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The measurement of membrane potential during photosynthesis and during respiration in intact cells of *Rhodopseudomonas capsulata* by both electrochromism and by permeant ion redistribution

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1. The membrane potential in intact cells of *Rhodopseudomonas capsulata* during photosynthesis and during dark respiration has been measured by two independent methods. 2. The light-induced and O_2-induced shifts in the carotenoid absorption spectrum were measured in the intact cells. The shift was calibrated with K^+-diffusion potentials in chromatophores derived from those cells. The light-induced and O_2-induced membrane potentials were −290mV and −230mV respectively. 3. The energized uptake of butyltriphenylphosphonium ions was measured in the same batch of cells. The light-induced and O_2-induced membrane potentials calculated from the Nernst equation were −160mV and −120mV respectively. 4. It is concluded that the two kinds of probe measure the electric potentials across different domains of the cytoplasmic membrane, but it is difficult to reconcile the existence of such domains with simple electrical analogues of the membrane and aqueous phases.

In many bacterial cells and subcellular vesicles, the only practical way of measuring the membrane potential is to follow the distribution of permeant ions between the aqueous phases on either side of the membrane (Rottenberg, 1980). Independent methods of measurement are generally unavailable, although there has been some progress with microelectrodes (Felle *et al.*, 1980). Several species of photosynthetic bacteria possess membrane-located pigments, particularly carotenoids, which are electrochromic, i.e. their absorption spectra are sensitive to electric fields (Jackson & Crofts, 1969). Experiments with chromatophores from these organisms have supported the notion that the carotenoid absorption band shifts are reliable indicators of delocalized membrane potential (Jackson & Crofts, 1971; Wraight *et al.*, 1978; Packham *et al.*, 1980). Quantitative evaluation of membrane potentials in chromatophores by the electrochromic method has generally given higher values (Jackson & Crofts, 1969; Baccarini-Melandri *et al.*, 1977) than those obtained by permeant ion re-distribution (Schuldiner *et al.*, 1974; Ferguson *et al.*, 1979; Michels & Konings, 1978). Symons *et al.* (1979) have explained this discrepancy on the basis that the calibration curve for the carotenoid shift was not properly corrected for changes in surface potential, ionophore concentration and specific ion effects.

In the present paper we describe experiments in which the membrane potential of intact cells of *Rhodopseudomonas capsulata*, generated during both anaerobic photosynthesis and dark respiration, was measured by electrochromism and by ion distribution. The experimental conditions were similar for both kinds of measurement. The carotenoid shift was calibrated in chromatophores prepared from the same batch of cells. Under the conditions employed, the correction procedures described by Symons *et al.* (1979) were not required. The electrochromic method indicated larger membrane potentials in the intact cells than did ion distribution during both respiratory and photosynthetic electron flow. Possible explanations for these findings are discussed.

Abbreviations used: FCCP, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone; $BTPP^+$, butyltriphenylphosphonium.

Experimental

The green mutant strain N22 of *Rps. capsulata* was grown in the medium described by Weaver *et al.* (1975) in conditions described by Cotton *et al.* (1981). Late-exponential-phase cells were harvested and then washed and resuspended in a solution containing 10mM-Na_2HPO_4 adjusted to pH7.0 with H_3PO_4 (henceforth called 10mM-sodium phosphate

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buffer). The harvested cells were stored on ice and used within 10h. Bacteriochlorophyll was estimated by extraction with acetone/methanol (7:2, v/v) using $\varepsilon_{772} = 75\,mM^{-1}\cdot cm^{-1}$ (Clayton, 1963). The relationship, 1 μmol of bacteriochlorophyll:22 mg of protein:54 mg dry wt. of cells may be used for comparison with non-photosynthetic organisms (Cotton *et al.*, 1981).

The internal (cytoplasmic) space of the bacterial cells was determined by double centrifugation in [U-^{14}C]sucrose/^3H_2O, essentially as described by Kell *et al.* (1978). The cells, containing 0.8 μmol of bacteriochlorophyll, were suspended in 5 ml of sodium phosphate buffer, 30 mM-sodium malate, pH 7.0, 40 mM-[U-^{14}C]sucrose (0.2 μCi of ^{14}C) and ^3H_2O (0.4 μCi of ^{3}H), incubated at room temperature for 1–2 min and centrifuged at 10000 *g* for 30 min. Portions of the supernatant were taken for counting and the pellet was resuspended in 5 ml of the same medium but deficient in radioisotopes. Samples of supernatant from a second centrifugation step were also taken for counting radioactivity. Radioactivity was measured on a dual-channel scintillation counter. The internal space was estimated from the difference in ratios of the ^{14}C and ^{3}H for the two supernatants by using the relationship derived by Kell *et al.* (1978).

