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OXYGEN-INDUCED INHIBITION OF LIGHT-DEPENDENT UPTAKE OF TETRAPHENYLPHOSPHONIUM IONS AS A PROBE OF A DIRECT INTERACTION BETWEEN PHOTOSYNTHETIC AND RESPIRATORY COMPONENTS IN CELLS OF RHODOPSEUDOMONAS CAPSULATA

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Light-generated and oxygen-dependent membrane potentials by heterotrophycally grown cells of Rhodopseudomonas capsulata have been investigated by using a tetraphenylphosphonium ion-selective electrode. The results show that respiratory electron transport affects the magnitude of photogenerated membrane potential while photosynthesis seems to either inhibit or stimulate respiration in coupled or uncoupled cells,respectively. These observations have been considered as evidence that the intracytoplasmic membrane system of R.capsulata contain respiratory and photosynthetic apparatuses which are strictly intermingled.

Rhodopseudomonas capsulata is a typical facultative photosynthetic bacterium that may perform both oxidative and photosynthetic ATP synthesis.The respiratory chain appears to be mainly located in the peripheral cytoplasmic membrane system,while the photosynthetic apparatus is associated with the elaboration of an intracytoplasmic membrane array which is regulated by external factors such as oxygen tension and light-energy flux.This process of adaptation by either chemotrophyc or phototrophyc conditions is reversible(reviewed in Refs. 1,2).

The question of whether intracytoplasmic membranes are capable of both respiration and photosynthesis,and therefore redox components of both apparatuses are intermingled,has not been resolved.

Light-induced oxygen reduction in membrane fragments from photosynthetically grown R.capsulata indicates that electrons can be transported from photosynthetic to respiratory electron carriers,i.e.oxidases,so to support the concept that these systems are located on the same membrane fragments(3).On the other hand,illumination of intact cells(4) or chromatophores(5)during substrate dependent respiration,causes an inhibition of oxygen consumption rather than an enhancement.The light-generated membrane potential,which is higher than that

Abbreviations:TPP^+,tetraphenylphosphonium ion;FCCP,carbonyl cyanide p-trifluoromethoxyphenylhydrazone.

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produced by respiration both in cells(6) and chromatophores(7),would be responsible for this indirect photosynthetic control of respiratory electron transport. Alternatively,it has been postulated that a light-induced change in the oxidation-reduction state of a sensitive electron carrier(s),e.g.cytochrome c,causes the inhibition(8).This latter interpretation implies a direct interaction between photosynthetic and respiratory apparatuses.

To verify these alternatives we have sought to determine whether the respiratory electron flow may affect the light-induced membrane potential in cells of heterotrophycally grown R.capsulata.This event would suggest a priori a redox interaction between photosynthesis and respiration,but only if membrane potential generated by oxygenation were lower than that induced by illumination.

In the present study we report that indeed the membrane potential measured by the redistribution of tetraphenylphosphonium(TPP^+)ions is higher when induced by photosynthesis than dark respiration.As expected the photogeneration of a membrane potential is coupled to a substantial inhibition of the endogenuous oxygen consumption.However,we have also been able to observe that the magnitude of the photosynthetic membrane potential is markedly affected by respiratory electron transport.This latter finding has been interpreted as evidence that respiration and photosynthesis directly interact and therefore a consistent part of the intracytoplasmic membrane system must carry both photosynthetic and respiratory electron transport components.

MATERIALS AND METHODS

Cell-growth and harvesting.Rhodopseudomonas capsulata,strain Kb1(a gift of Prof.J.Klemme,Univ. of Bonn,W.G.)was grown heterotrophycally in the dark in a fermenter(VirTis Mod.43-100) at a controlled oxygen tension of 1%(v/v) in RCV (malate plus ammonium)medium(9). The cells were grown to late exponential phase at +2°C and washed once in the cold with 50mM TES-buffer,pH 7.5.The washed cells were gently resuspended in a minimal volume of the same buffer and stored on ice.All experiments were carried out within 6 h of harvesting.

