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The relation between electron transfer, proton-motive force and energy-consuming processes in cells of *Rhodopseudomonas sphaeroides*

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In the phototrophic bacterium *Rhodopseudomonas sphaeroides* the two components of the proton-motive force (the $\Delta\psi$ and the ΔpH) were measured with ion-selective electrodes. Simultaneously, the rate of energy-consuming processes such as alanine transport or ATP-synthase activity was measured at increasing electron-transfer activity. The results indicate that the activity of the electron-transfer system determines the activity of the energy-consuming processes. The proton-motive force can increase or decrease with the activity of the electron-transfer chain depending on the experimental conditions. Consequently, under certain conditions the activity of secondary transport increases while the proton-motive force decreases. A fixed minimal rate of electron transfer is required before secondary solute transport can occur, independent of the magnitude of the proton-motive force. A threshold value of the proton-motive force for secondary solute transport is not required.

Introduction

The central dogma of the chemiosmotic hypothesis states that cytochrome-linked electron transfer in mitochondria, chloroplasts or bacteria is coupled to solute transport and ATP synthesis by an electrochemical gradient of protons (Δp) across the energy-transducing membrane [1]. However, also direct interactions between electron transfer systems and the membrane-bound ATP-synthase have been reported. In a variety of energy-transducing systems it was observed that under condition that the Δp remained constant or decreased only slightly the rate of ATP synthesis depended linearly on the rate of electron transfer. Such observations have been reported for rat liver mitochondria [2,3], bovine heart submitochondrial particles [4], plant mitochondria [5], chromatophores of *Rhodopseudomonas sphaeroides* [6,7] and of *Rhodopseudomonas capsulata* [8–10].

In our studies on the mechanism of solute transport in bacteria we also observed a direct interaction between the electron transfer chains and solute transport systems, both in *Rps. sphaeroides* and in *Escherichia coli* [11–13]. The activity of the solute transport systems was a linear function of the rate of cyclic or linear electron transfer at constant Δp value. In chromatophores of *Rps. capsulata* is shown that the rate of turnover of cyclic electron transfer is a monotonic saturating function of the light intensity [14]. We assume that the same relation between light intensity and rate of cyclic electron transfer exists in *Rps.*

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Abbreviations: Δp, proton-motive force; $\Delta\psi$, electrical potential; ΔpH, chemical potential of protons; TPP^+, tetraphenylphosphonium ion; DCCD, *N,N'*-dicyclohexylcarbodiimide; FCCP, carbonyl cyanide-*p*-trifluoromethoxyphenylhydrazone; V_{ala}, the initial rate of uptake of ^{14}C-L-alanine.

sphaeroides. The analogy in the regulation of ATPase activity and solute transport activity indicates that both energy-consuming processes are controlled by the same mechanism.

The flow-force relations obtained from these results showed at higher Δp values a steep increase in ATP-synthase activity or solute transport activity upon only a slight increase of the Δp. When the Δp was decreased with uncouplers, while the rate of electron transfer remained constant different flow-force relations were found. Also here the analogy between ATP synthase activity and solute transport activity existed. Especially the analogy in flow-force relations for photophosphorylation [9] and alanine transport [11] energized by cyclic electron transfer in *Rps. sphaeroides* was remarkable.

In this report the Δp was studied in detail in cells of *Rps. sphaeroides* at different external pH values and at various other experimental conditions. Since the development of different types of ion-selective electrodes in our laboratory it became possible to study the two components of the Δp simultaneously. The electrical potential ($\Delta\psi$) was measured with an ion-selective tetraphenylphosphonium (TPP^+)-electrode and the chemical potential of protons (ΔpH) with an ion-selective salicylate-electrode [15].

The results presented demonstrate that the activity of the electron-transfer system determines the activity of the energy-consuming processes. Under certain conditions these direct interaction processes can lead to a decrease of the Δp, while secondary transport activity increases. Preliminary reports of some results have been presented previously [16–18].

Materials and Methods

Pretreatment of Rhodopseudomonas sphaeroides cells. Rps. sphaeroides strain 2.4.1. was grown anaerobically under high light intensity in the medium described by Sistrom [19] at 30°C. Cells were harvested at an absorbance at 660 nm of approx. 2. 'Intact cells' were washed twice with 50 mM potassium phosphate (pH 6.7 or 8), containing 5 mM $MgSO_4$ and chloramphenicol (50 μg/ml), and were resuspended in this buffer to a final protein concentration of 10–15 mg/ml. 'EDTA/DCCD-treated cells' were washed twice with 50 mM potassiumphosphate (pH 6, 7 or 8) containing 5 mM sodium ethylenediaminetetraacetate (EDTA) and chloramphenicol (50 μg/ml) and resuspended to a concentration of 0.1 g wet weight/ml in this buffer to which 1 mM EDTA was added. To this suspension 50 μM *N,N'*-dicyclohexylcarbodiimide (DCCD) was added and after 1 h incubation at 30°C the cells were washed once with 50 mM potassiumphosphate (pH 6, 7 or 8), containing 5 mM $MgSO_4$ and chloramphenicol (50 μg/ml) and finally resuspended in this buffer to a protein concentration of 10–15 mg/ml.

