Biochimica et Biophysica Acta 850 (1986) 256–263
Elsevier

BBA 42037

Two modes of interaction between photosynthetic and respiratory electron chains in whole cells of *Rhodopseudomonas capsulata*

P. Richaud [a], B.L. Marrs [b] and A. Verméglio [a,*]

[a] *A.R.B.S., CEN Cadarache, 13108 Saint-Paul-lèz-Durance, Cedex (France), and*
[b] *Central Research and Development Department, E.I. Du Pont de Nemours and Company, Wilmington, DE 19898 (U.S.A.)*

(Received January 13th, 1986)

Key words: Photoinhibition; Respiration; Cytochrome c_2 oxidase; Alternative oxidase; Electron transport; (*Rps. capsulata*)

(1) The inhibition of respiration by continuous or short flashing light has been studied in whole cells of *Rhodopseudomonas capsulata*, wild type and mutants M_6 and M_7, which lack the alternative and the cytochrome c_2 oxidases, respectively. Continuous illumination inhibits oxygen uptake for the three strains studied. This inhibition is prevented in the presence of carbonyl cyanide *m*-chlorophenyl hydrazone (CCCP) (10 μM). (2) Upon excitation by flashes at low frequency (1 Hz), respiration is inhibited after each flash, but stimulated after even flashes for wild-type and M_6 strains. This oscillatory pattern is not influenced by the presence of CCCP. On the other hand, the strain M_7 exhibits no change in respiratory activity under flash excitation unless the flash frequency is higher than 10 Hz. (3) Addition of both antimycin A and low concentration (0.4–1 μM) of CCCP prevents the light inhibition of respiration, whereas addition of these chemicals separately does not impair the light inhibition in agreement with previous results (Cotton, N.P.J., Clark, A.J. and Jackson, J.B. (1983) Eur. J. Biochem. 130, 581–587). This synergistic effect is, however, observed only in wild-type and M_7 strains. For M_6, addition of both antimycin A and CCCP in a wide range of concentration (1–10 μM) does not impair the inhibition of respiration by light. (4) These results are taken as evidence that two types of light inhibition occur in whole photosynthetic bacteria. The first type, predominant under flashing light, is due to the diversion of electrons from the respiratory chain, at the level of cytochrome c_2, to the photooxidized reaction center. The second type, prevalent under continuous illumination, is a control of respiration by the photoinduced proton electrochemical gradient at the dehydrogenase level. Finally, in presence of antimycin A, CCCP, and light, the respiratory activity is sustained by the alternative oxidase.

Introduction

The Rhodospirillaceae family of photosynthetic bacteria is probably the most flexible group of microorganisms in the world. These bacteria are capable of growing either phototrophically or aerobically in the dark [1]. They can use alternative electron acceptors in anaerobic respiration, such as nitrate [2], or live on substrate fermentation [3,4].

When grown anaerobically in the light, these photosynthetic bacteria develop both respiratory and photosynthetic transport chains operating on the same continuous cytoplasmic membrane. The

* To whom correspondence should be addressed.

Abbreviations: CCCP, carbonyl cyanide *m*-chlorophenylhydrazone, DCIP, dichlorophenolindophenol, TMPD, *N,N,N',N'*-tetramethyl-*p*-phenylenediamine dihydrochloride.

respiratory chain is located mainly on the peripheral part, while the photosynthetic apparatus is associated with intracytoplasmic invaginations [5]. Light is seen to stimulate respiration in membrane fragments isolated from these cells, but in intact cells there is a reversible light-induced inhibition of respiration [6,7]. Both these observations suggest interactions between the respiratory and photosynthetic electron transport systems. Two types of hypothesis, which are not exclusive, have been proposed to explain this light-induced inhibition:

(1) The proton-motive force (Δp) generated by photosynthetic electron transport exerts a thermodynamic control upon the rate of respiration [8,9].

(2) The photosynthetic and respiratory chains share redox components. Upon illumination, oxidation of a common carrier prevents subsequent reaction in the respiratory chain [10,11].

