Biochimica et Biophysica Acta, 764 (1984) 226–232
Elsevier

BBA 41459

LIGHT-INDUCED ABSORPTION CHANGES IN INTACT CELLS OF *RHODOPSEUDOMONAS SPHAEROIDES*

EVIDENCE FOR INTERACTION BETWEEN PHOTOSYNTHETIC AND RESPIRATORY ELECTRON TRANSFER CHAINS

A. VERMEGLIO [a] and P. JOLIOT [b]

[a] *Association pour la Recherche en Bioénergie Solaire, Centre d'Etudes Nucléaires de Cadarache, B.P. 1, 13115 St.-Paul-Lez-Durance and* [b] *Equipe de Recherche No. 16 du Centre National de la Recherche Scientifique, Institut de Biologie Physico-chimique, 13, Rue P. et M. Curie, 75005 Paris (France)*

(Received June 27th, 1983)
(Revised manuscript received October 5th, 1983)

Key words: Respiration; Bacterial photosynthesis; Carotenoid bandshift; (Rps. Sphaeroides)

Absorption changes, following a series of actinic flashes, linked to oxidoreduction states of ubiquinone, cytochrome c_t together with the carotenoid bandshift, have been measured for intact cells of *Rhodopseudomonas sphaeroides* under aerobic conditions. Binary oscillations are observed for these different contributions: (1) about one molecule of ubisemiquinone and fully reduced quinone are formed on odd and even flashes, respectively; (2) cytochrome c_t re-reduction is faster ($t_{1/2} \approx 50$ ms) after an even number of flashes than after an odd number; ($t_{1/2} \approx 100$ ms); (3) a slow-rising phase ($t_{1/2} \approx 5$ ms, antimycin A-insensitive) of the carotenoid bandshift is observed after each even flash. These results are compared to the respiratory activity of the cells under flash excitation and discussed in relation to a model, in which respiratory and photosynthetic electron chains interact at the level of cytochrome c_2 and where the terminal oxidase is supposed to have electrogenic properties.

Introduction

Several species of photosynthetic bacteria, in particular the non-sulphur group (*Rhodospirillaceae*), can grow at the expense of energy, either from light or from oxidoreduction reactions of substrates by O_2 [1]. In light-grown cells, the photosynthetic and respiratory electron transfer chains appear to be present in the same membrane [2]. Several examples of similarities and interactions between the photosynthetic and respiratory apparatus can be found in the literature. An important part of the electron transport chain from ubiquinone 10 [3] to cytochrome c_2 [4] shows great likeness in their chemical, thermodynamic and immunological properties for membranes obtained either from photosynthetically- or aerobically-grown cells. One of the most clear-cut pieces of evidence of interaction is the reversible light-induced inhibition of respiration of light-grown intact cells [5,6].

In the preceding paper [7], we show, by an amperometric method, that whole cell respiration can be modulated by light, i.e., inhibited on every odd flash and stimulated after an even number of flashes. All these approaches suggest that the same electron carriers can be shared by respiratory and

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

photosynthetic chains [3,8,9]. However, no kinetic data have been reported so far to demonstrate this type of interaction. One main reason is that almost all flash-induced absorbance change measurements have been done with chromatophores, organelles which present a very low rate of respiration. The few experiments dealing with intact photosynthetic bacteria were performed mainly under anaerobic conditions [10,11].

In the present paper, we have investigated the relationship between photosynthetic and respiratory components by absorption changes measurements on *Rhodopseudomonas sphaeroides* 2-4-1 whole cells under respirating conditions. The characteristic oscillatory pattern detected by the amperometric method makes it possible to correlate respiratory activity and oxido-reduction state of different electron carriers. Moreover, the possibility to inhibit or reactivate respiratory electron flow in a series of flashes, together with the presence of an intrinsic probe of the membrane potential in the photosynthetic bacteria, namely the carotenoid pigments, give us the opportunity to investigate the possible electrogenic properties of the terminal oxidase.

Materials and Methods

Rps. sphaeroides strain 2-4-1, grown in degassed Hutner medium at 30°C, were harvested after 24 h. To reduce light-scattering and sedimentation, the bacteria were suspended in fresh-growing medium with 7% w/w Ficoll. Absorption changes were measured as previously described [12]. The cells were kept in the dark at least 15 min before measurements. An air flow was gently bubbled in the reservoir to ensure aerobic conditions. Excitation was provided by a Ruby laser (G.K.). Dark time between actinic flashes was 20 s. CCCP, purchased from Sigma, and tri-*N*-butyl tin, from Aldrich, were added in ethanolic solution.

