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The effect of illumination and oxygen concentration on respiration and metabolites content in pea leaves.

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Abstract

The leaves and mesophyll protoplasts of pea did not show the light-enhanced dark respiration (LEDR) when the O_2 concentration during the period of illumination was low. The rate of glycine oxidation by mitochondria from illuminated leaves was twice higher as that from darkened leaves. Illumination had no effect on malate oxidation. The content of glycine and serine was markedly higher in mitochondria of leaves illuminated than darkened. Independently of the CO_2 concentration in the air (21% O_2) the ATP/ADP ratio in illuminated leaves was significantly higher than in that illuminated at low O_2(1%). Presented results suggest that both the tricarboxylic acid cycle and the electron transport chain are capable of operation with the rate higher in leaves illuminated at 21% O_2 than at 1% O_2 or in the darkened.

Introduction

Respiration in leaves is higher after a period of photosynthesis than before. This phenomenon, termed light-enhanced dark respiration (LEDR) has been demonstrated as both, the transient increase in CO_2 release or O_2 uptake (Raghavendra et al. 1994). It has originally been suggested that postillumination increase in respiration may result from the oxidation of photorespiratory produced glycine (Azcón-Bieto and Osmond 1983). Recently it is postulated (Atkin et al. 2000) that LEDR may reflect an increased supply of non-photorespiratory substrates (e.g. malate and/or pyruvate) formed during photosynthesis. The several previous experiments (Graham and Chapman 1979) have indicated that respiration of leaves proceeded more intensive following photosynthesis at photorespiratory (21% O_2) than at non-photorespiratory (1-2% O_2) conditions. The results of our recent study (Parys and Romanowska 2000) have shown that atmospheric concentration of oxygen during the period of photosynthesis of tall fescue leaves was necessary not only for occurrence of LEDR but also for production of substrate(s) for this phenomenon. The present study with leaves, mesophyll protoplasts and mitochondria of pea provided further evidences on the involvement of photorespiratory metabolism in the occurrence of LEDR.

Material and methods

Plants of pea (*Pisum sativum* L. cv. Ilowiecki, Poland) were grown in Knop nutrient solution under a 14 h photoperiod and a day/night regime of 25/20º C. Photosynthetic photon flux density (PPFD) on the plant level was ca 900 μmol m^{-2} s^{-1} supplied by a hologen lamps. The fully expanded leaves of 3-4 week-old plants, illuminated or darkened for 23-24 h if necessary, were used for CO_2 exchange, isolation and assays

of mesophyll protoplasts, and mitochondria, and for determination of adenylates. The CO_2 exchange of detached leaves was measured in a closed circuit system with an infrared gas analyzer (Beckman 865) filled with either 1% O_2 balanced by nitrogen or 21% O_2 (air), as described previously (Parys and Romanowska 2000). Mesophyll protoplasts were isolated from leaves detached from plants immediately after the dark period (10 h) and assayed for O_2 exchange as described earlier (Parys et al. 1998). Mitochondria from leaves of plants illuminated (L) or darkened (D) for 24 hours were isolated, purified and assayed according to Vianello et al. (1997). Oxygen uptake by mitochondria was measured at 25º C using the oxygen electrode as for protoplasts. The free amino acids in mitochondria were determined by using amino acid analyzer "System 6300 High Performance Analyzer", Beckman. ATP in leaves was determined by the firefly luciferase method.

Results

Illumination of both the leaves and the mesophyll protoplasts at normal O_2 concentration (21%) caused the transient increase in respiration (LEDR) by about

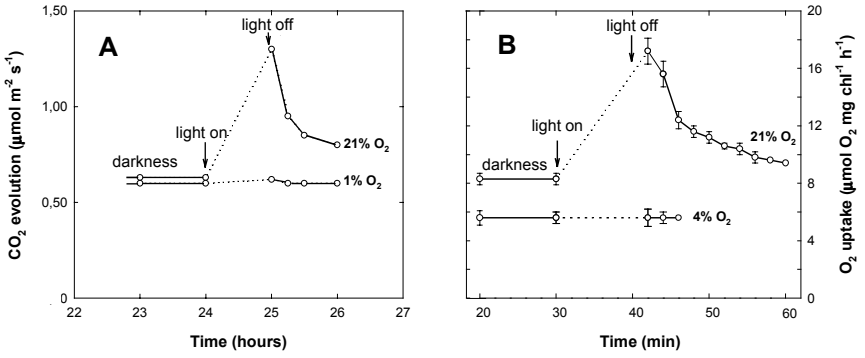

Fig. 1 Respiration rate of leaves (A) and mesophyll protoplasts (B) of pea before and after illumination in relation to oxygen concentration

Table. 1 Substrate oxidation by mitochondria of pea leaves. Substrates concentration were 10 mM. Malate oxidation was in the presence of 10 mM glutamate. State 3 was obtained by adding 100 µM ADP. L, D – mitochondria from plants illuminated or darkened 24 hours.

