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**Communication**

# Light-Enhanced Dark Respiration in Mesophyll Protoplasts from Leaves of Pea[1]

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## ABSTRACT

**The respiratory oxygen uptake by mesophyll protoplasts of pea (*Pisum sativum* cv Arkel) was stimulated up to threefold after 15 minutes of illumination at an intensity of 1250 microeinsteins per square meter per second in the presence of 5 millimolar bicarbonate at 30°C. The extent of light-enhanced dark respiration (LEDR) increased progressively with duration of preillumination. The LEDR exhibited two phases. The initial high rate of respiration decreased in about 10 minutes to a lower steady value similar to that before illumination. The promotion of LEDR by the presence of bicarbonate and inhibition by glyceraldehyde or 3-(3,4-dichlorophenyl)-1,1-dimethylurea suggested that LEDR was dependent on products of photosynthetic carbon assimilation/electron transport. Thus, the photosynthetic products exert a markedly quick influence on dark respiration in mesophyll protoplasts.**

The carbon and energy economy of a plant depend not only on photosynthesis but also on respiration. The interaction between photosynthesis and respiration, therefore, is very important. Photosynthesis over long periods of illumination leads to an accumulation of carbohydrates, which can stimulate dark respiration (3). The lowering of photosynthetic efficiency at lower light intensities, a phenomenon called the Kok effect (12), is believed to be primarily due to dark respiration (11, 16, 17).

The magnitude of mitochondrial (dark) respiration in leaves of higher plants during photosynthesis is not yet clearly established (7, 8). There is a large variation in the reported reduction in dark respiration on illumination, ranging from 0 to 100% (2, 4, 13). Most of the studies to date have been made with intact leaves or leaf discs, which have certain inherent problems, such as recycling of assimilated/released $CO_2$ within the leaf (5), making it difficult to establish the interaction between photosynthesis and respiration (14).

We have used isolated mesophyll protoplasts from pea (*Pisum sativum* cv Arkel) leaves to reevaluate the effect of light on dark respiration. Protoplasts are useful tools for studying plant metabolism because they form homogeneous suspensions; pose no problem of recycling of gas, as within the intercellular spaces of the leaf; and further allow an evaluation of externally added metabolites/inhibitors. Recently, we (20) have demonstrated a strong interaction between photosynthesis and respiration in mesophyll protoplasts of pea.

Our results indicate that there is a marked upsurge in respiratory $O_2$ uptake after even short periods of illumination. This phenomenon, termed LEDR,[2] appears to be related to photosynthetic carbon metabolism/electron transport. As this article was under preparation, LEDR was described in leaves (19). This article is the first report of LEDR in protoplasts.

## MATERIALS AND METHODS

The first and second fully expanded leaves from 8- to 10-d-old plants of pea (*Pisum sativum* L. cv Arkel) grown outdoors (natural photoperiod of approximately 12 h; average daily temperatures of 30°C day/20°C night) were used. The leaves were picked from the plants between 9:00 and 10:00 AM, by which time the plants were exposed to sunlight for about 3 or 4 h.

The abaxial (lower) epidermis of the leaves was stripped off carefully with the help of a forceps. The stripped leaves were cut into pieces of about 1.0 × 0.5 cm and were placed in a preplasmolysis medium of 0.3 M mannitol and 1 mM $CaCl_2$. Illumination was provided at an intensity of 50 $\mu E\ m^{-2}\ s^{-1}$. The plasmolyzed leaf pieces were then digested in Petri dishes with Cellulase Onozuka R-10 and Macerozyme R-10 (Yakult Honsha, Nishinomiya, Japan), as described in detail elsewhere (20). The preparation had about 90 to 95% intact protoplasts, as indicated by the uptake of neutral red and exclusion of Evans blue. Chl was determined by extraction into 80% (v/v) acetone (1).

The suspension of protoplasts containing 200 to 300 $\mu g$ Chl $mL^{-1}$ was either kept in darkness or illuminated at an intensity of 1250 $\mu E\ m^{-2}\ s^{-1}$ (measured with a quantum/radio/photometer, Li-Cor Instruments, Lincoln, NE). About 200 to 300 $\mu L$ of protoplast suspension was illuminated with gentle stirring in a chamber maintained at 30°C. After the required period of illumination, a small aliquot of protoplast suspension (equivalent to 15 $\mu g$ Chl) was added to the reaction mixture to monitor respiration.

The respiratory $O_2$ uptake or photosynthetic $O_2$ evolution by protoplasts was monitored at 30°C using a Clark-type

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[2] Abbreviation: LEDR, light-enhanced dark respiration.

oxygen electrode (21). The reaction mixture of 1 mL contained 0.4 M mannitol, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM sodium bicarbonate (unless otherwise specified), 10 mM Hepes-KOH, pH 7.8, and protoplasts equivalent to 15 μg Chl.

