Plant Physiol. (1971) 47, 373–379

The Kok Effect in *Chlamydomonas reinhardi*[1]

Received for publication September 21, 1970

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ABSTRACT

A Haxo-Blinks rate-measuring oxygen electrode together with a modulated light source gave an average current signal (change in net O_2 exchange) and a modulated current signal (photosynthetic O_2 evolution). Using this apparatus, net O_2 exchange and photosynthetic O_2 evolution at low intensities have been studied in the green alga, *Chlamydomonas reinhardi*. At both 645 nm and 695 nm, the curves of net O_2 exchange as a function of light intensity were steeper at lowest intensities than about compensation, indicative of the Kok effect. The effect was greater at 695 nm than at 645 nm. The corresponding curves of photosynthetic O_2 evolution, on the other hand, showed no Kok effect; here, the slope was lowest at lowest intensity. The absence of the Kok effect in O_2 evolution, together with its sensitivity to monofluoroacetic acid, show that it is due to an interaction of photosynthesis and respiration. The effect was exaggerated by 3-(3,4-dichlorophenyl)-1,1-dimethylurea. In the presence of concentrations of this inhibitor sufficient to inhibit O_2 evolution completely, a light-induced change in net O_2 exchange remained. This was interpreted as a system I dependent depression of respiratory O_2 uptake. The Kok effect remained undiminished in concentrations of carbonyl cyanide *m*-chlorophenylhydrazone and 2,4-dinitrophenol which partially uncoupled either oxidative phosphorylation alone or both oxidative and photosynthetic phosphorylations. The above results can be explained within a model of the Kok effect in which O_2 uptake is depressed by diversion of reductant away from respiratory electron transport and into photosystem I. The same photodepression of O_2 uptake also appears to account for a transient in net O_2 exchange seen in several algae upon turning off the light.

Attention was first drawn to a nonlinearity in the curve of net O_2 exchange as a function of light intensity by Kok (29), who studied it in *Chlorella* sp. Now known as the Kok effect, this phenomenon has also been observed in *Haematococcus* sp. (29), *Osillatoria* sp. and *Symploca* sp. (42), *Anacystis nidulans* and *Anabaena variabilis* (24, 27), *Porphyridium* sp. (15), *Fragillaria sublinearis* (5), *Chlorella fusca* (39), and under certain conditions in leaves of higher plants (12, 29). The effect is absent or very small in *Chlorella pyrenoidosa* (9, 35) and *Phaeodactylum tricornutum* (32).

Kok (29) suggested that the effect may be due to a light-dependent depression of respiration. A depression in the rate of uptake of isotopic O_2 in the light has been seen in *Anacystis nidulans* (24) and *Fragillaria sublinearis* (5). The results of both Hoch *et al.* (24) and of Jones and Myers (27) show that preferential activation of photosystem I exaggerates the Kok effect. Activation of system I in excess of activation of system II could depress respiratory O_2 uptake in at least two ways. Hoch *et al.* (24) proposed that cyclic phosphorylation driven by system I could raise the ratio of cellular ATP to ADP sufficiently to restrict respiratory electron transport. Alternately, preferential activation of system I could shift the flow of respiratory reductant away from respiratory electron transport and into system I of photosynthetic electron transport, as suggested by Jones and Myers (27). Recent observations on anaerobic, DCMU[3]-resistant $^{14}CO_2$ fixation by *Chlamydomonas reinhardi* (47), and on photoevolution of H_2 by *Scenedesmus obliquus* (45) and *Chlamydomonas moewusii* (18) show that the latter pathway may operate in anaerobic cells. The observation of a marked Kok effect in *C. moewusii* and *C. reinhardi* led to a study of the phenomenon in the latter alga in an attempt to gain a fuller understanding of its mechanism.

MATERIALS AND METHODS

Stock cultures of *Chlamydomonas reinhardi* Dangeard (Indiana culture collection No. 89, 44) were maintained in liquid media by weekly transfers. Cultures for experimental use were inoculated from week-old stock cultures and harvested during exponential growth about 40 hr later. Both stock and experimental cultures were grown in tubes containing 25 ml of FW-2 medium (18), aerated with 1% CO_2 in air at a rate of about 1 liter per hour. The cultures were maintained at 25 C in a water bath with constant illumination by fluorescent tubes from two sides giving about 3000 lux from each side. The cells were harvested by centrifugation and resuspended in FW-2 medium lacking trace elements and buffered at pH 6.8 to 7.0 with 0.02 M phosphate (unless otherwise mentioned). Absolute measurements of O_2 exchange were made in a 2.1-ml cuvette with 1-cm light path. A Beckman oxygen electrode, polarized at −0.65 v and placed in circuit with a Keithley Model 417 picoammeter, gave a sensitivity of about 1.6 μl O_2/ml full scale on a recorder. Relative measurements made on a Haxo-Blinks rate-measuring electrode formed the major part of the study. In conjunction with a modulated light beam, this apparatus gave an average current signal (change in net O_2 exchange) and a modulated current signal (photosynthetic O_2 evolution). Joliot (26) has presented evidence that the modulated signal is due to photosynthetic O_2 alone. In

[1] This study was supported by Grant GM11300 from the National Institutes of Health.

