Plant Physiol. (1982) 69, 107–111
0032-0889/82/69/0107/05/$00.50/0

Effects of Iron and Oxygen on Chlorophyll Biosynthesis[1]

I. *IN VIVO* OBSERVATIONS ON IRON AND OXYGEN-DEFICIENT PLANTS

Received for publication March 31, 1981 and in revised form July 31, 1981

SUSAN C. SPILLER, ANN M. CASTELFRANCO[2], AND PAUL A. CASTELFRANCO[3]
Department of Botany, University of California, Davis, California 95616

ABSTRACT

Corn (*Zea mays*, L.), bean (*Phaseolus vulgaris* L.), barley (*Hordeum vulgare* L.), spinach (*Spinacia oleracea* L.), and sugarbeet (*Beta vulgaris* L.) grown under iron deficiency, and *Potamogeton pectinatus* L, and *Potamogeton nodosus* Poir. grown under oxygen deficiency, contained less chlorophyll than the controls, but accumulated Mg-protoporphyrin IX and/or Mg-protoporphyrin IX monomethyl ester. No significant accumulation of these intermediates was detected in the controls or in the tissue of plants stressed by S, Mg, N deficiency, or by prolonged dark treatment. Treatment of normal plant tissue with δ-aminolevulinic acid in the dark resulted in the accumulation of protochlorophyllide. If this treatment was carried out under conditions of iron or oxygen deficiency, less protochlorophyllide was formed, but a significant amount of Mg-protoporphyrin IX and Mg-protoporphyrin IX monomethyl ester accumulated.

These results are consistent with the presence of an O_2, Fe-requiring step between Mg-protoporphyrin IX monomethyl ester and protochlorophyllide.

Iron and O_2 are essential to a vast array of biological functions. In this investigation, we have attempted to study those effects that are directly related to the Chl biosynthetic pathway.

In higher plants, Fe deficiency results in chlorosis. This relationship was carefully documented by DeKock *et al.* (6) and by Evans (8). Marsh *et al.* (15) noted an inhibition of ^{14}C incorporation from citrate, succinate, and α-ketoglutarate into Chl in Fe-deficient cowpeas. In photosynthetic bacteria, Fe deficiency results in the accumulation and excretion of intermediates in the tetrapyrrole biosynthetic pathway, particularly coproporphyrin (14). This finding led to the hypothesis that coproporphyrinogen oxidase (EC 1.3.3.3) is an Fe-requiring enzyme; and in fact the activity of the enzyme isolated from tobacco leaves was inhibited by chelating agents and stimulated by added Fe^{2+} (11).

Recently, aquatic plants, notably rice and *Echinochloa crusgalli* (L.) Beauv., were shown to possess metabolic adaptations that allow them to germinate and grow under essentially complete anaerobiosis (20). The shoots produced under anaerobic conditions were chlorotic. Wang (22) reported that *E. crusgalli* germinated under anaerobic conditions produced shoots devoid of both Chl and Pchl(ide). The ultrastructure of the plastids was very abnormal (19).

O_2 is required in higher plant tissues for the accumulation of ALA[4] (3). It is probable that this *in vivo* O_2 requirement, in part, reflects the need for molecular O_2 in aerobic respiration, which is necessary to generate ATP in the common test plants (*e.g.* cucumber, bean, barley).

In the present paper we are reporting on: (a) the accumulation of Mg-Proto(Me) *in vivo* by Fe and O_2-deficient plants; (b) the effect of Fe and O_2 deficiency on the conversion of exogenous ALA to Pchlide by plant tissue segments. The following article by Chereskin and Castelfranco (5) deals with the inhibition of ALA synthesis by Fe- and Mg-containing tetrapyrroles, and the effects of Fe-chelators and anaerobiosis on the conversion of Mg-Proto to Pchlide by cell-free preparations of greening plastids.

MATERIALS AND METHODS

Corn (*Zea mays L.* NC hybrid No. (904) 305 59 F5 M-159), barley (*Hordeum vulgare* L. USH 10, Lot 3102), spinach (*Spinacia oleracea* L. Burpee 5058-3 America, Lot 1980), sugarbeet (*Beta vulgaris* L. cv. USH10, Lot 3102), and bean (*Phaseolus vulgaris* L. Red Kidney Light) were cultured hydroponically in quart jars with aeration. Sugarbeet, spinach, barley, and beans were grown in a controlled environment chamber: 24 to 26°C, 16 h light, 8 h dark, approximately 400 $\mu E\ m^{-2}s^{-1}$ PAR. Corn, barley, and beans were also grown under lights at room temperature (approximately 200 $\mu E\ m^{-2}s^{-1}$ PAR, 12 Sylvania Lifeline F96712 WW and 12 Sylvania 25 w incandescent bulbs).

