Biochimica et Biophysica Acta, 767 (1984) 618–626
Elsevier

BBA 41668

EVIDENCE FOR REGULATION IN VIVO OF THE ATP SYNTHASE IN INTACT CELLS OF THE PHOTOSYNTHETIC BACTERIUM *RHODOPSEUDOMONAS CAPSULATA*

N.P.J. COTTON and J.B. JACKSON *

Department of Biochemistry, The University of Birmingham, P.O. Box 363, Birmingham, B15 2TT (U.K.)

(Received July 5th, 1984)

Key words: ATP synthase; Bacterial photosynthesis; Photophosphorylation; Membrane potential; (Rps. capsulata)

(1) The cytoplasmic membrane potential ($\Delta\psi$) of intact cells of *Rhodopseudomonas capsulata*, measured either from the uptake of butyltriphenylphosphonium cation or from the electrochromic carotenoid band shift, increased upon illumination (negative on the cytoplasmic side) and then, within the next 20 s, partly declined while the light was still on. In the presence of the F_0 inhibitor venturicidin the light-induced $\Delta\psi$ was increased by 30% and the partial decline was abolished. (2) From the ionic current/$\Delta\psi$ curves for the bacterial membranes it was concluded that the slow, partial decline of $\Delta\psi$ after the onset of illumination was the result of an increase in membrane conductance. The conductance increase seen in the ionic current/$\Delta\psi$ curves was blocked by venturicidin suggesting that it was caused by increased proton flux through the ATP synthase. (3) Analysis of the light-induced changes in adenine nucleotide levels in intact bacterial cells showed that the apparent increase in ATP synthase activity was not the result of a decrease in phosphorylation potential. The data were consistent with either an increase in the catalytic activity of the ATP synthase or with an increase in H^+ flux through the enzyme without a proportionate increase in the rate of phosphorylation (increased 'slip'). (4) This slow change in the properties of the ATP synthase, as judged by the venturicidin-sensitive partial decline of $\Delta\psi$, required a minimum initial value of $\Delta\psi$. When $\Delta\psi$ was reduced, either by decreasing the actinic light intensity or by adding carbonylcyanide trifluoromethoxyphenylhydrazone the partial decline in $\Delta\psi$ was abolished. (5) The slow change in ATP synthase properties reversed upon darkening the bacterial cell suspension. A second illumination period shortly after the first elicited a smaller initial $\Delta\psi$ and a smaller $\Delta\psi$ decline. The relaxation of the ATP synthase in the dark was measured from the dependence of the initial increase in $\Delta\psi$ after the second illumination period upon the dark-time between the two illumination periods.

Introduction

The ATP synthase of mitochondrial, chloroplast and bacterial membranes is responsible for the conversion of ADP and P_i into ATP using free energy released during electron transport. The enzyme can also catalyse ATP hydrolysis. Owing to its central position in energy metabolism, the ATP synthase in vivo is probably highly regulated. The properties of the enzyme are most easily studied in membrane vesicles in which the catalytic site is exposed on the outer membrane face, or in solubilized F_1 preparations. Many factors are known to influence the enzyme activity, especially in the direction of ATP hydrolysis, although not all of these factors may be significant in regulation in

* To whom correspondence should be addressed.

Abbreviations: $\Delta\psi$, cytoplasmic membrane potential; BTPP, butyltriphenylphosphonium; FCCP, carbonylcyanide trifluoromethoxyphenylhydrazone; S13, 2′,5-dichloro-3-*t*-butyl-4′-nitrosalicylanilide.

the intact organism. First, the enzyme substrates and divalent cations are known to have regulatory effects on ATP synthase in the direction of both hydrolysis and synthesis (see Ref. 1 for a review). Several classes of nucleotide binding sites have been characterised and these may represent distinct catalytic and regulatory functions [2]. Second, the rate of ATP synthesis has a complex dependence on its probable driving force, the electrochemical proton gradient (Δp). The rate increases disproportionately as Δp is increased [3–8]. This nonlinear dependence of the flux on the force may be a consequence of the catalytic properties of the enzyme (for example, see Refs. 3 and 7) but it might also arise from a purely regulatory interaction between Δp and the ATP synthase [5]. The lag in the rate of ATP synthesis in thylakoids irradiated with short flashes [9] was ascribed to a Δp-induced dissociation of inhibitor protein from the ATP synthase. The release of tightly-bound nucleotides, slow on the time-scale of catalysis, may also be symptomatic of a Δp-induced activation of the ATP synthase [10]. Finally, there are some indications that the activity of ATP-synthase can be influenced by redox interaction with electron-transport components. For instance, the rate of ATP synthesis in various membranes was shown to depend not only on the value of Δp but also on the way in which Δp was modified, whether by reducing the rate of electron transport with inhibitors or through the action of uncoupling agents [6,11]. The identity of the electron transport carrier(s) which might mediate an effect on the ATP synthase has not been established. For many years it has been known that the thylakoid enzyme, operating in the direction of ATP hydrolysis can be activated by reduced thiols [12]. More recently, it has been shown that the activation is effective in the direction of ATP synthesis although it remains to be established whether this is of physiological significance [13].