Electrode measurements of TPP^+-accumulation.A polyvinylchloride membrane selectively permeable to $TPP^+$was formed,essentially according to Kamo et al.(10),by allowing a solution containing 0.34mg tetraphenylboron(Na^+-salt),16mg plyvinylchloride(high molecular weight),57µl dioctylphthalate and tetrahydrofuran(to a final volume of 500µl) to evaporate on a glass plate constrained by a glass cylinder of 1.9cm diameter.The resulting membrane was glued in the place of an exhausted conventional membrane of Radiometer Type 2112a Ca-selective electrode. The internal filling solution was 10mM TPP^+ and 10mM NaCl.The electrode was assembled into a perpex chamber of 1.7ml capacity containing a bridge to KCl reference electrode.The incubation vessel possessed a Clark-oxygen electrode in addition to the tetraphenylphosphonium selective electrode.The vessel was closed except for an addition port for micro-syringe. The TPP^+uptake by intact

cells was recorded continuously and the current amplified by a Philips PW414 digital ion-activity meter.The accumulation of TPP^+(expressed as the ratio of the overall concentration of the cation within the cells to the residual concentration in the medium)was calculated assuming a Nernstian responce for the electrode over the range of 0.2-1μM. Since the aim of the present study is to report changes in potential rather than absolute values no-correction was made for both binding-phenomena and the activity coefficient of the TPP^+in the internal cell-matrix with the result that membrane potentials will be somewhat overestimated (11).The cell-internal volume was assumed of 102μl/μmol bacteriochlorophyll (12). Continuous illumination of the reaction chamber was provided with a 100 Watt quartz-halogen lamp filtered through a Wratten 88a gelatin-filter.
Determination of protein and bacteriochlorophyll concentration.Bacteriochlorophyll concentration was determined on each batch of cells by extraction into acetone/methanol(7:2 v/v) using $\epsilon_{mM}^{775}=75cm^{-1}$(13).Proteins were assayed by using the method of Lowry et al.(14).An equivalence of 1μmol BChl:25mg of protein was normally obtained.

RESULTS

The ion-exchange electrode technique for the measurements of lipid soluble ions uptake by intact cells is now commonly used to give a quantitative estimate of membrane potential(15,16).This approach has the great advantage of continuous monitoring the variations of the concentration of ions in the suspension medium and hence of the membrane potential.

Shown in Fig.1 is the tetraphenylphosphonium(TPP^+)uptake by heterotrophycally grown cells of Rhodopseudomonas capsulata Kb1 during illumination or respiration. Assuming an internal volume of the cells of 102μl/μmol bacteriochlorophyll(12) the light- and oxygen-induced membrane potentials may be calculated from the Nerst equation to be of -150mV and -120mV,respectively(for details see MATERIALS and METHODS). Cotton et al.(15)have reported values of -160mV and -120mV for light- and oxygen-driven changes in $\Delta\Psi$ of photoheterotrophycally grown R.capsulata B10. In this latter case butyltriphenylphosphonium($BTPP^+$)as lipid soluble ion was used.

To gain information of the possible control exerted by respiration on light-induced membrane potential, R.capsulata Kb1 cells were illuminated while actively respiring. Traces in Fig.2 clearly show that a steap decline in the initial level of light-induced $\Delta\Psi$ is apparent during respiration. Cyanide 10^{-4}M,previously shown to block cytochrome c oxidase activity of R.capsulata(17,18),partially removed the oxygen inhibition of light-driven change in membrane potential. A complete restoration of light-dependent TPP^+uptake was obtained after the onset of anaerobiosis. The oxygen-induced inhibitory effect could also be observed