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[3] Abbreviations: CCCP: carbonyl cyanide *m*-chlorophenylhydrazone; CMU: 3-(*p*-chlorophenyl)-1-,1-dimethylurea; DCMU: 3-(3,4-dichlorophenyl)-1,1dimethylurea; DNP: 2,4-dinitrophenol; MFA: monofluoroacetic acid.

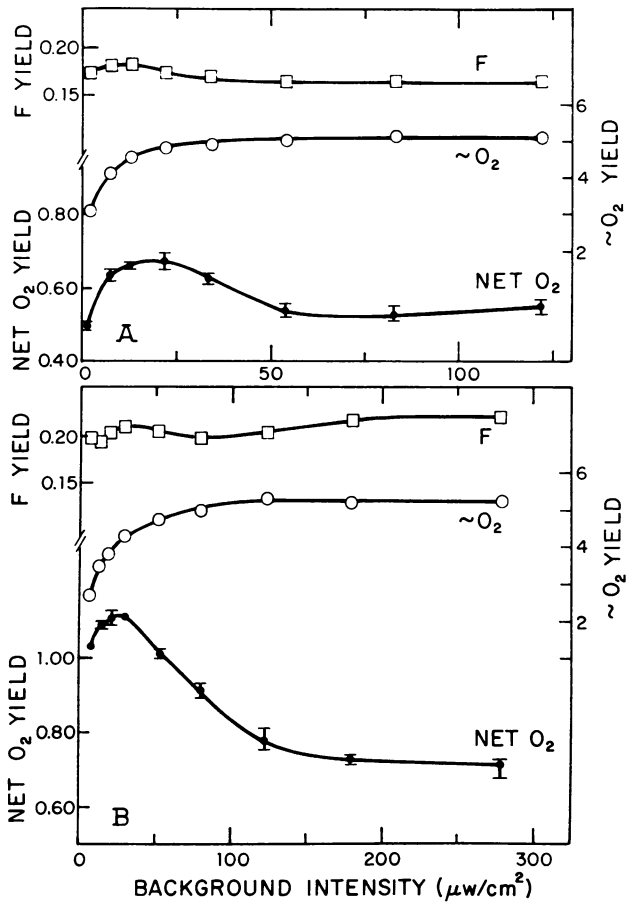

FIG. 1. Relative yield of net O_2 exchange, modulated O_2 evolution ($\sim O_2$) and fluorescence (F) supported by a standard low intensity modulated probe imposed on nonmodulated backgrounds of varying intensities. Each point is the mean of three successive measurements; the range of those diverging from one another is included by a bar. A: Both probe and background at 645 nm; probe = 4.0 μw/cm^2; fluorescence measured at 687 nm; compensation at 250 μw/cm^2. B: probe and background at 695 nm; probe = 15.0 μw/cm^2; fluorescence measured at 735 nm; compensation at 480 μw/cm^2.

simplest terms, the rationale behind the technique is this: the release of photosynthetic O_2 presumably begins and ceases with the same frequency as the modulated light because the reaction chain between photosystem II and the release of O_2 is very short. Similar fluctuations are not expected in reactions further removed from the light reactions of photosynthesis because of the buffering action of intermediate pools and reactions. The apparatus was the same as that described by Bonaventura and Myers (3), except that a larger electrode (6-mm diameter) was used and the optics were adjusted accordingly. Less than half of the cells in our cultures were motile. The cultures were enriched in nonmotile cells by allowing them to settle in a tube lighted only from above and decanting the motile cells. Microscopic examination showed that only a few per cent of the cells on the electrode were motile. The layer of algae on the electrode surface was never more than one cell thick. For use with the rate electrode, 10 mM NaCl was added to the medium, which was aerated with 1% CO_2 in air. All measurements were made at 25 C.

Light intensities received at both electrodes were measured with a calibrated large surface thermopile or a calibrated thermocouple with a 2-mm diameter receiver. In order to correct for light reflected by the platinum electrode and minor errors in sampling by the small thermocouple receiver, normalized curves of net O_2 exchange measured on both electrodes were compared. The two curves were superimposable when all light intensities measured at the rate electrode were multiplied by 1.8. This correction was applied to all intensities measured at the rate electrode. Scattered light received by the photomultiplier was less than 2 and 10% of the fluorescence activated by 645 and 695 nm, respectively.

