Interactions between Mitochondria and Chloroplasts in Cells

I. ACTION OF CYANIDE AND OF 3-(3,4-DICHLOROPHENYL)-1,1-DIMETHYLUREA ON THE SPORE OF FUNARIA HYGROMETRICA

Daniel Chevallier and Roland Douce
Laboratoire de Physiologie Végétale, Université Scientifique et Médicale de Grenoble, B.P. 53-Centre de Tri. 38041 Grenoble-Cédex France

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ABSTRACT

The effects of cyanide and 3-(3,4-dichlorophenyl)-1,1-dimethy lurea (DCMU) on photosynthesis and respiration of intact chlorophyllic moss (Funaria hygrometrica) spore were investigated. Thirty micromolar cyanide strongly inhibited dark respiration, without effect on photosynthesis at high light intensities (above the saturation plateau values), and stimulated photosynthesis at low light intensities (below the saturation plateau values). Three hundred nanomolar DCMU inhibited the photosynthesis and was without effect, even under light conditions, on the dark respiration. It seems likely, therefore, that in the chlorophyllic moss spore the cytochrome oxidase pathway is not functioning under high light intensities unless the photosynthesis is inhibited by DCMU.

It is now well established that during photosynthesis dihydroxyacetone-P and glyceraldehyde-3-P are rapidly transferred across the chloroplast envelope (2, 9, 10, 17, 22, 23). This shuttle mechanism induces a rapid increase in the cytoplasmatic ratio ATP/ADP which is transmitted to the mitochondria by the mitochondrial adenylate translocator (5, 9, 21). Under these circumstances, the Cyt oxidase pathway is probably inhibited in the light. Effects of light on respiration of autotrophic green cells have repeatedly been described but have been clouded with conflicting reports (6, 7, 9, 12, 19).

In the present study, we describe the effects of KCN and DCMU on the chlorophyllic spores of the moss Funaria hygrometrica in order to elucidate under light or dark conditions the possible interactions between mitochondria and chloroplasts.

MATERIALS AND METHODS

Shoots of Funaria hygrometrica Hedw. bearing mature sporophytes were collected in the spring of 1974 near Grenoble. The sporophytes were stored dry until needed.

The basic nutrient medium used for the culture of the spores was made up according to Kofer (15). The medium which contained 12 g/l of Noble agar Difco was poured into sterilized Petri dishes. The agar surface was covered with a sterilized cellophane membrane (cellulose membrane, Société “La Cellophane”, IIO Bd Haussman, Paris) (16). The Petri dishes were placed in a growth chamber at 24 C under continuous white light (10 w·m⁻²). The spores were rapidly collected by filtration on a Millipore filter (25 mm diameter, pore size: 5 μm).

Photosynthetic O₂ production and respiratory O₂ uptake were measured at 25 C in a 3 ml stirred cell, using an O₂ electrode. The O₂ concentration in air-saturated medium was taken as 240 μM. Photosynthetic CO₂ uptake and respiratory CO₂ production were measured at 25 C in the same cell, using a glass electrode (Beckman, Century SS pH meter). The velocity of the change of pH between 6.9 and 7.2 was recorded. The scale was calibrated by known quantities of HCl. Light was provided by a 300-w projector lamp through a 10-cm layer circulating water. Except when otherwise stated, the light intensity was 7.5 × 10⁶ ergs sec⁻¹ cm⁻². All reaction mixtures for spores were prepared and used at 25 C and contained: 0.3 mM mannitol and 0.5 mM NaHCO₃, pH 7.2.

Spores were prepared for ultrastructural studies by fixation for 1 hr with 4.5% glutaraldehyde in cacodylate buffer at pH 7.4. Following several washes in cold buffer, the samples were post-fixed with 1% OsO₄. Dehydration in an ethyl alcohol series was followed by embedding in Araldite. The Araldite blocks were trimmed and sectioned with a Sorval Porter-Blum MT-1 ultramicrotome. Specimens were stained with uranyl acetate and Reynolds' lead citrate. Electron microscopy was performed with a Siemens Elmskopp 1 A microscope.

Chlorophyll was extracted from spores in 80% acetone and measured according to Arnon (1). Protein was determined by the Folin-Ciocalteau phenol reagent (18).

