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The transmembrane electrical potential in intact bacteria: simultaneous measurements of carotenoid absorbance changes and lipophilic cation distribution in intact cells of *Rhodobacter sphaeroides*

Wim Crielaard [a], Nick P.J. Cotton [b], J. Baz Jackson [b], Klaas J. Hellingwerf [a] and Wil N. Konings [a]

[a] *Department of Microbiology, University of Groningen, Haren (The Netherlands) and* [b] *Department of Biochemistry, The University of Birmingham, Birmingham (U.K.)*

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The electrical potential across the cytoplasmic membrane of *Rhodobacter sphaeroides* has been measured in intact cells by two independent techniques: the uptake and release of tetraphenyl phosphonium ions and the carotenoid absorbance band-shifts. Simultaneous measurements show that these two procedures give different membrane potentials. Upon energization with either light or during respiration tetraphenylphosphonium-distribution indicates a depolarization of the membrane while the electrochromic carotenoid band-shift indicates a hyperpolarisation. Treatment of the cells with venturicidin resulted in an increased light-induced membrane potential indicated by the carotenoid band-shift and led to a reversal in the polarity of the tetraphenylphosphonium response. The presence of ethylene diaminetetraacetic acid had no effect on the light-induced carotenoid absorbance change, but it decreased the light-induced membrane depolarisation indicated by the tetraphenylphosphonium ions. These results show that at least one of these methods is seriously in error.

Introduction

In bacteria primary H^+-pumps transform chemical, redox or light energy into an electrochemical potential difference of protons ($\Delta\tilde{\mu}_{H^+}$) across the cytoplasmic membrane. According to the chemiosmotic hypothesis for energy coupling introduced by Mitchell [1], the protonmotive force ($\Delta p = \Delta\tilde{\mu}_{H^+}/F$) is the driving force for ATP-synthesis and secondary transport of solutes. To test this hypothesis many experiments have been performed to correlate the magnitude of Δp with the thermodynamic and kinetic properties of ATP-synthesis and solute transport [2–4]. Most conclusions drawn from these experiments depend heavily on the reliability of the methods used to determine the magnitude of the protonmotive force. The protonmotive force consists of two components: the concentration gradient of the protons (ΔpH) and the difference in electrical potential between the two aqueous bulk phases ($\Delta\psi$). Such that:

$$\Delta p = \frac{\Delta\tilde{\mu}_{H^+}}{F} = \Delta\psi - Z\Delta\text{pH}$$

Abbreviations: TPP^+, tetraphenylphosphonium ion; FCCP, carbonylcyanide *p*-trifluoromethoxyphenylhydrazone; EDTA, ethylenediaminetetraacetate; BChl, bacteriochlorophyll.

Correspondence: W.N. Konings, Department of Microbiology, University of Groningen, Kerklaan 30, 9751 NN Haren, The Netherlands.

($Z = 2.3\ RT/F$, where R is the gas constant, T the absolute temperature and F the Faraday constant).

Since, at present, there is no single method for determining the total Δp, estimations of Δp require separate measurements of both $\Delta\psi$ and ΔpH. For the estimation of ΔpH values several techniques are available which appear to be reliable [5]. Since independent methods, ^{31}P-NMR and the distribution of weak acids, give essentially the same values [5,6], both methods can be regarded as correct.

Methods for determining $\Delta\psi$, however, appear to be less reliable. The most direct method for measuring membrane potentials, the use of microelectrodes [7], is usually not applicable in bacteria and therefore it is necessary to use less direct methods. One of the methods most often used for the estimation of $\Delta\psi$ is based on distribution measurements of lipophilic ions between the two bulk phases. It has been argued that after correction for binding [8,9] the $\Delta\psi$ can be calculated from the distribution via the Nernst equation. A method which is commonly used to determine the membrane potential in photosynthetic membranes is the measurement of electrochromic absorbance changes. The absorption spectrum of the carotenoids in the membrane of phototrophic bacteria shifts to longer wavelengths upon imposition of a membrane potential [10]. The absorbance changes resulting from the carotenoid bandshift in bacterial chromatophores and in thylakoids from higher plants are reported to be linear with the membrane potential [10,11]. By applying potassium-diffusion potentials the carotenoid band-shift in these bacteria can be calibrated [10,12]. The calibration curve is subsequently used to estimate the membrane potential in chromatophores and intact bacteria from carotenoid absorbance changes observed upon energization. Estimates of the membrane potential based on the carotenoid band-shift generally lead to larger values than those obtained with the distribution of lipophilic ions [13,14]. However, in some cases it was observed that both methods yielded essentially the same results [15].