RESULTS

The Kok Effect. Relative yield curves of net O_2 exchange, modulated O_2 evolution, and fluorescence as a function of intensity were obtained by superimposing a light beam of constant low intensity chopped at 13 Hz on an unchopped background of variable intensity. Representative curves at 645 nm and 695 nm are shown in Figure 1. In both cases, the yield or slope of net O_2 exchange was greater at a low intensity than it was at intensities near and above compensation, indicative of the Kok effect. In several experiments at 695 nm, the ratio of maximal slope to that at compensation fell between 1.5 and 2, while at 645 nm, the same ratio varied between 1.1 and 1.3. The slope of modulated O_2 evolution showed the opposite pattern; at both wavelengths, the slope at the lowest intensity measured was between one-half and two-thirds that obtained by superimposing the modulated probe on a background of compensating intensity or higher. The yield of fluorescence, on the other hand, showed little or no variation at intensities below compensation.

Compensation occurred at 250 μw/cm^2 at 645 nm and 480 μw/cm^2 at 695 nm. At intensities exceeding about twice compensation, the slopes of both net and modulated O_2 began to decline slowly, while that of fluorescence rose. In the intensity interval of two to five times compensation, the decline in slope of net O_2 was 1.5- to 2-fold greater than that of modulated O_2. This may have been due to a photostimulation of O_2 uptake, such as has been observed in other algae at intensities higher than those causing the Kok effect (4, 16, 24, 48).

An estimate of the magnitude of the reaction or reactions causing the Kok effect was obtained by measuring the divergence between light intensity plots of net O_2 exchange and modulated O_2 evolution. The plots of modulated O_2 evolution were normalized to have the same slopes as the corresponding curves of net O_2 exchange at intensities above compensation. The normalizing factor was chosen as the ratio of the slope of net O_2 exchange to that of modulated O_2 evolution between compensation and twice compensation, an interval in which there was little or no change in either slope with intensity. Curves obtained at 645 nm and 695 nm are plotted in Figure 2A. A plot of the difference between net and modulated O_2, representing the reaction or reactions responsible for the Kok effect, is shown in Figure 2B. In several experiments, the rate of this reaction varied from 10 to 30% of respiration at 645 nm and 30 to 50% at 695 nm.

The presence of the Kok effect in net O_2 exchange, contrasted with its absence in modulated O_2 evolution, supports earlier evidence that the effect is due to an interaction between photosynthesis and some other aspect of metabolism, probably respiration. This suggestion was further studied with the use of monofluoroacetic acid, an inhibitor of aconitase in the citric acid cycle. Increasing concentrations of this inhibitor at pH 6.8 resulted in a depression of O_2 uptake in the dark to about 50% at 0.3 to 1.0 mM. At an intensity of three times compensation, the same concentrations inhibited gross photosynthesis (the difference between net O_2 exchange in the light and that in subsequent darkness) only slightly or not at all. Higher concentrations did not depress respiration further, but inhibition of

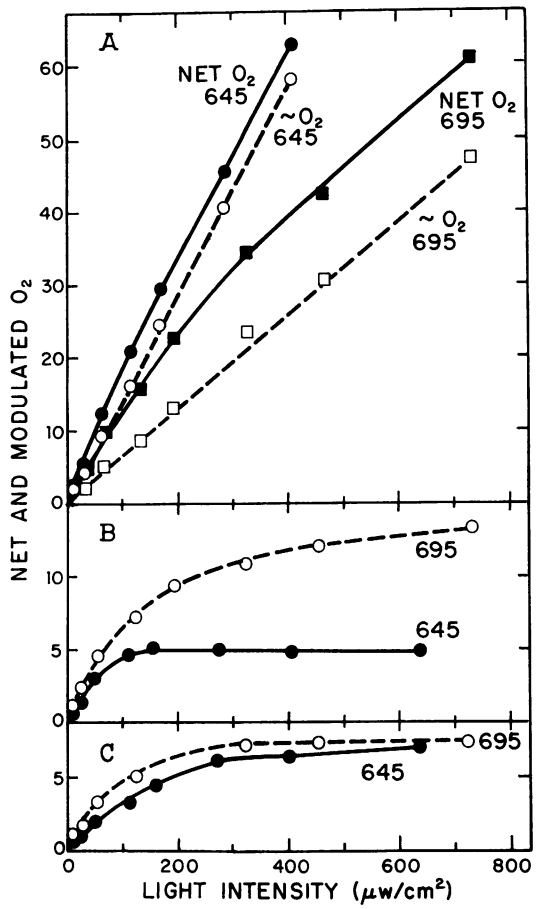

FIG. 2. A: Light intensity dependence of net O_2 exchange and modulated O_2 evolution ($\sim O_2$) at 645 nm and 695 nm. The modulated O_2 curve was normalized to have the same slope as that of net O_2 at intensities above the Kok effect. B: Light intensity dependence of the difference between net O_2 exchange and modulated O_2 evolution, calculated from the data in part A. Note change in ordinate scale. C: Light intensity dependence of the light-induced change in net O_2 exchange in the presence of 30 μM DCMU.

gross photosynthesis increased with increasing concentration. One millimolar MFA largely to completely eliminated the Kok effect while having little effect on modulated O_2 evolution or fluorescence (Fig. 3). Similar results were obtained at 645 nm.