In this report evidence is presented that in intact cells of the photosynthetic bacteria there is a transient change in the value of the cytoplasmic membrane potential ($\Delta\psi$), after the onset of illumination, which can be attributed to a lag in the development of ATP synthase activity. The transient decrease in $\Delta\psi$ was detected by either permeant cation redistribution [14] or by electrochromism [15]. The latter but not the former technique has a sufficiently rapid and linear response also to permit ionic current measurements on the bacterial membrane. The dependence of the dissipative membrane ionic current on membrane potential has been shown on earlier occasions to be non-ohmic [16]. It was found in the present series of experiments that the transient decrease in $\Delta\psi$ was the result of a time-dependent increase in membrane conductance. The transient decrease in $\Delta\psi$ and the conductance changes were prevented by treatment of the bacterial cells with venturicidin, an inhibitor of the F_0 component of the ATP synthase [17]. The significance of these observations is discussed in terms of in vivo regulation of the enzyme.

Methods

The phototrophic growth conditions for *Rhodopseudomonas capsulata* strain N22, the bacteria used in the present work, and the harvesting and washing procedures have been described [17]. The redistribution of butyltriphenylphosphonium cation [$BTPP^+$] was followed with an ion-exchange electrode [18,19]. Carotenoid absorbance changes one a time-scale of seconds and minutes were measured with a chopped, dual wavelength Perkin Elmer 356. On a more rapid time-scale, the ionic-current versus membrane-potential experiments were performed on a dual-wavelength crossed-beam spectrophotometer. Fuller descriptions of the spectrophotometer design, the experimental design and the precautions taken to ensure anaerobiosis have been described elsewhere [8,19]. Adenine nucleotide levels in the intact cells were determined as follows. After harvesting and washing, the bacterial cells were resuspended in argon-sparged fresh growth medium (pH 6.8) to a bacteriochlorophyll concentration of 50 μM. The suspension was drawn into a set of clear plastic syringes fitted with narrow gauge needles and the volume of each was adjusted to 3.0 ml. The filled syringes were preincubated in the dark at room temperature for 45 min. Where shown, illumination was provided with a 150 W quartz halogen bulb focused with a 4 cm diameter clear perspex cylinder on the syringe. The reaction was stopped by rapidly injecting the contents of the syringe

into 1.5 ml of an ice-cold solution of 14% perchloric acid, 9 mM EDTA. After centrifuging at 20000 × 15 *g* min at 0°C, 3 ml of the supernatant were neutralised with 1.5 ml of 1.0 M KOH/1.0 M $KHCO_3$ and frozen. The samples were thawed and centrifuged again before assay. Crude firefly extract was prepared from 0.25 g Sigma FFT (firefly tails) by extraction in the cold in 25 ml 100 mM sodium phosphate/1 mM EDTA (pH 7.4) followed by centrifugation. Light emission was assayed in a laboratory-constructed device using an EMI 9892B/350 photomultiplier. ATP was assayed in 0.1 ml of sample by addition to 2 ml 20 mM Tris-SO_4, 6 mM $MgSO_4$ (pH 7.7) and 0.5 ml crude firefly extract. The system was calibrated by a subsequent addition of 10 µl of 10 µM ATP. For assay of ADP and AMP, 0.2 ml of sample was incubated with 0.2 ml 20 mM Tris-SO_4 (pH 7.7)/6 mM $MgSO_4$/1 mM phosphoenolpyruvate/7 µg Sigma P9136 pyruvate kinase (and for AMP, 9 µg Sigma M-3003 myokinase, washed to remove $(NH_4)_2SO_4$) for 30 min at 30°C, then for 2 min at 100°C and then stored on ice. 0.2 ml samples were then assayed for ATP.

Results

Transients in the generation of membrane potential at the onset of illumination of intact cells of the photosynthetic bacteria

We have commented elsewhere that changes in $\Delta\psi$ across the cytoplasmic membrane of *Rps. capsulata* at the onset of illumination are complex and depend on the substrate and ionic composition of the medium, pH, etc [17]. In the present work we have confined our experiments with harvested and washed intact cells to anaerobic suspensions in fresh growth medium at pH 6.8. The $\Delta\psi$ was measured by $BTPP^+$ redistribution and by following electrochromic carotenoid absorption band shifts.