Plant Culture Solutions. All seeds were germinated in vermiculite or sand moistened with modified half-strength Hoagland solution of the following composition: in mmol l^{-1}, 2.5 $Ca(NO_3)_2$, 2.5 KNO_3, 1 $MgSO_4$, 0.5 KH_2PO_4; and in mg l^{-1}, 0.14 B, 0.25 Mn, 0.025 Zn, 0.01 Cu, 0.0055 Mo, 2.4 Fe supplied as Sequestrene Fe 330 (CIBA-GEIGY, Fe^{+3}-Na^+ EDTA complex). The amount of Fe supplied to control corn plants was doubled because of their rapid growth tending to make Fe less available by raising the pH of the nutrient medium. After 5 to 7 days of growth, the seedlings were transplanted to pint jars containing the half-strength Hoagland solution. After 2 to 3 more days, the plants were transferred to quart jars, 1 or 2 plants per jar, the jars being covered with aluminum foil or brown paper bags to limit the exposure of the roots to light and lined with polyethylene bags to limit exchange of ions with the glass surface. Compressed air, humidified by passing through H_2O, was used to aerate the roots.

Iron stress was obtained by eliminating the Sequestrene Fe 330 from the culture solution, Mg stress, by eliminating $MgSO_4$ and supplying an equivalent amount of S as Na_2SO_4. Sulfur-stressed plants were grown in solutions where equivalent amounts of Mg,

[1] Supported by National Science Foundation Grant PCM 7813250.

[2] Present address: Division of Mathematical Sciences, University of Iowa, Iowa City, IA 52242.
[3] To whom reprint requests should be addressed.

[4] Abbreviations: ALA, δ-aminolevulinic acid; Mg-Proto(Me), a mixture of Mg-Proto and Mg-Proto Me of unknown proportion; Mg-Proto Me_2, Mg-protoporphyrin IX dimethyl ester; Mg-Proto, Mg-protoporphyrin IX; Mg-Proto Me, Mg-protoporphyrin IX monomethyl ester; α,α′-DP, α,α′-dipyridyl.

Zn, and Cu were supplied as chlorides instead of sulfates. All plants were harvested when deficiency symptoms were apparent (up to a maximum of 11 days stress).

For the dark treatment, 8-day-old bean seedlings, cultured as described above, were transferred to the dark at 29°C for 11 days.

Plants for O_2 Stress Studies. Aquatic plants, *Oryza sativa* L., *Echinochloa crusgalli* (L.) Beauv., *Hydrilla verticillata* Casp., *Potamogeton pectinatus* L., and *Potamogeton nodosus* Poir. were grown successfully under anaerobic conditions (Hinchee, Castelfranco, and Castelfranco, unpublished). The two *Potamogeton* species were used in this study. Winter buds of *P. nodosus* and tubers of *P. pectinatus* with the attached growing points, were cultured in one-eighth strength Hoagland solution agitated by a stream of air or N_2. Commercial N_2, 99.995% pure, was scrubbed first through a solution obtained by mixing equal volumes of A (10% NaOH and 15% K_2CO_3, w/v) and B (59 g pyrogallol dissolved in 100 ml H_2O), and then through H_2O. The plant material was grown up to a maximum of 15 days. Since the cultures were not kept sterile, the medium was changed every two or three days to avoid excessive bacterial growth.

Incubation of Fe-Stressed Leaf Tissue with ALA. Iron-stressed corn or beans were grown as described above. The chlorotic and the control leaf tissues were then cut into approximately 1-cm squares and floated in four Petri dishes containing one-eighth strength Hoagland solution, except that Sequestrene Fe 330 was omitted from the two dishes containing the chlorotic (−Fe) tissue. Ten mM ALA was added to one of the −Fe dishes and to one of the +Fe dishes. The pH was adjusted to 5.4 with KOH. The dishes were incubated in the dark or in the light at room temperature for 20 h. The tissue was extracted, either immediately or after storage at −15°C, and the extract was assayed spectrofluorimetrically for Mg-porphyrins.

ALA Incubation of Tissue from Aquatic Plants. *Potamogeton* tissue that had been grown under either aerobic or anaerobic conditions was transferred to 250-ml Erlenmeyer flasks containing 125 ml of 10 mM ALA in one-eighth strength Hoagland solution. The ALA solution was adjusted to the same pH as the control Hoagland solution (pH 5.4). The flasks were covered with aluminum foil and gassed continually with air or N_2 for 20 h.

Pigment Extraction and Assay Procedure. Segments of leaf tissue from the mineral deficiency series were excised and weighed. After grinding with mortar and pestle in liquid N_2 with powdered $MgCO_3$ the tissue was extracted in 80% acetone, 24 mM NH_4OH. With the aquatic plants, the new tissue produced during growth in one-eighth strength Hoagland solution was detached from the older tissue, blotted, weighed, and ground with a Polytron tissue homogenizer in the ammoniacal acetone solution. The Polytron was also used to grind the leaf tissue in the ALA feeding experiments. The supernatant was collected after centrifuging for 5 min at 43,500*g*. The pellet was repeatedly extracted by adding 0.5 ml of 0.12 M NH_4OH, mixing, and adding acetone to 2.5 ml. All supernatants were combined. Total Chl in the supernatant was calculated from the equations of Arnon (2) or Anderson and Boardman (1). The Chl, lipids, and carotenes were removed from the acetone-water phase by three extractions with equal volumes of Hexanes (Mallinckrodt, boiling range 68–70°C). One-half volume of saturated NaCl and 0.5 ml of 0.25 M monosodium maleate were added to the acetone-water phase (the total volume was usually 20–30 ml); the pH was adjusted to 6.8 with HCl, and the mixture was repeatedly extracted with an equal volume of diethyl ether until the ether phase no longer fluoresced orange-red under UV light. The ether extracts were combined.

