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PHOTOINHIBITION BY FLASH AND CONTINUOUS LIGHT OF OXYGEN UPTAKE BY INTACT PHOTOSYNTHETIC BACTERIA

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The inhibition of respiration by continuous or flashing light has been studied in intact cells of different species of photosynthetic bacteria. For *Rhodopseudomonas palustris*, *Rhodopseudomonas sphaeroides* and *Rhodopseudomonas capsulata*, the inhibition by short actinic flashes shows a remarkable periodicity of two: each flash induces an inhibition of respiration, but a stimulation is observed after an even number of flashes. On the other hand, no oscillation is observed for *Rhodospirillum rubrum* and *Rhodopseudomonas viridis* cells. These different behaviours are explained by a difference in the redox state of the secondary electron acceptor as shown by the effect of ortho-phenanthroline on the amperometric signal. Addition of uncouplers (carbonyl cyanide *m*-chlorophenylhydrazone) or of an ATPase inhibitor (tri-*N*-butyl tin), has little effect on the oscillatory pattern induced by flash excitation. However, inhibition of respiration by continuous light is suppressed in the presence of carbonyl cyanide *m*-chlorophenylhydrazone. In the presence of tri-*N*-butyl tin, the steady-state level is reached more rapidly than in the control experiment for a given light intensity. These results are interpreted as evidence of two modes of light inhibition of respiration in photosynthetic bacteria. A first type of inhibition, clearly shown under flash excitation, is due to interaction between respiratory and photosynthetic chains at the level of electron carriers. After each flash, an electron is diverted from the respiratory chain to the photooxidized reaction center. Because of the gating mechanism at the level of the secondary acceptor, the respiration is stimulated after an even number of flashes. The second mode of inhibition prevails under continuous illumination. Under these conditions, the rate of respiration is controlled essentially by the photoinduced proton electrochemical gradient.

Introduction

The *Rhodospirillaceae* family of photosynthetic bacteria (*Rhodopseudomonas* and *Rhodospirillum genera*) is probably the most flexible group of microorganisms in the biological world. Besides the photoautotrophic and the photoheterotrophic conditions of growth, these bacteria adapt to aerobiosis by developing a respiratory electron transfer chain. Different examples of interaction between respiratory and photosynthetic apparatus in facultative bacteria have been reported. The partial or total reversible inhibition of respiration by light in intact cells of several species of Rhodospirillaceae [1,2] has been shown for many years. Two types of hypothesis, which are not exclusive, have been proposed to explain this light-induced inhibition. In the first, at least one electron carrier is common to both photosynthetic and respiratory electron transport chains [3,7]. Upon illumination, oxidation of this electron car-

Abbreviation: CCCP, carbonyl cyanide *m*-chlorophenylhydrazone.

rier would prevent subsequent reaction with molecular O_2 or its immediate electron donors. In the second hypothesis, two separate electron-transport phosphorylating chains interact via some soluble metabolites [8,9]. Very recently, McCarthy and Ferguson [10] proposed a modern version of this second situation. They identified the intermediate between the phosphorylating chains as the proton electrochemical gradient. In favour of these last ideas is the fact that uncouplers like carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) prevent the light-inhibition of respiration of whole cells or chromatophores [6,8,10]. Such a result is difficult to reconcile with the first hypothesis, since CCCP does not impair electron transfer.

In the present paper, we investigate light-inhibition of respiration of whole cells of different species of photosynthetic bacteria under short actinic saturating flashes and continuous illumination. Preliminary results have been published elsewhere [11].

Material and Methods

Rps. sphaeroides strain 2-4-1, *Rps. capsulata* strain 2-3-1, *Rps. palustris* strain 2-1-6, *Rps. viridis* and *R. rubrum* strain S_1, light grown in degassed Hutner medium, were harvested after 24 h. O_2 concentration was measured with a home-built platinum electrode [12] on which 0.6 ml of cell suspension (between 1 and 3 A/cm at 870 or 1010 nm for *Rps. viridis*) were deposited. The cells were allowed to settle for at least 45 min before measurements. Although the medium layer (1.5 mm thick) was in direct contact with the air, partial anoxia might have occurred in the suspension due to the lack of agitation. Excitation was provided by a Yag laser (Quantel, model 481C, 12 ns, 500 mJ at 532 nm) coupled with a dye laser (Quantel, model TDLIII, 10 ns, 100 mJ at 590 nm, using Rhodamine 590). The laser was fired at a frequency of one flash per second. The laser light was homogenized by a frosted glass and its intensity kept twice saturating by appropriate grey filters. A xenon flash (EG and G, FX 201, 2 μs duration) was also used for excitation. Continuous illumination was obtained by a fibre-optic illuminator (Fiberlite, Model 190), filtered through a Corning CS 7-69 filter. Each light-excitation cycle was preceeded by a dark-adaptation time of 15 min.

