INCOMPLETE MEMBRANE-BOUND PHOTOSYNTHETIC ELECTRON TRANSFER PATHWAY IN AGRANAL CHLOROPLASTS

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Summary. Photosystems I and II are not linked in agranal chloroplasts isolated from C_4-plants.

In chloroplasts of leaves the lamellae are frequently closely appressed to form stacks (grana). The precise physiological significance between grana formation and photosynthetic capacity has been the subject of much speculation. Lyttleton et al. (1) recently observed that in Amaranthus palmeri, a plant possessing the C_4-pathway of photosynthesis (2), grown under normal greenhouse conditions, the chloroplasts of the mesophyll cells contained grana while the chloroplasts of the bundle sheath cells contained unstacked lamellae. In contrast, at very high light intensities neither the mesophyll nor the bundle sheath chloroplasts contained grana while at low intensities grana were found in both. It would therefore appear that the presence or absence of grana in the chloroplasts of C_4-pathway plants is not necessarily

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related to the particular pathway of photosynthetic CO_2 fixation. A similar conclusion has been reached by Laetsch (3).

We have examined the photochemical activities of agranal and granal chloroplasts isolated from two C_4-plants, maize and sorghum, and find that while photosystems I and II are present in the agranal chloroplasts, they are not linked by a membrane-bound electron transfer pathway, as is the case in grana-containing chloroplasts. These observations are discussed in relation to the recent report by Woo et al. (4) that photosystem II is absent in agranal chloroplasts.

RESULTS

Fig. 1 compares the capacities of the mesophyll (granal) chloroplasts and bundle sheath (agranal) chloroplasts for photoreduction of NADP. Mesophyll chloroplasts reduced NADP at a rate of 1.2 (maize) and 0.56 (sorghum) μmoles NADP/min/mg chlorophyll. When mesophyll chloroplasts were subjected to the more vigorous procedures required to break the bundle sheath cells they did not lose this activity. DCMU abolished the reduction of NADP, but reduction resumed at a reduced rate after the further addition of ascorbate and DCIP.

In contrast, the bundle sheath chloroplasts did not photoreduce NADP. When ascorbate and DCIP were included in the reaction mixture containing bundle sheath chloroplasts, a light-dependent, DCMU-independent, reduction of NADP was observed. The addition of bundle sheath chloroplasts to mesophyll chloroplasts did not diminish the activities of the latter.