Simultaneous measurements of transport activity, transmembrane electrical potential and pH gradient. A thermostated polyvinylchloride vessel of 5 ml was constructed, in which both an ion-selective tetraphenylphosphonium ion (TPP^+) electrode and an ion-selective salicylate electrode were inserted from the side. Construction of the salicylate electrode was as described by Hellingwerf and Van Hoorn [15]. The TPP^+ electrode was constructed in the same way, with tetraphenylboron (TPB^-) incorporated in the electrode-membrane according to Shinbo [20]. All measurements were carried out anaerobically at 30°C. The incubation medium was kept anaerobically by a constant flow of water-saturated oxygen-free nitrogen over the surface of the incubation mixture. Light supplied by a projector lamp (24 V, 150 W) was transferred to the incubation vessel by a light-guide. The incubation medium contained 50 mM potassium phosphate of pH 6, 7 or 8, 5 mM $MgSO_4$, 50 μg/ml chloramphenicol, 4 μM TPP^+, 100 μM salicylate, 1.0–2.5 mg cell protein/ml and if simultaneously transport activity was measured, L-[^{14}C]alanine at a concentration of 50 μM. For uptake studies samples of 50 μl were rapidly taken from the incubation mixture with a Hamilton syringe at indicated time intervals and further handled as described [11].

Simultaneous measurements of membrane potential and ATP-synthase activity. From the vessel described above samples were taken at distinct time intervals for the measurement of the ATP content of the cells. The incubation medium was the same as above. Samples of 100 μl were rapidly diluted in 50 μl ice-cold 14% (w/w) $HClO_4$ + 9 mM Na_2EDTA. After centrifugation 100 μl of the

solution was neutralized with 50 μl 1 M KOH/$KHCO_3$, and stored at −20°C. ATP was determined with the luciferine-luciferase assay [21].

Simultaneous measurements of membrane potential and rate of oxygen consumption. Another 5 ml vessel was used in which both a TPP^+ electrode and a Clark-type oxygen electrode were inserted. Unlike the vessel described above no gas-phase was present above the incubation medium. The volume of the vessel was set with a screwcap cover. In this vessel light inhibition of respiration was studied. Light was supplied as described above. Before the start of the experiment the incubation medium was saturated with air. The incubation medium contained 50 mM potassiumphosphate (pH 8), 5 mM $MgSO_4$, 50 μg/ml chloramphenicol, 4 μM TPP^+ and 1–2.5 mg cell protein/ml.

Measurement of a reversed Δ pH. Reversed ΔpH was measured using the automated flow-dialysis technique as described by Hellingwerf and Konings [22] with [^{14}C]methylamine as distribution probe.

Analytical procedures. Protein was determined according to the method of Lowry et al. [23].

Calculations. The $\Delta\psi$ was calculated with the Nernst equation from the distribution of TPP^+ between the bulk phase of the medium and the cytoplasm determined with the ion selective TPP^+ electrode. A correction for TPP^+ binding was applied as described by Lolkema et al. [24,25]. Binding of TPP^+ was measured in cells deenergized by incubation in 1% chloroform for 1 h at 37°C. The ΔpH was calculated with the Nernst equation for the distribution of salicylate between the bulk phase of the medium and the cytoplasm as determined with a salicylate-selective electrode. A correction for non-linearity of the electrode response was applied based on a polynomal fit of the calibration curve of the electrode and calculated with a basic program run on an Apple II microcomputer as described by Hellingwerf and Van Hoorn [15].

Materials. L-[^{14}C]alanine (s.a. 10 Ci/mol) and [^{14}C]methylamine HCl (s.a. 40–60 Ci/mol) were obtained from the Radiochemical Centre (Amersham, Buckinghamshire, U.K.). All other chemicals were of analytical grade.

Results

The Δp in Rps. sphaeroides as a function of light intensity

The Δp in *Rps. sphaeroides* has been measured before [26], but here the two components of the Δp, the $\Delta\psi$ and the ΔpH were measured for the first time simultaneously. Fig. 1 shows an example of the continuous registration of the external TPP^+ and salicylate concentration. Upon addition of cells, anaerobically in the dark, the external TPP^+ concentration decreased due to: (i) the dilution of the medium by the cell suspension; (ii) aspecific TPP^+ binding to the different cell components [24,25]; (iii) a rest or dark potential. A distinction between a membrane potential and aspecific binding could be made by deenergizing the cells with 1% chloroform or by collapsing the rest membrane potential with valinomycin (5 nmol/mg protein). The external salicylate concentration decreased only by dilution of the medium upon addition of the cell suspension. No binding of salicylate to the cells was observed and no rest or dark ΔpH was

