A Study of Chloroplast Membrane Polypeptides from Mineral-Deficient Maize in Relation to Photosynthetic Activity[1]

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Abstract

Photosynthetic activity and the chloroplast protein distribution patterns on acrylamide gels were studied in micronutrient-deficient maize chloroplasts including boron, copper, iron, manganese, molybdenum, zinc, and all-micronutrient deficiencies. It was found that the chlorophyll concentration was reduced in all deficiencies compared to normal maize chloroplasts. The reaction rates of Photosystem I activity varied among the deficients but Photosystem II activity was reduced in all deficiencies. Non-cyclic photophosphorylation rates were affected most by manganese, zinc, copper, and all-micronutrient deficiencies, but cyclic photophosphorylation rates were most severely reduced by copper, manganese, and all-micronutrient deficiencies. The protein profiles of micronutrient-deficient maize chloroplast proteins were basically similar, with the exception of zinc-deficiency which showed less bands than normal maize chloroplasts and iron and all-micronutrient deficiencies which showed more bands than control chloroplasts. Of the 16-20 chloroplast proteins, five were found to be glycoproteins. The function to structure concept is discussed in relation to maize chloroplasts.

Introduction

Chloroplast proteins have been studied by gel electrophoresis by many investigators (5, 7, 10, 11, 12, 13), but the identification of stained bands with particular components of the electron transport chain has in most cases been tentative. Klein and Vernon (9) using Weber and Osborn's (14) sodium dodecyl sulfate system classified spinach chloroplast proteins according to molecular weight. On this basis they tentatively assign the names of known proteins to gel protein bands within a particular molecular weight range. Thus the correlation between functional chloroplast proteins and electrophoresed protein profiles is only in the beginning stages. In this study we chose a system which would allow us to correlate the knowledge between structure and function with more precision. By studying photosynthetic reactions in various types of micronutrient deficiencies in maize and correlating putative differences in function to differences in protein profiles on gels, we hoped to gain more knowledge about the photosynthetic apparatus in plants.

Materials and Methods

Micronutrient-deficient maize was grown in liquid culture according to Hoagland and Arnon's methods (6) in a controlled climate chamber for 5 weeks prior to chloroplast preparation from leaves with maximum expression of deficiency symptoms. Photosynthetic activity and photophosphorylation assays were performed with freshly prepared chloroplasts, but proteins were isolated from frozen chloroplasts by precipitation with 80, 90 and 100% acetone. Proteins were quantitated by the double biuret assay (15), chlorophyll by Arnon's method (1).

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Photosynthetic reactions (ascorbate and TMPD→methyl viologen for Photosystem I and water→indophenol dye or diphenylcarbazide→ indophenol for Photosystem II) were performed as described by Baszynski *et al.* (3) for macro-deficient maize chloroplasts.

Photophosphorylation rates were measured according to Dilley (4) by the acid→base transition method using a pH meter. Chloroplasts for photophosphorylation were prepared in a special medium containing ascorbate and albumin. Non-cyclic photophosphorylation was measured using methyl viologen, cyclic photophosphorylation with phenazine methosulfate in the reaction mix.

Leaf spectra of microdeficient maize leaves (Figs. 1 and 2) were taken with a Unicam SP spectrophotometer against an opal glass standard to reduce scattering as described by Barr *et al.* (2).

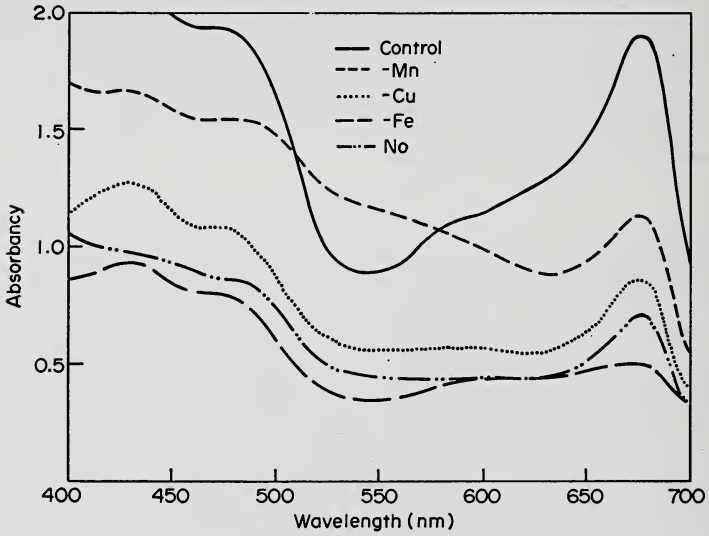

FIGURE 1. *Spectra of normal maize leaves compared to spectra of micronutrient-deficient maize leaves.*

