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STUDIES ON LIGHT-INDUCED INHIBITION OF RESPIRATION IN PURPLE BACTERIA: ACTION SPECTRA FOR *RHODOSPIRILLUM RUBRUM* AND *RHODOPSEUDOMONAS SPHEROIDES**

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SUMMARY

1. A reversible light-induced inhibition of respiration has been studied in the purple bacteria *Rhodospirillum rubrum* and *Rhodopseudomonas spheroides* by measuring O_2 uptake with a Teflon-covered Pt electrode. The action spectra follow the absorption of bacteriochlorophyll and show a partial participation of carotenoids which was higher in Rhodopseudomonas than in Rhodospirillum.

2. Light saturation of the inhibition effect occurs at a much lower light intensity than saturation of photosynthesis. Respiration stimulation after the end of illumination, similar to that occurring in red algae, could be observed. The measurements suggest an intimate coupling between photosynthesis and respiration. The inhibition effect seems to be brought about by a competition for electrons (or H^+) by intermediates common to both processes.

3. *N*-Methylphenazonium methosulfate stimulates dark O_2 uptake and inhibits the light suppression of respiration.

INTRODUCTION

There are various indications of an interaction between photosynthesis and respiration in photosynthesizing cells containing chlorophyll *a*[1–4]. Photosynthesis in purple bacteria does not result in evolution of O_2 (ref. 5). Here the changes in O_2 concentration are primarily due to changes in respiration. This makes these bacteria especially suitable for measurements of the interaction of respiration and photosynthesis.

The observation of a light-induced suppression of respiration in a purple bacterium reported by NAKAMURA[6] has since been confirmed and extended by others[5,7–11]. KATOH[10] has recently shown the inhibition effect to be located in the chromatophore. HORIO AND KAMEN[11] suggested that a chain of electron-transport carriers operating

Abbreviations: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; PMS, *N*-methylphenazonium methosulfate.

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in cyclic fashion with the photoactive pigments and heme proteins provide competitive hydrogen acceptors. If substrate hydrogen which normally reduces O_2 via the respiratory chain is diverted, upon illumination, to the reduction of CO_2 via the photochemical apparatus then the action spectrum for this effect would be expected to follow the absorption of the photosynthetically active pigments. This expectation was borne out by the action spectra determined here.

With green plants two pigment systems are known to be needed to perform complete photosynthesis. With photosynthetic bacteria there is as yet no indication of participation of more than one pigment system in photosynthesis. Action spectra for light-induced inhibition of respiration might give an indication of a possible participation of more than one pigment system in bacterial photosynthesis.

MATERIALS AND METHODS

Rhodospirillum rubrum cultures were grown in a 1 % peptone–0.5 % NaCl medium at pH 7, and *Rhodopseudomonas spheroides* in 0.5 % yeast extract, 0.5 % $MgSO_4$, 0.3 % L-malic acid and 0.02 M phosphate buffer at a pH of 6.8. Both media were made with tap water. The anaerobic cultures were grown with continuous incandescent illumination around 25°. Cells were used for action spectra determinations after 1 day's growth.

Respiratory changes were followed by means of a Teflon-covered Pt electrode used in conjunction with the liquid-circulating and gas-exchange system described previously[12]. The liquid-circulating system was not used for action spectra determinations. Instead, the bacteria after centrifugation were resuspended as a thin suspension in a fresh sample of medium. A drop of this suspension was placed on the electrode and held in place with another piece of 6 μ thick Teflon-covered membrane. Air was passed at a constant rate over the bacteria on the electrode.

For action spectra measurements the bacteria were illuminated with a 500-mm focal length Bausch and Lomb monochromator having a 100 × 100-mm grating ruled with 600 grooves/mm. Each action spectrum was done in three parts with the slits set to pass a beam having a half width of 3.3 mμ. From 940 to 740 mμ, a 600-mμ cut-off filter to remove second-order wavelengths plus a 48 % transmission neutral density filter were inserted in the monochromator beam; from 650 to 550 mμ only the 48 % transmission filter was used; and from 550 to 450 mμ no filters were used in the monochromator beam. The precision of measurement was lower in this region than for the other portions of the action spectrum because the light intensities were low and the resulting responses small. The wavelength dial was turned manually at 1 mμ per 10 sec while inhibition of respiration was being recorded. The resulting record was then corrected for equal incident quanta and for loss of activity with time (if any) and replotted at 5-mμ intervals.