In the experiments to calibrate the carotenoid shift, chromatophores were prepared from the washed cells by sonic irradiation of the suspension (about 50 μM-bacteriochlorophyll) in a medium containing 10 mM-$MgCl_2$, 40 mM-Na_2HPO_4 (pH adjusted to 7.4 with H_3PO_4). Unbroken cells and debris were removed by centrifugation at 20000 *g* for 30 min and the chromatophores were sedimented at 75000 *g* for 210 min. The pellet was resuspended in the same medium without further washing. The same medium was also used during the diffusion-potential calibration of the carotenoid shift.

The fast, double-beam spectrophotometry, the chopped double beam spectrophotometry and the butyltriphenylphosphonium potentiometry were carried out using the apparatus described by Cotton *et al.* (1981) and McCarthy *et al.* (1981) under the conditions described in the Figure legends. Anaerobiosis in the optical and electrode vessels was obtained with a stream of argon ($O_2 < 10$ vol./10^6 vol. of argon) directed over the surface of the suspension. A slight positive pressure of gas was maintained by passing the output through a few centimetres of water. Butyl rubber tubing and stainless steel needles were used throughout. The samples were magnetically stirred in the steady-state experiments (Figs. 1 and 2) but not in the short-flash experiments (Figs. 3 and 4). Oxygenation of the suspension was achieved by adding microlitre quantities of 0.1-volume H_2O_2 (the cells possessed catalase activity). Continuous illumination from the side of the vessels was from a 150 W projector lamp filtered through 3 cm of water and one layer of Wratten 88A gelatin. Short-flash excitation (20 μs half peak-width) was from below the cuvette.

In preliminary experiments we found that a bacterial cell concentration equivalent to about 13 μM-bacteriochlorophyll gave satisfactory data with both the ion-exchange electrode and spectrophotometer. Attenuation with neutral density filters showed that at 13 μM-bacteriochlorophyll the projector lamps gave saturating light-induced responses for both $BTPP^+$ uptake and carotenoid band shifts. This cell concentration was used routinely in our experiments unless noted otherwise.

$BTPP^+$ was obtained from Aldrich Chemical Co., Milwaukee, WI, U.S.A. Valinomycin and FCCP were obtained from Sigma. Other reagents were of analytical grade.

Results

Estimation of membrane potentials in intact cells by electrochromism

The carotenoid absorption changes of an intact cell suspension in response to a period of illumination and then oxygenation in the dark are shown in Fig. 1. Both the photosynthetic and the respiratory

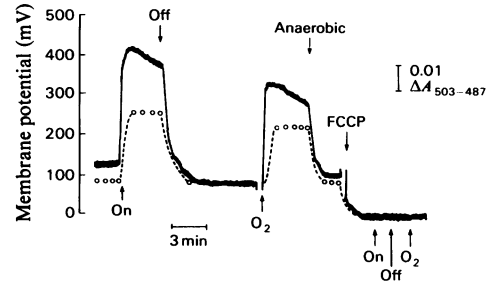

Fig. 1. *Measurement of membrane potential in intact cells of Rps. capsulata during photosynthesis and respiration*

O, Calculated from $BTPP^+$ uptake; —— calculated from carotenoid absorption band shift (see vertical bar for actual absorption changes). Data were taken on the same day from the same batch of cells. In each case the bacteriochlorophyll concentration was 13 μM and the cells were incubated in the appropriate vessel under argon in 10 mM-sodium phosphate buffer, 30 mM-sodium malate, 7 mM-$(NH_4)_2SO_4$, pH 7.0, for 20 min before starting the experiments. The suspension volume was 10.0 ml in the $BTPP^+$ ion-exchange electrode vessel and 2.5 ml in the spectrophotometer cuvette. The membrane potentials were calculated with respect to zero defined by the addition of 2 μM-FCCP. See the text for further details.

electron-transport systems gave rise to a shift in absorption towards the red. The detailed kinetics of the absorption changes were dependent on the content of the suspending medium. The slow decline during continuous illumination or oxygenation was almost complete in the 3–4 min period shown in Fig. 1. Light-scattering changes in the sample were largely eliminated by the dual-wavelength measurement. No correction was made for wavelength-dependent scattering (Thorne *et al.*, 1975), which should be small.