In agreement with this last proposal, we have recently presented evidence that, at least under flashing light, inhibition of respiration is due to channeling of electrons from the respiratory chain towards the photosynthetic reaction center at the level of cytochrome c_2 [12,13].

The situation can be, however, much more complex, since the respiratory chain branches at the level of UQ-*b*/*c* complex into distinct pathways going to different oxidases, a cytochrome c_2 oxidase sensitive to low concentration of KCN ($5 \cdot 10^{-5}$ M) and an alternative oxidase inhibited by high KCN concentration (10^{-3} M) and CO [11,14]. Moreover, Keister and Minton [15] and Cotton et al. [9] have shown that separate addition of antimycin A, an electron transport inhibitor at the UQ-*b*/*c* complex level, and CCCP, an uncoupler, does not impair light inhibition of respiration. On the other hand, concomitant addition of both compounds prevents the inhibition of respiration in the light. This synergistic effect is difficult to reconcile with a scheme where photosynthetic and respiratory chains interact only at the cytochrome c_2 and the UQ-*b*/*c* complex levels.

In the present paper we report on the inhibition by flashing and continuous light of the respiratory activity of two mutants of *Rhodopseudomonas capsulata* M_6 and M_7, deficient respectively in the alternative oxidase and in the cytochrome c_2 oxidase.

Our aim was to determine relative contribution of each mechanism of inhibition to the respiration catalyzed by each of the two types of terminal oxidase.

Materials and Methods

Rps. capsulata strain St. Louis (wild type) and the mutants M_6 and M_7 were grown in the light in degazed Hutner medium. The cells harvested after 24 h, were suspended in either fresh growth medium at pH 7 or in a medium adjusted at pH 7 by addition of H_3PO_4, containing 10 mM Na_2HPO_4/30 mM disodium malate/7 mM ammonium sulfate as described by Cotton et al. [9]. Respiration initiated by injection of 2 mM H_2O_2, was monitored with a Clark oxygen electrode (Rank brothers), thermostated at 25°C. Actinic illumination was provided by a 150 W quartz halogen lamp filtered through 4 cm of water and two Wratten 88 A gelatin film. Fast changes in respiratory activity following a series of flashes were measured as described previously [12] using a home-built platinum electrode. Absorbance changes related to membrane potential induced by illumination and/or oxygenation were measured with an Aminco DW7 spectrophotometer in the split beam mode: 20 μM CCCP was added to the reference cuvette to entirely collapse the membrane potential. Light excitation was provided by the set-up used with the Clark electrode. The maximal light intensity obtained was 140 $J \cdot m^{-2} \cdot s^{-1}$. Reversion of M_6 and M_7 mutants was checked by titration of the respiratory activity in the dark with KCN. Typically for strain M_6 50% of inhibition of whole cell respiration was obtained with $2.5 \cdot 10^{-6}$, while $2.5 \cdot 10^{-4}$ M KCN was necessary for M_7 strain. CCCP and antimycin A, purchased from Sigma, were added in ethanolic solution.

Results and Discussion

Fig. 1 shows the variation of the amperometric signal induced by a series of saturating flashes fired every 1 s for whole cells of *Rps. capsulata* wild type, and the mutants M_6 and M_7 lacking the alternative oxidase and the cytochrome c_2 oxidase, respectively. Since photosynthetic bacteria do not evolve oxygen, the variation of the amperometric signal reflects direct changes in their re-