RESULTS

A time course for inhibition of respiration of Rhodospirillum exposed to 880-mμ light is given in Fig. 1. Decreased respiration is indicated by deflection of the pen above the dark baseline because more O_2, diffusing from the circulating medium, can be reduced at the electrode when the respiratory uptake is lower. The electrode measures only changes in respiratory O_2 uptake since these bacteria do not evolve O_2 (ref. 5).

That these bacteria are not evolving O_2 is also seen by the disappearance of the inhibition of respiration effect when the cells are made anaerobic (unlike green-plant O_2 evolution which may continue under anaerobic conditions). Also, adding the inhibitor of green-plant O_2 evolution, DCMU, to a final concentration of $6.5 \cdot 10^{-5}$ M did not have an appreciable effect on light-induced inhibition.

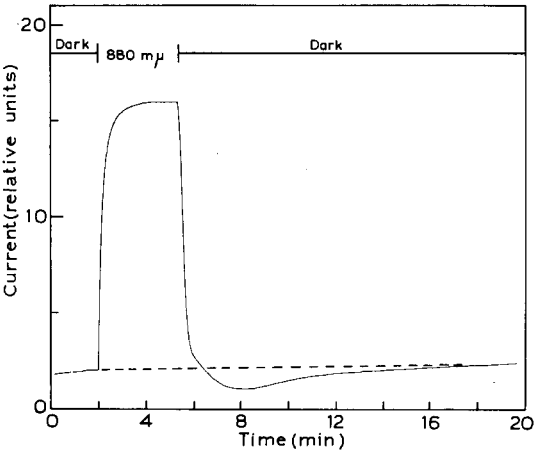

Fig. 1. Time course of inhibition of respiration in *Rhodospirillum rubrum* upon exposure to 880-mμ light having an intensity of 497 ergs·cm^{-2}·sec^{-1} and a half band width of 10 mμ. The cells, harvested after 2 days' growth, were resuspended in fresh medium and gassed with 5 % CO_2 in air.

JOHNSTON AND BROWN[5] found maximum light-induced respiratory inhibitions to range from 60 to 85 %. A similar magnitude of inhibition was noted in the present study. The time course of inhibition given in Fig. 1 shows that the inhibition becomes constant after about 2 min in 880-mμ light. Darkening the cells causes the recorder tracing to dip below the dark baseline established previously. It regains its former level in about 12 min which suggests that an exposure to 880-mμ light causes a temporary respiratory stimulation (compare respiratory stimulation observed in the red alga *Porphyridium cruentum* by FRENCH AND FORK[2]). A similar stimulation of

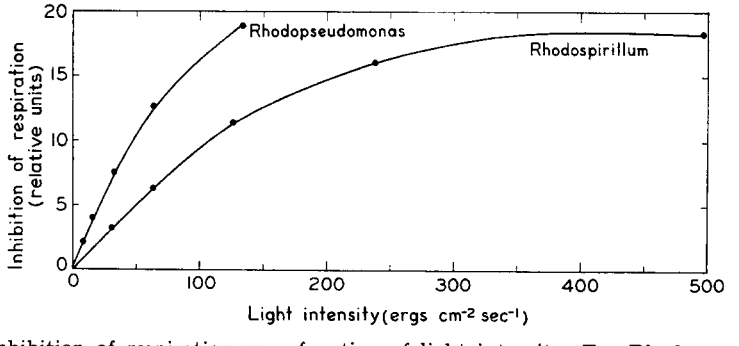

Fig. 2. Inhibition of respiration as a function of light intensity. For Rhodopseudomonas the 850-mμ light used had a half band width of 3.3 mμ. Cells from a 1-day-old culture were resuspended in fresh medium and gassed with air. This sample was used for determination of the action spectrum. For Rhodospirillum the 880-mμ light used had a half band width of 10 mμ. 2-day-old culture gassed with 5 % CO_2 in air.

respiration following illumination of Rhodopseudomonas with 850-mμ light has also been observed. In some instances no stimulation of respiration follows illumination.