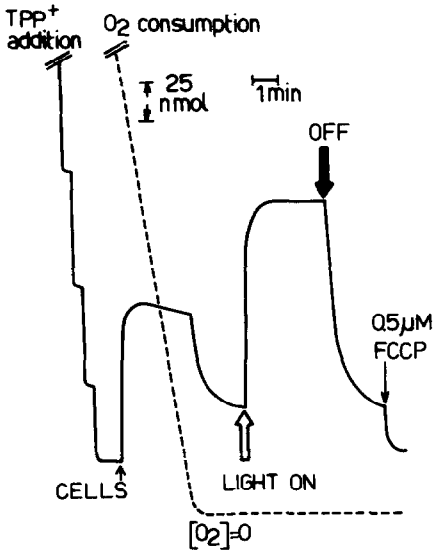

Fig.1.Light-induced and oxygen-dependent uptake of tetraphenylphosphonium(TPP^+) ions in intact cells of Rhodopseudomonas capsulata.Following the calibration addition of TPP^+(1µM final concentration)the bacterial cells(0.25µmol of bacteriochlorophyll)were added to 1.7ml of air-saturated reaction medium containing:TES-buffer,50mM pH7.5,30°C. Un upward deflection of the solid line indicates the uptake of TPP^+ ions as a consequence of the development of a negative potential inside the cells.A dawnward deflection of the dotted line represents the oxygen consumed by respiration.

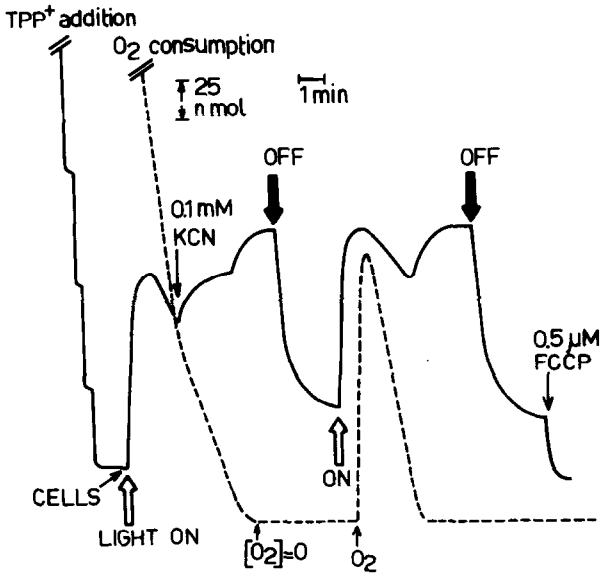

Fig.2.Oxygen-induced inhibition of light-dependent uptake of tetraphenylphosphonium in intact cells of Rhodopseudomonas capsulata.Reaction conditions and designation lines as for Fig.1. To produce a second period of oxygenation after the onset of anaerobiosis,20µg/ml catalase(EC.1.11.1.6)plus a few µl of diluted hydrogen peroxide were added.

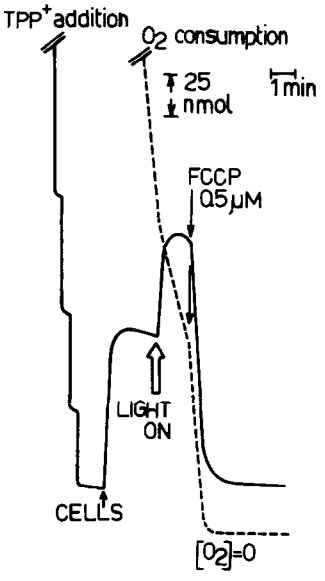

Fig.3. Light-induced inhibition of respiration and relief by uncoupling agent in intact cells of Rhodopseudomonas capsulata. Reaction conditions and designation lines as for Fig.1.

upon oxygenation of anaerobic illuminated cell suspension(Fig.2,second period of oxygenation).

The zero membrane potentials of the experiments reported in Fig.1 and Fig.2 was defined by the TPP^+ uptake levels after the final addition of the protonophore uncoupler carbonylcyanide-p-trifluoromethoxyphenylhydrazone(FCCP). The small decrease observed in the dark upon 0.5μM FCCP addition,was due to the residual oxygen in the suspension permitting a very low basal rate of respiration since the same effect was observed upon addition of sodium dithionite(data not shown).Unfortunately this condition of micro-aerobiosis could not be eliminated.