Fig. 1 shows the uptake of $BTPP^+$ during brief periods of illumination. The calibration of $\Delta\psi$ from $BTPP^+$ uptake is difficult due to contributions from binding of the phosphonium ion to cellular components. Correction factors can be applied if assumptions are made about the site and nature of the binding [20]. In the experiment shown in Fig. 1 two additions of the uncoupling agent,

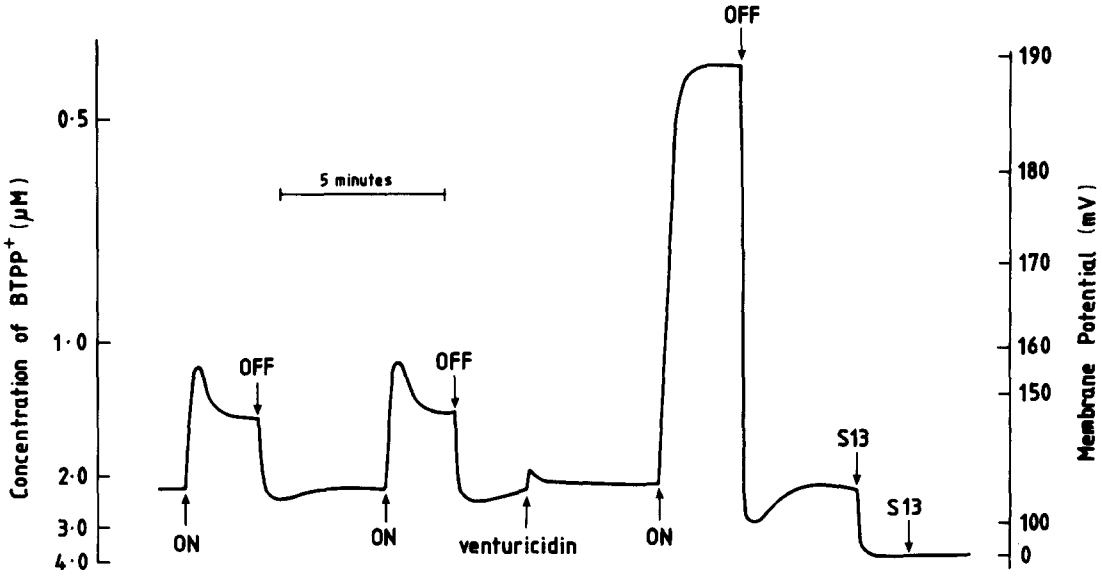

Fig. 1. Transients in the uptake of butyltriphenylphosphonium ion upon illumination of intact cells of *Rps. capsulata* and the effect of venturicidin. Harvested and washed cells of *Rps. capsulata* were added to fresh growth medium at pH 6.8 under anaerobic conditions at 30°C to give a final volume of 4.2 ml and a final concentration of 50 µM bacteriochlorophyll. The total BTPP concentration in the medium was 3.80 µM. The scale on the left indicates the BTPP concentration in the external medium as given by the calibrated ion-exchange electrode. The scale on the right indicates the membrane potential calculated as described in the text and using an internal cell volume of 102 µl per µmol bacteriochlorophyll. Venturicidin was added where indicated to a final concentration of 5 µg/ml. Each of the additions of S-13 were of 2 µM.

S13, were used to show that the degree of binding in completely deenergised cells was quite small (approx. 4 nmol $BTPP^+/\mu$mol bacteriochlorophyll). $\Delta\psi$ was calculated from the Nernst equation on the basis that there are no binding changes upon energisation and that $\Delta\psi = 0$ in the presence of S13. The rapid increase in $\Delta\psi$ at the onset of illumination was followed by a decline to a new steady state. The uptake rate of $BTPP^+$ probably limits the rate of response but the measured extent of the decline was about 10% of the initial $\Delta\psi$. After a short incubation with the F_0-inhibitor venturicidin, the character of light-induced $BTPP^+$ uptake was changed. $\Delta\psi$ reached a level 30% higher than in the absence of venturicidin and the slow decline during the illumination period was abolished.

The $\Delta\psi$ measured by the carotenoid band shift is also difficult to calibrate in intact cells. Where comparisons have been made the carotenoid shift gives larger values for $\Delta\psi$ than ion redistribution but reasons for this remain unclear [19]. Because the carotenoid shift is a linear indicator, the light-induced absorbance changes shown in Fig. 2 should be directly proportional to $\Delta\psi$. These experiments, performed in similar conditions to those in Fig. 1, showed the same features in the light-induced changes in $\Delta\psi$. The membrane potential rose initially to a high value and then fell by about 25% to a new steady state. The time taken for 50% of this decline was about 8 s which in contrast to the $BTPP^+$ determination is not limited by the response time of the probe. Venturicidin prevented the decline in $\Delta\psi$ and increased the maximum value by 30%.

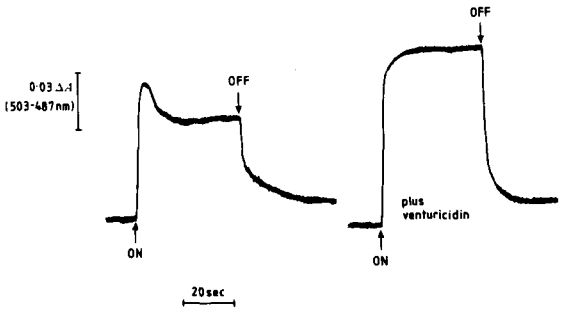

Fig. 2. Transients in the electrochromic absorption change upon illumination of intact cells of *Rps. capsulata* and the effect of venturicidin. Harvested and washed cells were resuspended to 20 μM bacteriochlorophyll in 2.5 ml fresh growth medium at pH 6.8 under anaerobic conditions in the spectrophotometer cuvette at 30°C. The cells in the experimental trace on the right had been pre-incubated in the dark with 4 μg/ml venturicidin.