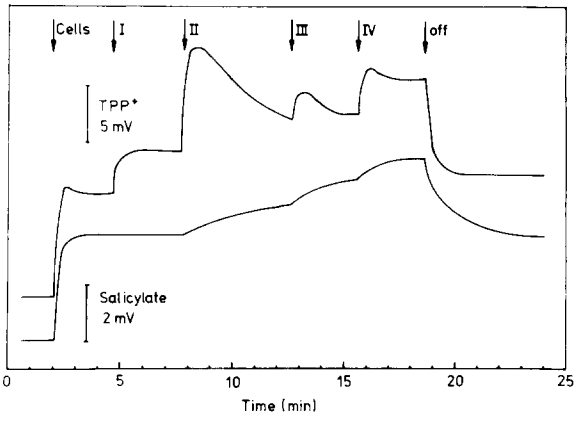

Fig. 1. Simultaneous registration of the external TPP^+ and salicylate concentration in *Rps. sphaeroides* cells. The external TPP^+ and salicylate concentration were recorded continuously in EDTA/DCCD-treated cells at an external pH of 6. The incubation medium contained: 50 mM potassium phosphate (pH 6)/5 mM $MgSO_4$/50 μg/ml chloramphenicol/4 μM TPP^+/100 μM salicylate. At $t = 2$ min cells were added to a final protein concentration of 2.5 mg/ml. The electrode response upon addition of the cells indicates a decrease in external TPP^+- and salicylate concentration. At the arrows the light intensities were increased from I–IV. At the last arrow the light was turned off. I: 130; II: 460; III; 700, and IV: 1300 $J \cdot m^{-2} \cdot s^{-1}$.

measurable in *Rps. sphaeroides*. When the light was turned on at low intensity (130 $J \cdot m^{-2} \cdot s^{-1}$) the $\Delta\psi$ increased immediately, but no ΔpH was generated. At higher light intensities the $\Delta\psi$ increased further followed by a partial decrease. Simultaneously, a ΔpH was generated. When the light was turned off both $\Delta\psi$ and ΔpH decreased again to the levels observed in the dark at the start of the experiment. Although all Δp measurements were carried out continuously as in Fig. 1, $\Delta\psi$ and ΔpH values were only calculated at 1.0 or 0.5 min intervals. In Fig. 2 the $\Delta\psi$ and ΔpH values are shown of intact cells at pH 6, 7 and 8, respectively. Again, no ΔpH was present at anaerobic conditions in the dark. A ΔpH was also not measurable upon illumination with low light intensity at any external pH value. At an external pH of 6 at higher light intensities a ΔpH was generated up to -88 mV (Fig. 2C). When the light was turned off this ΔpH collapsed slowly. In contrast, a ΔpH was not generated at pH 8 and at pH 7 at any light intensity (Fig. 2A and B). A $\Delta\psi$ on the other hand was always present at the three pH values also when no external energy source was available. The highest $\Delta\psi$ in the dark was found at pH 8 (Fig. 2A). This $\Delta\psi$ increased slightly upon illumination with low light intensities. At higher light intensity the $\Delta\psi$ initially increased during approx. 20 s and then decreased to a steady-state level lower than the $\Delta\psi$ in the dark. This steady-state $\Delta\psi$ decreased even further at still higher light intensities. Turning off the light led to an initial decrease of the $\Delta\psi$ followed by an increase to the dark level. The effect of different light intensities on the $\Delta\psi$ at pH 7 resembled the response at pH 8, but the absolute $\Delta\psi$ levels were much lower (Fig. 2B). At pH 6 the $\Delta\psi$ in the dark could reach a very low level (Fig. 2C). It is obvious that the energy obtained after a period of illumination could be used to generate a $\Delta\psi$ in the dark.

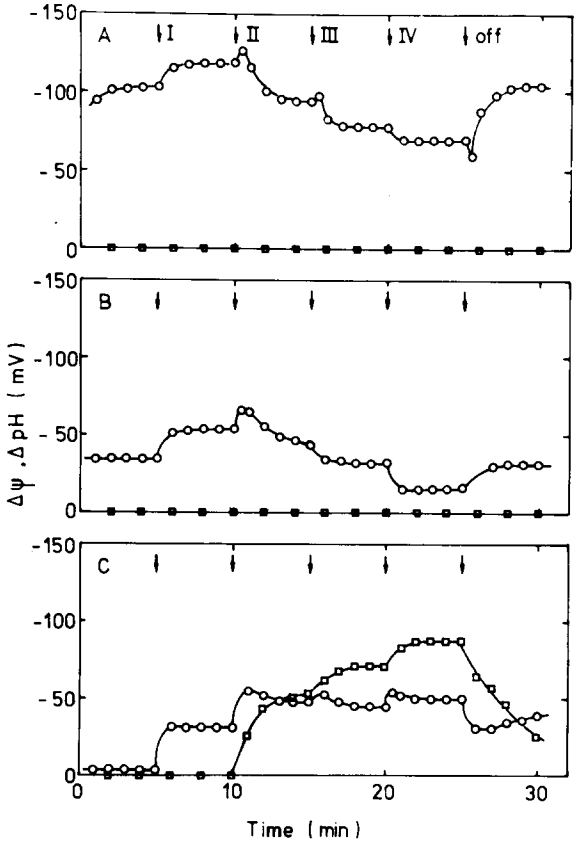

Fig. 2. Simultaneous measurement of $\Delta\psi$ and ΔpH in intact *Rps. sphaeroides* cells. From the continuous registration of the external TPP^+ and salicylate concentration the $\Delta\psi$ and ΔpH were calculated at 1 and 0.5 min intervals. The incubation medium was as in Fig. 1. The light intensity was increased from I to IV. O——O, $\Delta\psi$; □——□, ΔpH; A, pH = 8; B, pH = 7; C, pH = 6.

