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Mitochondrial oxidative phosphorylation participating in photosynthetic metabolism of a leaf cell

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Oligomycin was used for the selective inhibition of mitochondrial oxidative phosphorylation in barley leaf protoplasts. The addition of oligomycin inhibited the photosynthesis of intact protoplasts by 40–60%, this being accompanied by a pronounced decrease in cellular ATP content. When the protoplasts were ruptured in a way leaving the chloroplasts intact, the photosynthesis of the released chloroplasts was not inhibited by oligomycin. It is therefore concluded that in illuminated protoplasts, ATP formation by oxidative phosphorylation is required for utilization of the photosynthate by the cell. Thus, mitochondrial oxidative phosphorylation appears to serve an essential function for supplying the cytosol with ATP during photosynthesis.

Oxidative phosphorylation; Photophosphorylation; Plant respiration; Photosynthesis; (Barley protoplast)

1. INTRODUCTION

Green leaf cells have two organelles for electron transport-coupled ATP synthesis; chloroplasts and mitochondria. Whereas the role of mitochondrial ATP synthesis in maintaining plant metabolism in the dark is obvious, it has been a matter of debate as to whether mitochondrial oxidative phosphorylation also contributes to metabolism in the light. Although substantiated evidence is lacking, as it is difficult to measure respiration in the presence of photosynthesis [1], it has been widely assumed that in the light the ATP required in the cytosol is obtained mainly from the chloroplasts via the triose phosphate-phosphoglycerate shuttle, and that mitochondrial oxidative phosphorylation may be suppressed by an elevated cytosolic ATP/ADP ratio due to classical respiratory control [2,3]. The measurement of cytosolic adenylate levels, carried out with plant leaf protoplasts, however, did not support this concept, as the cytosolic ATP/ADP ratios in the light were found to be similar or even lower than in the dark [4–6].

In a C_3 plant, mitochondria participate in the photorespiratory cycle in converting glycine to serine. This reaction, occurring at high rates in the light, results in the formation of NADH in the mitochondrial matrix. In the photorespiratory cycle, an equimolar amount of NADH is required for the reduction of hydroxypyruvate to glycerate occurring in peroxisomes. There is an efficient malate-oxaloacetate shuttle to transfer redox equivalents from the mitochondrial matrix to the peroxisomes [7]. Due to the reduction equilibria, the presence of hydroxypyruvate in the peroxisomes results in the complete oxidation of mitochondrial NADH. Therefore, NADH formed in the mitochondrial matrix from the oxidation of glycine would be totally withdrawn to the peroxisomes and thus be unavailable as a substrate for mitochondrial electron transport unless there is an additional source of redox equivalents in the plant cell. Photosynthesis appears to be such an addi-

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tional source. Also, chloroplasts were shown to have a high capacity for transferring redox equivalents from the stroma to the cytosol via the malate-oxaloacetate shuttle [8]. The stromal NADP-malate dehydrogenase involved in this shuttle is under the control of the NADPH/NADP ratio [9]. In this way, excessive redox equivalents occurring in the stroma can be exported from the chloroplasts and be made available for peroxisomal hydroxypyruvate reduction. The equivalent amount of redox equivalents would not be withdrawn from the mitochondrial matrix and thus be available as a substrate for mitochondrial electron transport. This creates a mechanism by which surplus NADPH in the chloroplast stroma can ultimately be oxidized by mitochondrial electron transport.

Although these considerations make it seem likely that mitochondrial oxidative phosphorylation does have a function in photosynthesis, it remained to be elucidated whether this is indeed the case. Here, we investigate the role of oxidative phosphorylation in photosynthetic metabolism of barley leaf protoplasts. As shown below, selective inhibition by oligomycin of mitochondral oxidative phosphorylation in illuminated protoplasts results in the partial inhibition of photosynthesis and a decrease of cellular ATP content. This indicates that oxidative phosphorylation makes an important contribution to the synthesis of ATP in the light.

2. MATERIALS AND METHODS

Protoplasts from 10—14-day-old barley (*Hordeum vulgare*, var. 'Roland' obtained from R. Wolters, Göttingen) plants were prepared according to [4]. For preparation of spinach chloroplasts see [8] and of pea leaf mitochondria see [7]. Photosynthesis was measured using an oxygen electrode. Metabolite assays were carried out as in [10].