The dependence of membrane ionic current on $\Delta\psi$ after short and after long illumination times

The rate of change of $\Delta\psi$ when membrane capacitance is constant is a measure of the net ionic current across the membrane. According to the chemiosmotic hypothesis $\Delta\psi$ is generated by electron-transport-driven proton translocation and is dissipated by way of the ATP synthase, substrate translocators and leaks. We have followed the dependence of the dissipative ionic current (J_{dis}) on $\Delta\psi$ by measuring the dependence of the initial rate of decay of the carotenoid band shift upon darkening a cell suspension on the steady-state extent of the shift before switching off the light [16]. Fig. 3A shows the results of such an experiment in the presence and absence of venturicidin. In this experiment the illumination time was kept as short as possible: as soon as $\Delta\psi$

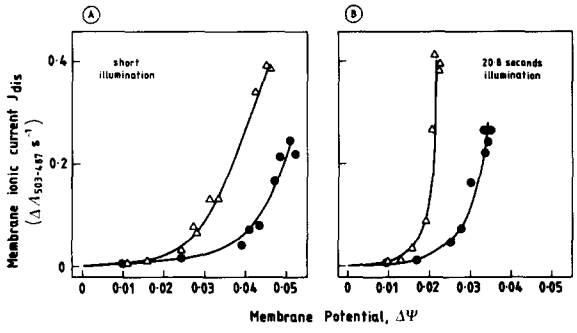

Fig. 3. The dependence of the membrane ionic current on $\Delta\psi$ in intact cells in the presence and absence of venturicidin. Conditions as Fig. 2, except that a more rapidly responding spectrophotometer was used (see Methods). Each data point was obtained from a single illumination period. In experiment A the illumination time was kept as short as possible – when an apparent steady-state value of $\Delta\psi$ was reached, the light was extinguished. For high illumination intensities (high values of $\Delta\psi$) the light period was 500 ms and for the lowest intensity the period was 5.0 s. In experiment B, the illumination time was kept to 20.8 s for all intensities. The membrane ionic current was recorded from the initial rate of decay of the electrochromic absorption change upon darkening and $\Delta\psi$ from the extent of the absorption change before darkening. Open symbols, no further additions. Closed symbols, plus 4 μg/ml venturicidin.

became constant, the light was extinguished. The nonohmic nature of the current/voltage curve and the dominating influence of the ATP synthase on the membrane conductance are confirmed [8,16]. In untreated cells at the maximum light intensity the proportion of the total membrane ionic current proceeding through the ATP synthase was approx. 70%. In Fig. 3B a similar experiment was carried out on a bacterial suspension from the same culture except that prolonged illumination periods were used. The decline in $\Delta\psi$ (in the absence of venturicidin), which is shown in Figs. 1 and 2, is also apparent from the comparison between Figs. 3A and B. The main feature of interest is that the increase in membrane conductance with increasing $\Delta\psi$ in untreated cells was even more pronounced after prolonged illumination: for equivalent values of $\Delta\psi$ the dissipative ionic current was greater. The experiments with venturicidin reveal that the increased ionic current after long illumination times was largely a result of a greater flux through the ATP synthase. For example at the maximum light intensity for 20.8 s, the proportion of J_{dis} proceeding through the ATP synthase was approx. 90%. Also of interest is the observation that the maximum value of J_{dis} after prolonged illumination at high light intensity was no greater than that after short illumination times despite the lower $\Delta\psi$ and greater membrane conductance.

Changes in adenine nucleotide levels at the onset of illumination

The level of adenine nucleotides assayed in extracts of bacteria can only be taken as a guide to cytoplasmic concentrations because the degree of binding to cellular components is unknown. With this reservation in mind, the mass action ratio of the adenylate kinase reaction, [ATP] [AMP]/[ADP]^2 in dark-adapted, anaerobic cells of *Rps. capsulata* (Table I) was close to equilibrium (K_{eq} = 0.44). In contrast, that for the ATPase reaction, [ADP] [P_i]/[ATP], assuming a cytoplasmic volume of 102 µl per µmol bacteriochlorophyll [19] and assuming 10 mM P_i (the concentration of phosphate in the growth medium), was far from equilibrium ($K_{eq} \simeq 10^{-5}$).

Upon illumination the ATP level in the cells rose during the first 5 s (Fig. 4). The level remained constant during the next 30 s (Fig. 4 and Table I). The increased level of ATP was mainly at the expense of AMP, although the ADP level decreased slightly, presumably through the combined action of ATP synthase and adenylate kinase. The most significant finding for present purposes was that the [ADP]/[ATP] ratio decreased during the first 5 s of illumination and thereafter remained fairly constant. It can also been seen from Table I that the reaction catalysed by adenylate kinase moved away from equilibrium during the first 5 s of illumination and then relaxed towards an apparent (new) equilibrium during the next 25 s.