Here we show with simultaneous measurements of carotenoid absorbance changes and TPP^+-distribution, that in intact cells of *Rb. sphaeroides* large quantitative and even qualitative differences are found between the membrane potentials measured with the two indicators. Circumstances are described in which increased illumination gives rise to an increase in $\Delta\psi$ (negative inside the cell, i.e., hyperpolarisation) when measured by electrochromism but a decrease in $\Delta\psi$ (depolarisation) when measured by TPP^+-distribution.

Methods

Pretreatment of cells. Rb. sphaeroides, strain 2.4.1, was grown anaerobically at moderate light intensity (equidistant from two banks of 100 W tungsten lamps placed 50 cm apart) in the medium described by Sistrom [16] at 30 °C. Exponentially growing cells were harvested, washed twice in 50 mM potassium phosphate (pH 8.0), 5 mM $MgSO_4$ and resuspended in this buffer at a concentration of 300–700 μM BChl. Cells were stored on ice and used within 10 h of preparation.

Analytical procedures. Bacteriochlorophyll (BChl) was estimated at 772 nm in acetone/methanol extracts according to Clayton [17]. Protein was determined according to the method of Lowry et al. [18].

Carotenoid absorbance changes. Carotenoid absorbance changes were measured at 528 minus 511 nm with a chopped, dual-wavelength Perkin Elmer 356 spectrophotometer. Calibrations were performed on chromatophore membranes prepared from the batch of cells which had been used to record the absorbance changes [13]. K^+-diffusion potentials in the presence of 2 μM antimycin A in darkened chromatophore suspensions yielded a linear relationship with the carotenoid absorbance change. The slope of this relationship ($0.289 \cdot 10^{-4}$ Abs/mV · μM BChl) was used to calculate the membrane potential resulting from the illumination and oxygenation of intact cells.

TPP^+-distribution measurements. TPP^+-distribution was measured simultaneously with carotenoid absorbance changes by placing an ion-selective TPP^+ electrode (constructed as described in Refs. 6 and 19) in the spectrophotometer cuvette. A remote calomel electrode which served as a reference was connected to the cuvette via a salt bridge (containing 3 M choline chloride, 3% agar). The membrane potential as measured by

TPP^+-distribution was calculated from the Nernst equation. A correction for TPP^+ binding was applied as described for *Rb. sphaeroides* cells by Lolkema et al. [9]. Binding of TPP^+ was determined in de-energized cells. De-energization was achieved by incubating the cells for 1 h at 30 °C with 1% toluene and 1% chloroform.

Experimental conditions. Experiments were carried out anaerobically under argon at 30 °C in a glass-cuvette with continuous stirring. Butyl rubber tubing and stainless steel gas trains were used to minimise leakage of oxygen. Where shown the cuvette was illuminated with a 150 W quartz halogen bulb focussed on the cuvette through 9 cm of water and one layer of Wratten 88A gelatine filter. The maximum light intensity at the cuvette surface was in excess of 100 $J \cdot m^{-2} \cdot s^{-1}$ as measured with a silicon photo-diode calibrated against a Hewlett-Packard thermopile. The light intensity was reduced where shown by neutral density filters.

In experiments with oxygen small quantities of H_2O_2 were added to the medium in the cuvette. The cells have a high endogenous catalase activity which promptly converts the added H_2O_2 to O_2. In all experiments the bacteriochlorophyll concentration was adjusted to 15 μM.

Results

Response of the carotenoid band-shift and TPP^+-distribution to light and oxygen

The membrane potential as indicated by the carotenoid band-shift and by TPP^+-distribution in *Rb. sphaeroides* is shown in Fig. 1. Anaerobically in the dark both methods indicated a resting potential of approx. −95 mV. Upon illumination (Fig. 1A) the carotenoid band-shift showed an initial hyperpolarization to −290 mV and a subsequent decay within about 1 min to a steady-state potential of approx. −185 mV. Addition of H_2O_2 also led to an apparent hyperpolarisation according to the carotenoid absorbance change (Fig. 1B). Following an initial 'spike' which might have been obscured during mixing, a steady-state potential of around −175 mV was reached. However, upon either illumination or oxygenation, the electrode recordings showed that TPP^+ was expelled from