Results

Light-induced difference spectra in intact cells of Rps. sphaeroides under aerobic conditions

Fig. 1A and B shows light-induced absorption changes occurring on flash numbers 1 and 2, respectively, for *Rps. sphaeroides* intact cells under aerobic conditions. The amplitude of the photoinduced absorption changes, for wavelengths between 440 and 570 nm, were measured at different times (10 μs, 4 ms, 70 ms and 1 s) after the actinic flashes. The main features of these difference absorption spectra have already been observed in chromatophores and correspond to: (i) the carotenoid bandshift with its characteristic contributions, positive at 525, 490 and 460 nm and nega-

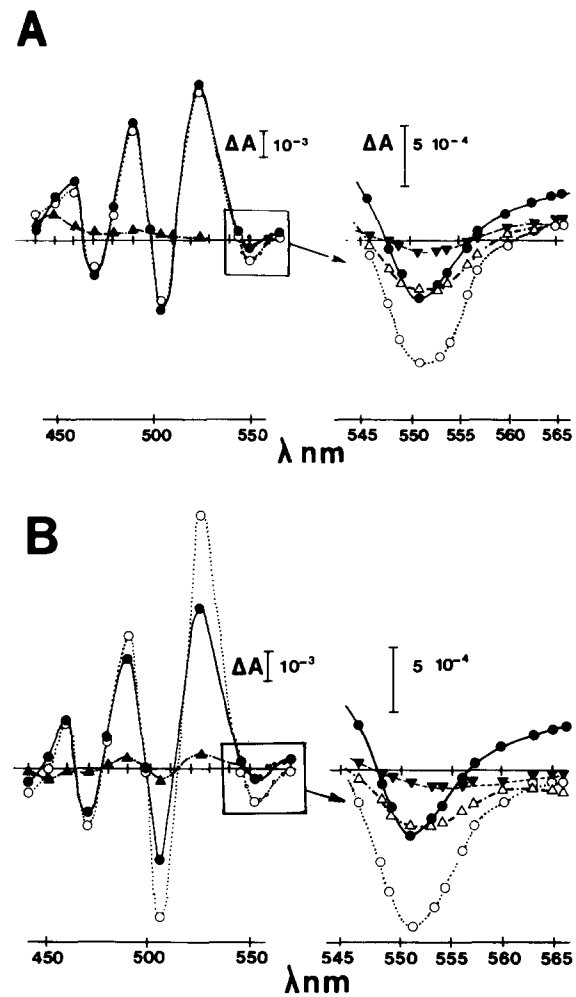

Fig. 1. (A) Flash-induced difference spectra measured between 440 and 570 nm for *Rps. sphaeroides* cells suspended in 7% w/w Ficoll under aerobic conditions at different times after the actinic flash number 1 (● 100 μs, ○ 10 ms, △ 70 ms, ▼ 150 ms, ▲ 1 s). BChl concentration approx. 10 μM. (B) Same as (A), but for flash number 2. Dark time between the two actinic flashes, 20 s. The 545–570 nm region has been enlarged for a better appreciation of the absorption changes related to cytochromes.

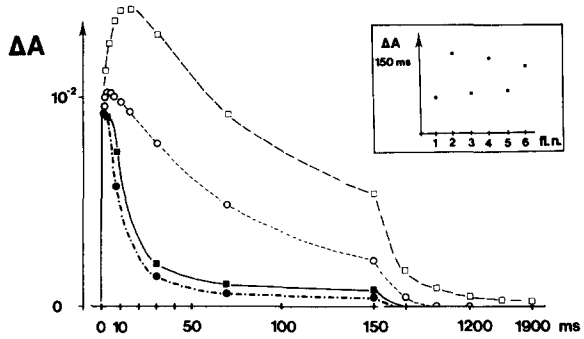

Fig. 2. Kinetics of the carotenoid bandshift measured at 525–507.5 nm under aerobic conditions. No addition: flash number 1 ●, flash number 2 ■; + tri-*N*-butyl tin (16 μM): flash number 1 ○, flash number 2 □. Other conditions, as in Fig. 1. The inset shows the amplitude of the signal measured at 150 ms in function of the actinic flash number.

tive at 507 and 470 nm; (ii) changes in the oxidoreduction state of cytochromes in the 540–570 nm region (see inset to Fig. 1A and B); and (iii) the formation of ubisemiquinone around 450 nm.