At pH 7.0, 3 mM acetate stimulated dark O_2 uptake of nonstarved cells about 50% with little effect on gross photosynthesis. The presence of 3 mM acetate did not alter the Kok effect at 695 nm or 705 nm.

Anaerobic conditions, obtained by gassing the medium circulating over the rate electrode with argon containing 2% CO_2, resulted in sigmoid-shaped rate *versus* intensity curves for both net and modulated O_2, similar to those previously reported for net O_2 exchange (15, 42). In the case of both net and modulated O_2, the sigmoid shape of the curves may have been due to either an inhibition of O_2 evolution by anaerobiosis at low intensities or to consumption of photosynthetic O_2 by the anaerobic cells at low intensities. However, the similarity between curves of net and modulated O_2 under anaerobic conditions suggests that anaerobiosis eliminates the Kok effect.

The greater magnitude of the Kok effect at 695 nm compared with that at 645 nm supports previous evidence that only system I is involved in the reaction. This was further tested by examining the effect of DCMU on the phenomenon. Figure 4 shows the effect of increasing concentrations of DCMU on reactions in *C. reinhardi* below compensation. Modulated O_2 evolution fell with increasing concentration to over 99% inhibition at 30 μM. The light-dependent change in net O_2 exchange decreased with concentration of DCMU to 0.3 μM, but was not further decreased by increasing concentration. Fluorescence increased about 2.5-fold over the same range as modulated O_2 evolution fell.

These results demonstrate a light-dependent change in net O_2 exchange which is insensitive to DCMU. Since O_2 evolution was completely inhibited, this change in net O_2 exchange was

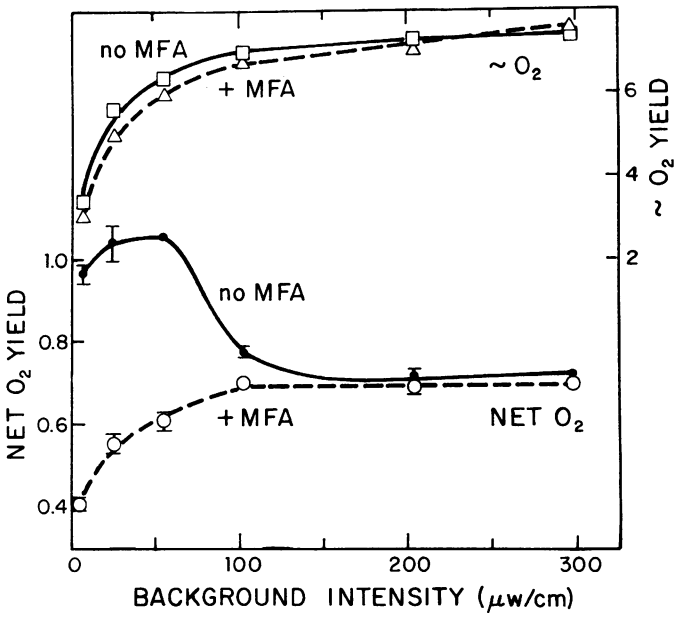

FIG. 3. Relative yield of net O_2 exchange and modulated O_2 evolution ($\sim O_2$) due to a standard low intensity modulated probe imposed on nonmodulated backgrounds of varying intensities at 695 nm in the presence and absence of 1 mM MFA at pH 6.9. Each point is the mean of 3 successive measurements, with vertical bars indicating the range of points which diverged from one another. Probe = 12.1 μw/cm^2.

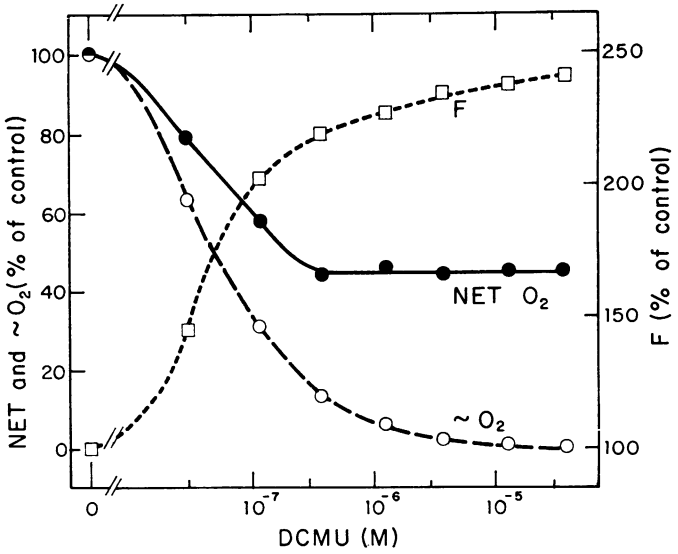

FIG. 4. Effect of concentration of DCMU on net O_2 exchange, modulated O_2 evolution ($\sim O_2$) and fluorescence (F) at 695 nm. Fluorescence emission measured at 735 nm; modulated light with average intensity of 245 μw/cm^2; control = no DCMU.