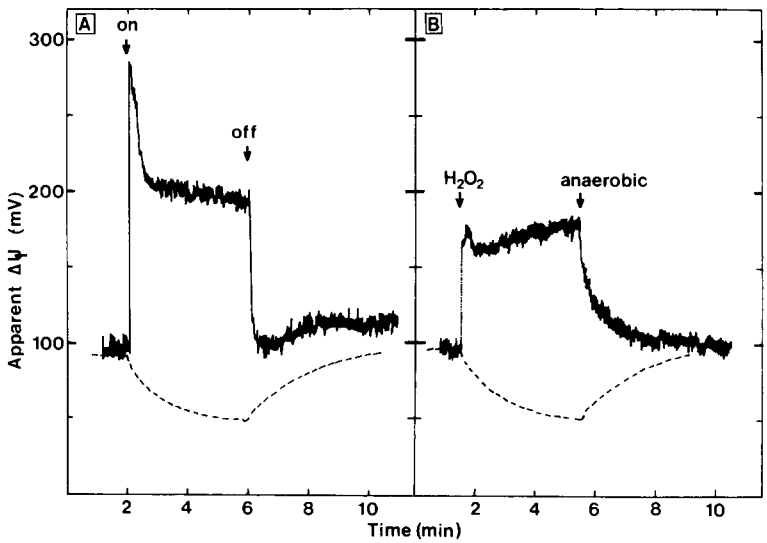

Fig. 1. Simultaneous measurements of TPP^+ distribution and carotenoid band-shift changes in intact cells of *Rb. sphaeroides* 2.4.1. The effect of energization (light, Fig. 1A or oxygen, Fig. 1B) on both indicators. Harvested and washed cells of *Rb. sphaeroides* were added to 50 mM potassium phosphate (pH 8.0)/5 mM $MgSO_4$ under anaerobic conditions to give a final concentration of 15 μM BChl. The total TPP^+ concentration in the medium was 4.2 μM. Illumination and H_2O_2 additions were done as described in Materials and Methods. The carotenoid band-shift was set to zero by dissipating the dark potential (as measured with the carotenoid band-shift) with 1 μM FCCP. Dashed line: TPP^+ distribution. Solid line: carotenoid band-shift with signal-to-noise ratio.

the organism indicating a depolarisation of the potential from −95 mV anaerobically in the dark to a value of −50 mV (Fig. 1A and 1B). Similar values were measured when the initial TPP^+-concentration was varied between 2 and 8 μM. In control experiments it was established that TPP^+ in this concentration range had no effect on either light- or oxygen-induced carotenoid absorbance changes.

Effect of an ATPase inhibitor

The magnitude of the steady state $\Delta\psi$ is always a reflection of the balance between $\Delta\psi$-generating and $\Delta\psi$-dissipating processes. When this balance is disturbed by an inhibition of one of these processes this should be reflected in the response of the membrane potential indicators. The effect of venturicidin on the response of both indicators was therefore studied. Venturicidin, an inhibitor of the F_0-component of the membrane bound F_0F_1-ATPase is very effective in intact cells of *Rhodobacter capsulatus* even at a concentration of 1.3 μM (= 1 μg/ml; 190 μg/mg BChl) [20]; it is considerably less effective in intact cells of *Rb. sphaeroides*. To observe any effect it was necessary to preincubate the cells with venturicidin at concentrations between 100 and 200 μM before diluting the samples for further assay. At these concentrations venturicidin does not inhibit electron transfer or uncouple ATP synthesis in *Rb. sphaeroides* (data not shown).

The venturicidin treatment led to an increase in the initial light-induced hyperpolarisation indicated by the carotenoid band-shift and to an increase in the apparent steady-state value of $\Delta\psi$ to approx. −225 mV (Fig. 2A). At the highest concentrations used, a decay from the initial spike of hyperpolarisation was largely prevented so that a steady-state of approx. −325 mV was estimated (Fig. 2B).

Venturicidin treatment, at the lowest concentrations used, decreased the light-induced depolarisation indicated by TPP^+ release (Fig. 2A). At higher concentrations (21 μM), the inhibi-