A complete light-dependent inhibition of respiration by cells of Rhodospirillum rubrum,largely removed by the photophosphorylation uncoupler FCCP has been reported(4). In Fig.3 it is shown that light-induced inhibition of respiration was less pronounced in heterotrophycally grown cells of R.capsulata(40% inhibition of the endogenuous dark-respiration),but it was totally abolished by 0.5μM FCCP in agreement with Ref.4. Moreover,it is important to remark that the addition of this uncoupling agent produced a stimulatory effect(20% of the control) of the endogenuous respiration during aerobic,illuminated conditions(this is shown in Fig.3).

DISCUSSION

The experiments reported in the present work suggest that multiple mechanisms are involved in light-control of respiration by intact cells of Rhodopseudomonas capsulata since differential effects in either coupled or uncoupled cells are observed,i.e.inhibition or stimulation of respiration,respectively.

An earlier suggestion by Ramirez and Smith(4),which has recently been favoured by Mc.Carthy and Ferguson(5),indicated the high-energy state common to both photosynthesis and respiration to be responsible for the light-induced inhibition of respiration.Our results seem to confirm such a conclusion since FCCP eliminated both the inhibition of respiration and the extent of membrane potential. However,we have also been able to observe that photosynthesis was unable to sustain a steady-level of $\Delta\Psi$ during active respiration.Since it is also apparent that the magnitude of the membrane potential is lower when generated by respiration than illumination,we are forced to conclude that the oxygen-control of photosynthesis cannot be accounted for a similar basis.Our explanation for this phenomenon is clearly related to an earlier indication by Keister and Minton(8) that a change in the oxidation-reduction state of electron transfer components common to both cyclic and respiratory chains causes the respiratory control of photosynthesis.This conclusion is nicely supported by the partial relief of the oxygen-induced inhibition of light-generated $\Delta\Psi$ which is consequent the repression of the cytochrome c oxidase activity by 10^{-4}M cyanide. This latter finding also indicates that the cytochrome c containing pathway plays a role in the direct redox-control of photosynthetic components.Additionally,it may be proposed, that the alternate oxidase as well,i.e.cyanide-resistant pathway,affects in a similar way the photocyclic electron flow,since a complete recovering of light-induced membrane potential takes place only under anaerobic conditions(this is shown in Fig.2).

Previous work(3)showed that under aerobic,illuminated conditions,two electron transport pathways operate in chromatophores derived from R.capsulata: a cyclic and a non-cyclic electron flow.They branch at the ubiquinone-10-cytochrome b level and the non-cyclic pathway is linked to an oxygen consumption.It has also been reported that respiratory electron flux by both photo-(17) and hetero-trophycally(18) membrane-fragments of R.capsulata is prevalently channelled through the alternate pathway,i.e.cyanide-resistant oxidase. If this is true it would be likely to expect a stimulation of the oxygen consumption upon illumination

also in intact cells of R.capsulata but only under experimental conditions in which the interplay between photosynthesis and respiration were reduced to a mere redox interaction.This is the case of FCCP-treated cells because of the sup pression of any membrane potential.Indeed, a light-induced stimulation,although restricted to about 20% of the endogenuous control,could be observed in heterotrophycally dark-grown cells of R.capsulata after addition of 0.5μM FCCP(shown in Fig.3).

The above reported considerations taken altogether sustain the concept that during illumination of respiring cells of R.capsulata, two processes are simultaniuously present:an oxygen-uptake stimulation,resulting from a direct interaction between photosynthetic and respiratory components,i.e.non-cyclic oxygen consumption, and an oxygen-uptake inhibition which is induced by photosynthetic control of respiration.The second phenomenon must prevail over the photorespiration and the net-oxygen consumption observed under illumination would be a balance between the two processes. It is interesting to report that under conditions in which no-endogenuous respiration is present,e.g.rotenone-treated cells, light-induced oxygen reduction can be observed(B.J.Jackson,personal communication).

In summary the present study has shown that the in vivo interaction between photosynthesis and respiration of R.capsulata takes place through both a direct exchange of electrons and an indirect energetic coupling between the two apparatuses .

Work in progress aims at defining the kinetic of cytochrome c oxidation-reduction induced by either flashes of light or oxygenation during stopped-flow experiments.

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