The $\Delta\psi$ and ΔpH values were also determined in cells which were treated with EDTA and DCCD (Fig. 3). EDTA-treatment facilitates the incorporation of inhibitors and ionophores in the cytoplasmic membrane. DCCD at a concentration of 50 μM blocks completely the membrane bound ATPase activity and possibly inhibits also other membrane functions [27]. The ΔpH levels in these EDTA/DCCD-treated cells were higher than in intact cells. At pH 7 the highest ΔpH value was -52 mV and at pH 6 the ΔpH went up to -96 mV. A pattern quite different from intact cells was observed for the $\Delta\psi$ in the EDTA/DCCD treated cells. The $\Delta\psi$ in the dark was much lower than in intact cells and upon illumination an increase of the $\Delta\psi$ was observed at every light intensity applied. These results indicate that the (partial) inhibition of energy-consuming processes by DCCD prevented the decrease of the $\Delta\psi$ at higher light intensities. Since energy-consuming processes cannot take place in the dark, despite a high Δp [11], the decrease of $\Delta\psi$ upon illumination in intact cells appeared to be correlated with the consumption of energy for ATP synthesis, solute transport

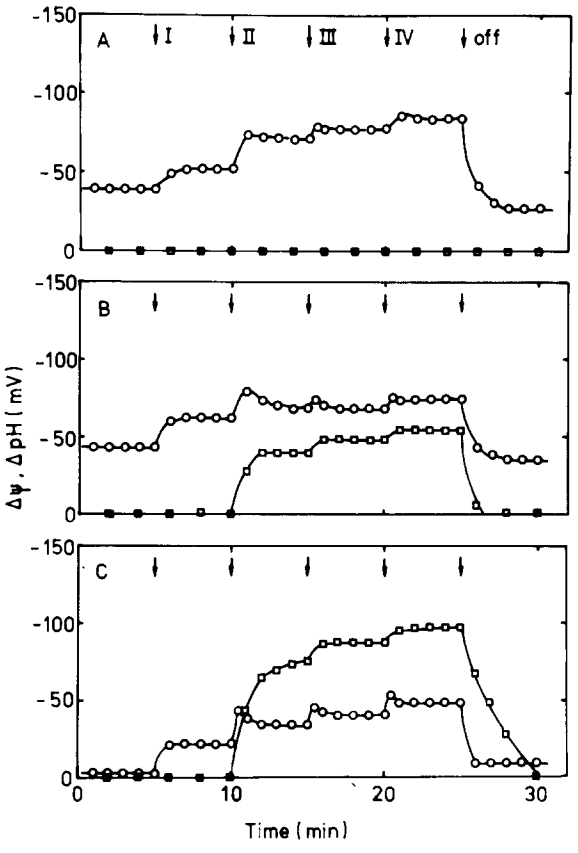

Fig. 3. Simultaneous measurement of $\Delta\psi$ and ΔpH in EDTA/DCCD-treated *Rps. sphaeroides* cells. Legend as in Fig. 2.

and possibly also other processes. A similar decrease of $\Delta\psi$ was observed when oxygen was allowed to penetrate the incubation vessel and the linear electron-transfer chain was activated (data not shown).

It should be noted that there were quantitative variations in the actual $\Delta\psi$ and Δ pH values between the different cell preparations, but the qualitative pattern of the $\Delta\psi$ and Δ pH value was always the same. The $\Delta\psi$ in the dark was also not constant in one and the same cell preparation, but depended on the treatment of the cells as is clearly illustrated in Fig. 2C. It was sometimes possible to measure a ΔpH in intact cells at pH 7, but in that case the decrease of the $\Delta\psi$ in the light was less pronounced. Intact cells tended to behave more like EDTA/DCCD treated cells after storage for one or more days at 0°C.

In general, the lowest Δp values were found in freshly harvested cells which were treated gently and resuspended in potassium phosphate in the presence of Mg^{2+} ions. In such cells the $\Delta\psi$ decreased dramatically in the light and no ΔpH values were measurable at pH 7. In potassium free buffers the decrease of the $\Delta\psi$ in the light was not observed or was much less pronounced. In the presence of EDTA always a light-dependent increase of the $\Delta\psi$ was observed. Addition of Mg^{2+}, Ca^{2+} or Mn^{2+} could restore the decrease of the $\Delta\psi$ in the light.

A reversed ΔpH was not measurable by flow dialysis with [^{14}C]methylamine as distribution probe. Intact cells of pH 7 or 8 were concentrated to 50 mg protein/ml and the distribution of methylamine was followed at alternating dark and light conditions, at stepwise increasing light intensities. The noise of the flow-dialysis recording is such that the detection limit of the ΔpH was 11 mV.

The relation between the proton-motive force and solute transport

More direct evidence that the decrease of the $\Delta\psi$ upon illumination is due to activity of energy-consuming processes was obtained by a simultaneous analysis of $\Delta\psi$ and transport of alanine. At an external pH of 8 the $\Delta\psi$ and uptake of alanine were measured simultaneously in the dark and at increasing light intensities, both in intact and in EDTA/DCCD-treated cells (Fig. 4). Exactly at the same moment that the $\Delta\psi$ in intact cells started to decrease alanine could be detected inside the cells. The rate of alanine transport increased with higher light intensities and consequently with decreasing $\Delta\psi$. When the light was turned off alanine leaked out of the cells, while the $\Delta\psi$ increased to its original dark level. In EDTA/DCCD-treated cells uptake of alanine started at exactly the same light intensity as in non-treated cells, in spite of a lower $\Delta\psi$ value (Fig. 4). This indicates that the rate of turnover of the cyclic electron-transfer chain controls the activity of the alanine transport carrier (see below).