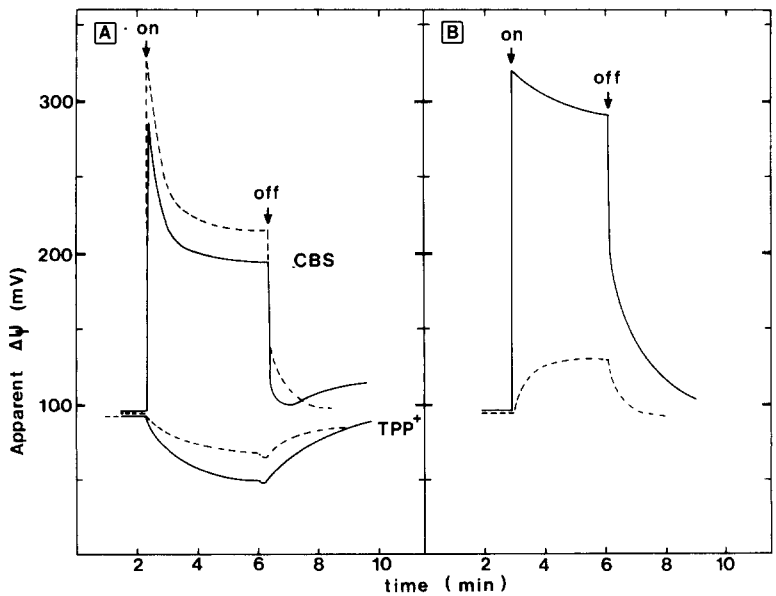

Fig. 2. Simultaneous measurements of TPP^+ distribution and carotenoid band-shift changes in intact cells of *Rb. sphaeroides* 2.4.1. The effect of venturicidin on the light-induced responses. Harvested and washed cells were preincubated with venturicidin (5 min, 0 °C) with a 40× concentrated venturicidin solution to give final concentrations of 2.5 μM (Fig. 2A) and 21 μM (Fig. 2B) in the cuvette. (A) Solid lines: no venturicidin added; dashed lines: +2.5 μM venturicidin. (B) Solid line: carotenoid band-shift; dashed line: TPP^+ distribution. See for further information legend to Fig. 1. CBS, carotenoid band-shift.

tor gave rise to a light-induced uptake of TPP^+ and a calculated $\Delta\psi$ of -125 mV (Fig. 2B).

The effect of EDTA on both $\Delta\psi$ signals

In earlier work it was shown that TPP^+-redistribution in *Rb. sphaeroides* cells upon illumination was affected by treatment with a combination of EDTA and dicyclohexylcarbodiimide (DCCD) [21]. In the present experiments the effect of EDTA on light-induced TPP^+-distribution and on the carotenoid band-shift was examined. First, it was confirmed [21] that removal of divalent cations from the buffer (50 mM potassium phosphate/0.2 mM EDTA, pH 8.0) led to a light- (or oxygen-) induced uptake of TPP^+, indicating membrane hyperpolarisation. Further addition of EDTA and $MgSO_4$ showed that the polarity of the TPP^+ response to light was dependent on which of the two compounds was present in excess. The presence of $MgSO_4$ and EDTA at these levels did not affect the apparent 'resting' potential indicated by TPP^+ in anaerobic cells in the dark.

In strong contrast to the effects on TPP^+ distribution, the carotenoid absorbance change in the dark or upon either illumination or oxygenation was not significantly influenced by whether Mg^{2+} or EDTA was present in excess. Illumination or oxygenation always led to an apparent hyperpolarization of the membrane. The hyperpolarization during illumination was slightly depressed by millimolar concentrations of EDTA (Fig. 3A).

Fig. 3 describes the results of an experiment in which extracellular TPP^+ concentration and carotenoid absorption were measured simultaneously at a range of actinic light intensities, in either the standard medium alone, containing 5 mM $MgSO_4$, or when supplemented with 5 mM EDTA. First, in the absence of the chelator (Fig. 3A) at low light intensities (below 9% of the maximum), both signals indicated membrane hyperpolarisation. However, an increase in light intensity from 1% to 9% caused a release of accumulated TPP^+, indicating depolarisation of the membrane potential, whereas the carotenoid signal showed a rapid hyperpolarisation followed by a slower depolarisation resulting in a slightly increased steady-state