interpreted as a light-dependent depression of respiratory O_2 uptake. This presumed respiratory depression offered a second estimate of the rate of the reactions responsible for the Kok effect. A plot of this parameter against light intensity at 645 nm and 695 nm is shown in Figure 2C. Both measurements of light-dependent depression in respiratory O_2 uptake, the difference between net and modulated O_2 and the DCMU-insensitive net O_2 exchange, rose more rapidly with intensity at 695 nm than at 645 nm; the rate of photosynthetic O_2 evolution, on the other hand, increased over two times as fast at 645 nm as at 695 nm. This is further evidence that the respiratory depression is activated through system I. At 695 nm the magnitude of this DCMU-resistant reaction was less than the reaction represented by the difference between net O_2 exchange and modulated O_2 evolution (Fig. 2B). A possible reason for this discrepancy will be discussed below.

The preceding results support previous suggestions that the Kok effect is due to a light-dependent depression of respiration, but do not differentiate between the two possible mechanisms suggested in the introduction. In an attempt to differentiate between these, some experiments with uncouplers were performed. While both CCCP and DNP appear to act on respiratory electron transport primarily as uncouplers (17, 22), both have been shown to uncouple and inhibit photosynthetic electron transport. However, studies involving both isolated chloroplasts and intact algae show that uncoupling occurs at lower concentrations than inhibition of electron transport (8, 25, 36, 37). Upon this basis, we have attributed stimulation of dark O_2 uptake to uncoupling of oxidative phosphorylation and inhibition of O_2 evolution primarily to uncoupling of photosynthetic phosphorylation.

Dark respiration was maximally stimulated (1.6- to 2-fold) by 1 to 2 μM CCCP, with little effect on gross photosynthesis. As shown in Figure 5, 1 μM CCCP caused no loss of the Kok effect. At higher concentrations of the uncoupler, dark respiration fell back to its control rate at 4 μM. Inhibition of gross photosynthesis increased from negligible at 1 μM to 90% at 3 μM, when measured with the Beckman electrode in a glass container. Measurements with the rate electrode in a Lucite housing required higher concentrations of the uncoupler to achieve the same inhibition. This may have been due to loss of the uncoupler into the Lucite. Figure 6 shows that concentrations of CCCP sufficient to inhibit O_2 evolution partially also did not alter the Kok effect.

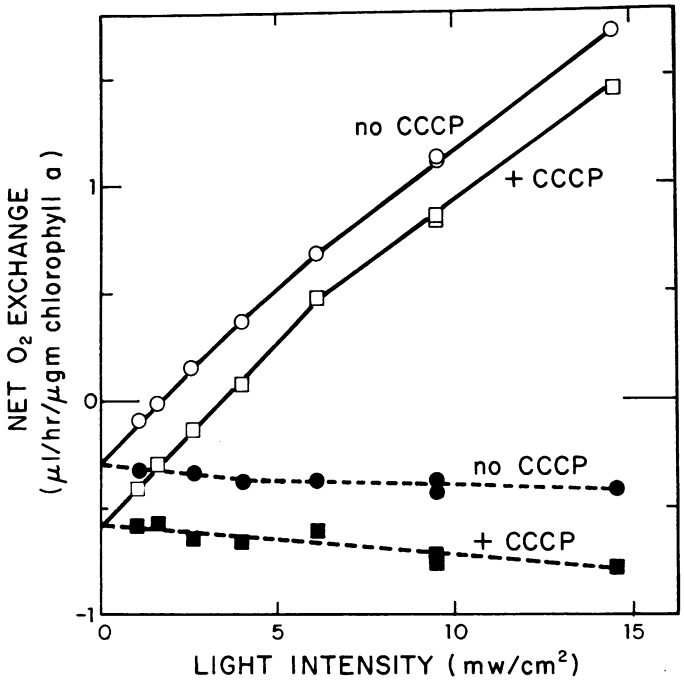

FIG. 5. Light intensity dependence of net O_2 exchange at 705 nm (solid lines) and O_2 uptake in subsequent darkness (broken lines) with and without 1.0 μM CCCP.