The flow-force relations determined from the data in Fig. 4 are shown in Fig. 5. For EDTA/DCCD-treated cells alanine transport apparently depends on $\Delta\psi$ with a threshold value of -90 mV below which no uptake of alanine occurs. At higher

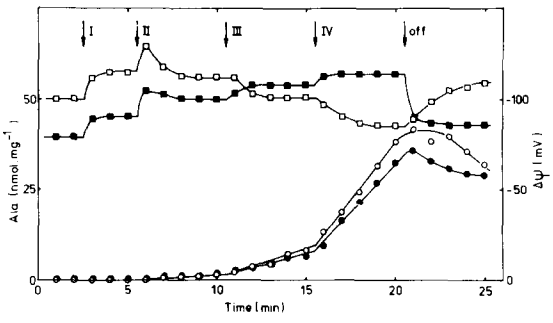

Fig. 4. Simultaneous measurement of $\Delta\psi$ and alanine uptake in intact and EDTA/DCCD-treated cells of *Rps. sphaeroides*. Non-treated and EDTA/DCCD-treated cells were added to the incubation medium to a final protein concentration of 1.1 and 1.6 mg/ml, respectively. The incubation medium contained 50 mM potassium phosphate (pH 8)/5 mM $MgSO_4$/50 μg/ml chloramphenicol/50 μM [^{14}C]alanine. The light intensity was increased from I to IV. ○——○, alanine uptake in intact cells; ●——●, alanine uptake in EDTA/DCCD-treated cells; □——□, $\Delta\psi$ in intact cells; ■——■, $\Delta\psi$ in EDTA/DCCD-treated cells.

$\Delta\psi$ values the transport activity increases steeply with the $\Delta\psi$. The flow-force relation for intact cells, however, clearly shows that the rate of uptake is determined in addition to the Δp by another factor. Between the two extremes shown in Fig. 5

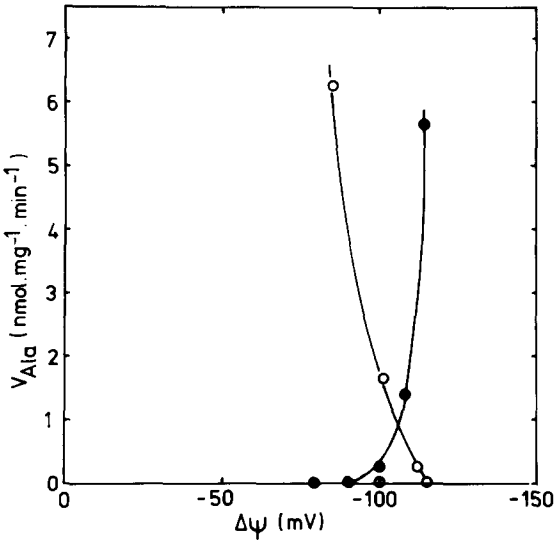

Fig. 5. The reaction between $\Delta\psi$ and V_{ala} in intact and EDTA/DCCD-treated cells of *Rps. sphaeroides*. Data of Fig. 4 were plotted as a flow-force relation.

any flow-force relation could be obtained by varying the pretreatment of the cells.

Similar relationships were found between the Δp and photophosphorylation. Under anaerobic dark conditions no phosphorylation was measurable in *Rps. sphaeroides* cells. At light intensity I of Fig 4 (130 $J \cdot m^{-2} \cdot s^{-1}$) the rate of photophosphorylation was very low. The net increase in ATP inside the cells was 0.2 nmol ATP/min per mg protein and proceeded linearly for approximately 10 min. At light intensity II (see Fig. 4) and at higher light intensities a steady-state level of ATP inside the cells was reached within 10 s.

The initial increase of the $\Delta\psi$ upon illumination with light intensity II and the initial decrease in $\Delta\psi$ when the light was turned off (Fig. 2A) indicates that the activity of the secondary pumps is delayed with respect to the activity of the primary pump, the cyclic electron-transfer chain. Upon illumination of dark-adapted cells after a lag phase the uptake of alanine was linear in time. When uptake was started by switching on the light the rate of alanine transport increased for 10–30 s. When the uptake was started by the addition of [^{14}C]alanine to preilluminated cells no lag-phase was observed and uptake was immediately linear in time. These observations indicate that maximal transport activity requires turnover of the electron-transfer chain for a certain period of time. When the light was turned off alanine transport did not stop immediately. This phenomenon was studied in more detail. Cells of *Rps. sphaeroides* were illuminated for 5 min with high light intensity and transport of alanine was measured for 30 s (Fig. 6). In a parallel experiment after 5 min preillumination the light was turned off and simultaneously [^{14}C]alanine was added to the cell suspension, and uptake of alanine was followed for 2 min. After 10 s, when the first sample was taken, a significant uptake of alanine was found in the dark which corresponded to the uptake in the light during 5 s at maximal transport activity. In a third experiment after 5 min preillumination the cells were kept in the dark for 2 min, then [^{14}C]alanine was added and the uptake of alanine followed for 2 min in the dark. No uptake of alanine was observed. These results indicate that the transport carrier remains active for a few seconds after electron transfer stops.