The addition of 2 μM-FCCP at the end of the experiment led to a small decrease in the dark, anaerobic absorption level and to the complete abolition of light- and O_2-induced responses. The small decrease on FCCP addition was probably due to residual O_2 in the suspension permitting a very low basal rate of respiration since the same effect was observed on addition of sodium dithionite. It was minimized by using metal and butyl rubber gas trains but could not be eliminated completely. In our initial experiments with crudely constructed gas trains the FCCP-induced decrease was much larger and the light-induced charge was smaller (by about 30%). It is clear that stringent anaerobic conditions are required to observe the complete development of the light-induced membrane potential. The carotenoid shifts shown in Fig. 1 are expressed as both an absorbance change and as a calibrated membrane potential.

The carotenoid shifts of intact cells cannot be calibrated directly because the cells are resistant to ionophores such as valinomycin and because it is difficult to set precise ionic gradients across the cytoplasmic membrane. The procedure that was therefore adopted was to prepare chromatophores from the same sample of cells that had been used to generate the data shown in Fig. 1 and then to calibrate the carotenoid shift in these chromatophores with K^+ diffusion potentials (Jackson & Crofts, 1969). This relies on the supposition that the addition of K^+ to valinomycin-treated chromatophores leads to the prompt formation of a membrane potential whose magnitude is described by the Nernst relation. The straight-line relationship between the logarithm of the added K^+ and the induced shift supports this contention and permits calibration (Jackson & Crofts, 1969; Baccarini-Melandri *et al.*, 1977). The data obtained with the chromatophores isolated from the batch of cells used in Fig. 1 is shown in Fig. 2.

Symons *et al.* (1979) found that they needed to apply three correcting procedures in the diffusion potential calibration: (1) to compensate for changes in surface potential during the KCl or RbCl pulse; (2) to ensure that the concentration of valinomycin was optimal (3); to use Rb^+ instead of K^+. To avoid problems of changing surface potential we

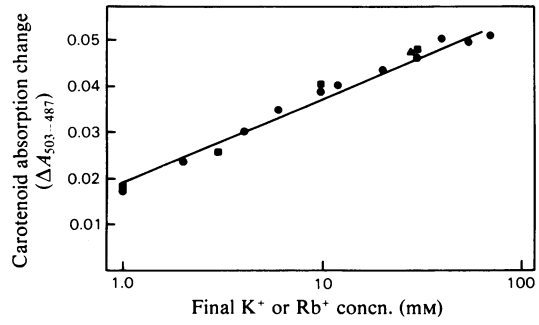

Fig. 2. *Calibration of the carotenoid absorption band shift*

Chromatophores were prepared from the batch of cells used in Fig. 1. For each point the chromatophores (26 μM-bacteriochlorophyll) were incubated aerobically in K^+-free medium (see the text) in the magnetically stirred spectrophotometer cuvette for 10 min. Then 0.24 μM- (●, ■) or 0.024 μM-valinomycin (▲) was added. The resulting blue shift in carotenoid absorption (Jackson & Crofts, 1969) was allowed to subside during another 10 min incubation. The prompt absorption change after the addition of microlitre amounts of concentrated solutions of KCl (●, ▲) or RbCl (■) corrected for dilution artefact is plotted as a function of the final K^+ or Rb^+ concentration.

performed our experiments in the high salt medium described in the Experimental section. The addition of 50 mM-NaCl to chromatophores in this medium did not produce significant carotenoid absorption changes. In contrast with Symons *et al.* (1979) we found that the K^+-induced carotenoid shifts were absolutely dependent on the presence of valinomycin. Moreover, a 10-fold lower concentration of valinomycin than that routinely used in our experiments still gave rise to an identical K^+-diffusion potential (see Fig. 2). We can only conclude that there were differences between our chromatophores and those used by Symons *et al.* (1979). In fact it has been shown (M. Symons & J. B. Jackson, unpublished work) that as chromatophores age, their intrinsic K^+ permeability increases. The chromatophores used in the present report were less than 3 days old. We also obtained different results from Symons *et al.* (1979), when we used RbCl instead of KCl. The latter group found unexpectedly that the two cations gave different slopes for the calibration curves. They argued that the Rb^+ data should be more reliable. In our hands RbCl and KCl gave identical results (Fig. 2).