Upon switching off the light the adenylate kinase equilibrium was rapidly restored (Table I). Fig. 4B shows that the initial rate of decrease in the ATP level in the dark was greater after a long (30 s) than a short (2 s) period of illumination.

TABLE I

ADENINE NUCLEOTIDE LEVELS IN INTACT CELLS OF *RPS. CAPSULATA* NANOMOL NUCLEOTIDE PER MICROMOL BACTERIOCHLOROPHYLL

Figures in brackets refer to a duplicate experiment performed under identical conditions on cells from a different culture of bacteria. The total adenine nucleotide levels are shown as an indication of the reliability of the experiments. The ratios shown in the two right-hand columns are averaged over the two experiments.

Illumination Regime	ATP	ADP	AMP	AdN (total)	$\frac{ADP}{ATP}$	$\frac{[ATP][AMP]}{[ADP]^2}$
Dark	20 (28)	66 (66)	126 (113)	211 (208)	2.75	0.65
2 s light	77 (114)	55 (44)	90 (71)	222 (229)	0.52	3.13
5 s light	165 (201	35 (9)	28 (36)	228 (246)	0.12	12.1
30 s light	150 (225)	48 (15)	20 (23)	218 (264)	0.17	4.06
30 s light 5 s dark	103 (149)	92 (60)	30 (27)	225 (237)	0.60	0.62

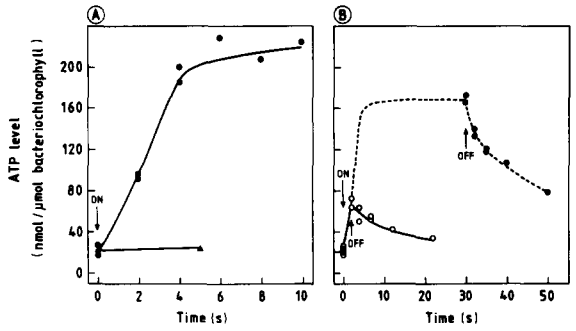

Fig. 4. ATP level in intact cells of *Rps. capsulata*. Experimental conditions were as described in Methods. The points at zero time are for 45 min dark pre-incubation and no illumination. (A) ●, washed, harvested cells in fresh growth medium at pH 6.8 and no further addition; ▲, plus 5 µg/ml venturicidin. (B) ●, the cells were illuminated for 30 s and then darkened. The dotted line shows, for the purpose of continuity, the typical time-course of ATP levels during a 30 s illumination period (e.g., compare with A); ○, the cells were illuminated for 2 s and then darkened.

Factors affecting the venturicidin-sensitive decline of $\Delta\psi$

When the bacterial cell suspension was irradiated with very low actinic light levels, the $\Delta\psi$, measured by electrochromism rose to a low level (Fig. 5B) and did not then decline (compare Fig. 5A). When the actinic light intensity was then raised to its full value $\Delta\psi$ rose to its maximum value and then fell by the same extent as was observed in the absence of low intensity preillumination (compare Fig. 5A and B). Even when the low intensity preillumination was extended to about 90 s the $\Delta\psi$ kinetics upon subsequent high intensity illumination were unchanged. Thus there was no evidence for the accumulation of a photoproduct capable of causing the transition responsible for the venturicidin-sensitive membrane potential decline. In experiments at higher intensity pre-illumination levels, similar results were observed but only when the $\Delta\psi$ reached during pre-illumination failed to exceed a minimum value (Fig. 5C). This minimum value was approximately the same as that reached in the steady state (after the $\Delta\psi$ decline) at maximum light intensity. However, when this value was exceeded during preillumination (Fig. 5D) then $\Delta\psi$ decayed during pre-illumination. In this case the $\Delta\psi$ decline following the shift to maximum intensity was less pronounced. Clearly, the venturicidin-sensitive $\Delta\psi$ decline was additive and it appeared to require a minimum $\Delta\psi$ before it was triggered.