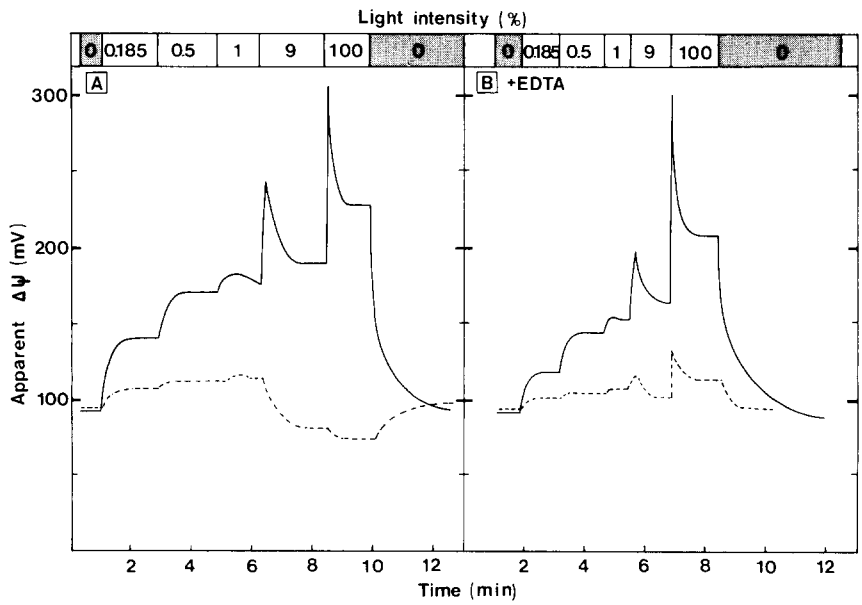

Fig. 3. Simultaneous measurements of TPP^+ distribution and carotenoid band-shift changes in intact cells of *Rb. sphaeroides* 2.4.1. The effect of increasing light intensities on both indicators in the absence (Fig. 3A) and presence of EDTA (Fig. 3B). Cells in 50 mM potassium phosphate (pH 8.0), 5 mM $MgSO_4$, were added to 50 mM potassium phosphate (pH 8.0)/5 mM $MgSO_4$ (Fig. 3A) or 50 mM potassium phosphate (pH 8.0)/5 mM EDTA (Fig. 3B). The light intensities were increased by placing various neutral density filters in the light pathway. Solid lines: carotenoid band-shift; dashed lines; TPP^+ distribution. Further as in legend to Fig. 1.

value. Interestingly, the period of depolarisation indicated by both probes had similar kinetics. Further increase of the light intensity, from 9% to the maximum, led to responses which exaggerated the differences between the two signals.

At low light intensities Mg^{2+} chelation had little effect on either the carotenoid absorbance changes or on the TPP^+ distribution (Fig. 3B), nor was an effect on the carotenoid signal observed at higher light intensities. However, in the presence of EDTA, the release of pre-accumulated TPP^+ upon increasing light intensity to 9% (Fig. 3B) was much less marked than it had been in the presence of free Mg^{2+}: consequently, a net depolarisation was not observed. Moreover, the increased light intensity from 9% to 100% produced qualitatively, if not quantitatively, a similar response from the two probes.

Discussion

It is clear from the data presented that measurements of membrane potentials from the distribution of phosphonium cations and the carotenoid band-shift in intact cells of *Rb. sphaeroides* display not only quantitative differences, as was found in *Rb. capsulatus* [13], but even, under some circumstances, they show differences in the direction of membrane potential propagation during illumination and respiration. These differences cannot simply be a result of inaccurate calibration of one of the two probes. In the present report we have emphasized conditions in which the response of the carotenoid pigments and the TPP^+ cation give conflicting results. Our objective was to highlight this discrepancy. In other situations the two probes give qualitatively a similar response. For example at high salt concentrations and particularly at lower pH (Jackson and Cotton; unpublished results; see also Ref. 21) a hyperpolarisation of the membrane is indicated by both the carotenoid band-shift and the TPP^+ electrode. If it can be accepted that there is only one cytoplasmic membrane in *Rb. sphaeroides* (i.e., the 'intracytoplasmic membranes' are contiguous with the cytoplasmic membrane – see below), then the conclusion is inescapable that either one or both indicators of membrane potential are seriously in error. This has implications in conclusions which have been reached on earlier occasions concerning the nature of energy-coupling reactions in intact cells of this and related species of bacteria.

The cytoplasmic and intracytoplasmic membrane of Rhodobacter sphaeroides

Before discussing the possible short-comings of the two probes we shall consider that TPP^+ and carotenoid band-shifts might accurately reflect electric-potential changes in different membranes within the organism. The generally accepted view that the cytoplasmic and the intracytoplasmic membrane of *Rb. sphaeroides* are in fact one contiguous membrane has arisen from demonstrations that connections can be seen on electron micrographs [22]. It has not been possible, of course, to show that in any one cell all membranes are connected. Moreover, the complexity of micrographs of related organisms [23] make it highly unlikely that there is one highly convoluted membrane system and this suggests that the function of the membrane does not require that it be a single operating unit. Indeed, Holmqvist [24] showed that ferrous gluconate did not penetrate into the lumen of the chromatophores of *Rb. sphaeroides* cells and concluded that the chromatophores were discrete entities. The electrochromically active carotenoid pigments are associated with the B800–850 pigment-protein complexes of *Rb. sphaeroides* [25] and these photosynthetic pigments are concentrated in the intracytoplasmic membrane and excluded from the cytoplasmic membrane. It would therefore follow from this reasoning that electrochromic signals would arise predominantly from the putative intracytoplasmic vesicles. On the other hand, TPP^+ distribution as measured with electrodes in the extracellular medium will be dominated by membrane potential changes across the cytoplasmic membrane, since the vesicles – negative outside – can hardly be recorded by the distribution of phosphonium cations.