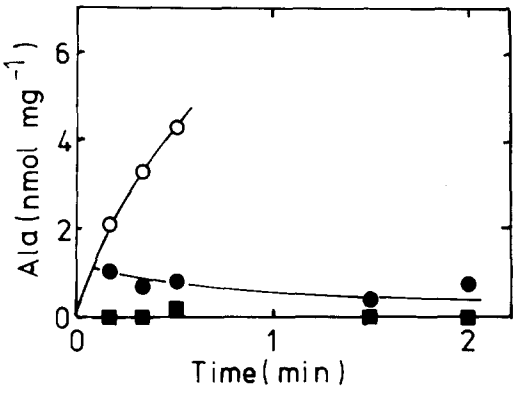

Fig. 6. Alanine uptake in *Rps. sphaeroides* cells in the light and in the dark after preillumination. EDTA/DCCD cells at a protein concentration of 0.63 mg/ml were preilluminated for 5 min in a medium as in Fig. 4. Hereafter: 50 μM [^{14}C]alanine was added and uptake was measured in the light (○———○); the light was turned off and simultaneously 50 μM [^{14}C]alanine was added and uptake was measured in the dark (●———●); the light was turned off and 2 min later 50 μM [^{14}C]alanine was added and uptake was measured in the dark (■———■).

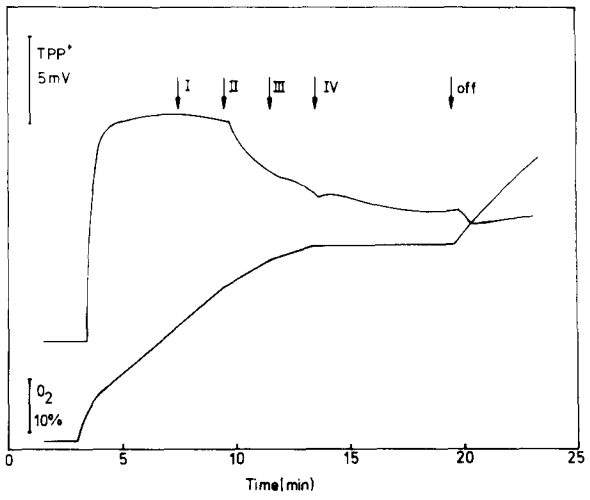

Fig. 7. Simultaneous recording of oxygen consumption and TPP^+ uptake in *Rps. sphaeroides* cells. Intact cells were added to an air-saturated incubation medium at a final protein concentration of 0.5 mg/ml and the disappearance of oxygen and TPP^+ were directly recorded. The incubation medium was as in Fig. 4. The light intensity was increased from I to IV.

Light inhibition of respiration is not due to a back pressure of the Δp on the rate of linear electron transfer

The observed phenomenon that under certain conditions the Δp decreased upon an increase of electron transfer activity has consequences for the assumed role of the Δp in different membrane-linked processes. Recently, it has been reported that inhibition of linear electron transfer upon illumination is caused by a back pressure of the Δp on the linear electron-transfer chain, an effect comparable with respiratory control [28,29]. The observation that in intact cells the Δp decreased upon an increase of electron-transfer activity forced us to reinvestigate the effect of light on the respiration rate. For this purpose the $\Delta\psi$ was measured simultaneously with the rate of oxygen consumption at increasing light intensities. Fig. 7 shows a direct recording of the oxygen and TPP^+ concentrations in intact cells at an external pH of 8. When the cells were added to the incubation vessel an initial rapid disappearance of oxygen was observed, due to the dilution of the oxygen saturated medium with the anaerobic cell suspension. Then the rate of oxygen consumption proceeded linearly, while the $\Delta\psi$ increased to steady state level of -95 mV. When the light was turned on at low intensity (130 $J \cdot m^{-2} \cdot s^{-1}$) no effect was observed on the respiration rate nor on the $\Delta\psi$. When the light intensity was increased further both the respiration rate and the $\Delta\psi$ decreased (see also Table I). At the highest light intensity (1300 $J \cdot m^{-1} \cdot s^{-1}$) respiration was completely blocked and the $\Delta\psi$ was -74 mV. When the light was turned off respiration continued and the $\Delta\psi$ very slowly increased after an initial decrease. In

TABLE I

SIMULTANEOUS MEASUREMENT OF OXYGEN CONSUMPTION AND $\Delta\psi$ IN *RPS. SPHAEROIDES* CELLS AT INCREASING LIGHT INTENSITIES

The respiration rate and $\Delta\psi$ were calculated from the data of Fig. 7. The lowest $\Delta\psi$ value obtained at every light intensity is given.

	Light intensity ($J \cdot m^{-2} \cdot s^{-1}$)	Respiration rate (nmol O_2 per mg protein per min)	$\Delta\psi$ (mV)
Dark	0	17.7	−95
I	130	17.7	−95
II	460	12.0	−82
III	700	6.6	−78
IV	1300	0	−74

Table I the respiration rates and $\Delta\psi$ values calculated from Fig. 7 are shown. These results clearly show that the respiration rate is a function of the light intensity and is not controlled by the $\Delta\psi$.