Our experimental arrangement measures the intensity of the electrochemical current, which is limited by the rate of oxygen diffusion to the electrode. This rate is proportional to the oxygen concentration in steady-state conditions. However, after flash or continuous illumination, one measures a mixture of both rate- and O_2-concentration values. We therefore emphasize that no kinetic parameter can be simply deduced from our measurements.

ortho-Phenanthroline, CCCP, antimycin A, tri-*N*-butyl tin were used dissolved in ethanol. 2-Heptyl-4-hydroxyquinoline *N*-oxide and thenoylotrifluoro acetone were purchased from Sigma.

Results

Amperometric signal following a series of saturating flashes

The variation of O_2 concentration at the electrode level are direct changes in the respiration activity of the photosynthetic bacteria, since these organisms do not evolve oxygen [13]. An increase in the amperometric signal, i.e., more O_2 reduced by the electrode, indicates a diminution of the bacterial respiratory activity. On the other hand, stimulation of respiration will induce a decrease.

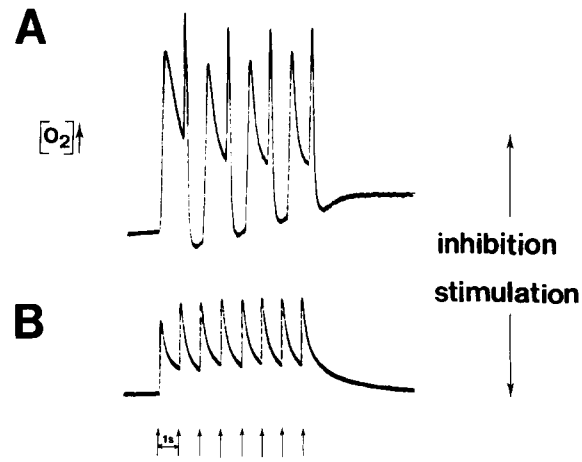

Fig. 1. Amperometric signal of the platinum electrode, on which *Rps. palustris* (A) and *R. rubrum* (B) whole cells have been deposited. The bacteria were subjected to a series of saturating laser flashes, fired every second.

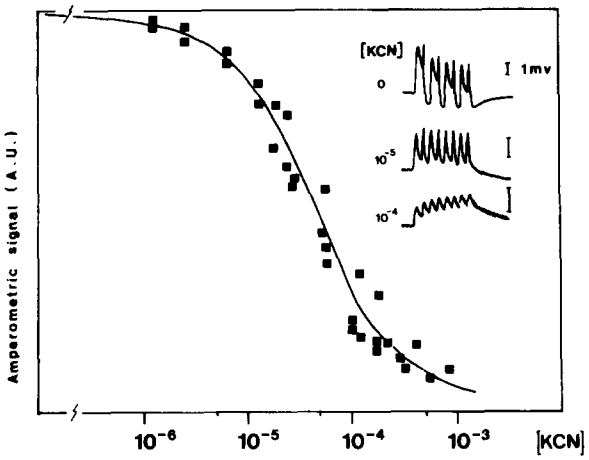

Fig. 2. Amplitude of the amperometric signal, measured on the first flash, in function of the concentration of added KCN. The insert shows the actual recording for the control experiment and in presence of 10^{-4} and 10^{-5} M KCN for *Rps. palustris* cells. A.U., arbitrary units.