Substrate		O_2 uptake nmol O_2 min^{-1} mg^{-1} protein		RCR	ADP/O
		State 3	State 4		
Glycine	L	153±18	54±6	2.7±0.4	2.5±0.2
	D	70±6	24±4	2.9±0.3	2.6±0.2
Succinate	L	135±17	50±7	2.7±0.7	2.1±0.1
	D	96±9	35±4	2.7±0.5	1.9±0.2
Malate	L	74±6	35±3	2.2±0.5	2.7±0.2
	D	75±8	33±3	2.3±0.4	2.7±0.1

twice (Fig. 1). When O_2 concentration during the period of illumination was low (1% and 4% for leaves and protoplasts, respectively) no increase in respiration was noted. The rate of glycine oxidation in both 3 and 4 states of mitochondria from

iluminated leaves was twice as high as that from darkened ones (Tab. 1). Smaller effect of illumination was with succinate (about 40% stimulation) and not any with malate. The content of glycine and serine was 2.5-fold higher in mitochondria of leaves illuminated than darkened (Fig. 2). The content of glycine in mitochondria of both the illuminated and darkened leaves was also higher (by about 2.7-fold) than serine. When the leaves were illuminated for 1 hour at 1% O_2 and CO_2 concentration of 350 µl l^{-1}, the ATP/ADP ratio in the total extract decreased by about 20%, as compared to normal air (21% O_2, 350 µl CO_2 l^{-1}, Fig. 3).

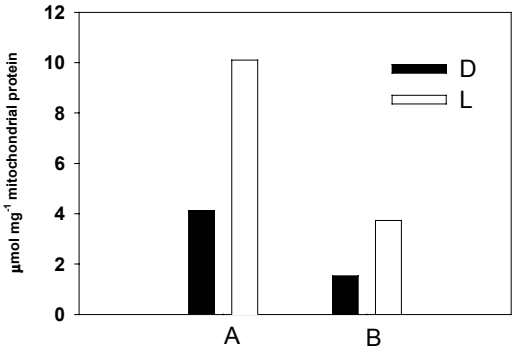

Fig. 2 Content of glycine (A) and serine (B) in mitochondria of illuminated (L) or darkened (D) pea leaves.

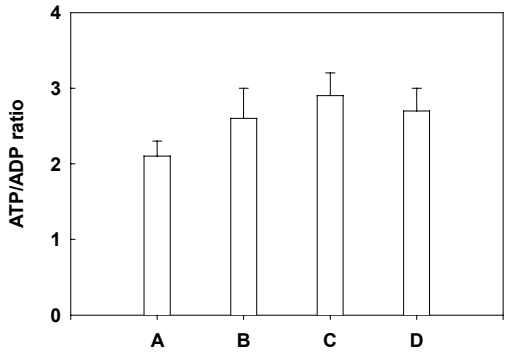

Fig. 3 ATP/ADP ratios in pea leaves illuminated in the atmosphere of: A, 1% O_2, 350 µl CO_2 l^{-1}; B, C, D, 21% O_2 and 350, 40 or 1600 µl CO_2 l^{-1}, respectively.

The decrease of CO_2 concentration in air (21% O_2) to very low value (40 µl l^{-1}) caused slight increase (about 10%) in the ATP/ADP ratio whereas the increase of CO_2 to very high value (1600 µl l^{-1}) produce the ATP/ADP ratio practically the same as noted in air.

Discussion

The stimulation of LEDR in both the leaves and the mesophyll protoplasts of pea following photosynthesis at 21% O_2 (air), as compared with low O_2 (Fig. 1) suggests that LEDR may to reflect the level of photorespiratory metabolite(s) available to the mitochondria after a period of illumination. The recent study (Parys and Romanowska 2000), have shown that LEDR in tall fescue leaves was decreased significantly when the O_2 concentration was lowered from 21% to 1% during the illumination period and when the rate of LEDR was measured again at 21% O_2. The glycine solution introduced into the tall fescue leaves stimulated LEDR (but only at 21% O_2) much more (above 70%) than other respiratory substrates (malate and pyruvate – by about 50%). Opposite results noted Atkin et al. (1998) since LEDR in tobacco leaves was markedly higher at low O_2 (2%) than at normal (21%). They postulated involvement of non-photorespiratory substrates formed during photosynthesis (e. g. malate and/or pyruvate), in occurence of LEDR. Our assumption that LEDR in pea leaves resulted mainly from an increased oxidation of glycine was confirmed by markedly higher rate of its oxidation than other respiratory substrates, greater level of glycine and serine (Fig. 2), and other free amino acids (not shown) in mitochondria isolated from illuminated than from darkened leaves (Tabl. 1). High content of malate in illuminated pea leaves (not shown) could also stimulate oxidation of glycine (Wiskich et al. 1990).

Independently of the CO_2 concentration in the air (21% O_2) during the illumination of leaves, the ATP/ADP ratios were higher (in the range 25-40%) than in that illuminated at low O_2 (1%), (Fig. 3). Presented results suggest that both, the tricarboxylic acid cycle and the electron transport chain are capable of operation with the rate higher in leaves illuminated at 21% O_2 than at 1% O_2 or in the darkened. It seems that rate of LEDR may reflect the rate of respiration in the preceding light period.

Acknowledgments

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