Support for the requirement of a minimum

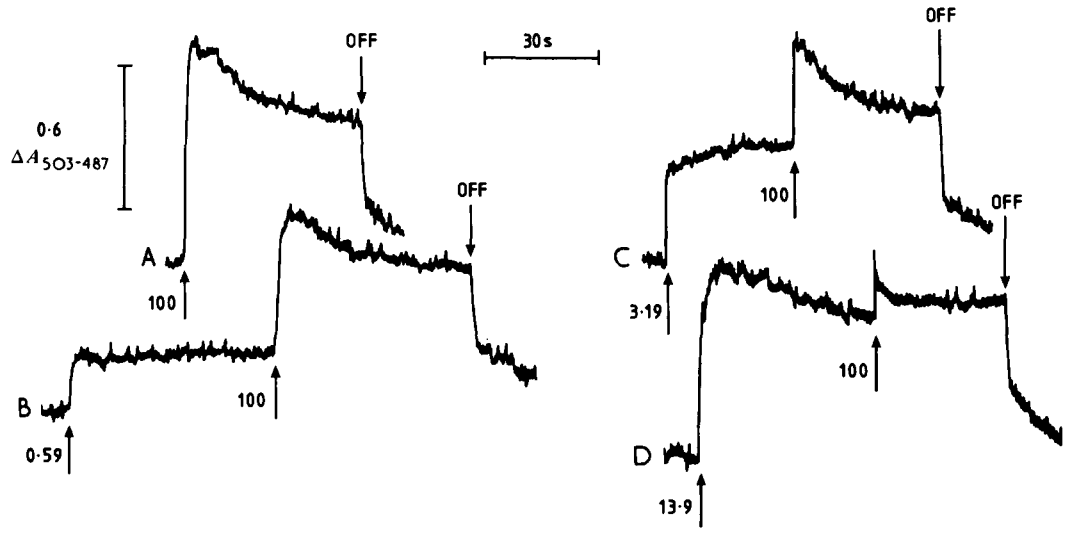

Fig. 5. The effect of low intensity pre-illumination on the kinetics of the electrochromic absorption changes in intact cells of *Rps. capsulata*. Conditions as Fig. 2 in the absence of venturicidin. The upward-pointing arrows indicate when the suspension was illuminated. The number by the side of the arrows gives the transmittance value of neutral density filters used to attenuate the light intensity. The value of 100 (no filter) is the collimated beam from a 150 W quartz-halogen lamp passed through 5 cm water and 1 thickness of Wratten 88A gelatin filter.

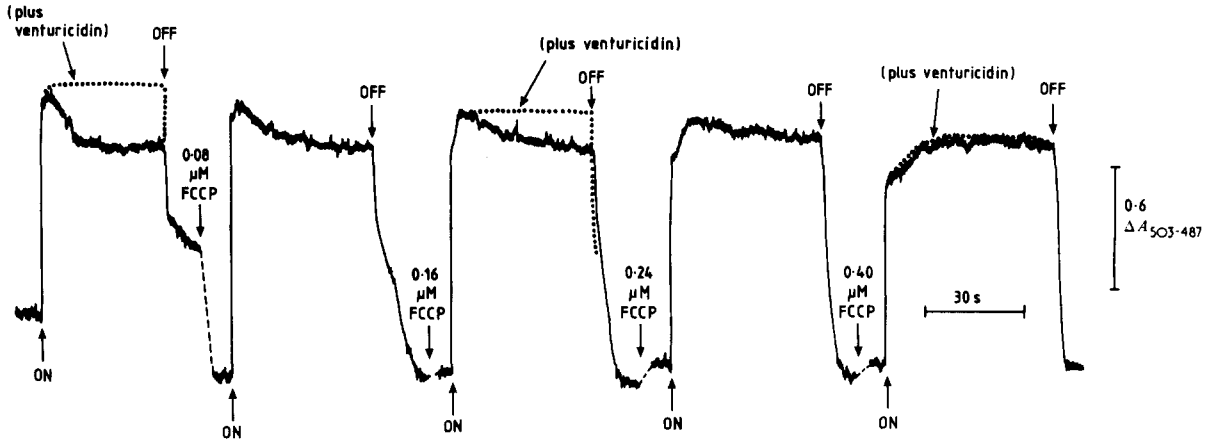

Fig. 6. The effect of FCCP on the kinetics of the electrochromic absorption changes in intact cells of *Rps. capsulata*. Conditions as Fig. 2. The dashed line after the FCCP additions (final concentrations are shown) represents a time-period of approx. 2 min. The dotted lines show the effect of 4 μg/ml venturicidin in the presence of similar FCCP concentrations from parallel experiments. The change in base line level after the first addition of FCCP has been observed in earlier work and is believed to be due to the collapse of a residual $\Delta\psi$ which is believed to arise from a combination of extremely low rates of respiratory electron flow due to oxygen contamination of the argon (even at less than 3 ppm) and the very low conductance of the membrane at low $\Delta\psi$ (see Ref. 21).

value of $\Delta\psi$ is shown in Fig. 6. In this experiment the light-induced $\Delta\psi$ was lowered progressively with the uncoupling agent, FCCP. The venturicidin-sensitive decline in $\Delta\psi$ was more sensitive to FCCP than was the initial light-induced increase in $\Delta\psi$. When sufficient FCCP was added to reduce the initial rise in $\Delta\psi$ to a minimum value (a value which was approximately the same as that reached after the decline in $\Delta\psi$ in the absence of FCCP) then no $\Delta\psi$ decline was observed.

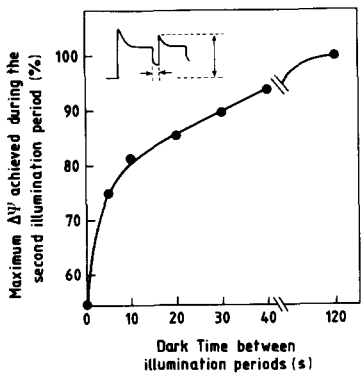

Fig. 7 The dark relaxation of the process responsible for the venturicidin-sensitive decay of $\Delta\psi$ during illumination. Conditions as Fig. 1. The inset illustrates the principle behind the method. The extent of $\Delta\psi$ reached at the start of a second illumination period is plotted as a function of dark-time between the two illumination periods.