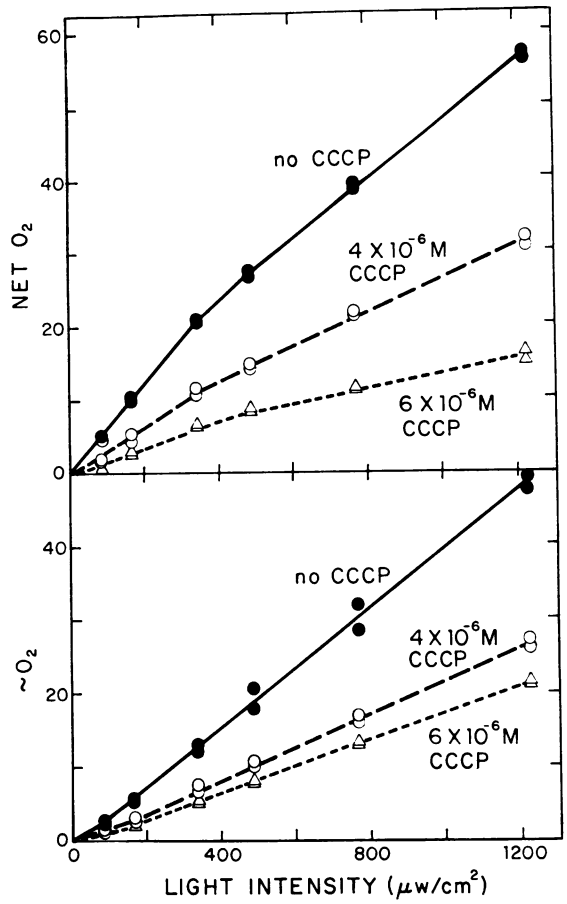

FIG. 6. Net O_2 exchange (above) and modulated O_2 evolution (~O_2, below) as functions of light intensity at 695 nm with increasing concentrations of CCCP.

At pH 6, DNP stimulated dark O_2 uptake maximally (30–40%) at 0.1 mM, without affecting gross photosynthesis; higher concentrations depressed respiration to about 70% of the control rate and inhibited photosynthesis completely at 0.3 mM. Like CCCP, this uncoupler did not alter the Kok effect at concentrations uncoupling oxidative phosphorylation alone or both oxidative and photosynthetic phosphorylations.

Arsenate inhibited dark O_2 uptake and photosynthesis over the same concentration range; in the absence of intentionally added phosphate at pH 7 (buffered with 1.0 gm/liter tris), 1 μM arsenate inhibited respiration about 40% and gross photosynthesis about 60%. The lack of stimulation of respiration by arsenate suggests that it may not have been acting as an uncoupler. However, both respiration and photosynthesis were about 100 times less sensitive to arsenate in the presence of 2 mM phosphate than in the absence of phosphate. This suggests that arsenate was interfering with some aspect of phosphorus metabolism. Concentrations of arsenate inhibiting photosynthesis 25 to 50% either did not change or exaggerated the Kok effect.

Persistence of the Kok effect under conditions where phosphorylation must have been partially or largely uncoupled

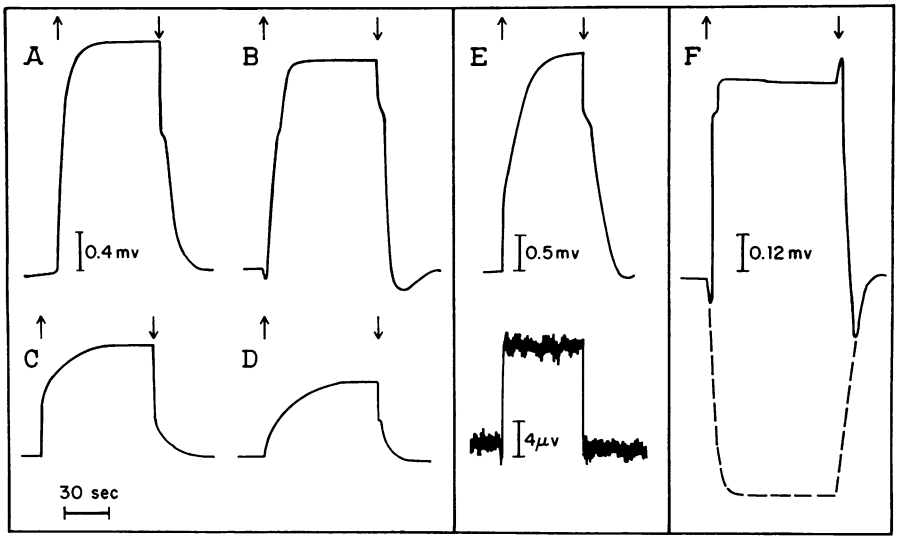

FIG. 7. Transients in net O_2 exchange and modulated O_2 evolution on turning light on (↑) and off (↓). The time scale in the lower left corner refers to all figures. The vertical (rate) scale with A refers also to B, C, and D, which have been normalized to be comparable with one another. A: Net O_2 at 645 nm, 14.0 μw/cm²; B: net O_2 at 695 nm, 16.1 μw/cm²; C: net O_2 at 695 nm, 108 μw/cm², under anaerobic conditions; D: net O_2 at 695 nm, 16.1 μw/cm², with 1 mM MFA (pH 6.9); E: net O_2 (above) and modulated O_2 (below) at 695 nm, modulated light of 9.9 μw/cm² turned on and off with a modulated background of 4.1 μw/cm² on continuously; F: observed net O_2 exchange at 645 nm (14.0 μw/cm²) in presence of 30 μM DCMU (——) together with suggested downward signal (— —).

argues against at direct dependence of the effect on phosphorylation.