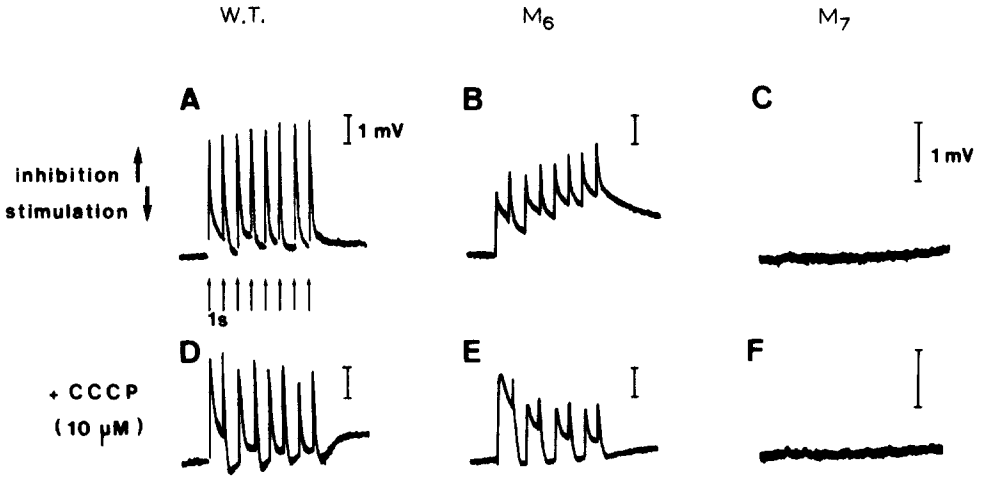

Fig. 1. Fast changes of respiratory activity following a series of actinic flashes fired every second. An increase of the amperometric signal indicates a diminution of the respiratory activity, while a decrease corresponds to a stimulation. Traces A, B, C were obtained for a suspension (3 absorbance at 850 nm) of whole cells of *Rps. capsulata* wild-type M_6 and M_7 strains respectively. Traces D, E, F same as A, B, C but in presence of CCCP (10 μM). W.T., wild type.

spiratory activity. An increase of the amperometric signal, i.e., more O_2 reduce by the electrode, indicates a diminution of the bacterial respiratory activity, while a decrease reflects a stimulation. As already reported [12], the flash-induced amperometric signal shows a remarkable periodicity of two for wild-type cells (Fig. 1A) as a function of flash number: each flash induces an inhibition of respiration, but a stimulation is observed after an even number of flashes. Similar oscillations of the flash-induced amperometric signal are observed for the strain M_6 (Fig. 1B). The oscillatory pattern is even more clear in presence of CCCP (10 μM) for both wild-type and M_6 strains (Fig. 1D and E). For the strain M_7 no signal is observed at all, without (Fig. 1C) or with (Fig. 1F) addition of CCCP. These experiments nicely confirm our previous hypothesis [12,13] that a first type of inhibition of respiration by light is due to the photooxidation of cytochrome c_2. This inhibition is followed by a stimulation of respiration after even flashes because of the operation of the gating mechanism at the level of the secondary acceptor [16,17]. This inhibition is present only in the strains possessing the cytochrome c_2 oxidase, i.e., wild type and M_6, and is not suppressed by addition of CCCP (Fig. 1).

A second type of inhibition, not involving the photooxidation of cytochrome c_2, is demonstrated by the complete arrest of respiratory activity in strain M_7 under continuous illumination (Fig. 2C). This inhibition of respiration can also be obtained upon excitation by flashes at high frequency (20 Hz) as seen by the increase of the amperometric signal of the fast electrode (Fig. 3C). Addition of CCCP (10 μM) completely suppressed this light inhibition (Fig. 2F and Fig. 3F). Similar behaviours are observed for the wild-type (Fig. 2A and D and Fig. 3A and D) and the M_6 (Fig. 2B and E and Fig. 3B and E) strains.

Before discussing its possible origins, we have to comment on the apparent discrepancy between the light inhibition we observed for whole cells of M_7 (Fig. 2C) and the light-stimulated oxygen uptake reported by Zannoni et al. [18] for chromatophore membranes isolated from the same strain. The experiments with whole cells (this work) and chromatophores [18] have been performed under quite distinct conditions: illumination of chromatophores was done in presence of a high concentration ($2 \cdot 10^{-4}$ M) of exogenous electron donor (DCIP), while in the condition of Fig. 2 only a relatively small amount of endogenous electron donors was available in whole cells. In presence of exogenous electron donor (reduced TMPD) a large stimulation of respiration is ob-