Gel electrophoresis of chloroplast proteins was done by Hoober's methods (8). Protein bands were stained with Coomassie brilliant blue and destained according to Weber and Osborn (14) in acetic acid, methanol and water. Glycoproteins were identified on gels by the procedure of Zacharius and Zell (16). The reddish glycoprotein bands were found to become obscured by the background within 30 min after destaining. Gel protein band profiles seen in Figures 3, 4, and 5 were obtained by scanning with a Beckman Acta III spectrophotometer equipped with a special gel holder.

Results

Figures 1 and 2 present leaf spectra of normal maize leaves compared to spectra from leaves of various micronutrient deficiencies. The

670-680 nm region shows chlorophyll a peaks. In all deficiencies including boron, copper, iron, manganese, molybdenum, zinc and all-micronutrient deficiencies, the chlorophyll a content is reduced in varying degrees.

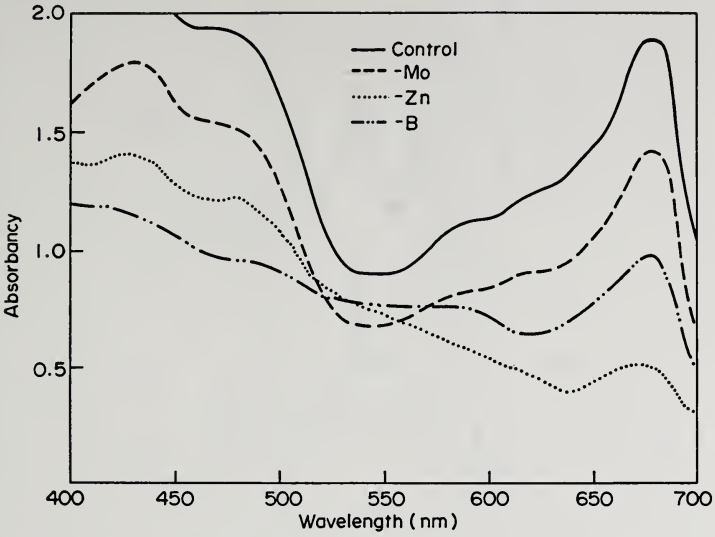

FIGURE 2. *Spectra of normal maize leaves compared to spectra of micronutrient-deficient maize leaves.*

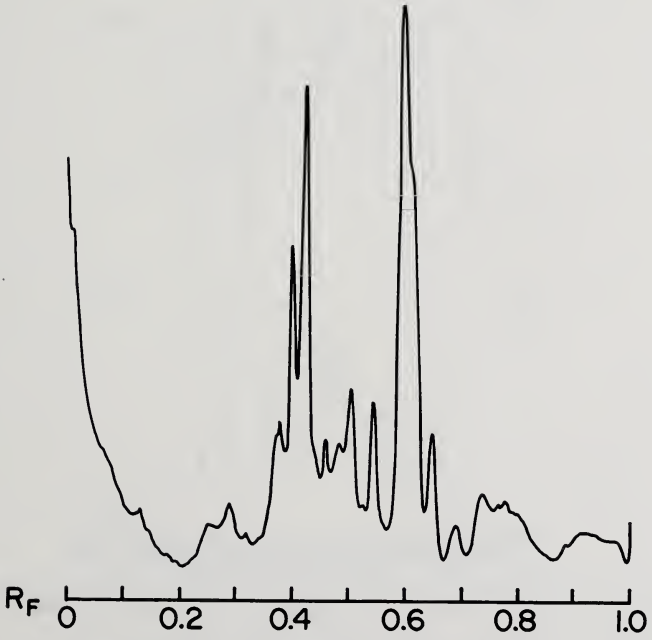

FIGURE 3. *A spinach chloroplast protein profile made by densitometer tracing of protein bands after electrophoresis on an acrylamide gel*