Fig. 7 shows the results of an experiment designed to follow the dark relaxation of the process responsible for the venturicidin-sensitive decline in $\Delta\psi$. The strategy was to illuminate the bacterial cell suspension for 30 s until the $\Delta\psi$ decline was complete, then to darken the suspension and measure the initial extent of the rise in $\Delta\psi$ upon subsequent re-illumination (see inset to Fig. 7). If the process responsible for the venturicidin-sensitive decline in $\Delta\psi$ had insufficient time to relax in the intervening dark period, then the initial $\Delta\psi$ on the second illumination would be low. When the two periods of illumination were separated by more than 2 min, then the $\Delta\psi$ kinetics were indistinguishable. The recovery in the dark period had a half-time of approx. 10 s.

Discussion

The decline in $\Delta\psi$ from its initial maximum value at the onset of illumination (Figs. 1 and 2) could in principle be a result of a decrease in rate of electron-transport-driven H^+-translocation (J_{gen}), or an increase in the ionic conductance of the membrane, or both. Evidence against a signifi-

cant decrease in J_{gen} is provided by a comparison between Fig. 3A and B. At the highest light intensity used in these experiments the value of J_{dis} was similar after short or after long illumination periods (i.e., before and after the decline in $\Delta\psi$). Since J_{dis} must be equal to J_{gen} ($\Delta\psi$ was constant in the period before measurement) it follows that J_{gen} did not decrease during prolonged illumination. Since the value of H^+/e^- ratio is independent of $\Delta\psi$ [22] this shows that the rate of electron transport was unchanged.

Evidence that the decline in $\Delta\psi$ after the onset of illumination was due to a membrane conductance increase was also provided by Fig. 3A and B. At maximum light intensity the membrane conductance ($J_{dis}/\Delta\psi$) was 2.1-times higher after the decline in $\Delta\psi$ than before. The conductance increase at a comparable value of $\Delta\psi$ (i.e., at comparable driving force) was even larger than this: at the point on the $J_{dis}/\Delta\psi$ curve in Fig. 3A at which $\Delta\psi$ was equal to that reached at maximum light intensity in Fig. 3B ($\Delta\psi = 0.022$ $\Delta A_{530-487\ nm}$) there was a 14-fold difference in the value of membrane conductance. This large difference must result either from an increase in the number of ion-conducting channels in the cell membrane or from an increase in the conductance of ions through existing channel.

Experiments with the F_0 inhibitor, venturicidin, suggest that the increase in conductance during the first 20 s of illumination was associated with an increased activity of the ATP synthase. This is apparent from Figs. 1 and 2 in which venturicidin abolished the slow decay in $\Delta\psi$ during illumination but is more clearly evident in the current/voltage curves shown in Fig. 3. At $\Delta\psi = 0.022$ $\Delta A_{503-487\ nm}$ the venturicidin-sensitive conductance was approx. 30-fold greater after 20.8 s illumination than after short illumination. The conclusion arising from these experiments is that inward proton flux through the ATP synthase increases during the first 20 s of illumination of dark-adapted cells without any accompanying increase in the driving force. The result is a drop in the value of the cytoplasmic membrane potential.

Increased H^+-flux through the ATP synthase could arise from an increase in the activity of the enzyme itself or from a reduction in the value of the cytoplasmic phosphorylation potential against which the ATP synthase has to work, due to an increase rate of ATP utilisation, e.g., by a slow induction of the biosynthetic reactions of the cell. Evidence against the latter possibility is the data on the adenine nucleotide levels shown in Table I. The ADP/ATP ratio declined during the first 5 s of illumination and thereafter remained fairly constant up to 30 s. This suggests that the value of the phosphorylation potential actually increased (became more negative) during the period in which the increased ATP synthase conductance was recorded. The simplest explanation for the data is that after a dark preincubation period the ATP synthase exists in a low activity form. This is consistent with the observation (Table I and Results) that, despite the low value of Δp in these conditions, the ATP is not extensively hydrolysed. During the first seconds of illumination we propose that photophosphorylation proceeds at less than the maximum rate and $\Delta\psi$, because it is not being rapidly consumed by ATP synthesis, rises to a high value. The enzyme is subsequently activated, the photophosphorylation rate is enhanced and the consumption of $\Delta\psi$ is increased. The photophosphorylation rate is difficult to measure in intact cells, not least because of the simultaneous utilisation of high-energy phosphate by metabolism. There is no evidence for an upturn in the net rate of ATP synthesis (Fig. 4A and Table I) or in the net rate of ~ P formation (taken as [2 × ATP] plus [ADP] from Table I) during the first seconds of photosynthesis, so the postulated increased rate of photophosphorylation (above) must be accompanied by an increased rate of ~ P utilisation. A measure of support for this is provided in Fig. 4B where it is shown that the rate of ATP disappearance upon darkening an illuminated cell suspension was greater after long than after short illumination times.