For measurement of mitochondrial respiration mitochondria were kept in a medium containing 0.3 M sucrose, 10 mM KH_2PO_4 (pH 7.2), 10 mM KCl, 5 mM $MgCl_2$, 0.1% (w/v) bovine serum albumin, 0.5 mM NAD and 20 mM glycine. Respiration was measured using the oxygen electrode after the addition of 1 mM ADP; temperature, 25°C.

For measurement of chloroplast photosynthesis, chloroplasts were maintained in a medium containing 0.33 M sorbitol, 50 mM Hepes (pH 7.6), 1 mM $MgCl_2$, 1 mM $MnCl_2$, 2 mM EDTA and 10 mM $NaHCO_3$. The reaction was initiated by illumination with white light (1×10^5 erg/s per cm^2); temperature, 20°C. Oxygen evolution was measured after linear kinetics had been attained. Measurement of protoplast photosynthesis was made in the same way, but the medium contained 0.4 M sorbitol, 50 mM Hepes (pH 7.6), 10 mM EDTA and 1 mM $MgCl_2$. These conditions were also employed for the determination of stromal metabolite levels (table 2). For this samples were deproteinized using 2% $HClO_4$ 8 min after the onset of illumination.

3. RESULTS

Oligomycin strongly inhibits mitochondrial oxidative phosphorylation at the site of ATPase [11]. As shown in fig.1 the coupled respiration of pea leaf mitochondria with glycine as substrate is inhibited by about 70% at an inhibitor concentration of 0.05 μg/ml. In comparison, the inhibitory effect on photophosphorylation by chloroplasts is very low [12]. As shown in fig.2, an oligomycin concentration as high as 20 μg/ml inhibited the photosynthesis of intact chloroplasts by only 20%. Similar results were also obtained in photophosphorylation measurements with thylakoids from spinach chloroplasts, where the inhibition by 1 μg/ml oligomycin was less than 10% (not shown). These results show that oligomycin at about 0.1 μg/ml can be used in a plant cell to inhibit selectively mitochondrial ATP synthesis without markedly affecting the ATP synthesis by chloroplasts.

The addition of oligomycin to barley protoplasts resulted in the partial inhibition of photosynthesis (fig.3). Half-maximal inhibition was obtained at an oligomycin concentration of about 0.05 μg/ml. The striking similarity between the oligomycin sensitivity of plant protoplast photosynthesis and of mitochondrial ATP synthesis is obvious. The inhibition of photosynthesis by oligomycin, however, is only observed with intact protoplasts. This is shown in table 1. When protoplasts, in which photosynthesis was inhibited by oligomycin, were disrupted by forcing them through a 5 μm

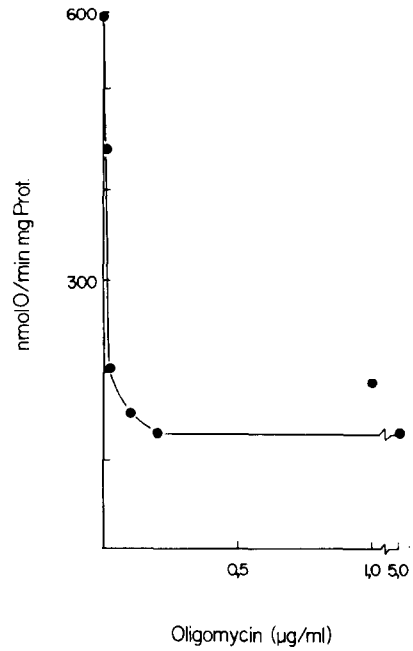

Fig.1. Pea leaf mitochondria. Effect of oligomycin on coupled respiration with glycine as substrate. Concentration of mitochondria, 120 μg protein/ml.