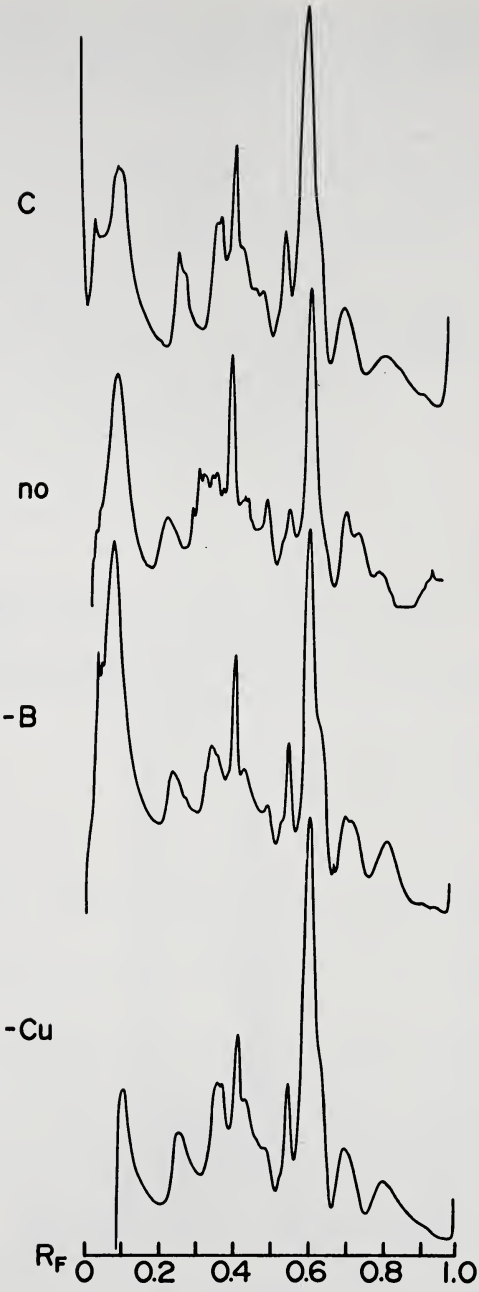

FIGURE 4. *A maize chloroplast protein profile from normal and micronutrient-deficient maize leaves.*

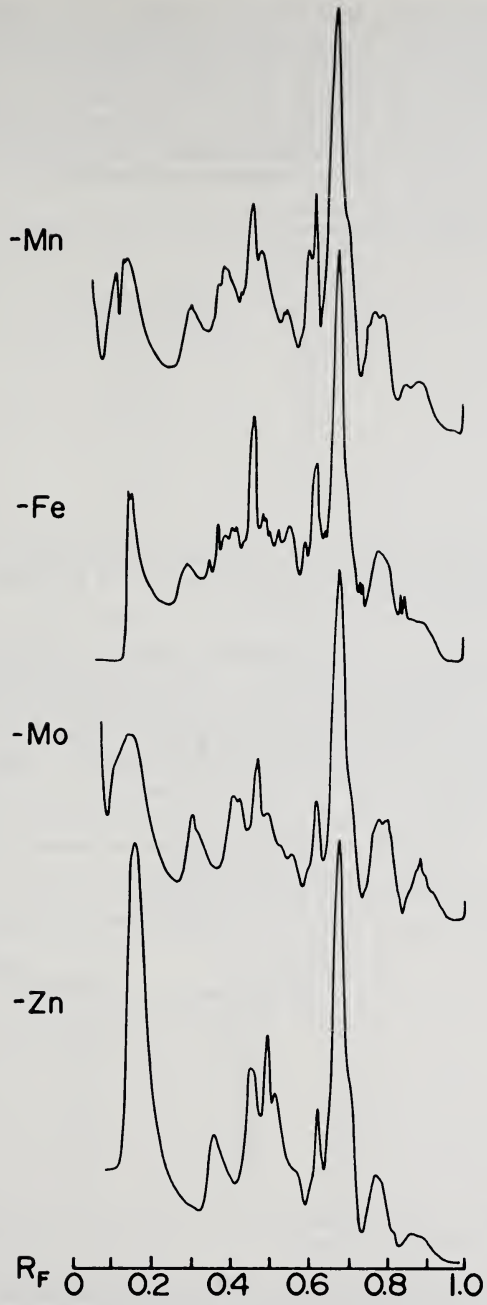

FIGURE 5. *A maize chloroplast protein profile from micronutrient-deficient maize leaves.*

Table 1 shows the chlorophyll a/b ratios and protein/mg chlorophyll concentration in normal and micronutrient-deficient chloroplasts. Only iron deficiency affects the chlorophyll a/b ratio because less chlorophyll a is synthesized under these conditions. On total chlorophyll basis, the protein concentrations of chlorotic or slightly chlorotic chloroplasts therefore increase giving higher mg protein/mg chlorophyll values in iron, zinc and all-micronutrient deficiencies.