## RESULTS

The rate of respiration in mesophyll protoplasts was stimulated nearly threefold after 15 min of illumination (Fig. 1). The respiratory rate was not altered if the protoplasts were kept in continuous darkness for similar periods. The extent of LEDR progressively increased with longer duration, with the maximum LEDR after a 15 min preillumination.

The high respiratory rate following illumination persisted for about 2 to 4 min (depending on duration of preillumination) and subsided subsequently in the next 10 min to reach a lower rate similar to that in the control (Fig. 2). The maximum duration of LEDR, which was about 4 min, occurred after 15 min of preillumination. Further increase in the period of preillumination decreased not only the extent

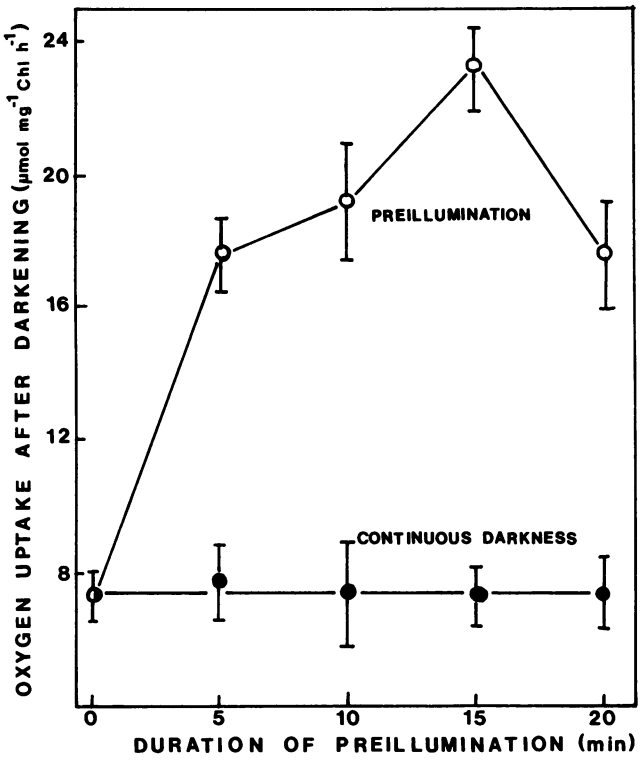


**Figure 1.** Stimulation of dark respiration in mesophyll protoplasts by varying periods of preillumination. The rate of oxygen uptake in darkness was monitored in pea mesophyll protoplasts kept under continuous darkness or immediately after preillumination (in the presence of 5 mM bicarbonate) for 5 to 20 min at 30°C. The dilution of the protoplast aliquot during assay of respiration excluded the possibility of an increase in $O_2$ level due to illumination. The photosynthetic rate during preillumination was linear up to 10 to 12 min, but decreased slightly afterward. The rates (μmol $O_2$ evolved $mg^{-1}$ Chl $h^{-1}$) were 102 ± 12 (until 12 min), 92 ± 8 (at 15 min), and 82 ± 10 (at 20 min). The results are averages ± SE of four experiments.

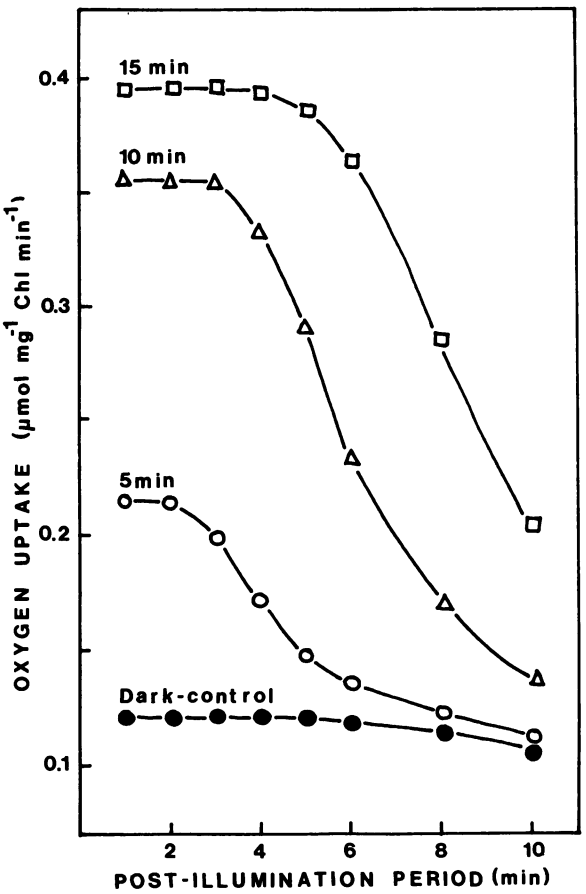


**Figure 2.** The decay kinetics of LEDR in mesophyll protoplasts in relation to the duration of preillumination. The high initial rate after a preillumination for 5, 10, or 15 min subsided over the next several minutes to a lower level similar to the rate of respiration in nonilluminated (control) sets. The maximum respiration was in protoplasts preilluminated for 15 min. The values are expressed as rates $min^{-1}$, in view of the quick decrease in the rate of LEDR. Other details are as in Figure 1.

of LEDR (Fig. 1), but also the duration of high initial rate (data not shown).