It seems to us that, at least in freshly prepared chromatophores, the correction procedures are unnecessary (see also Jackson & Clark, 1981). For comparison with previous work by various authors, light-induced membrane potentials of chromato-

Table 1. *The light-induced membrane potential measured by electrochromism in chromatophores*

Author	Medium	Organism	Light-induced membrane potential (mV) After 10s	Steady-state
Jackson & Crofts (1969)	100mM-Choline chloride/20mM-4-morpholine-ethanesulphonic acid (pH 6.4)	*Rps. sphaeroides*	430	190
Casadio *et al.* (1974)	50mM-KCl/40mM-glycylglycine (pH 7.8)	*Rps. capsulata*	310	190
Ferguson *et al.* (1979)	5mM-Magnesium acetate/10mM-P_i/Tris (pH 7.0)	*Rps. sphaeroides*	450	300
Symons *et al.* (1979) (corrections applied)	5mM-4-Morpholinepropanesulphonic acid/3.5mM-Na^+ (pH 7.0)	*Rps. capsulata*	210	70
The present work (correction procedure checked)	10mM-$MgCl_2$/40mM-sodium phosphate buffer (pH 7.4)	*Rps. capsulata*	400	250

phores estimated by electrochromism are listed in Table 1. In the present experiments, despite our taking note of the suggestions of Symons *et al.* (1979), the measured values were very similar to the earlier uncorrected estimates. We suppose that the low values reported by Symons *et al.* (1979) were due less to the correction procedures than to the high intrinsic ion permeability of their chromatophore preparations, perhaps due to storage in 10% dimethyl sulphoxide under liquid N_2.

The data of Fig. 2 were used to calculate the membrane potential achieved during illumination and oxygenation of the intact cells from which the chromatophores were derived (Fig. 1). Similar data from another cell preparation are shown in Table 2. In both cases the calibration was performed on chromatophores isolated from the same batch of cells. The values are all relative to a zero membrane potential defined by the level after the final addition of 2μM-FCCP.

Estimation of membrane potentials in intact cells by redistribution of BTPP$^+$

The internal volume of our cells of *Rps. capsulata* was measured as the sucrose-impermeable space by the method described by Kell *et al.* (1978). The results are shown in Table 3. The mean value of 102μl/μmol of bacteriochlorophyll (approx. 1.9μl/mg dry wt. or 4.6μl/mg of protein) is less than the value (4.5μl/mg dry wt.) determined by Miovic & Gibson (1973) for *Chromatium* that we had assumed in a previous report (Cotton *et al.*, 1981) and larger than the value (2.3μl/mg of protein) measured by Nicolay *et al.* (1981) for *Rps. sphaeroides.*

Intact cells of *Rps. capsulata* take up $BTPP^+$ from the medium during either illumination or respiration (Cotton *et al.*, 1981). The membrane potential may be calculated from the distribution of the ion according to the Nernst equation:

$$\Delta\psi = -\frac{RT}{zF}\ln\frac{[BTPP^+]_{in}}{[BTPP^+]_{out}}$$

Table 2. *The light-induced and O_2-dependent membrane potentials measured in intact cells of Rps. capsulata by electrochromism*

All values were measured with respect to the potential measured after addition of 2μM-FCCP. These values are approx. 100mV larger than the change in membrane potential from the dark, anaerobic value achieved under argon (see Fig. 1 and the text).

Cell preparation	Experiment	Membrane potential (mV) Light-induced	O_2-dependent
A	A1	405	315
A	A2	405	305
B	B1	405	315
B	B2	380	315

where $[BTPP^+]_{out}$, the external concentration of the ion, is determined directly from the calibrated electrode reading and $[BTPP^+]_{in}$, the cytosolic concentration, is calculated from the energized uptake of $BTPP^+$ and the internal volume of the cells. Fig. 1 shows the results of an experiment carried out on cells from the batch that had also been used for the electrochromic determinations. As with the carotenoid shift calibration procedure the membrane potential was assigned a value of zero after treatment of the cells with 2μM-FCCP. The light-induced and O_2-induced changes in membrane potential were about −160mV and −120mV respectively, each being about 60% of the values determined by electrochromism. The logarithmic uptake of the $BTPP^+$ leads to great precision for the membrane-potential measurements during energization but to large errors of the FCCP (or dithionite)-induced change. One consequence of this was that the calculation of the light-induced membrane potential from the $BTPP^+$ measurements was relatively independent of the degree of anaerobiosis achieved by the gas train (cf. the electrochromic measurements; see above).

The value of the light-induced membrane potential

Table 3. *The internal volume of intact cells of Rps. capsulata grown photosynthetically to late-exponential phase*
The sucrose-impermeable space was measured by the method of Kell *et al.* (1978) as described in the Experimental section. The mean value was 102 (S.D. 17) μl/μmol of bacteriochlorophyll.

^{3}H in second supernatant/ ^{3}H in first supernatant	^{14}C in second supernatant/ ^{14}C in first supernatant	Internal volume (μl/μmol of bacteriochlorophyll)
0.0531	0.038	85
0.0540	0.0351	111
0.0694	0.0546	87
0.0506	0.0332	102
0.0637	0.0426	124

determined in this manner was independent of initial $BTPP^+$ concentrations between 4 and 15 μM and of the cell concentration from 11.5 to 22.7 μM-bacteriochlorophyll (Table 4).