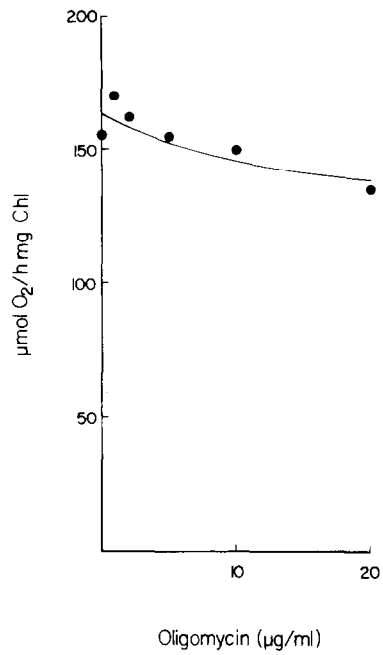

Fig.2. Spinach chloroplasts. Effect of oligomycin on photosynthesis. Concentration of chloroplasts, 45 μg Chl/ml.

nylon net, a procedure leaving chloroplasts and mitochondria intact, photosynthesis was found to be stimulated, recovering to the rate found without oligomycin in either intact or disrupted protoplasts. The oligomycin insensitivity of photosynthesis of chloroplasts released after rupture of protoplasts concurs with the oligomycin insensitivity of isolated spinach chloroplasts shown in fig.2.

It appears from these results that the strong inhibition of photosynthesis observed with these low concentrations of oligomycin was not due to an effect of oligomycin on chloroplast photosynthesis as such, but to an inhibition of the utilization of the photosynthate outside the chloroplasts in the cytosol. If an inhibition of mitochondrial oxidative phosphorylation were responsible for this, one should expect a decrease in cellular ATP content under these conditions. This is actually the case. As shown in table 2, the addition of 0.05 μg/ml oligomycin caused a drastic decrease in the cellular ATP/ADP ratio and a large increase in AMP content. Taking into account that about half of the total cellular adenylates are located in the chloroplasts [4], and that the chloroplast

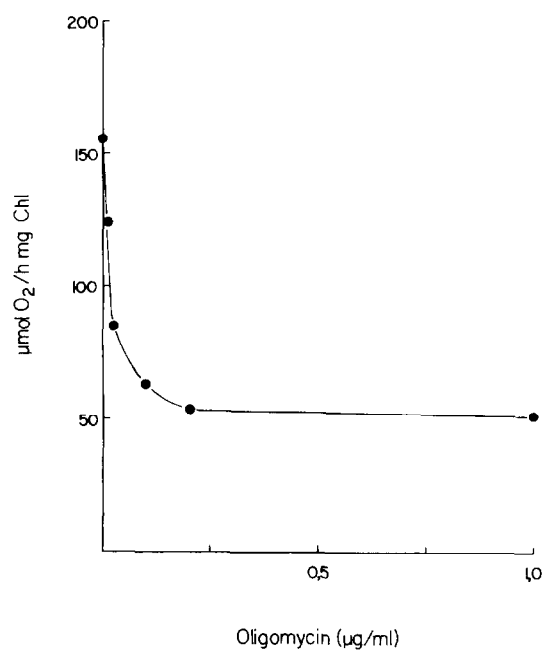

Fig.3. Barley leaf protoplasts. Effect of oligomycin on photosynthesis. Concentration of protoplasts, 18 μg Chl/ml.

Table 1

Effect of oligomycin on photosynthesis of protoplasts and chloroplasts

	Photosynthesis (μmol O_2 evolved/mg Chl per h)	
	Control	+ oligomycin (0.1 μg/ml)
Protoplasts, intact	139 ± 9	87 ± 5
Protoplasts, disrupted	132 ± 11	127 ± 17

Protoplasts were disrupted by forcing them through a 5 μm nylon net. Values are means from 4 different preparations ± SD

ATP/ADP ratio may be increased rather than decreased on addition of oligomycin to protoplasts, the decrease in cytosolic ATP/ADP ratio upon addition of oligomycin would be even larger than that of the overall ATP/ADP ratio. In plant protoplasts, most of the photosynthate is converted into sucrose, an ATP-consuming process located in the cytosol. The sharp decrease in photosynthesis of intact protoplasts upon addition of oligomycin may be therefore explained by a decrease in sucrose synthesis caused by metabolic regulation, e.g. of fructose bisphosphatase and sucrose-phosphate synthase [14] in response to the decreased availability of cytosolic ATP. This notion is supported by metabolite measurements. As shown in table 2, the cellular contents of triose and hexose phosphates, precursors of sucrose synthesis, are found to be increased upon addition of oligomycin.