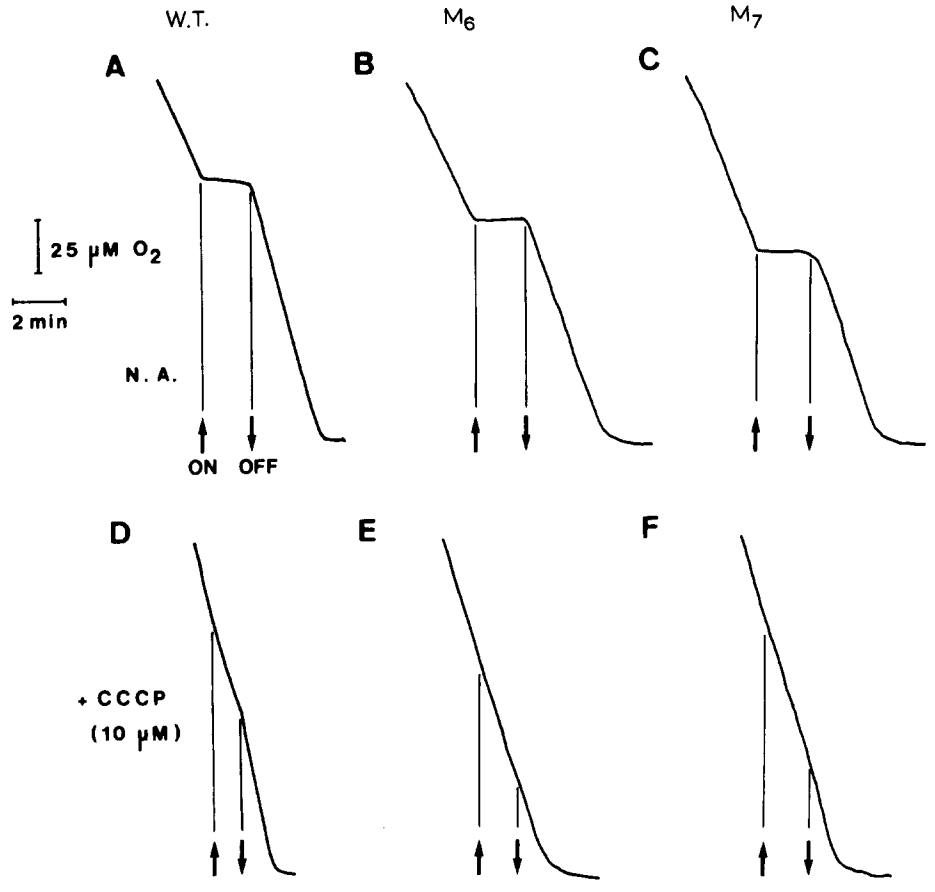

Fig. 2. Respiratory activity recorded with a Clark electrode in the dark or during continuous illumination of a suspension (3.0 absorbance at 850 nm) of whole cells of *Rps. capsulata* wild type (A), M_6 (B) and M_7 (C) – D, E, F same as A, B, C but in presence of CCCP (10 μM). The upwards arrows indicate the switching on of the excitation light while the downwards arrows corresponds to its cessation. W.T., wild type.

served upon illumination of whole cells (Fig. 4) in complete agreement with the results obtained with chromatophores [18]. At the cessation of illumination, the respiratory activity is first inhibited for approx. 90 s (Fig. 4) and then reaches a level twice as high as before light action. The period of inhibition of respiratory activity observed at the end of illumination corresponds to the time necessary to reduce the photooxidized TMPD (not shown). Following the scheme proposed by Zannoni et al. [18], these experiments can be easily rationalized by supposing that the quinone pool, largely reduced under continuous illumination at the expense of TMPD, serves as electron donor for the alternative oxidase. This implies that photosynthetic and respiratory electron chains can interact at the quinone level. Moreover, the fact that the photooxidized TMPD has to be re-reduced before the respiratory activity is restored (Fig. 4) suggests that electrons flow from substrates by-passes the alternative oxidase under these conditions. In other terms, reduced quinones have a much better affinity for the oxidized TMPD (or the UQ-*b*/*c* complex) than for the alternative oxidase.