Light-saturation curves of respiration inhibition, measured for Rhodopseudomonas with incident light of 850 mμ and for Rhodospirillum with light of 880 mμ, are given in Fig. 2. Since these curves start to bend even at low light intensities the action spectra were determined by keeping the intensities as low as possible. The Rhodopseudomonas sample used for the saturation curve given in Fig. 2 was also used to determine the action spectrum given in Fig. 3. At the 850-mμ peak in the action spectrum the intensity used was 64.3 ergs·cm^{-2}·sec^{-1}. At this intensity the effect per unit of intensity is 16% less than at very low intensity. Since the calculations were made by assuming that a linear relationship existed between inhibition of respiration and light intensity the action spectrum would be flattened somewhat in this region. The action spectrum for relative inhibition of respiration in Rhodopseudomonas has peaks at 850, 800, 590, 510, and 480 mμ and a shoulder around

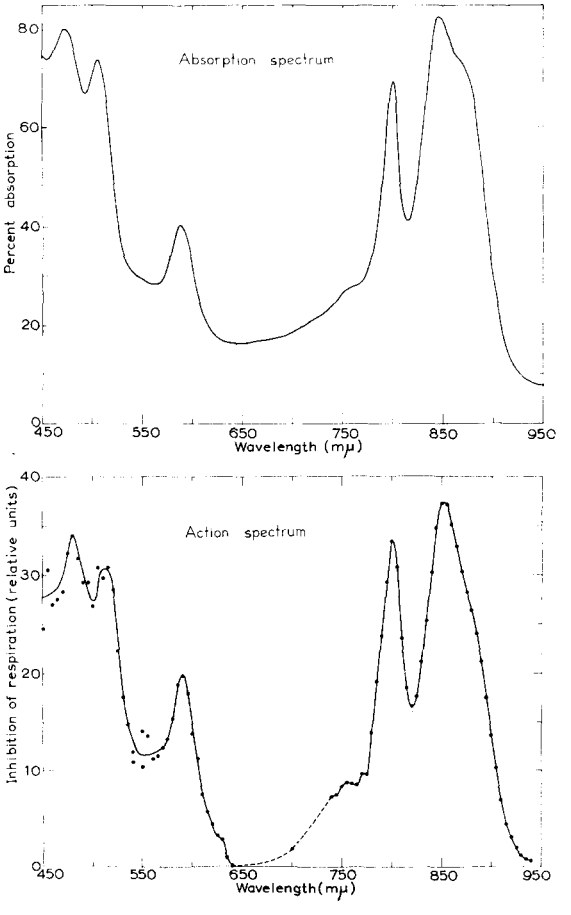

Fig. 3. Lower half: Action spectrum for relative inhibition of respiration by light in *Rhodopseudomonas spheroides*. 1-day-old culture in growth medium, gas phase air. Upper half: Absorption spectrum of chromatophores in phosphate buffer which were prepared from a different sample than used for action spectrum.

880 mμ. The action spectrum for Rhodospirillum (Fig. 4) has peaks at 880, 810, 595, approx. 520, and approx. 485 mμ.

KATOH[10] has studied the effect of a number of inhibitors on photoinhibition of respiration but found none which specifically affected the photoinhibition in question. He noted, however, that the effect was sensitive to high temperature and could be abolished by a 5-min treatment at 40°. HORIO AND KAMEN[11] discovered that 3 M methanol, 1 M ethanol, and 0.05 M isobutanol inhibited almost all of the light-sensitive respiration. This was attributed to a disruption in the coupling between the photoactive pigments.

A disruption of light inhibition of respiration was noted with the redox dye, PMS. Fig. 5 shows that in Rhodospirillum, PMS stimulates respiration in the dark and inhibits the effect of light on respiration. When cells in phosphate buffer and sodium butyrate are exposed to 880-mμ light a 75 % inhibition of respiration results. After the 880-mμ exposure a transient respiratory stimulation of 25 % results. Addition of