The most difficult problem with the analysis of membrane potentials by $BTPP^+$ distribution was the apparent non-specific binding of the ion to the cells. On adding *Rps. capsulata* cells to a solution containing $BTPP^+$, the free $BTPP^+$ concentration decreased. When the cells became anaerobic under argon the decrease in $BTPP^+$ concentration was only partly reversed. The residual 'lost' $BTPP^+$ was presumably bound to cell components. Typically 15 nmol/μmol of bacteriochlorophyll was bound from a solution containing 4 μM-$BTPP^+$. The bound $BTPP^+$ was not released on addition of sodium dithionite, 5 mM-KCN or 5 μM-FCCP. A similar extent of binding occurred when boiled bacterial cells were used and binding showed no signs of saturation up to $BTPP^+$ concentrations of 20 μM for cell suspensions of 16 μM-bacteriochlorophyll. In the calculations used above it was assumed that the extent of this $BTPP^+$ 'binding' did not change when the cells were energized. We shall return to this problem in the Discussion section.

The effect of $BTPP^+$ and FCCP on electrochromic absorption changes

The presence of sensitive electrochromic pigments in *Rps. capsulata* permits a simple demonstration of the ability of $BTPP^+$ to diffuse rapidly across the bacterial cytoplasmic membrane in response to membrane potential. In Fig. 3, the membrane potential was generated by two closely spaced 20 μs light pulses and was monitored by the carotenoid absorption changes. After the flashes the electrochromic signal decayed with half time of about 0.58 s. The addition of $BTPP^+$ led to an acceleration of the decay, presumably as the lipophilic cation moved electrophoretically into the cells. FCCP had a similar effect and is shown for comparison. This experiment demonstrates that the presence of $BTPP^+$ leads to dissipation of the membrane potential. If, however, permeant ions are to be used as a reliable probe for membrane potential then condi-

Table 4. *The dependence of membrane potential determined by $BTPP^+$ uptake on the concentration of the permeant ion (a) and on the density of the cell suspension (b)*
Conditions were as for Fig. 1, except that in (*a*) the bacteriochlorophyll concentration was 12.5 μM and $BTPP^+$ concentration was varied as indicated.

(*a*)

Initial concentration of $BTPP^+$ (μM)	Light-induced membrane potential (mV)
4	160
7	165
12	160
15	165

(*b*)

[Bacteriochlorophyll] (μM)	Light-induced membrane potential (mV)
11.5	166
16.9	170
22.7	167

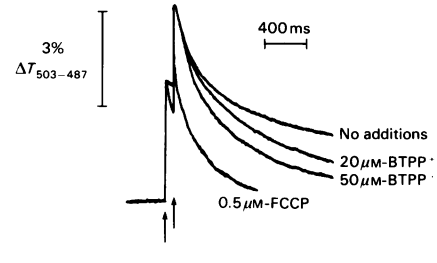

Fig. 3. *The effect of $BTPP^+$ and FCCP on the decay of the carotenoid absorption band shift after short flashes*
A suspension of cells (14.4 μM-bacteriochlorophyll) was incubated under argon for 20 min in the medium described in Fig. 1. The cells were excited by two 20 μs flashes fired at 80 ms apart (indicated by the arrows). The traces are an averaged recording over eight double-flashes each separated by 50 s. The experiments were repeated at the concentration of $BTPP^+$ and FCCP shown. Abbreviation used: *T*, transmission.

tions must be found in which membrane-potential dissipation by the ion is only transitory. Whether or not the probe lowers the membrane potential permanently depends on the electrical and chemical capacity of other ions in a rather complex manner. If the calculated membrane potential is not dependent on the concentration of the probing ion (e.g. see Table 4), then the method is usually judged to be reliable. In cells of *Rps. capsulata*, electrochromism provides an additional check. Table 5 shows that $BTPP^+$ at concentrations in the range used for membrane-potential determination do not affect the steady-state light-induced carotenoid absorption change. At slightly higher concentrations, the permeant ion does lead to a permanent lowering of the membrane potential.

Table 5. *The dependence of membrane potential, determined by electrochromism, on the concentration of added butyltriphenylphosphonium bromide ($BTPP^+Br^-$)*
Conditions were as for Fig. 1, except that the bacteriochlorophyll concentration was 12 μM.