Maury et al. [13] reported that the addition of Mg^{2+} to illuminated chloroplasts caused efflux of K^+ and acidification of the stroma, effects which could be reversed by the addition of K^+ to the medium. With 2 μg/ml oligomycin, however, K^+ did not prevent the acidification caused by Mg^{2+} addition. From these findings the authors concluded that there is an ATP-dependent proton pump in the envelope with the function of maintaining a pH gradient, and that this pump was being inhibited by oligomycin. The inhibitory effect of oligomycin on protoplast photosynthesis shown above cannot be explained in terms of the results of Maury et al. [13] for the following reasons: (i) the oligomycin sensitivity of protoplast photosynthesis is more than one order of magnitude higher than that of the postulated proton pump; (ii) an inhibition of photosynthesis by acidification of the chloroplast stroma is known to result in a large increase in stromal ATP/ADP ratio [15], whereas in our experiments a marked decrease in cellular ATP/ADP ratio is found. In control experiments (not shown) we also investigated the oligomycin sensitivity of photosynthesis in ruptured protoplasts in the presence of 1 mM Mg^{2+} and 1 mM Mg^{2+} plus 20 mM K^+. In both cases the inhibition of photosynthesis by 0.05 μg/ml oligomycin was less than 10%.

4. DISCUSSION

Our results demonstrate that the inhibition of mitochondrial ATP synthesis in the light has a severe effect on the metabolic performance of plant protoplasts. Although it remains to be elucidated whether this is a common feature of green plant cells, these data suggest that mitochondrial oxidative phosphorylation can also make an important contribution to supplying the cytosol of a plant cell in the light. In previous discussions about the role of oxidative phosphorylation in a green plant cell in the light, mitochondrial respiration was primarily connected with the oxidation of

Table 2

Effect of oligomycin on the metabolite content of illuminated barley protoplasts

[Oligomycin] (μg/ml)	Metabolite (nmol/mg Chl)						
	ATP	ADP	AMP	ATP/ADP	Triose P	Fru-1,6-P_2	Gluc-6-P
0	37	36	<4	1.0	32	62	189
0.05	18	39	21	0.45	42	92	220
0.1	8	32	29	0.26	46	91	245

Protoplast concentration, 40 μg Chl/ml

carbon compounds [1]. In fact, the release of CO_2 from substrate oxidation has often been used as a measure of respiration occurring in the light [1]. Oxidation of carbon compounds by the Krebs cycle for the sake of ATP synthesis proceeding in the same cell simultaneously with CO_2 fixation by the Calvin cycle would indeed be a waste of energy. If, on the other hand, the fuel for mitochondrial respiration is substrate hydrogen generated by noncyclic electron transport, as described in section 1, mitochondrial respiration in the light could be regarded as an energy-saving device. The cooperation of noncyclic photosynthetic and mitochondrial electron transport seems to be a much more efficient mechanism for the conversion of light energy into ATP than cyclic photophosphorylation. In cyclic photophosphorylation, according to the widely accepted stoichiometry, the absorption of two quanta of light leads to the formation of 0.66 molecules of ATP. If in noncyclic photosynthetic electron transport, four quanta of light yield 1.3 molecules of ATP and 1 molecule of NADPH, the oxidation of the latter by mitochondrial electron transport would yield in addition about 2.5 molecules of ATP. In this way the yield of ATP per four quanta of light absorbed, would be 3.8 molecules, which is about three times higher than the quantum yield of cyclic photophosphorylation.

Thus, mitochondrial oxidative phosphorylation appears to be an efficient way for providing the cytosol with ATP. In spinach leaf with a photosynthetic rate of 200 μmol CO_2/mg Chl per h (unpublished) and all photosynthate being converted to sucrose, the cytosolic ATP demand for sucrose synthesis alone would be 17 μmol/mg Chl per h, with an ATP/O ratio of 2.5 equivalent to a respiration rate of about 7 μgatom O/mg Chl per h. On top of this comes the ATP demand for other reactions, e.g. active transport and biosynthetic processes. In leaf discs from spinach, the average rates of dark respiration were 11 μgatom O/mg Chl per h [10]. If one assumes that this respiration may be necessary for the supply of the cytosol in the light, a total respiration rate of about 20 μgatom O/mg Chl per h might be required for meeting the ATP demand of the cytosol in the light. In spinach leaves the maximal capacity of mitochondrial respiration, as measured with glycine as substrate, is about 50 μgatom O/mg Chl per h [16]. This shows that in a leaf cell the capacity of mitochondrial oxidative phosphorylation is more than sufficient to supply the cytosol with ATP.

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