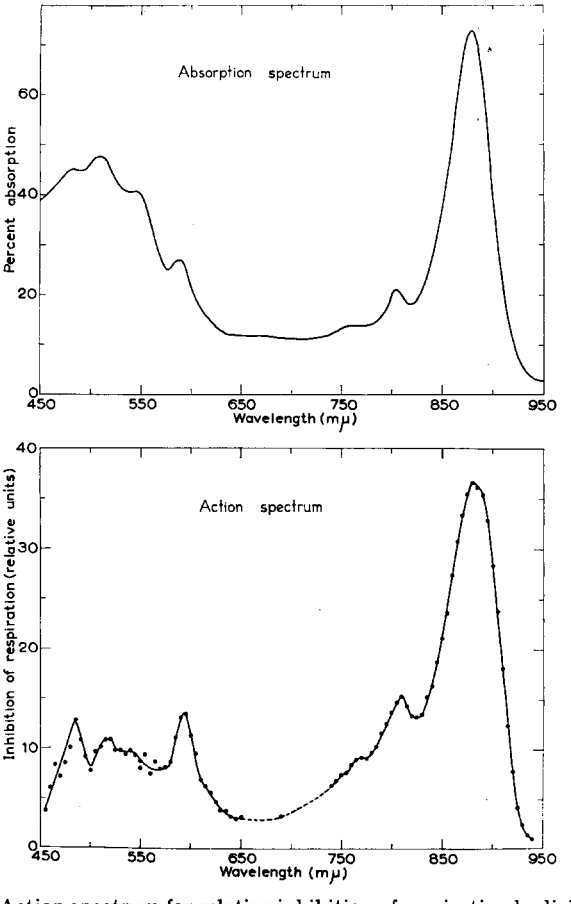

Fig. 4. Lower half: Action spectrum for relative inhibition of respiration by light in *Rhodospirillum rubrum*. 1-day-old culture in growth medium, gas phase air. A different sample used for the action spectrum from that used for the saturation curve of Fig. 2. Upper half: Absorption spectrum of chromatophores in phosphate buffer which were prepared from a different sample than used for action spectrum.

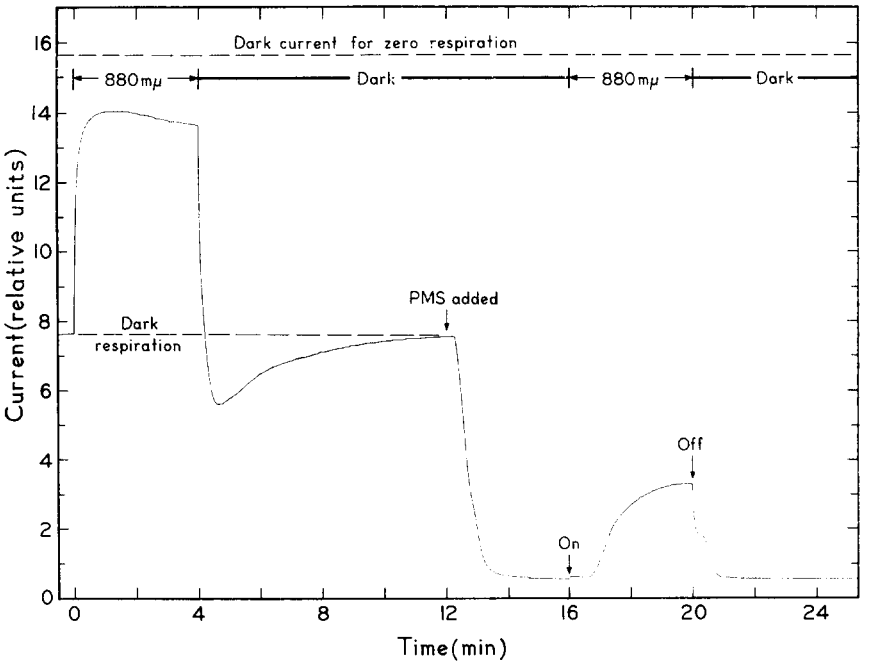

Fig. 5. Effect of PMS on inhibition of respiration by light in Rhodospirillum. Cells from a 7-day-old culture in 0.01 M sodium butyrate and 0.02 M $Na_2HPO_4-KH_2PO_4$ buffer (pH 7.5). Gas phase, air. The 880-mμ light (half band width 10 mμ) had an intensity of 497 ergs·cm^{-2}·sec^{-1}. The same intensity 880-mμ light used after addition of PMS (to a final concentration of $3.3 \cdot 10^{-4}$ M).