A more precise analysis of the kinetics of the absorption changes, measured at a few selected wavelengths, typical for the contributions mentioned above, is described in the following paragraphs. From the difference spectra depicted in Fig. 1, it should already be noted that changes,

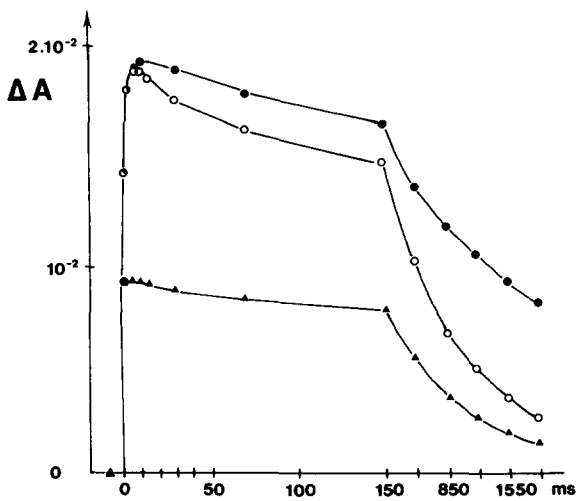

Fig. 3. As for Fig. 2 but in presence of KCN ($5 \cdot 10^{-4}$ M). No addition, except for KCN: ●; + tri-*N*-butyl tin (16 μM): ○; + tri-*N*-butyl tin (16 μM) and antimycin A (2 μM): ▲.

stable for at least 1 s after the first actinic flash and centred at 450 nm, are characteristic of the ubisemiquinone species [13].

Kinetics of the carotenoid banshift under aerobic and anaerobic conditions

The kinetics of the carotenoid bandshift, measured at 525–507.5 nm after flash numbers 1 and 2, under aerobic conditions are shown in Fig. 2. As already noted by Cotton and Jackson [11], a slow rising phase is only observed in presence of KCN ($5 \cdot 10^{-4}$ M) (fig. 3) or under anaerobic conditions (not shown). Moreover the decay is much faster under aerobic conditions than in presence of KCN. The carotenoid bandshift decay is, in part, due to inwards protons translocation through the ATPase complex during ATP synthesis. To slow down this relaxation process, phosphorylation reactions have to be inhibited. We used tri-*N*-butyl tin, a well-known inhibitor of phosphorylations in chloroplasts [14] and mitochondria [15]. Using luciferin luciferase assay, we have shown that ATP synthesis, induced either by light or by oxidative phosphorylation, is also inhibited in whole cells of *Rps. sphaeroides* if tri-*N*-butyl tin has been in contact with the cells in presence of O_2. (Vermeglio, A., Girault, G. and Galmiche, J.M., in preparation). Tri-*N*-butyl tin (16 μM) after 1 h of incubation appears to have two distinct effects on the carotenoid bandshift.

(1) Under aerobic conditions, the carotenoid bandshift decay is severely decelerated compared to the control experiment (Fig. 2). This allows the detection, on an even number of flashes, of a slow rising phase ($t_{1/2} \approx 5$ ms). Binary oscillations in the amplitude of the carotenoid bandshift, larger on every even than odd number of flashes, can be detected up to 1 s. This phenomenon is also observed in the control experiment, but in a much less marked fashion (Fig. 2).

(2) Under anaerobic conditions, or in presence of KCN (Fig. 3), addition of tri-*N*-butyl tin slightly accelerates the carotenoid bandshift decay.

We conclude from these experiments that tri-*N*-butyl tin can act as inhibitor of the ATPase, as has been by the deceleration of the carotenoid bandshift (Fig. 2), and also as a slight uncoupling agent (Fig. 3). Contrary to what has been observed in isolated chromatophores [16,19], antimycin A (2

μM) has no effect on the slow phase observed on an even number of flashes under aerobic conditions (not shown). The slow phase observed in presence of KCN (Fig. 3) is, however, completely suppressed by antimycin A (2 μM), as already reported [11].