Transients in O_2 Exchange. The present experiments revealed some interesting observations on light-dark transients in net O_2 exchange. These are summarized in Figure 7. Using uninhibited, aerobic *C. reinhardi*, net O_2 exchange did not fall smoothly to its dark rate on turning off the light; instead, it passed through a shoulder-like transient. Similar transients have been observed in other algae (10, 14, 39). This light-off transient was about equally prominent at 645 nm and 695 nm (Fig. 7, A and B) and occupied a larger fraction of the total net O_2 signal at lower intensities. No transient was seen in the modulated O_2 signal upon removing a low intensity modulated probe from a low intensity modulated background; however, it was present in the comparable net O_2 signal (Fig. 7E). In this case, the transient to a low intensity background rather than to darkness was studied because complete removal of the modulated light would have automatically resulted in loss of the modulated signal, obscuring any transient which may have been present. The light-off transient was eliminated under anaerobic conditions and greatly reduced by MFA (Fig. 7, C and D), but was unaffected by CCCP and DNP. At all concentrations of these uncouplers used, both the transient and photosynthesis were depressed to the same extent. These results suggest that the same repiratory anomaly involved in the Kok effect is also responsible for the transient.

Also shown in Figure 7F are the transients observed in the presence of DCMU. Here, a downward spike when the light was turned on, and an upward spike and a protracted downward transient when it was turned off were evident. Similar transients have been observed in *Porphyridium cruentum* exposed to 695 nm (11). Except for similar, but smaller light-on and light-off spikes seen only at the lowest intensities of 695 nm, these transients were not observed in the absence of DCMU. For measurements of the DCMU-insensitive change in net O_2 exchange, the net signal was used. As suggested by the dotted line in Figure 7F, the transients may indicate a simultaneous, but initially faster responding, signal moving in the opposite direction (O_2 uptake). This would result in the net signal being an underestimate of the gross upward reaction and may account for the plot of the DCMU-resistant net O_2 exchange giving a lower estimate of the photodepression of respiration than the difference between net and photosynthetic O_2 at 695 nm (Fig. 2).

DISCUSSION

A number of correlations seen in the literature and in the present study suggest that both the Kok effect and the light-off transient (which appears to be the same as T_3 of Ried [38]) are manifestations of a photodepression of O_2 uptake. Evidence that light can depress O_2 uptake in algae comes chiefly from experiments involving the uptake of isotopic O_2, conducted by Hoch and coworkers. In both *Anacystis nidulans* (24) and *Chlorella vulgaris* (16), the depression is activated more effectively by far red than by red light; in *Fragillaria sublinearis*, the effect can also be seen in light absorbed preferentially by system II but only at higher intensities than required for the same effect in system I light (5). In *Scenedesmus* sp., photodepression of O_2 uptake can be seen only in the presence of DCMU, which eliminates a photostimulation of O_2 uptake (24); this shows that the depression is insensitive to DCMU.

Like the photodepression of O_2 uptake, the Kok effect in *Anacystis nidulans* (24, 27) and in *C. reinhardi* is more readily apparent in far red than in red light; however, with the present method it can also be detected in the latter alga at wavelengths absorbed primarily into system II. The light-off transient in *C. reinhardi* can also be seen at both 695 nm and 645 nm, but occupies a larger fraction of the total signal at 695 nm than at 645 nm. This agrees with Ried's (39) observation that T_3 in *Chlorella fusca* has an action spectrum of system I.

Like the photodepression of O_2 uptake, the Kok effect is not inhibited by DCMU; rather, it becomes more exaggerated with increasing concentrations of DCMU in that the ratio between the slope of net O_2 exchange at lowest intensities to that above compensating intensities increases. With sufficient DCMU to completely eliminate photosynthetic O_2 evolution, a light-dependent change in net O_2 exchange remains. Like the photodepression of O_2 uptake in *Anacystis nidulans* (24), this reaction in both *Chlorella* sp. (30, 39) and *C. reinhardi* saturates at

a low intensity. In the presence of such concentrations of DCMU, the light-off transient occupies the entire net O_2 signal of both *Chlorella fusca* (39) and *C. reinhardi*. Similarly, DCMU does not inhibit T_2 in *Chlorella fusca*, which also saturates at low intensity (38).