PMS (arrow) in the dark increases O_2 uptake by 80 %. The time course of inhibition of respiration by light in the presence of PMS is markedly slowed down. After 4 min in the light, inhibition is only 18 % as compared to 75 % without PMS. The time course of recovery of respiration in darkness is complex and shows a fast component followed by a slower one. No stimulation of respiration follows illumination in the presence of PMS. Repeated exposures to 880-mμ light resulted in a gradual decrease in the amount of light-induced inhibition of respiration as well as a gradual retardation in the time course. (The zero-respiration line is the electrode dark current after the cells were killed by adding formaldehyde solution to a final concentration of about 4 % in the circulating system.)

DISCUSSION

A scheme proposed by NISHIMURA[13] suggests that electron transport for both photosynthesis and respiration passes through a common cytochrome. A similar idea has been proposed by HORIO AND KAMEN[11] who explained light-induced inhibition of respiration on the basis of a competition between the photoactive pigments and an intermediate in the respiratory electron-transport chain.

Action spectra for light-induced inhibition of respiration in *Rhodospirillum rubrum* and *Rhodopseudomonas spheroides* which we have determined in the range from 450 to 950 mμ indicate a close correspondence between this inhibition effect

and the spectral absorption of the photosynthetic pigments, and suggest an intimate coupling between photosynthesis and respiration. This substantiates the assumption of NAKAMURA[6] that under certain conditions it is reasonable to study some aspects of bacterial photosynthesis by measuring the light-induced changes of respiration of the organisms*. The inhibition effect, however, saturates at a light intensity of only a few per cent of that of photosynthesis. HORIO *et al.*[15] are reporting an action spectrum for light inhibition of respiration in *Rhodospirillum rubrum* in the 410–610-mμ region which appears to be similar to that reported here.

A comparison of the relative activity of the carotenoids in sensitizing inhibition in Rhodopseudomonas and Rhodospirillum shows carotenoid activity to be higher in Rhodopseudomonas. It is interesting to note, in this regard, that GOEDHEER[16] has found a higher efficiency in the transfer of energy from carotenoids to bacteriochlorophyll in Rhodopseudomonas than in Rhodospirillum. These action spectra are similar to the action spectra for phototaxis of a young culture of *Rhodospirillum rubrum* reported by DUYSENS[14].

The similarity of the spectra of inhibition effect and absorption in the near infrared, both with Rhodopseudomonas and Rhodospirillum, and the absence of a measurable "long-wavelength decline" indicate that all bacteriochlorophyll types "B 800, B 850 etc." participate in this reaction, either directly or via energy transfer. This appears to be another indication that bacterial photosynthesis acts via a single pigment system.

A disruption of photometabolism of Rhodospirillum by $5 \cdot 10^{-4}$ M PMS was noted by GEST *et al.*[17] who found it to inhibit completely the endogenous and substrate-dependent H_2 evolution. It also caused the cells to ferment their endogenous reserves with the formation of fatty acids even though they were in continuous light. This fermentation was attributed to an inhibition of photophosphorylation by PMS. However, KATOH[10] ruled out the possibility that the photoinhibition effect could be explained on the basis of a competition between photophosphorylation and oxidative phosphorylation for a common phosphate acceptor since added ADP had little effect. He also noted that *o*-phenanthroline or 2,6-dichlorophenolindophenol in concentrations effective in blocking photophosphorylation did not affect photoinhibition. PMS may mediate a more rapid passage of electrons to O_2 by acting as a "bypass" of that intermediate which is common to both photosynthesis and respiration, resulting in an increased dark respiration and a loss of the inhibitory effect of light on respiration.

GOEDHEER[16] has suggested an interaction of respiration in a two-pigment system for photosynthesis in green plants in order to explain chromatic transients, induction effects, and certain other aspects of luminescence. His scheme also proposes a cytochrome common to both photosynthesis and respiration and suggests that excitation of the long-wavelength chlorophyll reaction would result in an inhibition of O_2 uptake. It is interesting in this regard that HOCH *et al.*[3] noted an inhibition of respiration in the blue-green alga Anacystis when chlorophyll was excited and very little, if any, when phycocyanin was excited.

The light-stimulated respiration of these bacteria which persists for some minutes in the dark after the exposure is also analogous to respiratory stimulation observed

* A suggestion that action spectra of photosynthesis could be measured in this way has been made by L. N. M. DUYSENS[14] in his Stelling VI.

after excitation of long-wavelength chlorophyll in Porphyridium[2]. This may result from the accumulation of a reduced intermediate such as pyridine nucleotide in the light which is respired in the dark.

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