Kinetics of photoinduced oxido reduction changes of quinones and cytochromes

The kinetics measured at 450 nm (Fig. 4) clearly show that a stable semiquinone is formed on flash number 1 in whole cells under aerobic conditions, in absence or presence of tri-*N*-butyl tin. On flash number 2, the absorption decrease reflects the formation of fully reduced ubiquinone. Binary oscillations can be observed up to six flashes (Fig. 4B). Using an ϵ of 8.5 $mM^{-1} \cdot cm^{-1}$ for the semiquinone species at 450 nm [20] and of 19 $mM^{-1} \cdot cm^{-1}$ for cytochrome c_t at 551–545 nm [21] and taking into account that about 60% of the total photooxidizable cytochrome c_t is detected on the first flash in oxidizing conditions (see Fig. 1D in Ref. 21), we estimate that 0.9 electron is stabilized on the secondary acceptor, Q_B, after flash 1.

Cytochrome $c_t(c_2 + c_b)$ photooxidation measured at 551–545 nm is completed in less than 200 μs in control experiment (Fig. 5). The half-time of re-reduction in the dark oscillates with a periodicity of two, being of 25 and 15 ms on an odd and even number of flashes, respectively. Upon addition of tri-*N*-butyl tin, the photooxidation takes about 5 ms. The re-reduction half-time is also slowed down (Fig. 5) but oscillations are still

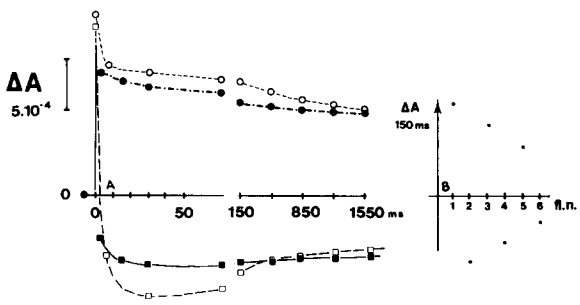

Fig. 4. Kinetics measured at 450 nm in absence (flash number 1 ●; flash number 2 ■) or in presence of tri-*N*-butyl tin (16 μM) (flash number 1, ○; flash number 2, □). Other conditions as in Fig. 1. The inset shows the amplitude of the signal measured at 150 ms after a different number of actinic flashes.

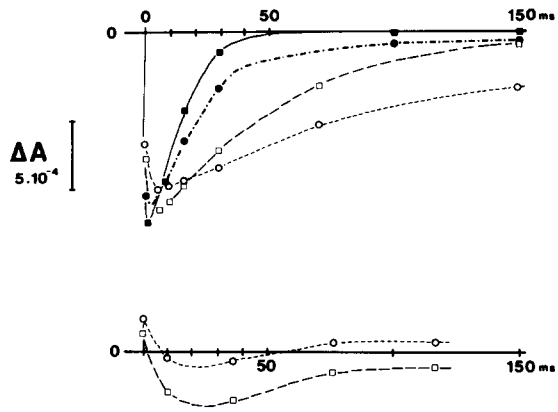

Fig. 5. Upper part: kinetics of cytochrome c_t measured at 551–545 nm. No addition: flash number 1 ●, flash number 2 ■; + tri-*N*-butyl tin (16 μM): flash number 1, ○; flash number 2, □. Lower part: kinetics measured at 560 nm in presence of tri-*N*-butyl tin (16 μM), occurring on flash number 1, ○, and flash number 2, □.

observed. Fig. 5 shows also the kinetics measured on flashes 1 and 2 at 560 nm, characteristic of cytochromes *b*.

Discussion

The two electron gate mechanism

It is now well established that a two-electron gate mechanism is operating on the acceptor side of isolated reaction centres [23,24]. In chromatophores, the situation is more complex. Full oscillatory behaviour, when low concentration of mediators are used (less than 10 μM), has only been observed [25,26] at redox potentials (greater than 300 mV) much higher than the $E_{m,7}$ (+100 mV) measured by Rutherford and Evans [27] for the $Q_B/Q_BH^{\cdot}$ couple. At higher concentration of redox mediators (approx. 100 μM), the oscillatory pattern [28,29] can be observed for E_h between 100 and 300 mV. Crofts et al. [30] suggest to explain these anomalies that Q_B^- can exist out of equilibrium with the chain at low concentration of mediators. O'Keefe et al. [26], on the other hand, propose to explain the appearance of Q_B oscillation at $E_h > 300$ mV by electrostatic interaction between the photooxidized primary donor $BChl_2^+$, stable for hundreds of milliseconds at this E_h, and Q_B^- preventing its subsequent oxidation or protonation.