The complete inhibition of respiratory activity observed under continuous illumination (Fig. 2) is certainly, at least for the strain M_7, mediated by the photoinduced $\Delta\psi$ as proposed by McCarthy and Ferguson [8] and Cotton et al. [9], since this inhibition is prevented in the presence of CCCP (Fig. 2D, E and F) and needs a high flash frequency (Fig. 3C). We propose that the membrane poten-

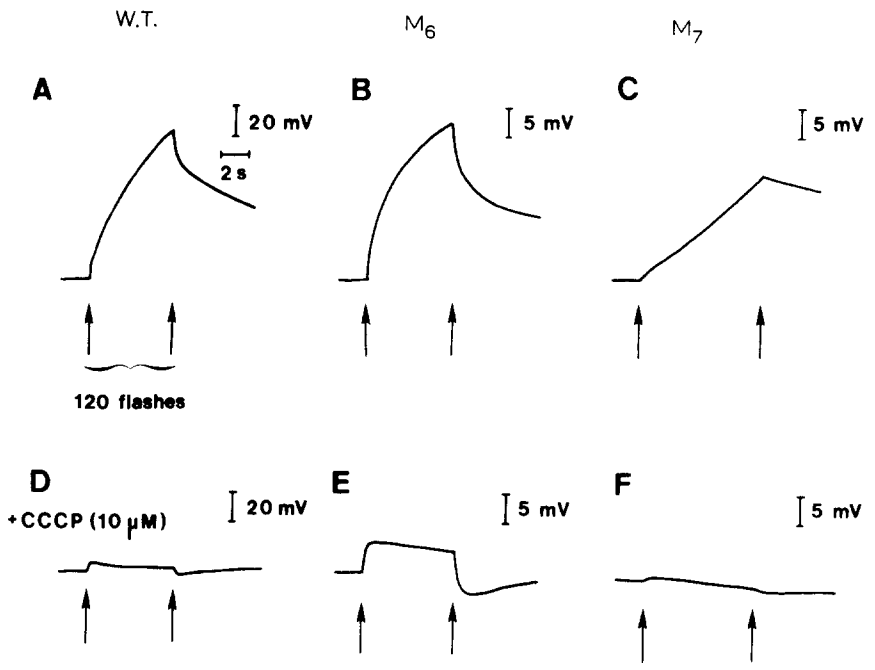

Fig. 3. Same as Fig. 1 but the flash frequency was set at 20 Hz. W.T., wild type.

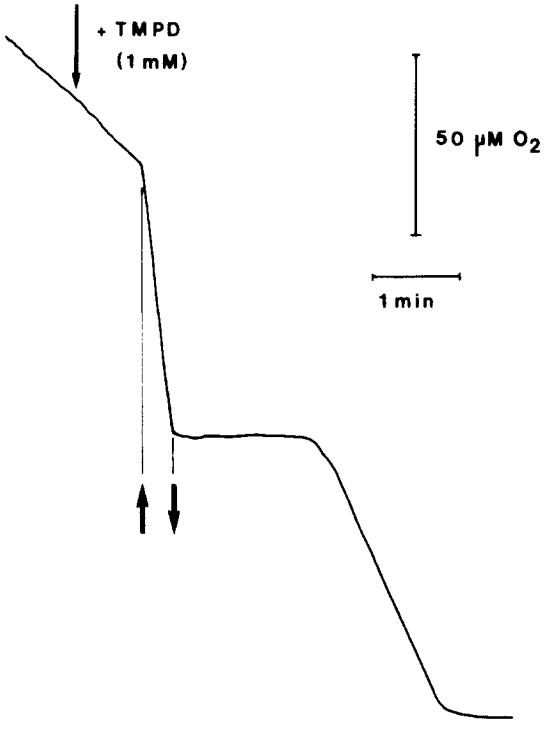

Fig. 4. Effect of a continuous illumination on the respiratory activity of mutant M_7 in presence of 1 mM reduced TMPD. Otherwise conditions as in Fig. 2.