Fig. 1 shows the variation of the amperometric signal induced by a series of saturating flashes, spaced by a dark time of 1 s for *Rps. palustris* (Fig. 1A) and *R. rubrum* (Fig. 1B) cells. In the case of *Rps. palustris* (Fig. 1A), each flash promotes a rapid increase of the amperometric signal. However, after an even number of flashes, the fast increase is followed by a rapid oxygen uptake. This induces a remarkable periodicity of two in function of the flash number: after each odd number of flashes, the O_2 level remains higher than in the dark and after each even number of flashes, the level rapidly reaches the dark baseline. Such an oscillatory pattern, also observed for *Rps. sphaeroides* and *Rps. capsulata* whole cells (not shown), indicates that each flash induces an inhibition of respiration but that this inhibition is followed by a stimulation after only even flashes. On the other hand, *R. rubrum* (Fig. 1B) and *Rps. viridis* (not shown) cells do not present such an oscillatory phenomenon. Two series of experiments show that the amperometric signals observed are related to the respiratory activity of the photosynthetic bacteria. First the polarogram profile of the photoinduced amperometric signals is typical of molecular O_2 (not shown). Secondly, addition of KCN or NaN_3 significantly decreases their amplitude. Fig. 2 shows the extent of the amperometric signal on the first flash as a function of KCN concentration for *Rps. palustris* cells. The insert shows the actual recording of the control and two selected concentrations of KCN. Note the progressive disappearance of the oscillatory phenomenon when increasing the KCN concentration. Similar results have been obtained for the other species studied. In presence of 10 mM NaN_3, the amplitude of the amperometric signal is reduced of about 80% (not shown). Several other inhibitors of the respiratory chains (amytal, thenoylotrifluoroacetone, rotenone, malonate, antimycin A, 2-heptyl-4-hydroxyquinoline *N*-oxide) have been found to have no effect on the amperometric signal. The effectiveness of some of these compounds can be due to their lack of penetration in the bacteria.

Effect of ortho-phenanthroline on the amperometric signal

The oscillatory pattern observed for *Rps. palustris, Rps. sphaeroides* and *Rps. capsulata* (Fig. 1A) species has to be related to the binary oscillations of the redox state of the secondary quinone Q_B observed in isolated reaction centres [14,15], chromatophores [16–18] and whole cells [19,20]. For *R. rubrum* and *Rps. viridis* species, which do not show oscillations (fig. 1B), one can assume that the secondary acceptor Q_B is in its semireduced form for 50% of the reaction centres. In order to check that hypothesis, we used *ortho*-phenanthroline which is known to specifically inhibit electron transfer between primary (Q_A) and secondary (Q_B) acceptors, but only when the latter

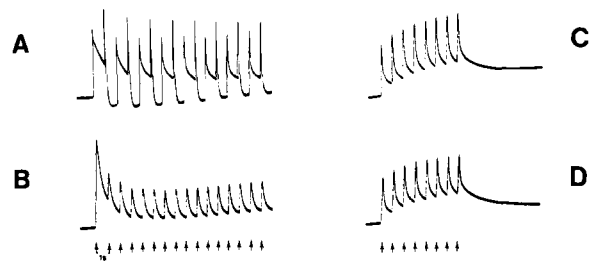

Fig. 3. Experimental conditions as in Fig. 1: (A), *Rps. sphaeroides* cells: control experiment; (B) *Rps. sphaeroides* cells: + *ortho*-phenanthroline (5 mM); (C) *R. rubrum* cells: control experiment; (D) *R. rubrum* cells: + *ortho*-phenanthroline (5 mM).

is in its fully oxidized state [21,22]. In the presence of 5 mM *ortho*-phenanthroline, the first actinic flash still promotes a large inhibition of respiration whatever the species considered (Fig. 3C and D). On subsequent flashes, the light-induced inhibition is, however, significantly decreased and no more oscillations are observed for the oscillating species (*Rps. palustris, Rps. capsulata* and *Rps. sphaeroides*) (Fig. 3C). For *R. rubrum* cells, addition of *ortho*-phenanthroline only slightly affects the amperometric signal, whatever the flash number (Fig. 3D). We deduce from the above-described experiments that for *Rps. palustris, Rps. sphaeroides* and *Rps. capsulata* species the secondary electron acceptor, Q_B, is mainly in its oxidized state, but that for *R. rubrum* and *Rps. viridis* Q_B is semi-reduced in about 50% of the reaction centres.

These conclusions are in marked contrast with those of De Grooth et al. [19] who observed strong oscillations at the secondary acceptor level for *R. rubrum* but not for *Rps. sphaeroides* cells under aerobic conditions. However, the quinone oxidoreduction state depends strongly on the O_2 concentration [19], which can be quite different in our experiments and those of De Grooth et al. [19]. In particular, the lack of special aeration of the medium in our experimental arrangement may induce partial anoxia.