'Gating' of the $\Delta\psi$ for transport activity does not exist

We have argued above that flow-force relations can be interpreted only when the turnover rate of the (cyclic) electron-transfer chain is kept constant. In Fig. 8A such a flow-force relation (alanine transport as a function of $\Delta\psi$) is shown. At three different light intensities the $\Delta\psi$ was titrated with

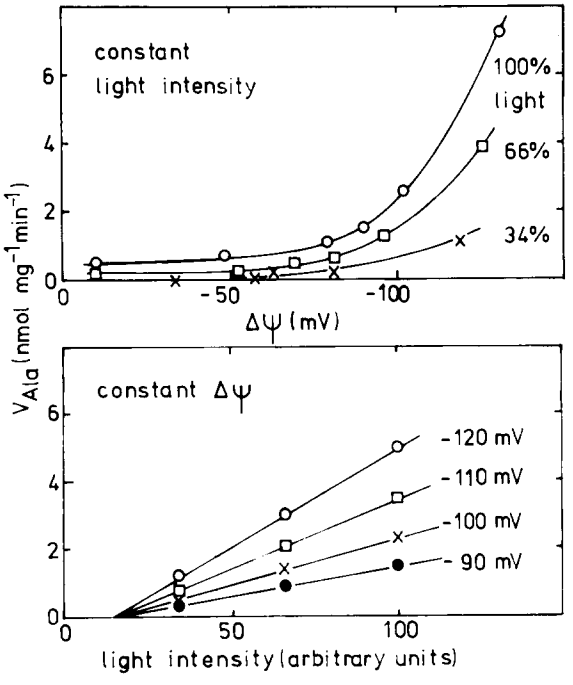

Fig. 8. The relation between the rate of alanine uptake and $\Delta\psi$ at constant light intensity and between the rate of alanine uptake and light intensity at constant $\Delta\psi$ in cells of *Rps. sphaeroides*. EDTA/DCCD-treated cells of *Rps. sphaeroides* were preincubated at a protein concentration of 16 mg/ml with 4 μM nigericin and increasing valinomycin concentration up to 32 μM. The incubation medium was the same as in Fig. 4. Alanine uptake and $\Delta\psi$ were measured simultaneously in the different cell preparations and at different light intensities after dilution of the cells to a final protein concentration of 0.63 mg/ml. (A) V_{ala} as a function of $\Delta\psi$ at constant light intensity. ○, 100% light intensity (2000 J·m^{-2}·s^{-1}); □, 66% light; × 34% light. (B) V_{ala} as a function of light intensity at constant $\Delta\psi$ values. Data from A were replotted. ○, 120 mV; □, 110 mV; ×, 100 mV; ●, 90 mV.

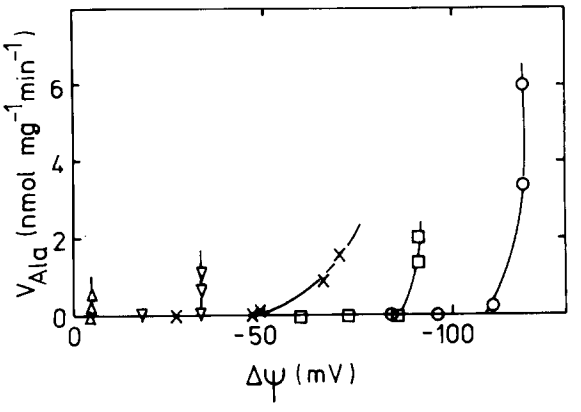

Fig. 9. The relation between V_{ala} and $\Delta\psi$ at varying light intensity for cells preincubated with different valinomycin concentrations. Legend as in Fig. 8. ○, no valinomycin added; □, preincubation with 2 μM valinomycin; ×, 8 μM valinomycin; ▽, 16 μM valinomycin; △, 32 μM valinomycin.

valinomycin in EDTA/DCCD-treated cells at pH 8 and in the presence of nigericin. The rate of alanine transport decreased with $\Delta\psi$ at every light intensity applied. The interpolated data of Fig. 8A are replotted in Fig. 8B as a function of the light intensity. At constant $\Delta\psi$ the rate of alanine uptake is a linear function of the light intensity. There is a fixed threshold value of the light intensity below which no alanine transport occurs. This threshold value is independent of the actual Δp.

In Fig. 9 the rate of alanine uptake was replotted as a function of the $\Delta\psi$ titrated by varying the light intensity at different valinomycin concentrations. The rate of electron transfer is then not constant. This figure clearly demonstrates that a threshold $\Delta\psi$ value for transport activity does not exist. At the lowest $\Delta\psi$ value (−5 mV) still a light-dependent increase in transport activity was measurable.

Discussion

The bulk Δp across the cytoplasmic membrane in *Rps. sphaeroides* is the result of the activity of all energy-producing and energy-consuming processes. As we have reported before [11,12] *Rps. sphaeroides* can maintain a significant membrane potential in the dark. Several processes can contribute to the generation and maintenance of the

dark potential: (i) ATP generation from reserve materials in the cells and subsequent ATP-hydrolysis via the membrane-bound ATPase complex; (ii) diffusion potentials of inorganic ions present in the suspension; (iii) efflux of metabolic endproducts [30] and (iv) reduction of traces of oxygen.