In whole cells of *R. rubrum* under aerobic conditions, De Grooth et al. [29] measured full oscillation.

In the present paper, we show that oscillations at the level of the secondary acceptor can also be observed in *Rps. sphaeroides* whole cells if aerobic conditions are ensured. Almost one equivalent electron is stabilized on Q_B after flash 1. The large oscillations observed for Q_B^- formation cannot be explained by the proposition of O'Keefe et al. [26], since under our conditions $BChl_2^+$ is re-reduced in less than 5 ms (not shown). On the other hand, the high respiratory activity of the whole cells in presence of O_2 keeps the quinone pool in the oxidized state in the dark. The lack of high oxidase activity in isolated chromatophores prevents reoxidation of the quinone pool or of the semi-reduced secondary acceptor. Only when oxidized mediators at high concentration can act as terminal acceptor is the quinone pool reoxidized and oscillations observed. It is worth noting that, in agreement with that explanation, the quinone oscillations disappear with an apparent E_m, very similar to the E_m (420 mV) of the ferri/ferrocyanide couple [26] or to the E_m (240 mV) of TMPD [29] when these two compounds were respectively by far the more concentrated mediators.

Interaction between respiratory and photosynthetic electron transfer chains

The main absorbance changes observed in the cytochrome region (Fig. 1) are related to cytochrome $c_t(c_2 + c_b)$, although small changes due to cytochromes *b* are also observed (Fig. 1 and 5). As already stated, about one electron is stabilized for seconds on the secondary acceptor Q_B after each odd number of flashes. The photooxidized cytochrome c_t is, however, rereduced with a half-time of about 100 ms in presence of tri-*N*-butyl tin. On an even numbers of flashes the half-time of re-reduction is shorter ($t_{1/2} \approx 50$ ms). These results imply that on an odd number of flashes cytochrome c_t is not rereduced via a cyclic electron pathway but via a substrate-linked electron transfer. Moreover, our amperometric studies [7] suggest that each odd number of flashes inhibits the respiration, but that respiratory activity is stimulated after an even number of flashes. These two lines of data, amperometric and spectrophotometric, can be qualitatively rationalized in a scheme in which respiratory and photosynthetic electron transfer chains interact at the level ubiquinone-cytochrome b/c_b oxido/reductases (UQ-b/c_b) (Fig. 6). In the dark, a continuous flow of electrons is going from succinate to the terminal oxidase through the UQ-b/c_b complex and cytochrome c_2. After each odd number of flashes reduced cytochrome c_2 reacts preferentially with $BChl_2^+$ formed on the flash rather than with the oxidase. The switching of one electron to the $BChl_2^+$ produces the lack of one electron for the reduction of O_2 by the oxidase, in other words inhibition of respiration. After an even number of flashes, and because of the gating mechanism at the level of the secondary acceptor, two electrons are injected into the UQ-b/c_b complex and are therefore available for the oxidase, stimulating the respiratory activity. At least two factors prevent a more quantitative comparison of our amperometric and spectroscopic data: first of all , it is difficult from our O_2 measurements to estimate whether all respiratory chains or only some of them are inactivated on odd flashes. Secondly, the kinetics of the amperometric signal, even under conditions where the electrode O_2 consumption is kept minimal, depend not only on the respiratory activity of the cells, but also on the rate of reequilibration of the O_2 concentration in the liquid layer.

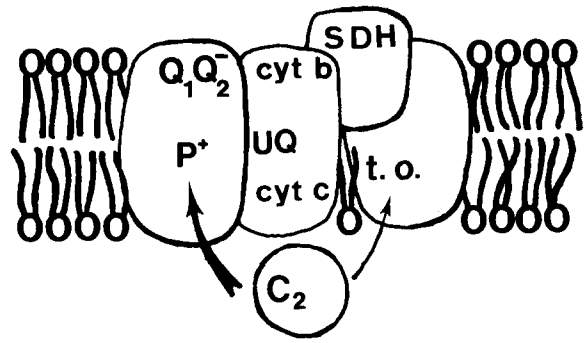

Fig. 6. Proposed scheme for interaction between photosynthetic and respiratory electron-transfer chains. The cytochrome c_2 reacts with the terminal oxidase (t.o.) or with, and preferentially, the photooxidized primary donor, P^+. This type of interaction does not imply a close proximity of the complexes (reaction centre, UQ-b/c and t.o.), since the cytochrome c_2 is a soluble protein.