Effect of various lengths of dark interval between two actinic flashes on the inhibition of respiration

To determine dark limiting steps in the photoinhibition process, we have measured the amplitude signal peak induced by a second actinic flash, fired at different dark times (Δt) after the first one. In such experiments, the time resolution is not determined by the response time of the O_2 electrode, but by the shortest available dark-time between two saturating actinic flashes, i.e., 3 ms in our conditions. Fig. 4 compares the amplitude of the amperometric signal peak induced by the second flash as a function of Δt and the kinetics of rereduction of $(BChl)_2^+$, measured at 790 nm, for *Rps. palustris* (Fig. 4B) and *R. rubrum* (Fig. 4C) cells.

A good similitude is observed between the half-time of rereduction of $(BChl)_2^+$, and the dark limiting step of the photoinhibition of respiration. For *Rps. sphaeroides* cells, the amplitude of the amperometric signal induced by the second flash is maximal for Δt as short as 10 ms (Fig. 4A), in good agreement with the time of rereduction of $(BChl)_2^+$ in this species [20]. This implies that for all the species studied, the rereduction of $(BChl)_2^+$ by cytochrome *c* is the limiting step of the photoinhibition process of respiration under flashing light.

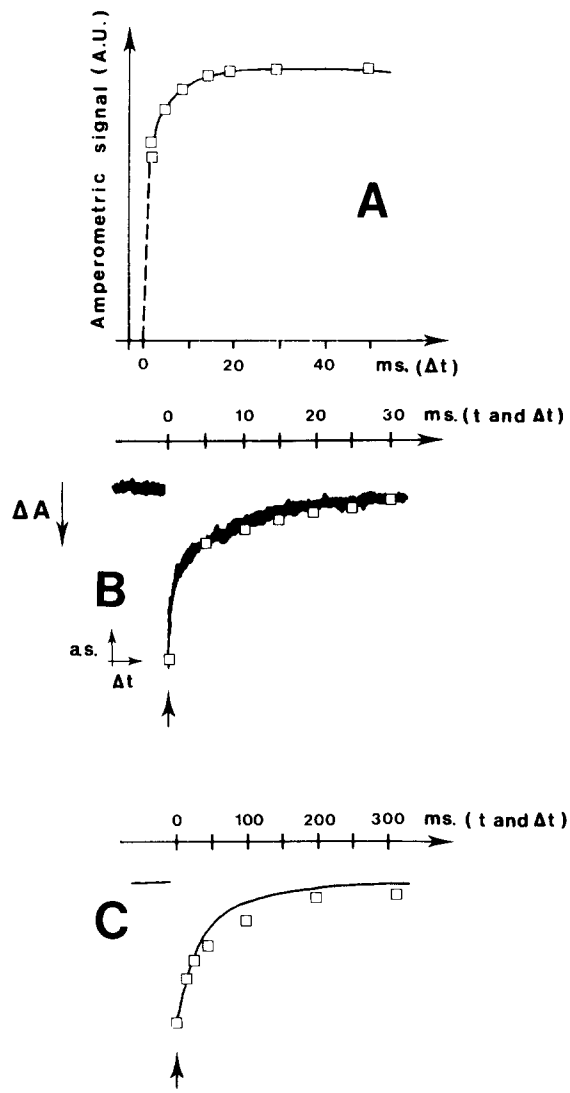

Fig. 4. (A) Amplitude of the amperometric signal measured for different dark-interval times (Δt) between two actinic flashes for *Rps. sphaeroides* cells. (B) Comparison between the flash-induced absorption changes of $(BChl)_2^+$ measured at 790 nm and the amplitude of the amperometric signal (a.s.) in function of Δt for *Rps. palustris* cells. (C) Same as (B) for *R. rubrum* cells.

Discussion

Because of their disappearance in presence of KCN (Fig. 2) and NaN_3, the flash-induced amperometric signals observed (Fig. 1) have to be related to the respiratory activity of the photosynthetic bacteria. As already stated, an increase and a decrease of the amperometric signal correspond to an inhibition and a stimulation of the respiration activity, respectively. The remarkable periodicity of two of the amperometric signals observed in *Rps. palustris* (Fig. 1A) suggests, therefore, that each flash induces an inhibition of the bacterial respiration, but that after an even number of flashes the respiratory activity is stimulated. A coupling between respiratory and photosynthetic electron transfer chains at the level of ubiquinone cytochrome *b/c* complexes and cytochrome c_2 can readily explain these flash-induced inhibition and stimulation of respiration. After each flash, an electron is diverted from the respiratory chain towards the photosynthetic reaction centre, i.e., cytochrome c_2 rereduces the $(BChl)_2^+$ formed on the flash instead of reacting with the terminal oxidase.