[$BTPP^+$ Br^-] (μM)	Light-induced carotenoid absorption change ($\Delta A_{503-487}$)
0	0.035
1	0.037
2	0.034
5	0.036
10	0.037
20	0.032
50	0.029

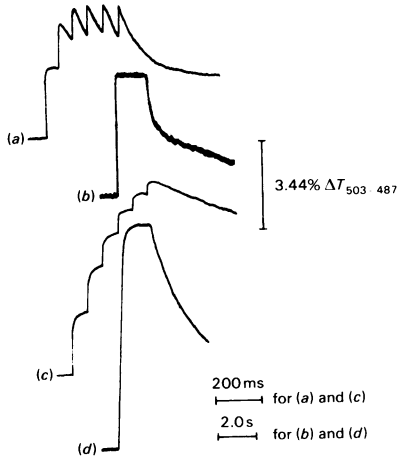

Fig. 4. *Electrochromic absorption changes in chromatophores and intact cells during continuous light or during flash excitation*
(*a*) and (*b*) Intact cells. A suspension of cells (7.4 μM-bacteriochlorophyll) was incubated under argon for 20 min in the medium described in the legend to Fig. 1. In (*a*) the cells were excited by a train of six 20 μs flashes 70 ms apart. The trace is an averaged recording over eight flash trains fired 50 s apart. In (*b*) the cells were excited by a short period of continuous illumination (not averaged). (*c*) and (*d*), Chromatophores. A suspension of chromatophores (7.9 μM-bacteriochlorophyll) was incubated under argon for 20 min in a medium containing 10 mM-$MgCl_2$, 40 mM-Na_2HPO_4 (pH 7.4 with H_3PO_4) and 1 mM NADH. In (*c*) flash excitation (see *a*) and in (*d*) (see *b*) continuous illumination were employed. Abbreviation used: *T*, transmission.

Membrane potential in intact cells and chromatophores after short flashes of light

Measurements of $BTPP^+$ redistribution cannot give information on the kinetics of membrane-potential generation because the ion moves rather slowly across the cytoplasmic membrane (response time in seconds; see Fig. 3). Electrochromism is, in contrast, an extremely rapid response.

It can be seen from a comparison of Fig. 1 and Table 1 that in intact cells the light-induced change in membrane potential measured by electrochromism was smaller than that in chromatophores. This is shown again in Figs. 4(*b*) and 4(*d*) in a different preparation (in this case, the membrane potential was not calibrated). In Figs. 4(*a*) and 4(*c*) the membrane potential was generated not by continuous light but by a train of closely spaced light flashes (each of 20 μs duration) in cells and chromatophores respectively.

Within 50–100 ms after the first short flash in the train, the carotenoid shift reached a similar amplitude in both cells and chromatophores. Particularly after subsequent flashes the decay of the carotenoid shift is much more rapid in the cells than in the chromatophores. Consequently the amplitude achieved during the flash train is lower in the intact organisms. In both cells and chromatophores, the peak value generated during the flash-train approaches that generated during 1–2 s of continuous light (compare Fig. 4*a* with Fig. 4*b* and Fig. 4*c* with Fig. 4*d*; but note the difference in time scales). Presumably it is the difference in the rate of decay of the membrane potential that accounts for the difference in steady-state values.

Discussion

The electrochromic method yielded larger values than the method of permeant ion redistribution for the membrane potentials generated during both respiration and photosynthesis in intact cells of *Rps. capsulata*. Although we encountered difficulties in the application of both methods the discrepancy is sufficient to suggest that larger membrane potential changes take place between the membrane interfaces than between the bulk aqueous phases.

The evidence that the absorption changes of

the carotenoid pigments in chloroplast and chromatophore membranes are electrochromic is extensive (e.g. Wraight *et al.*, 1978; Witt, 1979; Junge & Jackson, 1981). Most important is the data with ionophores, which leads to the conclusion that the electric fields to which the pigments respond are delocalized across the entire membrane (Junge & Witt, 1968; Saphon *et al.*, 1975; Packham *et al.*, 1980). In other words, the potential generated by an individual electron-transport reaction is sensed by responsive carotenoids without measurable delay and can be dissipated by ionophores in a remote area of the membrane. This argues for a rapid transmission of the electric field by ionic diffusion within the plane of the membrane interfaces.

For the reasons mentioned in the Results section the carotenoid shift cannot be calibrated directly in the intact cells. It was necessary to disrupt the cells and then to calibrate the carotenoid absorption bands in isolated chromatophores. In view of the fact that chromatophore membranes comprise a large fraction of the cytoplasmic membrane in the cell [electron micrographs (see Remsen, 1978) suggest more than 90%], we think that this is reasonable. The diffusion-potential calibration has yielded similar values of light-induced membrane potentials in chromatophores in several laboratories (see Table 1). Symons *et al.* (1979), however, incorporated three correction procedures and obtained values lower by more than a factor of 2. Our results (Fig. 2) show that in freshly prepared chromatophores these procedures need not be applied (see the Results section and also Jackson & Clark, 1981). Note that the calibration procedure relies on the assumption that a 10-fold increase in the concentration of K^+ or Rb^+ in the pulse leads to a diffusion potential that is larger by 58 mV. Unfortunately diffusion potentials in small vesicles cannot easily be measured by the distribution of other permeant ions but because of the proven effect of valinomycin in specifically increasing the K^+ permeability of chromatophore membranes (Jackson *et al.*, 1968) we think that the assumption is sound.