The electrogenic properties of the terminal oxidase

The slow-rising antimycin-A-sensitive phase of the carotenoid bandshift observed on even numbers of flashes at moderated redox potentials [29] or on each flash for potentials below +100 mV in isolated chromatophores [16,19] is usually interpreted as due to an electrogenic electron transport through the UQ-b/c_b complex and as evidence of a Q-cycle mechanism. The antimycin-A-insensitivity we observed for the slow phase under respiring conditions suggests that extension of this hypothesis to whole cells is not straightforward. One cannot invoke a lack of penetration of antimycin A in whole cells, since this compound suppresses the slow phase under reducing conditions (Ref. 11 and Fig. 3). A redox dependency of the action of antimycin A, active only at low redox potential, seems also to be excluded, since in chromatophores antimycin A effectively inhibits the slow phase for E_h between −100 and +300 mV (Refs. 19 and 29, see, however, Ref. 18). An 'ad hoc' hypothesis has, therefore, to be found to explain the antimycin-A-insensitivity of the slow phase under respiring conditions in whole cells in the framework of a Q-cycle mechanism. On the other hand, following the model (Fig. 6) that we developed in the preceding paragraph, a membrane potential will be induced on even flashes by the extra electron available for the terminal oxidase, if this protein has an electrogenic character, as proposed for the mitochondrial cytochrome oxidase [31]. After correction for the fast decay of the carotenoid bandshift, we estimate that the amplitude of the slow-rising phase on an even number of flashes is nearly equal to the very fast phase (see Fig. 2). This implies that only one charge is translocated per electron at the level of the terminated oxidase. Consistent with this is the lack of proton-pumping activity demonstrated in the recent work of Gennis et al. [33] on purified cytochrome oxidase from *Rps. sphaeroides* incorporated into phospholipid liposomes.

The hypothesis that the terminal oxidase has electrogenic properties can also explain the rapid decay observed on odd numbers of flashes, even in the presence of tri-*N*-butyl tin, for the carotenoid bandshift in whole cells under respiring conditions (Fig. 2). In the dark, a high membrane potential due to the respiration is present. We estimate, by double beam spectroscopy, this membrane potential to be 3–4-times larger than the one induced by one actinic flash under aerobic conditions. The total membrane potential reached just after each odd flash is therefore 4–5-times larger than the flash-induced one. Supposing that all respiratory chains are inactivated on odd flashes and that the rate of the carotenoid bandshift decay depends linearly on its amplitude *, the initial slope of the decay of the carotenoid bandshift will be 4–5-times steeper on odd flashes and under aerobic conditions, than for example, in the presence of KCN + antimycin A, where there is no background membrane potential, in agreement with our observations (compare Figs. 2 and 3). Within the time of re-reduction of cytochrome c_t (approx. 100 ms), respiration is restored and the membrane potential reaches its original dark level.

From the above discussion, we propose that the terminal oxidase in *Rps. sphaeroides* cells has electrogenic properties. The fast decay and the slow rising phase of the carotenoid bandshift, observed under respiring conditions on odd and even number of flashes, respectively, are then related to the photoinhibition and photostimulation of respiration, observed in a series of flashes. Having related the fast decay of the membrane potential in whole cells to photoinhibition of respiration, the much slower decay observed in chromatophores [34] has to be related to the low respiratory activity of these organelles.

* We rule out a non-linear dependence of the rate of the carotenoid bandshift decay on its amplitude, because decreasing the magnitude of the light-induced membrane potential, by a factor of 8, by varying the flash intensity, did not significantly affect the rate constant of its decay in presence of O_2 and tri-*N*-butyl tin.