In *C. reinhardi*, both the Kok effect and the light-off transient are eliminated or strongly reduced by anaerobiosis and MFA. Similarly, anaerobiosis eliminates the light-off transient in *Symploca* sp. and *Oscillatoria* sp. (43) and T_2 in *Chlorella fusca* (38), while sufficient antimycin A to inhibit respiration in *C. fusca* abolishes both the Kok effect and T_2 (39). In all of the above cases, either anaerobiosis or an inhibitor completely eliminated respiration or reduced it to the point where it was no longer affected by light.

The foregoing all suggest that both the Kok effect and the light-off transient are manifestations of a photodepression of respiratory O_2 uptake dependent on system I. Both the above results and the absence of a Kok effect in plots of modulated O_2 evolution against light intensity are at variance with a recent suggestion that the Kok effect is a characteristic of photosynthesis alone (21). However, the results discussed so far do not allow one to decide how system I depresses respiratory O_2 uptake. One proposal suggests that a lowering of the cellular ratio of ADP to ATP due to photophosphorylation could depress the rate of respiration (19, 24). This view is supported by apparent inhibition of glycolysis (19, 28) and the failure of recently fixed ^{14}C to enter the tricarboxylic acid cycle in the light (1, 2). However, other studies show that the turnover of intermediates of the tricarboxylic acid cycle is unaffected by light in some unicellular green algae (33, 34). Partial inhibition of respiratory CO_2 release in the light is not necessarily accompanied by a reduction in O_2 uptake as would be expected if photoinhibition of respiration were mediated through photosynthetic ATP (4, 48).

In view of the rapid equilibration of ATP between chloroplasts and cytoplasm (41), it would be surprising if light-induced changes in the ATP to ADP ratio did not affect respiratory processes. However, there is evidence both in the literature and in the present study suggesting that this is not the only, nor always the principal, effect of light on respiration mediated through photosynthesis. Recent observations (18, 23, 45, 47) strongly suggest that in anaerobic algae, respiratory reductant can move into the chloroplasts. If this occurred under aerobic conditions, the resulting diversion of reductant away from respiratory electron transport into photosystem I would cause a depression of respiratory O_2 uptake without a similar effect on carbon metabolism. The close association between mitochondria and chloroplasts often seen in *Chlamydomonas* sp. (13, 31, 40) may be important in this movement. Light-induced oxidation of reduced pyridine nucleotide in a mutant of *Chlamydomonas* sp. and to a lesser extent in *Chlorella* sp. (6) and of cytoplasmic NADH in leaf cells (20) under aerobic conditions could be explained on this basis. This mechanism could also explain the light-dependent fixation of $^{14}CO_2$ by *Anabaena cylindrica* in the presence of high concentrations of CMU (7), but does not explain the dependence of the analogous reaction in *C. reinhardi* on anaerobiosis and dyes (47). However, the fact that the ^{14}C-fixation method is less sensitive than the O_2 electrode, especially at low rates of photosynthesis, may account for this.

The inability of uncouplers to eliminate either the Kok effect or the light-off transient in *C. reinhardi* shows that the interaction between photosynthesis and respiration here does not depend on phosphorylation. In the present study, concentrations of CCCP and DNP giving maximal stimulation of respiration did not inhibit gross photosynthesis. This gave a situation in which no reactions required for photosynthesis were impeded; but respiration, and perhaps cyclic photophosphorylation, which may be more sensitive to some uncouplers than photosynthesis (37, 46), were partly to largely uncoupled. If the Kok effect were dependent on an interaction of cyclic and oxidative phosphorylations, it should have been decreased under these conditions, but it was not. Higher concentrations of both uncouplers, affecting both photosynthesis and respiration, also caused no loss of either the Kok effect or the light-off transient. The situation produced by arsenate was less clear since inhibition of both respiration and photosynthesis occurred over the same concentration range, but here again interference with phosphorus metabolism did not diminish the Kok effect. While phosphorylation does not appear to be directly involved in the Kok effect in *C. reinhardi*, it may be in *Chlorella fusca*. Although DNP did not affect T_2 here, CCCP eliminated it (38). Unlike the Kok effect in *C. reinhardi*, which was unaffected by acetate, that in *C. fusca* was eliminated by glucose (39), possibly through the increased ATP demand of glucose uptake (46). Differences in response to substrate and CCCP between these two algae may reflect different mechanisms.

In conclusion, the present results are in accord with previous observations suggesting that the Kok effect is caused by a system I mediated depression of respiratory O_2 uptake. However, they do not support previous proposals for an interaction between photosynthesis and respiration mediated solely through ATP levels. Rather, they can be explained on the basis of movement of reductant from respiratory carbon metabolism into photosystem I, in a manner similar to that proposed for the photoevolution of H_2 by *Chlamydomonas moewusii* (18). While this mechanism accommodates many observations on interactions of photosynthesis and respiration, it does not explain all of them. This should only emphasize that there are many potential ways in which photosynthesis and respiration can interact, and one can expect the mode and degree of interaction to vary with the organism and conditions used.

Acknowledgments—We acknowledge with pleasure the valuable assistance provided by Mr. Searle Crate.

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