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Short Communication

Some Biochemical Characteristics of Chloroplasts from Mineral-deficient Maize[1]

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The effects of mineral deficiencies on plant growth have been studied for many years, but little use has been made of mineral deficiency as a tool for understanding the photosynthetic process. Hill reaction rates of chloroplasts from mineral-deficient plants were measured by Spencer and Possingham (1), who found lower rates per unit of chlorophyll in all deficiencies except iron. Kessler's (9) study of manganese-deficient plants indicate a role for this element in photosystem II, and this work has proved to be an important wedge into a difficult area of investigation (6).

We have measured the activity of each of the two photosystems in mesophyll cell chloroplasts from maize deficient in macronutrient elements. The work of Hall *et al.* (7) shows that some mineral deficiencies cause a distortion in chloroplast morphology. The work described below is an attempt to relate changes induced in chloroplast structure by mineral deficiencies with alterations of the composition and function of the photosynthetic apparatus.

Maize seed, *Zea mays* (H55 × C 103 Rf), was obtained from the Agriculture Alumni Seed Association, West Lafayette, Indiana. Diphenyl carbazide was purchased from Aldrich Chemicals, DCIP[4] from Eastman Organic Chemicals, and methyl viologen from K and K Laboratories. DCMU was a gift of the E. I. DuPont de Nemours and Co.

Six major mineral deficiencies (S, Mg, Ca, N, P, and K) were induced by the methods of Hoagland and Arnon (8). The growth conditions have been described by Hall *et al.* (7). The pigment composition and gross morphological symptoms of these deficient plants have been described earlier by Barr *et al.* (3). The chloroplasts were isolated from normal and deficient leaves 8 weeks after transplanting. Growth on deficient media for 4 weeks did not cause any pronounced alterations in the biochemical parameters measured, as seen by photosynthetic reaction rates.

Chloroplasts from 10 g of fresh leaves were prepared by briefly (45 sec-1 min) homogenizing the leaves in 0.4 M sucrose with 25 mM Tricine buffer, pH 7.5, in a Waring Blendor. This brief homogenization broke a minimum of bundle sheath cells (1). The leaf homogenate was passed through four layers of cheesecloth and centrifuged at 600*g* for 2 min. The supernatant was centrifuged at 1500*g* for 10 min. The pellet containing the chloroplasts was suspended in a standard volume of the sucrose-Tricine homogenization medium and assayed immediately for photosynthetic activities. Subsequently, aliquots from the same preparation were used for chlorophyll and protein analyses.

Photosystem I activity was measured as the light-dependent transfer of electrons from reduced TMPD to methyl viologen, and photosystem II activity was measured by following the light-induced reduction of DCIP with electrons from either water or DPC. The details of these measurements have been published elsewhere (5). Chlorophyll measurements were done according to Arnon (2). The data presented in Table I represent chlorophyll determinations of a random sample from the leaves of at least six plants. Leaves were cut into 5- to 6-cm squares and thoroughly mixed before random selection of three 1-g samples. Thus the values in Table I are average amounts of chlorophyll for each type of deficiency. Protein concentrations were measured using a modified biuret assay as follows. A sample of chloroplasts from 5 g of leaves containing approximately 10 mg of chlorophyll was extracted with 80% acetone in water (v/v) to remove the chlorophyll. The chloroplast proteins were pelleted by centrifugation and resuspended in 10 ml of 50 mM phosphate buffer with deoxycholate added to give a final concentration of 10% and a pH of 9. A few drops of a 20% solution of hydrogen peroxide were added to decolorize completely the suspension, then an aliquot was mixed with biuret reagent (12) in which each of the components was present at twice the usual concentration. After incubation for 15 min at 36 C, the 540 nm absorbance was measured, and the protein concentration was calculated with reference to a standard curve.

The relative chlorophyll and protein contents of chloroplasts from normal and the six different kinds of mineral-deficient maize are presented in Table I. In nitrogen-deficient plants there is a slight elevation in the ratio of chlorophyll *a* to *b* and a very pronounced elevation in the ratio of chloroplast protein to chlorophyll.