TABLE 1. *Chloroplast protein and chlorophyll content.*

Deficiency	Chlorophyll a/b	Mg protein/mg chlorophyll[1]
Control	3.3	6.5
Boron	3.3	6.4
Copper	2.9	7.8
Iron	2.1	10.6
Manganese	3.2	8.6
Molybdenum	3.3	6.6
Zinc	2.9	11.5
No micronutrients	3.0	12.0

[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 2 reports the photosynthetic reaction rates of normal and micronutrient-deficient chloroplasts. Zinc and all-micronutrient deficiencies lead to decreased, Mn-, Mo-, and B-deficiencies to increased PSI rates. Photosystem II rates either from $H_2O \rightarrow$ indophenol or diphenylcarbazide→indophenol are reduced in all deficiencies compared to control chloroplast rates.

TABLE 2. *Photosynthetic reaction rates of normal and micronutrient-deficient maize chloroplasts.*[1]

	umoles acceptor reduced/mg protein/hr		
	PS I	PS II	
Deficiency	Asc. TMPD➤M.V.	H_2O➤DCIP	DPC➤DCIP
Control	100	45	51
Boron	119	17	19
Copper	85	11	13
Iron	106	11	14
Manganese	131	13	15
Molybdenum	124	14	17
Zinc	61	8	8
No micronutrients	21	5	7

[1] Data by T. Baszynski and J. Brand

Table 3 compares non-cyclic and cyclic photophosphorylation rates in normal and microdeficient maize chloroplasts. Non-cyclic photophosphorylation with methyl viologen is most severely curtailed by Mn-deficiency. Cyclic photophosphorylation, on the other hand, is most

severely affected by Cu-, all-micronutrient, or Mn-deficiency. The most interesting case is presented by Mo-deficiency in which non-cyclic photophosphorylation is decreased but the cyclic rate is increased.

TABLE 3. *Photophosphorylation rates in normal and micronutrient-deficient maize chloroplasts.*

	umoles ATP formed/mg protein/hr	
Deficiency	Non-cyclic with M.V.	Cyclic with PMS
Control	4.6	10.5
Boron	3.5	12.0
Copper	2.6	2.5
Iron	4.9	10.2
Manganese	1.2	2.9
Molybdenum	2.5	15.7
Zinc	2.3	5.5
No micronutrients	2.6	2.6

Figure 3 presents the chloroplast polypeptide profile obtained by densitometer scanning of a polyacrylamide gel on which spinach proteins have been separated by electrophoresis in an SDS-urea system. Since such a system separates polypeptides by molecular weight, low-molecular weight proteins migrate through the gel faster than high-molecular weight ones. Therefore, the R_F of low-molecular weight proteins approaches 1, while the reverse is true for high-molecular weight proteins. In Table 4 Klein and Vernon's (9) identification of some of the major peaks of a spinach chloroplast protein profile is compared with chloroplast polypeptides from maize within a given R_F range.

TABLE 4. *A Comparison of Klein and Vernon's spinach chloroplast proteins with polypeptides from maize.*

Spinach chloroplast component	Molecular wt. (Kilodaltons)	Maize chloroplasts (RF range)
Plastocyanin	10	0.9-1.0
Ribulose-diphosphate carboxylase	12	------
Coupling factor	13	------
Ferredoxin	14	0.8-0.9
Coupling factor	17.5	0.7-0.8
Structural protein	23	0.6-0.7
Cytochrome f	33	0.6-0.7
Coupling factor	37	0.5-0.6
Cytochrome b_6	42	0.5-0.6
Transhydrogenase	45	0.4-0.5
Ribulose-diphosphate carboxylase	56	0.3-0.4
Coupling factor	56	0.3-0.4
Coupling factor	59	0.3-0.4
Chlorophyll a-bearing protein	61	0.2-0.3
Unknown	--	0.1-0.2
Unknown	--	0-0.1

Four or five of the bands seen in Figures 4 and 5 are associated with the coupling factor. Two of the prominent peaks within the R_F range 0.3-0.4 belong to the coupling factor. This puts them in the range of molecular weights between 50-60,000.