RESULTS

Figure 1a shows a thin section of an ungerminated dry spore. The mitochondria are numerous, contain a number of well developed cristae, and range in length from 0.6 to 1 μm. The spheroidal typical proplastids range from 1.5 to 3.5 μm in diameter and contain several starch granules. The cytoplasm is rich in lipid bodies. Figure 1b shows a thin section of a 20-hr-old spore. The spore remains a single cell. There is, however, evidence of cracking of the outermost spore wall layer. The shape and the size of the mitochondria are unchanged. The chloroplasts are fully developed and contain a well developed grana fret-work system. Starch is present in the chloroplasts in relative abundance. At this stage, the chloroplasts are as numerous as the proplastids of the dry spore.

Photosynthesis appears very rapidly in moss spores (Fig. 2). Photosynthesis is perceptible after 10 hr of growth and reaches its maximum value at 20 hr. The spore presumably has low rates of photorespiration because net photosynthesis is not increased in 5 μM O₂ as compared with 240 μM (24).

Examples of O₂ electrode tracing of O₂ concentration are shown in Figure 3a. Upon illumination, O₂ production starts rapidly and proceeds at a remarkable constant rate for a long period of time (at least for 2 hr). The same is true for the dark respiration. Figure 3b shows that upon addition of 30 μM KCN to dark spores, a clear inhibition of the O₂ uptake rate occurs. The shape of the curve obtained seems to indicate that KCN passes through the cell wall very slowly. In fact, this hypothesis is unlikely. We have systematically observed that the final inhibi...
tion is strongly dependent on the O₂ concentration in the reaction medium. The half-maximal inhibition of O₂ uptake rate is attained at 10 μM KCN. Upon illumination, although the dark respiration is inhibited, the O₂ production remains unaffected and proceeds at a constant rate for a long period of time. It has to be pointed out that, above 60 μM KCN, the O₂ production is also affected (Fig. 3, c and d).

Figures 4 and 5 show that the effects of 30 μM KCN are dependent on the light intensity. At high light intensities above the saturation plateau values (7.5 × 10⁵ ergs sec⁻¹ cm⁻²), the addition of 30 μM KCN to the reaction medium is without effect on the O₂ production and CO₂ uptake rates (Figs. 4a and 5a). On the contrary, at low light intensities (1.5 × 10⁵ ergs sec⁻¹ cm⁻²), the addition of 30 μM KCN induces a marked increase of the O₂ production and CO₂ uptake rates (Figs. 4b and 5b). This effect is less pronounced at intermediate light intensities (results not shown).

Photosynthetic O₂ production by moss spores is inhibited by 300 nM DCMU (Fig. 6). On the contrary, O₂ uptake remains unaffected even after a long period of time and under light conditions. The rate of O₂ uptake is stimulated by an uncoupler (FCCP)¹ and is inhibited by 30 μM KCN.

**DISCUSSION**

These results indicate that, at least in moss spore, the light-saturated photosynthetic rate is not affected when the O₂ uptake catalyzed by the Cyt oxidase is specifically inhibited by 30 μM KCN (4, 13). Conversely, the Cyt oxidase pathway is not altered under light conditions when the electron flow between the two photosystems is specifically inhibited by DCMU. These results seem to be in good agreement with the work of, Heber (9) showing that in full light the Cyt oxidase pathway, which is under the control of the cytoplasmic ATP/ADP ratio, is not operative.

¹ Abbreviation: FCCP: carbonyl cyanide p-trifluoromethoxyphenylhydrazone.
However (Figs. 4 and 5), this hypothesis is not true at low light intensities. In this case, it is possible that the cytoplasmic ATP/ADP ratio is not high enough to permit an inhibition of the Cyt oxidase pathway.

Results give no information about the cyanide-insensitive respiration catalyzed by the mitochondria. Probably this pathway which is relatively insensitive to the energy conditions outside the chloroplasts (11, 20) is operative under light conditions. This could explain the fact that the Krebs cycle is operating during light conditions (19). Furthermore at the onset of photosynthesis the triose-P/glycerate-3-P shuttle transfer energy as ATP and NADPH (3, 8, 9, 14, 23). In the cytoplasm, the NADH formed is oxidized either by the oxaloacetate/malate shuttle (9) or by cyanide-insensitive respiration.

The fast growing spores represent a good material for the studies of the interactions between the chloroplasts and the mitochondria inside the cell. The arguments in favor of the use of this material are numerous. First, the O2 production and uptake proceed at a remarkable constant rate for a long period of time. Second, the cyanide insensitive O2 uptake is not considerable as it is often the case for the green leaves (Chevallier and Douce, unpublished data). Third, photorepiration does not seem to be operative.

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LITERATURE CITED