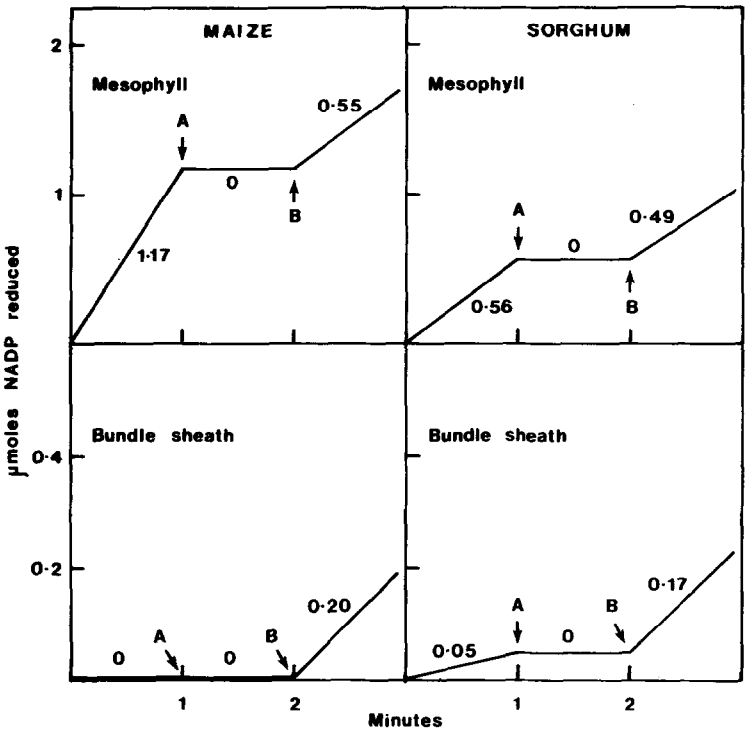

Fig. 1. Photoreduction of NADP by mesophyll and bundle sheath chloroplasts from maize and sorghum. The chloroplasts were isolated from the leaves of 12-to 16-day old maize (Zea mays var. DS 606A) and sorghum plants (Sorghum bicolor var. Texas 610) using the method described by Woo et al. (4) The plants were raised in a greenhouse during September. All chloroplast preparations were washed once with suspension medium before assay. NADP photoreduction was followed using an Aminco Chance Dual Wave-length Spectrophotometer fitted with a cross illumination attachment. The reaction mixture (1.5 ml) contained chloroplasts (5 µg chlorophyll) and sorbitol 300 mM, phosphate buffer pH 7.4 10 mM, $MgCl_2$ 1mM, NADP 0.67 mM and ferredoxin (from Anacystis nidulans) 3.3 µM. A, DCMU 1.25 µM added; B, ascorbate 2.5 mM and DCIP 67 µM added. The assay temperature was 25°C. The actinic light (tungsten light filtered with a Corning 2-60 red filter and two 1-69 infra-red filters; energy incident on sample was 6.9 X 10^4 ergs cm^{-2} sec^{-1}) and the ferredoxin concentration were both saturating for the chloroplast concentration used. All of the increases shown were light-dependent. The rates shown on the graphs are in µmoles NADP reduced/min/mg of chlorophyll.

Fig. 2 compares the Hill reaction activities of mesophyll and bundle sheath chloroplasts from maize and sorghum. Each of the three Hill oxidants tested, DCIP,

potassium ferricyanide and cytochrome c were reduced by both mesophyll and bundle sheath chloroplasts. The rate of photoreduction of cytochrome c by maize mesophyll chloroplasts,but not by maize bundle sheath chloroplasts, was stimulated by the addition of ferredoxin.

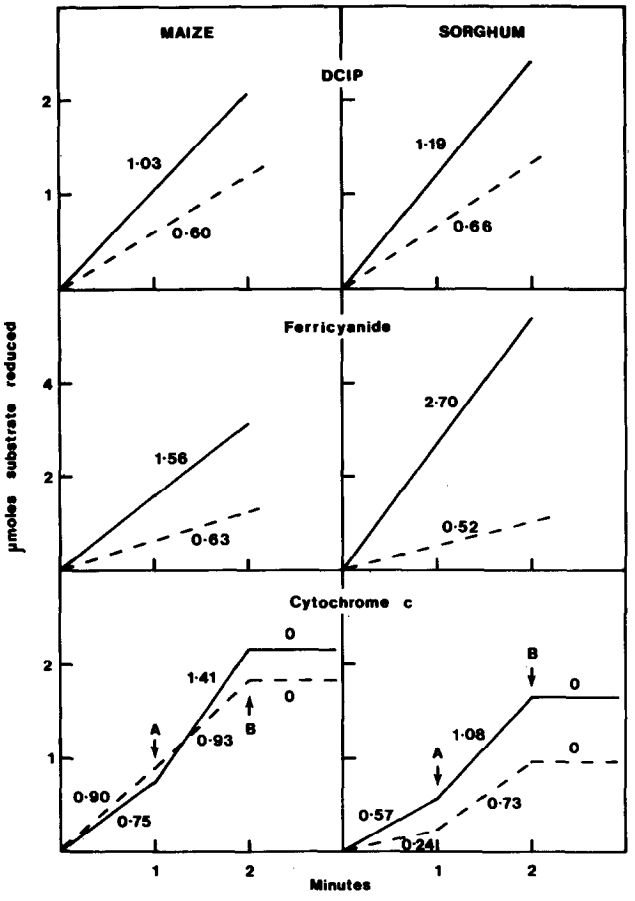

Fig. 2. Hill reaction by mesophyll and bundle sheath chloroplasts from maize and sorghum. Solid line, mesophyll chloroplasts, broken line, bundle sheath chloroplasts. The experimental conditions are described in Fig. 1. The reaction mixture (1.5 ml) contained chloroplasts (5 µg chlorophyll) and sorbitol 300 mM, phosphate buffer pH 7.4 10 mM, $MgCl_2$ 1 mM, and Hill oxidant (DCIP 17 µM, or $K_3Fe(CN)_6$ 330 µM or cytochrome c 50 µM). A, ferredoxin 3.3 mµM added; B, DCMU 1.25 µM added. The rates shown on the graphs are in µmoles substrate reduced/min/mg chlorophyll.