Working with intact chloroplasts, Schapendonk & Vredenberg (1979) found that a component in the signal that they called the 'P515 absorbance change' was insensitive to valinomycin. They proposed that the valinomycin-sensitive component was a response to transmembrane potential (Junge & Witt, 1968; Witt, 1979) but that the insensitive component was a response to alterations in fixed-charge distribution resulting from electron-transport-induced conformational changes. The conformational changes might take place on the membrane surface and influence the P515 pigment complex by way of surface-charge redistribution (Schapendonk *et al.*, 1980). Note that even such surface processes will be ionophore-insensitive. The carotenoid-absorption changes that we measure in bacteria are entirely sensitive to ionophores or permeant ions. This is true in both chromatophores (Packham *et al.*, 1980) and in intact cells (Fig. 3). Consequently, our calibration procedure should not suffer from this complication.

The diffusion-potential-induced carotenoid shift is linear over at least 2 logarithmic units of K^+ concentration. The practical limit to the size of potential that can be generated by diffusion potentials is about 180 mV. The calibration of the light- and respiration-induced shifts requires extrapolation beyond 180 mV, where linearity cannot be guaranteed. It is therefore possible that the membrane potentials determined by electrochromism are an overestimate. This argument, however, cannot explain the discrepancy with the ion redistribution method because the membrane potentials obtained with $BTPP^+$ are within the 180 mV limit.

For many organisms, observation of the redistribution of permeant ions such as phosphonium compounds is the only method available for the determination of membrane potential. It is assumed that when the uptake of the ion has reached completion, then the ion is at the electrochemical equilibrium described by the Nernst equation across the membrane (using concentrations for activities). This is difficult to confirm directly but is supported by the finding that the estimated membrane potential is independent of the starting concentration of permeant ion (e.g., Table 4). Unfortunately this control experiment must also be relied on to guard against energy-dependent changes in ion binding and to ensure that the presence of the permeant ion does not artefactually lower the membrane potential. In *Rps. capsulata* the last problem can also be ruled out from the lack of effect of $BTPP^+$ on the electrochromic change (Table 5).

The main difficulty in computing membrane potentials from our $BTPP^+$ data was how to deal with the apparent binding of the cation to the cells. In Fig. 1 it was assumed that the extent of the binding did not change when the cells were energized. This might not be the case. When the cells are in the dark under anaerobic conditions, the $BTPP^+$ concentration inside and outside is in the micromolar range. Under the influence of the membrane potential the concentration of $BTPP^+$ outside the cells decreased by only 2- or 3-fold, whereas the concentration inside increases by two or three orders of magnitude. If then, for instance, the $BTPP^+$ binds to the cytoplasmic membrane, the degree of binding would increase considerably (e.g. McLaughlin, 1977) and lead to an overestimate of membrane potential. If the binding sites can be identified and the binding parameters measured, the approach developed by Casadio *et al.* (1980) for SCN^- redistribution in chromatophores would lead to a refinement of this

method. For the moment it is sufficient to point out that an energy-dependent binding of $BTPP^+$ would probably mean that our values are overestimated and would widen the discrepancy with electrochromism.

These conclusions extend to intact cells the previous findings with chromatophores that electrochromic absorption changes lead to larger estimates for light-induced membrane potential than permeant ion redistribution (Michels & Konings, 1978; Ferguson *et al.*, 1979; but see Casadio *et al.*, 1980). In both types of measurement with intact cells we have used demonstrably saturating light intensities to counter a possible criticism of the chromatophore experiments, that the high chlorophyll concentrations required to detect SCN^- uptake resulted in diminished light levels.

Electrochromic measurements revealed that the light-induced membrane potential in intact cells was lower than that in chromatophores prepared from those cells (compare Fig. 1 and Table 1 and see Fig. 4). The reason for this seems to be that the rate of dissipation of the membrane potential is greater in the intact organism (Fig. 4). This is probably a simple reflection of the increased level of energy metabolism (transport, ATP synthesis) in the intact cells. The fact that the membrane potential measured soon after a single flash is similar in intact cells and chromatophores can also be easily explained; in both cases the same quantity of charge (2 electrons/photosynthetic reaction centre per single turnover) is translocated across the same area of membrane dielectric.