The occurrence of LEDR required the presence of bicarbonate in the medium (Table I). On the other hand, the extent of LEDR was suppressed by glyceraldehyde and DCMU, inhibitors of photosynthetic carbon metabolism and electron transport, respectively.

## DISCUSSION

The present article demonstrates for the first time the existence of LEDR in mesophyll protoplasts (Fig. 1). The threefold stimulation in dark respiration of mesophyll protoplasts makes LEDR a significant phenomenon. It has been observed in leaves that the rate of $O_2$ uptake in the dark, immediately after illumination, is greater than that under continuous darkness (21). Such an increase could be due to the increased availability of respiratory substrates (3). However, the marked stimulation within a few minutes of illumination (Fig. 1) suggests that the interaction between respira-

**Table I.** *The Extent of LEDR in Pea Mesophyll Protoplasts in Relation to Photosynthetic Carbon Metabolism*

Protoplasts were preilluminated in the presence of 5 mM bicarbonate, unless otherwise stated. The test compounds were included in the medium during preincubation in either darkness or light. An aliquot was examined for the rate of dark respiration. Results are averages ± SE of five experiments. The photosynthetic rate during preillumination was 97 ± 8 μmol $O_2$ evolved $mg^{-1}$ Chl $h^{-1}$.

| Modification during Preincubation | $O_2$ Uptake after Preincubation | | LEDR |
|---|---|---|---|
| | Darkness | After 15 min illumination | |
| | μmol $mg^{-1}$ Chl $h^{-1}$ | | % of control |
| None (control) | 7.9 ± 0.9 | 22.8 ± 4.2 | 289 |
| +5 mM glyceraldehyde | 8.1 ± 1.2 | 4.2 ± 0.6 | 52 |
| +10 μM DCMU | 7.8 ± 0.6 | 3.1 ± 0.5 | 40 |
| No bicarbonate | 7.2 ± 0.8 | 10.3 ± 1.7 | 143 |

tion and light is quite rapid and involves early photosynthetic products.

The increase in the extent of respiration immediately after illumination is not due to the removal of any limitation in the photosynthetic capacity of protoplasts, at least during our experimental periods. The rates of photosynthetic oxygen evolution were quite high during the preillumination period. The slight decrease in the photosynthesis of the protoplasts after 15 min of illumination, however, was followed by a decrease in respiration as well (Fig. 1).

The LEDR could be analogous to a photorespiratory $CO_2$ outburst (16) or could result from the well-known Mehler reaction (15) or the recently characterized "chlororespiration" (6, 22). The presence of saturating bicarbonate in the medium precluded the operation of photorespiration. Further, the extent of LEDR was 10 to 15 times more than the expected $O_2$ uptake due to a possible Mehler reaction (resulting from reoxidation of photosynthetic electron transport components). Similarly, the rate of $O_2$ uptake due to the recycling of NADH generated by a reversed glycolytic pathway during chlororespiration is several hundred times lower (22) and cannot account for the LEDR.

The promotion of LEDR in mesophyll protoplasts by the presence of bicarbonate (Table I) indicated that photosynthetic carbon assimilation facilitated high respiratory activity during subsequent darkness. The sensitivity of LEDR to glyceraldehyde and DCMU further confirmed that the upsurge of respiratory $O_2$ uptake was dependent on products of photosynthetic carbon assimilation/electron transport.

The relation between photosynthesis and respiration is rather complex. We have recently reported that the processes of photosynthesis and respiration are mutually beneficial (20). The movement of phosphoglycerate/dihydroxyacetone phosphate and oxalacetate/malate between the chloroplasts, cytosol, and mitochondria may help to maintain a favorable balance of adenine and pyridine nucleotides in these organelles for their optimal performance (10, 23). Our present observations demonstrate that the photosynthetic products exert a remarkably quick influence on respiratory $O_2$ uptake in mesophyll protoplasts.

Control of tricarboxylic acid cycle activity by photosynthesis may occur through changes in adenine/pyridine nucleotides (18, 23) or triose phosphates (9). Further studies on the pattern of carbon metabolism, partitioning of carbon, and the levels of adenine/pyridine nucleotides are necessary to establish the mechanism of upsurge in mitochondrial respiration after even short periods of illumination.

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