tial exerts its control on the first two coupling regions of the respiratory chain. A direct action of the membrane potential on the alternative oxidase can be ruled out, since its activity can be stimulated by light, i.e., in the presence of high membrane potential if substrate is not a limitation (Fig. 4). The situation in wild-type and M_6 strains is expected to be quite complex, since both types of inhibition, i.e., modulation of the respiratory activity by the redox state of cytochrome c_2 and by the membrane potential, could occur under continuous illumination (Fig. 2A, B). Additions of chemicals inhibiting either the electron transfer (antimycin A for example) or the built up of the membrane potential (uncouplers like CCCP) have been used by several authors to resolve that problem. In particular, Keister and Minton [15] and more recently Cotton et al. [9] described a synergistic effect of addition of antimycin A and CCCP in preventing the light-inhibition of respiration of whole cells. Addition of antimycin A at concentration high enough (5–10 μM) to inhibit photosynthetic electron transport has no (or little) effect on the respiration of intact bacteria or on its inhibition by light. Addition of the uncoupler CCCP at

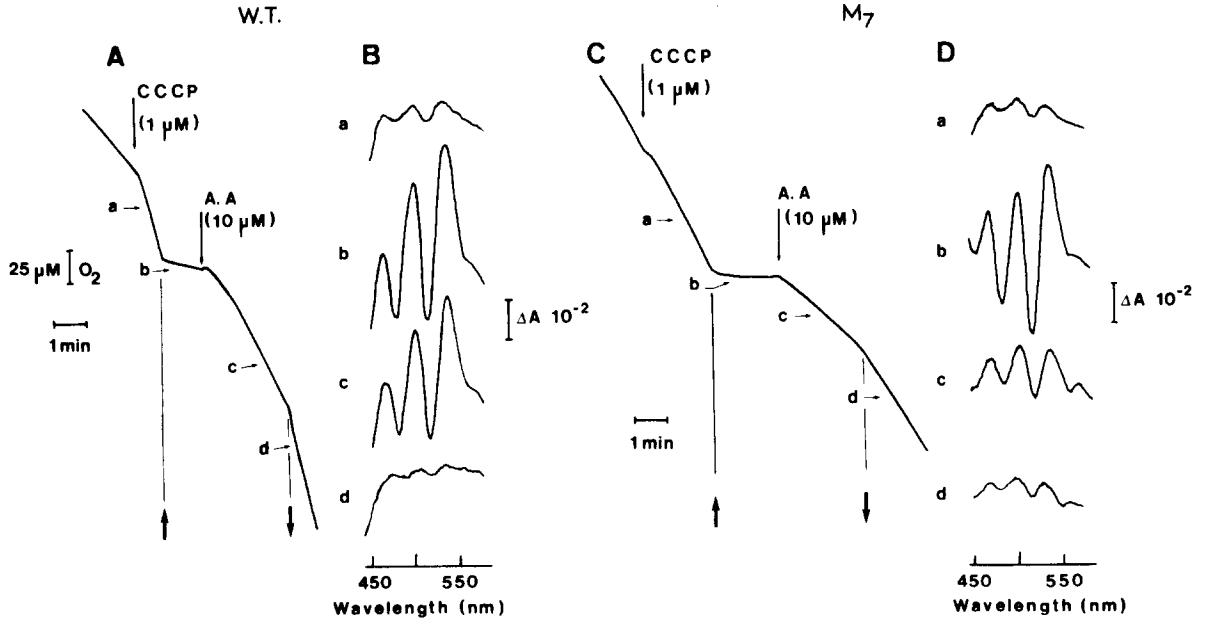

Fig. 5 (A) Respiratory activity recorded as in Fig. 2, for a suspension of whole cells of *Rps. capsulata* wild type (W.T.) subjected to successive different additions as indicated by the arrows: (a) dark in presence of CCCP (1 μM); (b) light in presence of CCCP (1 μM); (c) light in presenceo f both CCCP (1 μM) and antimycin A (20 μM) (A.A); (d) dark in the presence of these two compounds. (B) Difference absorption spectra, corresponding mainly to the carotenoid band shift, between a suspension of whole bacteria subjected to the different conditions (a, b, c, d) of the oxygraph trace (part A) and a suspension where the membrane potential has been collapsed by addition of 20 μM CCCP. (C) and (D), as A and B, respectively but for strain M_7.