The largest peak seen in Figures 4 and 5 belongs to "structural protein" (R_F 0.6-0.7). In spinach chloroplasts (Fig. 3), normal maize chloroplasts, B-deficient, and Cu-deficient chloroplasts (Fig. 4), as well as in Mn-deficient, Mo-deficient, and Zn-deficient chloroplasts (Fig. 5) there may be a double peak in this R_F range, a part of which appears to contain a glycoprotein (R_F 0.64 in Table 5). As Figures 4 and 5 show, most of the chloroplast polypeptides seen upon SDS gel electrophoresis can be found in varying proportions in all micro-deficient maize chloroplasts. The unique features to distinguish one deficiency from another by densitometer profiles of chloroplast proteins is the occurrence of more bands than normal in iron and all-micronutrient deficiencies, especially in the 0.3-0.5 R_F region. The most significant difference between spinach chloroplast polypeptidese (Fig. 3) and maize chloroplast polypeptides (Figs. 4 and 5) is finding large-molecular weight proteins in maize (R_F range 0-0.2). This is especially striking in Zn- (Fig. 5) and B-deficiencies (Fig. 4).

TABLE 5. *A comparison of normal and micronutrient-deficient maize chloroplast glycoproteins*[1]

Deficiency	R_F of major glycoprotein bands				
	0.32	0.49	0.60	0.64	0.67
Control	++	+++	+	+++	+
Boron	+	+++	+	+++	+
Copper	++	+	+	+++	+
Manganese	++	+++	?	+++	+
Molybdenum	+	+++	+	+++	+
Zinc	++	++	+	+++	+

[1] These data are contrary to McEvoy and Lynn (J. Biol. Chem. 248:4568, 1973) who found no glycoproteins in spinach chloroplasts.

Discussion

The purpose of this study—to correlate photosynthetic reactions with electrophoretic protein profiles on gels in micronutrient-deficient maize—has been realized only partially. The original premise depended on finding specific differences in photosynthetic reaction rates with each deficiency. As can be seen in Table 2, Photosystem I rates have been affected most severely by the absence of all micronutrients and in zinc and Manganese deficiencies, while photosystem II rates have been reduced in all deficiencies compared to rates in normal maize chloroplasts. Non-cyclic photophosphorylation rates (Table 3) are also greatly reduced in manganese and zinc deficiencies; copper, manganese and the absence of micronutrients influences cyclic photophosphorylation most. Such differences in photosynthetic activity would be expected to correlate with the chloroplast protein distribution patterns but this is not entirely

the case, as seen in Figures 4 and 5. Basically, the gel protein profiles of all micronutrient-deficient maize plants are similar—structural protein (R_F range 0.6-0.7), the major bands of the coupling factor (R_F range 0.3-0.4) the chlorophyll a-bearing protein (R_F range 0.2-0.3), and ribulose-diphosphate-carboxylase (R_F 0.4-0.5) are present in all cases. The most striking difference is shown by zinc-deficient chloroplasts which have less protein bands than normal maize, and by iron and all-micronutrient deficient chloroplasts which show more bands than normal maize. The glycoprotein distribution pattern (Table 5) is also basically the same in all micronutrient-deficient maize plants showing only quantitative differences among the 2 major and 3 minor glycoproteins found in maize. In spinach *versus* maize chloroplasts, maize shows the presence of large-molecular weight proteins (R_F 0-0.2) which are absent from spinach chloroplasts.

Major changes in photosynthetic activity are not correlated with any change in the major polypeptide components of the chloroplast membrane; minor components may change. The loss of function must be more specificially associated with proteins which do not make a large contribution to membrane bulk. Perhaps polypeptides can still be formed and incorporated into the membrane even if a micronutrient prosthetic group is not available. Other effects may be secondary as in the case of boron where a change in metabolic function of the cell may prevent formation of some essential chloroplast component. In this latter situation the micronutrient would not necessarily be found in or function within the chloroplast.

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