Since in *Rps. sphaeroides* in the dark and at low light intensity no Δp-consuming processes can take place (Refs. 11 and 12, Figs. 4 and 7) the Δp under these conditions can reach high levels (Fig. 2). When the light intensity is further increased not only the rate of the primary Δp-generating pump increased, but also the secondary Δp-consuming pumps are activated via a direct interaction. The net result may be an increase or a decrease of the Δp depending on the experimental conditions. In potassium free buffers the decrease of the $\Delta\psi$ in the light was not observed or was much less pronounced. Potassium ions are necessary for full activity of the alanine transport system (Elferink, M.G.L., unpublished results). The depolarization of the $\Delta\psi$ in the light at high potassium concentration could be due to potassium cycling (electrogenic K^+ uptake followed by electroneutral K^+/H^+ antiport). The omission of Mg^{2+} ions also prevented the decrease of the $\Delta\psi$ in the light. Mg^{2+} ions are essential for secondary solute transport and for ATP-synthase activity. Ca^{2+} or Mn^{2+} could substitute for Mg^{2+} ions. The complex pattern of the Δp obtained in intact cells after illumination at increasing light intensities reflects the delay in response of the secondary pumps to the primary pump. Also other secondary pumps will respond in the same way as the alanine transport carrier to cyclic electron-transfer activity. Cotton et al. reported that photophosphorylation steadily increased in *Rps. capsulata* cells during the first 20 s after illumination [31]. In cells treated with the ATPase inhibitor DCCD in the presence of EDTA always a light-dependent increase of the Δp was observed.

It is difficult to draw conclusions about the relative contribution of ATPase activity to $\Delta\psi$ consumption, since DCCD can affect also other membrane functions [27] and EDTA treatment alone without DCCD already had similar although less pronounced effects as treatment with EDTA plus DCCD.

A ΔpH, inside alkaline, was only measurable at light intensities above the threshold light intensity for transport. However, it cannot be excluded that at low light intensity a small ΔpH was generated, which was below the detection limit of the salicylate electrode (15 mV at our experimental conditions). Since no aspecific binding of salicylate occurs to the cells, the sensitivity of the salicylate electrode is much lower than the TPP^+ electrode.

A comparable flow-force relation as obtained for alanine-transport in EDTA/DCCD-treated cells (Fig. 5) has repeatedly been reported for phosphorylation in a variety of energy-transducing systems [2–10]. The parallel decrease of the phosphorylation rate with the electron-flow rate, while very limited effects can be detected on the Δp, has been taken as an argument for a more localized coupling in oxidative or photophosphorylation [3–7,10]. In contrast, Clark et al. [14] attributed the observed flow-force relation to the increase of the membrane conductance with the increase of $\Delta\psi$. As a consequence the rate of ATP synthesis increased approximately to the sixth power of the $\Delta\psi$. This explanation would be satisfactory as long as the $\Delta\psi$ increases with the rate of the secondary pump. However, our data (Fig. 5), which show a decrease of the $\Delta\psi$ with an increase of the rate of the secondary pump, cannot be explained in this way. A more localized coupling in which the Δp between the bulk phases represents only part of the driving force for transport is also difficult to conform with our experimental results. A local proton pool would equilibrate with the Δp in the aqueous bulk phases after a long period of incubation. However, secondary solute transport was never observed in the dark or at low light intensity despite the high Δp values. In the concept of localized chemiosmosis it is also difficult to explain why at low light intensity the protons would flow to the bulk phase (an increase in Δp is observed), while the local proton-motive force is not high enough to drive secondary transport.

Another possibility which we have discussed before [13] is that the direct interaction is exerted by an influence on the redox state of the transport protein via an interaction with one or more electron-transfer intermediates. It has been reported that the activity of solute transport carriers can be altered by changing the redox state of the dithiols in these carriers [32]. Such a dithiol-disulphide

interconversion in the carrier protein in response to electron-transfer activity is more in agreement with our experimental results. The absence of transport activity at low electron-transfer rates and the delay in response of the transport carriers after initiation and termination at higher rates of electron transfer, might be a consequence of the influence of the electron transfer chain on the redox state of the carrier. Possibly the ubiquinone pool could function as an intermediate between the electron-transfer chain and the transport carrier. No evidence for a direct energy transfer between the primary pump and secondary pumps are obtained, since alanine transport is dependent on Δp.

The simultaneous measurement of $\Delta\psi$ and respiration at increasing light intensities (Fig. 7) clearly shows that the level of the Δp cannot be the only parameter which determines the level of inhibition of respiration upon illumination. It is more likely that light inhibition of respiration is due to a competition between cytochrome oxidase and the photoreaction centre for electrons from cytochrome c_2, a system comparable to that described for light inhibition of respiration in chloroplasts [33]. Recently, two types of inhibition of respiration by light are described in *Rps. capsulata* [34]. The first type is due to the diversion of electrons from the respiratory chain, at the level of cytochrome c_2, to the photooxidized reaction centre. The second type is a control of respiration by the Δp.

When in *Rps. sphaeroides* the Δp is titrated with valinomycin in the presence of nigericin at constant light intensity the initial rate of alanine transport decreases as the Δp decreases, but at every measurable Δp value a significant uptake rate is measured if the light intensity is high. The valinomycin titrations clearly show that no threshold $\Delta\psi$ is necessary for solute transport. The apparent threshold value observed in alanine transport [11] and potassium transport [35] was the result of the threshold value of the turnover rate of the electron transfer chain. The same explanation could hold for other observed gating phenomena of the Δp in solute transport [36] where the role of the electron transfer chain was not taken into account.

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