low concentration (0.1–1 μM) slightly stimulates respiration in the dark (Fig. 5Aa), but does not impair its photoinhibition (Fig. 5Ab). When added together, these compounds have a synergistic effect in preventing the inhibition of respiration by light [9] (Fig. 5Ac, Cc). Cotton et al. [9], measuring the carotenoid band shift as index of the membrane potential, show a good correlation between its extent and the inhibition of respiration by light, i.e., the presence of antimycin A or CCCP (Fig. 5Bb and Db) only slightly lowered the total extent of the membrane potential in the light, while addition of both chemicals depressed the light-induced membrane potential of an aerobic suspension (Fig. 5Bc and Dc). These authors therefore concluded that the light inhibition of respiration is mediated by the proton-motive force. Indeed, this synergistic effect seems to rule out an important contribution of direct interaction between photosynthetic and respiratory chains at the cytochrome c_2 level. However, the respiratory activity in presence of CCCP and antimycin A could be sustained by the alternative oxidase, and therefore would not be influenced by the photooxidation of cytochrome c_2. To check that hypothesis we have studied the synergistic effect of antimycin A and CCCP for M_6 and M_7 strains. Fig. 5 (A, C) clearly shows the synergistic effect upon addition of antimycin A and CCCP for the strains wild type and M_7. This effect does not depend on the order of addition of CCCP and antimycin A. The photoinduced carotenoid band shift (Fig. 5Bc, Dc) is reduced in presence of both chemicals in agreement with the results of Cotton et al. [9]. For strain M_6, addition of both antimycin A and CCCP does not prevent inhibition of respiration by light (Fig. 6Ac). Even in the presence of a concentration of CCCP high enough (10 μM) to prevent completely inhibition of respiration by light (Fig. 6Cc) and the formation of the light-induced membrane potential (Fig. 6Dc), addition of antimycin A restores the light inhibition of respiration.

These experiments clearly demonstrate that only

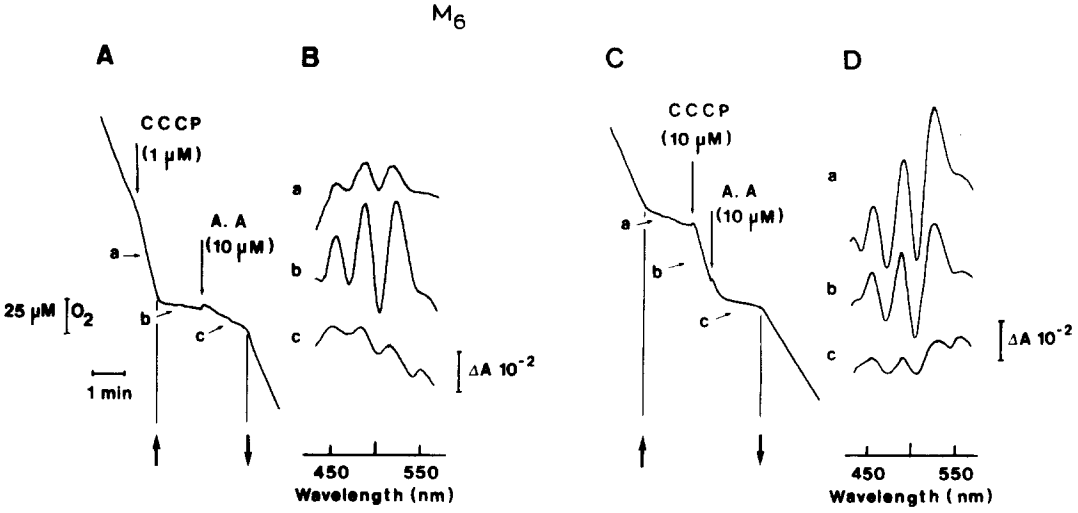

Fig. 6. Same as Fig. 5 but for strain M_6 and two concentrations of CCCP: (A), (B) 1 μM; (C), (D), 10 μM.

the alternative oxidase is working in the light after addition of antimycin A and CCCP. Moreover, when only the cytochrome c_2 oxidase is present, the respiration can be totally inhibited by the photooxidation of cytochrome c_2.