In our view this is the only way in which the results presented above can be explained without the need to invoke artefacts in the $\Delta\psi$ measurements. Therefore a re-evaluation of the organisation of the intracellular membranes in these cells would be useful. Furthermore, a comparison should be made between carotenoid absorbance

changes and TPP^+ distribution in phototrophic bacteria lacking intracellular membranes [26] and in bacteria like *Ectothiorhodospira halochloris* in which the intracytoplasmic membranes are very likely contiguous with the cytoplasmic membrane [27].

Since this conclusion is not altogether satisfactory we shall briefly consider other reasons why TPP^+ uptake and/or the carotenoid band-shift in intact cells of *Rb. sphaeroides* might deviate from ideal indicators of membrane potential.

Possibility A: the uptake/efflux of TPP^+ from intact cells of Rb. sphaeroides accurately reflects changes in $\Delta\psi$, whereas carotenoid absorbance changes are misleading

It is not in dispute that the carotenoid pigments can respond electrochromically. The clearest demonstration of this is that isolated carotenoids embedded between plates of a capacitor and exposed to large electric fields undergo absorbance band-shifts [28]. Furthermore, the response of the carotenoid pigments in chromatophore membranes to ionic diffusion potentials is very probably due to electrochromism [11]. Evidence against the possibility that other carotenoid absorbance changes contribute to the electrochromic effect during illumination relies heavily on the interpretation of the results of experiments with ionophores: valinomycin and related compounds, and protonophorous uncouplers modify the light-induced carotenoid response in a manner which fits quantitatively with their expected effect on ionic permeability [29]. For example, this kind of data has been used as evidence that local electric-potential changes, resulting from charge separations within the membrane dielectric, do not influence the carotenoid absorbance changes at appropriately selected wavelengths [29]. There is also independent evidence that on a rapid time-scale (10^{-6}–10^{-2} s) the kinetics of the carotenoid band shift are quantitatively consistent with (a) the location of redox carriers defined by the reaction centre crystal structure [30], (b) direct electrical current recordings of reaction centres reconstituted into lipid bilayers [31,32], (c) direct electrical recordings from macroscopic electrodes of intact bacteria irradiated with light gradients [33]. However, it is evident that these pieces of evidence, including the experiments with ionophores, are only pertinent to short periods of illumination: confidence that other absorbance changes are not involved diminishes with the time of illumination. It is conceivable that during prolonged illumination other, unknown parameters give rise to absorbance changes. If TPP^+ distribution in Figs. 1–3 accurately describes the behaviour of $\Delta\psi$ during illumination, then we could view the carotenoid response as follows. At the onset of illumination (before TPP^+ redistributes or before the TPP^+ electrode can respond) the carotenoid absorbance change properly indicates the $\Delta\psi$ arising from the electrogenic activity of the photosynthetic electron-transport chain. Within 1 or 2 s, however, direct redox interactions with the electron-transport chain [34] lead to activation of the consumers of $\Delta\psi$ (ATP synthesis and solute translocation) and results in an accelerated rate of $\Delta\psi$-dissipation. The eventual depolarisation leads to the observed TPP^+ efflux from the cell but, during the same period, the unspecified changes in the environment of the sensitive carotenoid mask the electrochromic response and the membrane depolarisation is inaccurately sensed. Difficult to reconcile with this view are the findings that (a) the spectrum of the carotenoid response is not altered during prolonged illumination on respiration, (b) the light-induced absorbance changes during prolonged illumination or respiration can be eliminated with uncouplers (intact cells and chromatophores) or valinomycin/K^+ (chromatophores).