DISCUSSION

Our data confirm those of Woo et al. (4) that chloroplasts isolated from the bundle sheath cells of maize and sorghum leaves were unable to photoreduce NADP. Reduction of NADP requires the co-operation of photosystems I and II. NADP was, however, reduced by these chloroplasts in the presence of DCIP and ascorbate and hence they must have contained photosystem I.

Since all of the Hill oxidants tested by us were reduced by the bundle sheath chloroplasts in the light, photosystem II was also present. We conclude from this that photoreduction of NADP did not occur because the two photosystems were not linked. Cytochrome c, which is reduced by photosystem II, can also be reduced by photosystem I via ferredoxin and the addition of ferredoxin to the reaction mixture containing mesophyll chloroplasts and cytochrome c resulted in a 2-fold stimulation of cytochrome reduction (Fig. 2). No stimulation was obtained by adding ferredoxin to maize bundle sheath chloroplasts, again suggesting that reductants formed in photosystem II are not available for photosystem I.

The small activity of the sorghum bundle sheath chloroplasts for NADP photoreduction and the stimulation of cytochrome c reduction by ferredoxin might be attributed to contamination by mesophyll chloroplasts although microscopic examination of the bundle sheath cells just prior to breakage indicated that contamination by mesophyll cells was no more than 1 to 2%. Alternatively, it is possible that the two photosystems were not completely disconnected in the chloroplasts obtained from the plants grown under our experimental conditions.

A photosynthetic deficient mutant of Chlamydomonas reinhardi with characteristics similar to those of the bundle sheath chloroplasts has been known for some time. Levine and Smillie (5) showed that chloroplasts from this mutant, ac-21, failed to photoreduce NADP. Nevertheless, both photosystems I and II were present and functional, but there appeared to be no flow of electrons from photosystem II to photosystem I.

In the experiments of Woo et al. (4), the agranal bundle sheath chloroplasts were characterized by altered ratios of chlorophyll a to chlorophyll b, a reduced cytochrome b-559 content and the absence of a fluorescence signal attributed to photosystem II. They concluded that the pigment-protein assembly of photosystem II was absent. Although they did not directly assay for photosystem II activity, their plants were grown in a greenhouse in summer (C.B. Osmond, personal communication) and the prevailing high light intensities may have resulted in the destruction of photosystem II in the agranal chloroplasts. It is of interest that the chlorophyll a/b ratios of our bundle sheath preparations (maize 2.05, sorghum 2.62) were only slightly higher than those of the corresponding mesophyll chloroplasts (maize 1.64, sorghum 2.10). Most preparations enriched for photosystem I have a chlorophyll a/b ratio in the range 5 to 7 (6), and if the bundle sheath chloroplasts were devoid of photosystem II, a higher ratio might have been expected.

Our results point to a novel concept, namely that the rate of photosynthesis in leaves may be controlled by the extent of coupling between photosystems I and II.

This in turn may be correlated with the degree of stacking. This concept probably does not apply only to C_4-plants since grana formation in other plants can be reversibly influenced by light (7). While it is tempting to postulate that grana formation results in the linking of the two photosystems such a suggestion is inconsistent with observations made on the Chlamydomonas mutant ac-21, whose chloroplasts show near normal stacking (8). Instead, we prefer the explanation already put forth by Goodenough et al. (9) that a deficiency in photosystem II may have the effect of curtailing normal membrane stacking. We would further suggest that the absence of a fully constituted membrane-bound electron transfer pathway linking photosystems I and II can, in some instances, prevent normal stacking. In the mutant ac-21 the deficiency is not so severe as to impair stacking, while in the bundle sheath chloroplasts the loss of some critical protein(s) or other lamellar components has the effect of blocking both electron flow between photosystems I and II and grana formation. Deficiencies in other regions of the photosynthetic electron transfer system may result in complete loss of activity of photosystem II as well as loss of the capacity to form grana as exemplified by a number of mutants of Chlamydomonas reinhardi (8) and tobacco (10). The stroma lamellae of grana-containing chloroplasts of higher plants appear also to fit into this last category (11).

While photosystems I and II are not linked in the isolated chloroplasts from bundle sheath cells of maize and sorghum, it should be emphasized that this is not necessarily the case in the intact cells. A connection

between the two light systems might be accomplished in vivo by means of a soluble "linking" protein. If this were the case the rate of photosynthesis could be limited by the concentration of such a linking protein.

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