Only after an even number of flashes because of the gating mechanism at the level of the secondary acceptor, two electrons are available for the terminal oxidase, stimulating the respiratory activity. Consistent with such a scheme is the observation that the limiting step of the photoinhibition of the respiration under flashing light is the rereduction of $(BChl)_2^+$ (Fig. 4). The addition of *ortho*-phenanthroline, which does not affect the inhibition of respiration on the first flash, is also readily explained by our scheme, since the first actinic flash, even in presence of this compound, induces the full oxidation of cytochrome c_2. Moreover, the effect of *ortho*-phenanthroline on the amperometric signal on the successive flashes (Fig. 3) indicates that Q_B is primarily oxidized in the oscillating species (*Rps. palustris, Rps. sphaeroides* and *Rps. capsulata*) and semi-reduced in 50% of the reaction centres in *R. rubrum* and *Rps. viridis* cells.

Two main objections can be made against our above proposed scheme for the mode of inhibition of respiration by light in photosynthetic bacteria. First, the preventive effect of CCCP [6,8,10,23], a well-known uncoupler, on the light-induced inhibition of respiration is difficult to reconcile with our scheme. The second objection one can make is that, following our hypothesis, no inhibition is expected under continuous illumination, since light induces both inhibition and stimulation of the respiratory activity. These inconsistancies let us suppose that, under continuous illumination, another mode of inhibition prevails over the diversion of electrons from one transport chain to the other. One obvious hypothesis is that, under continuous illumination, the rate of respiration is controlled by the photoinduced proton electrochemical gradient [10,23]. To check that possibility, we have compared the inhibition induced either by continuous or flashing light in presence of an uncoupler, CCCP, or an ATPase inhibitor, tri-*N*-butyl tin. Addition of CCCP (Fig. 5C) does not significantly affect the inhibition induced on an odd number of flashes, but accelerates the O_2 uptake on an even number of flashes. On the other hand, inhibition of respiration induced by continuous light is suppressed in the presence of CCCP (2 μM) as already reported [6,8,10,23], except for a transitory signal at the onset and end of illumination (Fig. 5F). Upon addition of tri-*N*-butyl tin (16 μM), an ATPase inhibitor, which slows down the carotenoid bandshift [20], the flash-induced oscil-

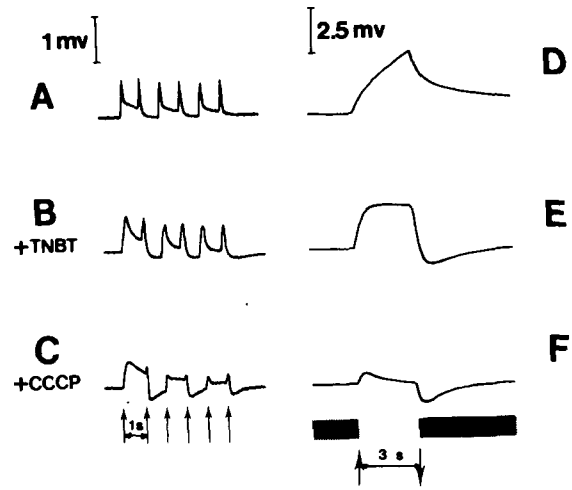

Fig. 5. Amperometric signals observed for *Rps. sphaeroides* cells: control experiment: (A) flash excitation, (D) continuous light excitation; + tri-*N*-butyl tin (16 μM): (B) flash excitation, (E) continuous light excitation; + CCCP (2 μM): (C) flash excitation, (F) continuous light excitation.

latory pattern is very similar to the control experiment (Fig. 5B). However, the shape of the amperometric signal induced by continuous light is significantly affected (Fig. 5E). The steady-state level is reached more rapidly than in the control experiment (compare Fig. 5D and E).

The above experiments are consistent with two distinct modes of inhibition of respiration by light in photosynthetic bacteria.

The first type of inhibition is predominant under flashing light. An electron is diverted on each flash from the respiratory chain to the photooxidized reaction centre. Respiration is then stimulated after an even number of flashes because of the gating mechanism at the level of the seccondary acceptor. Flash-induced spectroscopic data, presented in the following paper [20], are in favour of such a mechanism. The second mode of inhibition is prevalent under continuous illumination. The rate of respiration is then controlled by the photoinduced proton electrochemical gradient [10,23].

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