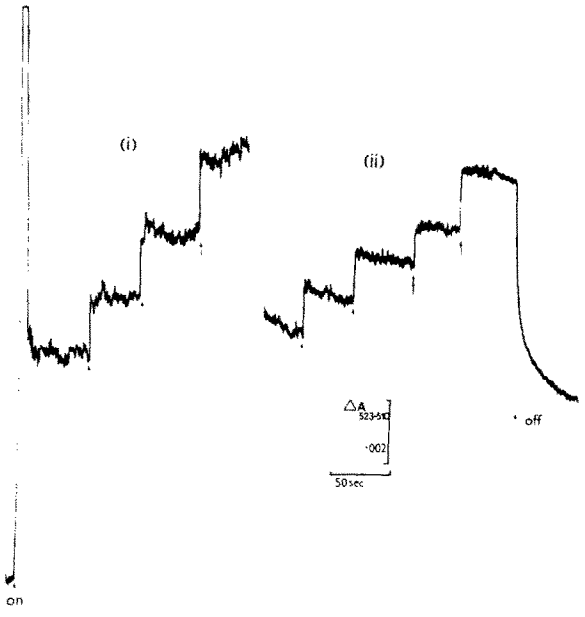

Fig.3. Response of chromatophores of *Rps. sphaeroides* to additions of 1.2 mM (i) D-glucose, L-aspartate, L-cysteine and (ii) D-glucose, L-cysteine, water and D-galactose. All measurements were made with half maximum light-induced membrane potentials.

taxis signalling occur at a normal level in bacteria without an outer cell membrane.

It should therefore be possible to investigate specific properties of the membrane and their role in chemotaxis using potential- and ion-sensitive dyes which cannot be used with intact bacteria. It should also be possible to use these dyes with spheroplasts of non-photosynthetic bacteria to investigate membrane potential and ion gradient changes in genetically well-characterised systems.

Acknowledgements

This work was supported by grants from the Science Research Council, Royal Society and the University of London Central Research Fund.

References

[1] Armitage, J. P. and Evans, M. C. W. (1979) FEBS Lett. 102, 143–146.
[2] Clayton, R. K. (1960) Biochim. Biophys. Acta 37, 503–579.
[3] Armitage, J. P., Keighley, P. and Evans, M. C. W. (1979) FEMS Microbiol. Lett. 6, 99–102.
[4] Markwell, J. P. and Lascelles, J. (1978) J. Bacteriol. 133, 593–600.
[5] Jackson, J. B. and Crofts, A. R. (1971) Eur. J. Biochem. 18, 120–130.
[6] Hazelbauer, G. L. and Adler, J. (1971) Nature New. Biol. 230, 101–104.