References

1 Baccarini Melandri, A. and Zannoni, D. (1978) J. Bioenerg. and Biomembranes 10, 109–138

2 Remsen, C.C. (1978) in The Photosynthetic Bacteria (Clayton, R.K. and Sistrom, W.R., ed.), pp. 31–60, Plenum Press, New York

3 Zannoni, D., Melanozo, B.A. and Baccarini Melandri, A. (1976) Biochim. Biophys. Acta 423, 413–430

4 Baccarini Melandri, A., Jones, O.T.G. and Hauska, G. (1978) FEBS Lett 86, 151–154

5 Van Niel, C.B. (1941) Adv. Enzymol. 1, 263–328
6 Nakamura, H. (1937) Acta Phytochim. 9, 189–234
7 Vermeglio, A. and Carrier, J.M. (1984) Biochim. Biophys. Acta 764, 233–238
8 Keister, D.L. (1978) in The Photosynthetic Bacteria (Clayton, R.K. and Sistrom, W.R., eds.), pp. 849–856, Plenum Press, New York
9 La Monica, R.F. and Marrs, B. (1976) Biochim. Biophys. Acta 423, 431–439
10 Van Grondelle, R., Duysens, L.N.M. and Van der Wal, H.N. (1976) Biochim. Biophys. Acta 449, 169–187
11 Cotton, N.P.J. and Jackson, J.B. (1982) Biochim. Biophys. Acta 679, 138–145
12 Joliot, P., Beal, D. and Frilley, B. (1980) J. Chim. Phys. 77, 209–216
13 Bensasson, R. and Land, E.J. (1973) Biochim. Biophys. Acta 325, 175–181
14 Kahn, J.S. (1968) Biochim. Biophys. Acta 153, 207–210
15 Rose, M.S. and Aldridge, W.N. (1972) Biochem. J. 127, 51–59
16 Jackson, J.B. and Dutton, P.L. (1973) Biochim. Biophys. Acta 325, 102–113
17 Crofts, A.R., Crowther, D. and Tierney, G.V. (1975) in Electron Transfer Chains and Oxidative Phosphorylation (Quagliariello, E., Papa, S., Palmieri, F., Slater, E.C. and Siliprandi, N., eds.), p. 233–241, North-Holland, Amsterdam
18 Bowyer, J.L. and Crofts, A.R. (1981) Biochim. Biophys. Acta 636, 218–233
19 Matsuura, K., O'Keefe, D.P. and Dutton, P.L. (1983) Biochim. Biophys. Acta 722, 12–22
20 Wraight, C.A., Cogdell, R.J. and Clayton, R.K. (1975) Biochim. Biophys. Acta 396, 242–249
21 Dutton, P.L., Petty, K.M., Bonner, H.S. and Morse, S.D. (1975) Biochim. Biophys. Acta 387, 536–556
22 Bowyer, J.R., Tierney, G.V. and Crofts, A.R. (1979) FEBS Lett. 101, 207–212
23 Wraight, C.A. (1977) Biochim. Biophys. Acta 459, 525–531
24 Vermeglio, A. (1977) Biochim. Biophys. Acta 459, 516–524
25 Bowyer, J.R., Tierney, G.V. and Crofts, A.R. (1975) FEBS Lett. 101, 201–206
26 O'Keefe, D.P., Prince, R.C. and Dutton, P.L. (1981) Biochim. Biophys. Acta 637, 512–522
27 Rutherford, A.W. and Evans, M.C.W. (1980) FEBS Lett. 110, 257–261
28 Barouch, Y. and Clayton, R.K. (1977) Biochim. Biophys. Acta 462, 785–788
29 De Grooth, B.G., Van Grondelle, R., Romijn, J.C. and Pulles, M.P.J. (1978) Biochim. Biophys. Acta 503, 480–490
30 Crofts, A.R., Meinhardt, S.W. and Bowyer, J.R. (1982) in Function of Quinones in Energy Conserving Systems (Trumpower, B., ed.), pp. 477–498, Academic Press, New York
31 Mitchell, P. (1972) FEBS Symp. 28, 353–370
32 Keister, D.L. and Minton, M.J. (1971) in Energy Transduction in Respiration and Photosynthesis (Quagliarello, E., Papa, S. and Rossi, C.S., eds.), pp. 375–384, Adriatica Editrice, Bari
33 Gennis, R.B., Casey, R.P., Azzi, A. and Ludwig, B. (1982) Eur. J. Biochem. 125, 189–195
34 Clark, A.J. and Jackson, J.B. (1981) Biochem. J. 200, 389–397