Conclusions

From the above experiments, we conclude that two different modes of interactions between respiratory and photosynthetic electron chains occur. First, even if fractionation studies have indicated that respiratory enzymes are mainly located in the cytoplasmic region of the membrane while photosynthetic components are in the intracytoplasmic part [5], there is overlap of redox components: soluble cytochrome c_2 and quinones. Participation of soluble cytochrome c_2 in both transport chains is clearly demonstrated by the modulation of the respiratory activity upon flash excitation for wild type and M_6 cells (Fig. 1) in agreement with other lines of evidence [19,20]. Respiratory and photosynthetic chains branche also at the quinone level as shown by the light stimulation of oxygen uptake in presence of exogenous electron donors for strain M_7 (Fig. 4). A second type of interaction is due to the thermodynamic control exerted by the photoinduced membrane potential on the electron transfer at the first two coupling sites of the respiratory chain. As proposed by Cotton et al. [9], this last control is particularly clear upon addition of both antimycin A and CCCP, which strongly lowers the membrane potential and prevents the light-inhibition. The observation of this synergistic effect for only the wild type and M_7 strains, demonstrates that the respiratory activity is sustained by the alternative oxidase under continuous illumination in the presence of antimycin A and CCCP. The cytochrome c_2 oxidase activity can be completely inhibited by continuous illumination if rereduction of cytochrome c_2 is slown down by addition of antimycin A even if the membrane potential has been completely suppressed by addition of high concentration of CCCP.

References

1 Baccarini-Melandri, A. and Zannoni, D. (1978) J. Bioenerg. Biomembranes 10, 109–138
2 Katoh, T. (1963) Plant Cell Physiol. 4, 199–215
3 Uffen, R.L. and Wolfe, R.S. (1970) J. Bacteriol. 104, 462–472
4 Yen, H.C. and Marrs, B. (1977) Arch. Biochem. Biophys. 181, 411–418
5 Lampe, H.H. and Drews, G. (1972) Arch. Microbiol. 84, 1–19
6 Van Niel, C.B. (1941) Adv. Enzymol. 1, 263–328
7 Nakamura, H. (1937) Acta Phytochim. 9, 189–234
8 McCarthy, J.E.G. and Ferguson, S.J. (1982) Biochem. Biophys. Res. Comm. 107, 1406–1411
9 Cotton, N.P.J., Clark, A.J. and Jackson, J.B. (1983) Eur. J. Biochem. 130, 581–587

10 Marrs, B. and Gest, H. (1973) J. Bacteriol. 114, 1045–1051
11 Zannoni, D., Melandri, B.A. and Baccarini-Melandri, A. (1976) Biochim. Biophys. Acta 423, 413–430
12 Verméglio, A. and Carrier, J.M. (1984) Biochim. Biophys. Acta 764, 233–238
13 Verméglio, A. and Joliot, P. (1984) Biochim. Biophys. Acta 764, 226–232
14 La Monica, R.F. and Marrs, B.L. (1976) Biochim. Biophys. Acta 423, 431–439
15 Keister, D.L. and Minton, M.J. (1971) in Energy Transduction in Respiration and Photosynthesis (Quagliariello, D., Papa, S. and Rossi, C.S., eds.), pp. 375–384, Adriatica Editrice, Bari
16 Verméglio, A. (1977) Biochim. Biophys. Acta 459, 516–524
17 Wraight, A. (1977) Biochim. Biophys. Acta 459, 525–531
18 Zannoni, D., Jasper, P. and Marrs, B. (1978) Arch. Biochem. Biophys. 191, 625–631
19 Baccarini-Melandri, A., Jones, O.T.G. and Hauska, G. (1978) FEBS Lett. 86, 151–154
20 Zannoni, D., Prince, R.C., Dutton, P.L. and Marrs, B.L. (1980) FEBS Lett. 113, 289–293