Possibility B: the carotenoid absorbance change accurately reflects $\Delta\psi$, whereas TPP^+ uptake/release is misleading

The distribution of TPP^+ across unilamellar liposome membranes in response to K^+-diffusion potentials in the presence of valinomycin is as predicted by the Nernst equation [35]. TPP^+ and its analogues have been used as probes for $\Delta\psi$ in a variety of natural membranes [8]. The importance of suitable corrections for probe binding in such systems has been emphasised [8,9]. De-energized, intact bacteria bind considerable quantities of TPP^+, but at low concentrations the binding is linear and so, provided that experiments are performed at low concentrations of the probe, changes

in binding which result from re-distribution of the TPP^+ during energization can be taken into account in the calculation of $\Delta\psi$ [8,9]. It is possible, however, that energization results in binding changes which are not predictable on the basis of curves derived for de-energized membranes. Several authors have described what they consider to be binding anomalies with phosphonium cations [36,37]. If the discrepancies in the present investigation are to be explained by such effects, then the binding change would need to be very large indeed to account for the observed release of TPP^+ from the cells during energization. Note that no light-induced or respiration-dependent changes in TPP^+ binding is observed in cells permeabilised with uncoupler or with chaotropic solvents and so a change in binding cannot be a simple consequence of redox changes in the electron-transport chain.

The release of phosphonium cations from intact cells upon energization has been observed in other species of bacteria by Midgley and colleagues [38,39]. These workers accept the likelihood of an increased $\Delta\psi$, negative inside the cells, upon energization, and so they conclude that the observed efflux of the probe must arise from the activity of an outwardly directed ion pump which can carry the phosphonium cation. In this case the distribution of TPP^+ across the cytoplasmic membrane would represent a balance of TPP^+-uptake driven by $\Delta\psi$ and TPP^+-efflux driven by the pump. Because evidence on the nature of the driving force for the putative TPP^+ pump is not forthcoming, it is difficult to predict how the distribution ratio, $[TPP^+]_{in}/[TPP^+]_{out}$, in steady state would depend on the starting concentration of TPP^+. We find that, after suitable corrections for binding, the distribution ratio is independent of the starting concentration of TPP^+ between 2 and 8 μM (the limits imposed by the sensitivity of the electrode and by the linearity of the binding). This result is consistent with a simple Nernstian distribution of the probe (see also Ref. 9), but at this stage does not fully rule out the possibility of interference from a TPP^+ efflux pump.

Experimental discrimination between A and B

In each of the two alternatives described above, it is supposed that one probe responds only to $\Delta\psi$ and that the other responds to both $\Delta\psi$ and another parameter. The results of the experiments with venturicidin can be accommodated by either of the two alternatives from the known effect of this inhibitor on the ATP synthase: venturicidin treatment should result in enhanced hyperpolarisation of the membrane during energization by reducing the rate of consumption of Δp during ATP synthesis.

Such a response was observed for both TPP^+ and the electrochromic absorbance change. In the present context the experiments with EDTA are more interesting, since a completely different result was observed for the two indicators. This reagent had a slightly inhibitory effect on the carotenoid response during illumination but a dramatic effect on the TPP^+ distribution in preventing the membrane depolarisation at high light intensities (Fig. 3). Unfortunately, EDTA is not a specific reagent and it might have multiple effects on the bioenergetics of intact bacterial cells. Its recognised effect on Gram-negative bacteria is to modify the permeability properties of the outer membrane. If this is the limit of its effect then the result in Fig. 3 would generally favour the carotenoid band-shift as the more accurate $\Delta\psi$ indicator. However, it seems likely that chelation of divalent cations could have a more disruptive influence on the bioenergetics of the cell. For example, it might result in the depletion of Mg^{2+} from the cytoplasmic compartment of the cell with a consequent effect on ATP synthesis. This would satisfactorily account for the finding that EDTA decreases the degree of depolarisation as indicated by the TPP^+ response and would in this case raise doubts over the relatively small effect on the carotenoid band-shift.

In the absence of a third undisputedly reliable measure of $\Delta\psi$ across the cytoplasmic membrane of intact cells of *Rb. sphaeroides,* the uncertainty over the accuracy of the carotenoid band shift and TPP^+ uptake/release will remain.