An alternative explanation for the data cannot be ruled out. It is possible that the increased flux of protons through the ATP synthase shortly after the onset of illumination is not accompanied by an increased rate of ATP synthesis, i.e., the enzyme might 'slip'. Conceivably the ATP requirement of metabolism is met during the first seconds of illumination and the cells cope with an unfavourably high $\Delta\psi$ by partially decoupling the H^+ current through the ATP synthase from its catalytic

conversion of ADP to ATP. In these circumstances the enzyme would operate with a lower H^+/ATP ratio. The increased rate of ATP consumption upon darkening after a long illumination period (Fig. 4B) is not explained by, but is not inconsistent with, the concept of increased slip as an explanation for the decline in $\Delta\psi$.

The factor(s) responsible for the change in ATP synthase activity at the onset of illumination cannot be identified from the present experiments. Of those factors mentioned in the Introduction which can affect ATP synthase activity in vitro, a measure of support for a role of $\Delta\psi$ on the enzyme activity in vivo is given in Figs. 5 and 6. The enzyme activation, as seen from the venturicidin-sensitive decline in $\Delta\psi$, requires a minimum value of $\Delta\psi$. This was evident when $\Delta\psi$ was decreased either by reducing the actinic light intensity or by titrating with FCCP. Incidentally, since these two treatments are expected to have opposing effects on the rate of photosynthetic electron transport (a decrease and an increase, respectively), this renders unlikely the possibility that the redox state of an electron transport carrier influences directly the ATP synthase activity.

If this view of an activation of ATP synthase by $\Delta\psi$ is correct then it is important to distinguish between two effects of $\Delta\psi$. The process of ATP synthase modification discussed in this report is relatively slow. Activation in the light (Fig. 2) and deactivation in the dark (Fig. 7) occur with reaction half-times of approx. 8 and 10 s, respectively. In contrast observations of the highly nonlinear dependence of ATP synthesis rate on $\Delta\psi$ in chromatophores [8] were carried out on time scales (less than 1 s) involving only the first few turnovers of the enzyme. The further effects observed in the present work on intact cells are probably superimposed upon this on a much slower time-scale and probably reflect a different kind of regulation.

Acknowledgement

This work was supported by a grant from the Science and Engineering Research Council.

References

1 Cross, R.L. (1981) Annu. Rev. Biochem. 50, 681–714
2 Rosen, G., Gresser, M., Vinkler, C. and Boyer, P.D. (1979) J. Biol. Chem. 254, 10654–10661
3 Portis, A.R. and McCarty, R.E. (1974) J. Biol. Chem. 249, 6250–6254
4 Pick, U., Rottenberg, H. and Avron, M. (1974) FEBS Lett. 48, 32–36
5 Graber, P. and Witt, H.T. (1976) Biochim. Biophys. Acta 423, 141–163
6 Baccarini-Melandri, A., Casadio, R. and Melandri, B.A. (1977) Eur. J. Biochem. 78, 389–402
7 Rumberg, B. and Heinz, T. (1980) Ber. Bunsenges. Phys. Chem. 84, 1055–1059
8 Clark, A.J., Cotton, N.P.J. and Jackson, J.B. (1983) Biochim. Biophys. Acta 723, 440–453
9 Harris, D.A. and Crofts, A.R. (1978) Biochim. Biophys. Acta 502, 87–102
10 Graber, P., Schlodder, E. and Witt, H.T. (1977) Biochim. Biophys. Acta 461, 426–440
11 Mandolino, G., De Santis, A. and Melandri, A. (1983) Biochim. Biophys. Acta 723, 428–439
12 Bakker-Grunwald, T. and Van Dam, K. (1984) Biochim. Biophys. Acta 347, 290–298
13 Mills, J.D. and Mitchell, P. (1984) Biochim. Biophys. Acta 704, 93–104
14 Rottenberg, H. (1980) Methods Enzymol. 55, 547–569
15 Junge, W. and Jackson, J.B. (1982) in 'Photosynthesis;, Vol. 1: Energy Conversion by Plants and Bacteria', pp. 589–646, (Govindjee, ed.), Academic Press, New York
16 Jackson, J.B. (1982) FEBS Lett. 139, 139–143
17 Cotton, N.P.J., Clark, A.J. and Jackson, J.B. (1981) Arch. Microbiol. 129, 94–99
18 Kamo, N., Muratsuga, M., Hongoh, R. and Kobatake, Y. (1979) J. Membrane Biol. 49, 105–121
19 Clark, A.J. and Jackson, J.B. (1981) Biochem. J. 200, 389–397
20 Lolkema, J.S., Abbing, A., Hellingwerf, K.J. and Konings, W.N. (1983) Eur. J. Biochem. 130, 287–292
21 Cotton, N.P.J., Clark, A.J. and Jackson, J.B. (1983) Eur. J. Biochem. 130, 581–587
22 Cotton, N.P.J., Clark, A.J. and Jackson, J.B. (1984) Eur. J. Biochem., in press