The experiments presented in Fig. 1 show that in intact cells the electrochromic response during respiration as well as during illumination yielded larger values of membrane potential than $BTPP^+$ uptake. In fact the proportion, O_2-induced/light-induced membrane potential was similar for both kinds of measurement. This finding helps to dispel the suspicion that because the carotenoids are located within the light-harvesting pigment-protein complexes they might be particularly sensitive to electric potentials localized within the photosynthetic apparatus. In chromatophores from *Rps. sphaeroides*, Ferguson *et al.* (1979) found that electrochromic measurements did lead to larger membrane potentials during illumination and smaller membrane potentials during respiration than did measurements using SCN^- uptake. The difference between intact cells (the present paper) and chromatophores (Ferguson *et al.*, 1979) could be explained by a degree of inhomogeneity in the chromatophore preparation (e.g. see McCarthy & Ferguson, 1981). In intact cells the entire cytoplasmic and intracytoplasmic membranes are believed to be contiguous (Holt & Marr, 1965).

Rumberg & Muhle (1976) were first to point out that electrochromic pigments and permeant ions do not necessarily respond to the same electric fields. The pigments are located in protein complexes within the membrane and are subjected to the sum of the difference in potential between the inside and outside membrane surfaces and the difference in potential between the bulk aqueous phases. Permeant ions, on the other hand, will distribute approximately according to the bulk-phase potential difference (deviations resulting from surface potentials should be small). Rumberg & Muhle (1976) further suggested that changes in surface potential resulting from the fall in internal pH of thylakoids during illumination could account for the discrepancy between electrochromic and ion-distribution estimates of membrane potential. However, this kind of argument cannot explain our results with bacterial cells, because ΔpH across the cell membrane is expected to be very small in the sodium phosphate malate $(NH_4)_2SO_4$ medium used in our experiments. Nicolay *et al.* (1981) have shown that illuminated cells of *Rps. sphaeroides* only develop a significant ΔpH (about 0.8 unit at pH 7) in the presence of high concentrations of permeant ions (100mM-KCl). Even this, by Rumberg's hypothesis, would be insufficient to make up the 130mV deficit shown in Fig. 1.

There have been several reports that bulk-phase electrochemical proton gradients are too low at acceptable, fixed H^+/ATP ratios to account for measured phosphorylation potentials in respiring mitochondria and bacteria (Azzone *et al.*, 1978; Van Dam *et al.*, 1978; Sorgato *et al.*, 1978; Rottenberg, 1980). For this and for other reasons variants of the chemiosmotic hypothesis have been proposed in which there is a preferred protonic pathway between the proton-translocating electron-transport chain and ATPase close to the membrane interface (Mitchell, 1977; Rottenberg, 1978; Van Dam *et al.*, 1978; Del Valle-Tascon *et al.*, 1978; Kell, 1979). In contrast with bulk-phase measurements, the electrical potential difference indicated by electrochromism is sufficient to poise the phosphorylation potential with $H^+/ATP = 2$ (Baccarini-Melandri *et al.*, 1977; Petty & Jackson, 1979). It could be proposed, in agreement with Rumberg & Muhle (1976), that the carotenoid shift is a response to the electrical potential between the interfaces of the membrane and that it senses more closely than do bulk-phase measurements the electrical potential difference experienced by the membrane-located proton translocators (see Kell, 1979).

This kind of model is not without its difficulties. Mitchell (1981) insists that in steady-state electron-transport phosphorylation, the averge protonic potential difference between the interface and bulk phase on one side of the coupling membrane must be zero. In the model of Van Dam *et al.* (1978), regions

of high protonic potential difference in the vicinity of the electron transport–ATPase supercomplexes are isolated by regions of low conductivity from the bulk-phase potentials, which are connected by way of ionic leaks. To suppose that the carotenoids respond to this kind of local potential in turn leads to inconsistencies with the long-established observation that the functional electrical (electrochromic) and phosphorylation unit of chloroplasts and chromatophores is the entire membrane and not an individual electron transport–ATPase complex (Junge & Witt, 1968; Saphon *et al.*, 1975; Witt, 1979). Another possibility is that macroscopic electrical analogue circuits (Mitchell, 1981; Van Dam *et al.*, 1978) are not appropriate for a description of the microscopic events taking place close to membrane surfaces (e.g. Azzone *et al.*, 1978; McClare, 1971). However, in the absence of a coherent theoretical framework to replace the more conventional view we should be reluctant to retreat to this position.

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