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References

1 Mitchell, P. (1961) Nature 191, 144–148.
2 Van Dam, K., Wiechmann, A.H.C.A., Hellingwerf, K.J., Arents, J.C. and Westerhoff, H.V. (1978) in Proceedings of the 11th FEBS Meeting Copenhagen 1977 (Nichols, P., Møller, J.V., Jørgensen, P.T. and Moody, A.J., eds), Vol. 45, pp. 121–132, Pergamon, Oxford.
3 Casadio, R. and Melandri, B.A. (1984) in H^+-ATPase (ATP-synthase). Structure, Function and Biogenesis. The F_0-F_1 Complex of Coupling Membrane (Papa, S., Altendorf, K., Ernster, L. and Packer, L., eds.), pp. 411–420, Adriatica Editrice, Bari.
4 Ramos, R. and Kaback, H.R. (1977) Biochemistry 16, 854–859.
5 Nicolay, K., Lolkema, J.S., Hellingwerf, K.J., Kaptein, R. and Konings, W.N. (1981) FEBS Lett. 123, 319–323.
6 Hellingwerf, K.J. and Van Hoorn, P. (1985) J. Biochem. Biophys. Meth. 11, 83–93.
7 Felle, H., Porter, J.S., Slayman, C.L. and Kaback, H.R. (1980) Biochemistry 19, 3585–3590.
8 Ritchie, R. (1984) Prog. Biophys. Molec. Biol. 43, 1–32.
9 Lolkema, J.S., Abbing, A., Hellingwerf, K.J. and Konings, W.N. (1983) Eur. J. Biochem. 130, 287–292.
10 Jackson, J.B. and Crofts, A.R. (1969) FEBS Lett. 4, 185–189.
11 Junge, W. and Jackson, J.B. (1982) in Photosynthesis, Vol. 1, Energy conservation in plants and bacteria (Govindjee, ed.), pp. 589–646, Academic Press, New York.
12 Symons, M., Nuyten, A. and Sybesma, C. (1979) FEBS Lett. 107, 10–14.
13 Clark, A.J. and Jackson, J.B. (1981) Biochem. J. 200, 389–397.
14 Ferguson, S.J., Jones, O.T.G., Kell, D.B. and Sorgato, M.L. (1979) Biochem. J. 180, 75–85.
15 Casadio, R., Venturoli, G. and Melandri, B.A. (1981) Photobiochem. Photobiophys. 2, 245–253.
16 Sistrom, W.R. (1960) J. Gen. Microbiol. 22, 778–785.
17 Clayton, R.K. (1963) Biochim. Biophys. Acta 75, 312–323.
18 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.
19 Shinbo, T. (1978) Arch. Biochem. Biophys. 187, 414–422.
20 Cotton, N.P.J., Clark, A.J. and Jackson, J.B. (1981) Arch. Microbiol. 129, 94–99.
21 Elferink, M.G.L., Hellingwerf, K.J. and Konings, W.N. (1986) Biochim. Biophys. Acta 848, 58–68.
22 Peters, G.A. and Celliarus, R.A. (1972) J. Bioenerg. 3, 345–359.
23 Remsen, C.C. (1978) in: The Photosynthetic Bacteria (Clayton, R.K. and Sistrom, W.R., eds.), pp. 31–60, Plenum, New York.
24 Holmqvist, O. (1979) FEMS Microbiol. Lett. 6, 37–40.
25 Scolnick, P.A., Zannoni, D. and Marrs, B.L. (1980) Biochim. Biophys. Acta 593, 230–240.
25 Miller, K., Jacob, J.S., Smith, U., Koloczkowski, S. and Bowman, M.K. (1986) Arch. Microbiol. 146, 111–114.
27 Wanner, G., Steiner, R. and Scheer, H. (1986) Arch. Microbiol. 146, 267–274.
28 Schmidt, F., Reich, R. and Witt, H.T. (1970) Naturwissenschaften 85, 414–415.
29 Packham, N.K., Greenrod, J.E. and Jackson, J.B. (1980) Biochim. Biophys. Acta 592, 130–142.
30 Chang, C.H., Tiede, D., Tang, J., Smith, U., Norris, J. and Schiffer, M. (1986) FEBS Lett. 205, 82–85.
31 Packham, N.K., Dutton, P.L. and Mueller, T. (1982) Biophys. J. 37, 465–473.
32 Gopher, E., Blatt, Y., Schönfeld, M., Okamura, M.Y. and Feher, G. (1985) Biophys. J. 48, 311–320.
33 Duprez, J., Trissel, H.W. and Breton, J. (1986) PNAS 83, 1699–1703.
34 Elferink, M.G.L., Friedberg, I., Hellingwerf, K.J. and Konings, W.N. (1983) Eur. J. Biochem. 129, 583–587.
35 De Vrij, W., Driessen, A.J.M., Hellingwerf, K.J. and Konings, W.N. (1986) Eur. J. Biochem. 156, 431–440.
36 Bakker, E.P. (1982) Biochim. Biophys. Acta 681, 474–483.
37 Zaritsky, A., Kihara, M. and Macnab, R.M. (1981) J. Membr. Biol. 63, 215–231.
38 Midgley, M., Iscander, N.S. and Dawes, E.A. (1986) Biochim. Biophys. Acta 865, 45–49.
39 Midgley, M., Iscander, N.S., Parish, M. and Dawes, E.A. (1986) FEMS Microbiol Lett. 34, 187–190.