The data in Table II indicate that photosystem I activity, as measured by the transfer of electrons from TMPD to methyl viologen, is hardly affected by any of these mineral deficiencies. However, the photosystem II activity of nitrogen- and sulfur-deficient plants is increased on a chlorophyll basis. This increased specific activity in photoreduction of DCIP is seen when either water or DPC is used as the electron donor. In all cases the photosystem II activities were found to be DCMU-sensitive. In no case did addition to the isolated chloroplast of the mineral which had been omitted from the growth medium have any effect on the measured activities.

The accompanying paper (7) describes the effects of mineral deficiencies on chloroplast morphology. Each deficiency results in characteristic morphological change. In the analysis of

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[4] Abbreviations: DCIP: 2,6'-dichlorophenol indophenol; DPC: diphenylcarbazide; TMPD: N,N,N',N'-tetramethyl-*p*-phenylene diamine.

Table I. *Chloroplast Protein and Chlorophyll Content*

These values are from randomized samples from the leaves of six plants.

Deficiency	Chlorophyll *a/b*	Mg Protein/mg Chlorophyll[1]
Control	2.7	6.6
S	2.7	10.1
N	3.4	29
Mg	2.9	10
Ca	2.8	12
P	2.7	11
K	3.1	9

[1] Ratio given in place of absolute chlorophyll or protein values on dry or wet weight basis because in case of deficiencies very small amounts of functional chloroplasts can be isolated from equal amounts of leaf tissue.

Table II. *Photosynthetic Electron Transport Activities of Chloroplasts from Control and Mineral-deficient Maize Plants*

The basic reaction mixture contained the following in 3 ml of total volume: sodium ascorbate (50 μmoles), *Mes*-Tricine, 50 mM, pH 8.0, DCIP (1 μmole), methyl viologen (0.4 μmole) and chloroplasts containing 30 μg chlorophyll with additions of KCl (10 μmoles), DCMU (5 nmoles), and plastocyanin (15 pmoles).

Deficiency	PS1	PS2	
	Asc. TMPD → M.V	H_2O → DCIP	DPC → DCIP
	μmoles acceptor reduced/mg chlorophyll/hr		
Control	650	290	330
S	520	495	550
N	650	700	810
Mg	520	290	320
Ca	594	280	260
P	650	230	265
K	650	285	310

chlorophyll and chloroplast protein content, only nitrogen deficiency causes a significant change from the values observed in control material. As noted earlier (3), the amount of chlorophyll present in these leaves is greatly reduced by nitrogen deficiency. Since chloroplasts from nitrogen-deficient plants show many alterations in the kinds and amounts of protein present, there is no obvious relation between reduced chlorophyll content and changes in specific proteins. In as much as the specific activity of photosystem II is increased in chloroplasts from nitrogen-deficient leaves, there may be a selective retention of catalysts which increases the activity per unit chlorophyll in photosystem II. Since the photosystem I specific activities in the nitrogen deficient and control preparations are quite similar, there may be a uniform loss of all constituents from photosystem I or a parallel loss in both chlorophyll and rate-limiting catalyst(s).

Sulfur-deficient plants yield chloroplasts with a chlorophyll to protein content quite like that found in control material although the sulfur-deficient leaves are quite chlorotic and show a very obvious diminution in total chlorophyll. Again the specific activity of photosystem II is high. The sulfur-deficient growth results in a marked reduction in sulfolipid and digalactosyl diglyceride content of these chloroplasts (4). This loss of membrane lipid might result in the decrease in stroma lamellae observed in these chloroplasts (7), which in turn might facilitate the reduction of DCIP.

The significance of increased grana stacking in N- and S-deficient plants which is also associated with increased photosystem II activity is further discussed by Hall *et al.* (7). From a functional point of view the data presented here show a correlation of high photosystem II activity with the increase in grana stack membranes seen by Hall *et al.* (7).

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