Porphyrins and metalloporphyrins in the diethyl ether extracts were assayed using a Perkin-Elmer MPF 44-A fluorescence spectrophotometer. The excitation wavelength was 420 nm with a 20 nm slit width. Emission peak heights for Mg-Proto(Me) and Pchlide were measured with a 2-nm slit width. The emission peak height for Mg-Proto(Me) was measured at 594 nm. The Pchlide emission peak was at 628 nm. Protoporphyrin IX, which also emits in this region, was absent from our extracts, as shown by TLC. Uncorrected spectra were obtained with a scan speed of 60 nm/min. Concentrations were determined by reference to standard Mg-Proto Me_2 and Pchlide measured under identical conditions.

Identification of Porphyrins by Thin Layer Chromatography. Diethyl ether extracts from the ALA feeding experiments with bean and corn leaf tissue were concentrated and spotted on cellulose plates (microcrystalline cellulose K2F 250-μm thickness, Whatman). They were developed in acetone: *n*-heptane:*n*-dodecane:acetic acid (30:68:2:0.1). The R_F values were compared with standards of Mg-Proto, biosynthetic Mg-Proto Me (16), and Pchlide on the same plate.

Spectrofluorimetric Standards. The standards were synthetic Mg-Proto Me_2, and Pchlide. Protochlorophyllide was prepared from cucumber cotyledons by Chereskin and Castelfranco (5) using a modification of a prior procedure for extraction of other Mg-porphyrins (4). The Pchlide concentration in diethyl ether was determined spectrophotometrically using the equations of Anderson and Boardman (1). This Pchlide preparation was used to prepared fluorimetric standard curves for the quantitation of Pchlide in our tissue extracts.

RESULTS

The accumulation of Mg-Proto(Me) in Fe-stressed leaf tissue was detectable by spectrofluorimetry (Fig. 1). In all cases, Fe stress increased the amount of Mg-Proto(Me) relative to Fe-sufficient (control) plants. As much as 55 pmol g^{-1} fresh weight accumulated in Fe-stressed, chlorotic leaf tissue, whereas, Fe-sufficient tissue usually contained less than 5 pmol g^{-1} fresh weight (Fig. 1). When chlorosis was induced by other manipulations (S, Mg, N deficiency, or an 11-day dark treatment) little or no Mg-Proto(Me) accumulated, and in general, the level of these metalloporphyrins bore no relationship to the degree of chlorosis (Fig. 2).

Potamogeton pectinatus L. and *Potamogeton nodosus* Poir. were grown under a nitrogen atmosphere and analyzed for porphyrin Chl precursors in experiments analogous to those with Fe-stressed plants. The anaerobically grown *P. pectinatus* accumulated 3 times more Mg-Proto(Me) than the air-grown tissue and only 7% as much Chl. The *P. nodosus* accumulated 10 times more Mg-Proto(Me) and 38% as much Chl as the control (Table I).

When the tetrapyrrole precursor, ALA, was supplied to Fe-stressed and anaerobic tissue, the accumulation of Mg-Proto(Me) was enhanced (Tables II, III, and IV, Fig. 3). Control tissue accumulated large amounts of Pchlide as expected on the basis of work reported by others (9). When ALA was fed to Fe-stressed corn and bean leaf tissue, the Pchlide accumulation tended to be less than in the Fe-sufficient controls. However, greatly increased amounts of Mg-Proto(Me) were accumulated (Table II, Fig. 3). In these experiments (Tables II, III, and IV, Fig. 3), the Chl content did not change appreciably during the 20-h incubation with ALA.

The ether extracts from the Fe-stressed leaf tissues, incubated with ALA were analyzed by TLC and HPLC (Hanamoto, Spiller, and Castelfranco, unpublished): Mg-Proto Me was the most abundant component, but some Mg-Proto was also detected.

Table III summarizes an experiment with *P. nodosus* tissue grown for 7 days under either aerobic or anaerobic conditions. The tissue was then incubated with or without ALA under the same gas phase used during growth. The Pchlide content was quite low unless the tissue was treated with exogenous ALA. If the tissue was treated with ALA, there was a moderate Pchlide accumulation in the anaerobic sample, and a very large accumulation in the aerobic control.

In subsequent experiments, *P. nodosus* tissue was grown under aerobic conditions, but was incubated either anaerobically or

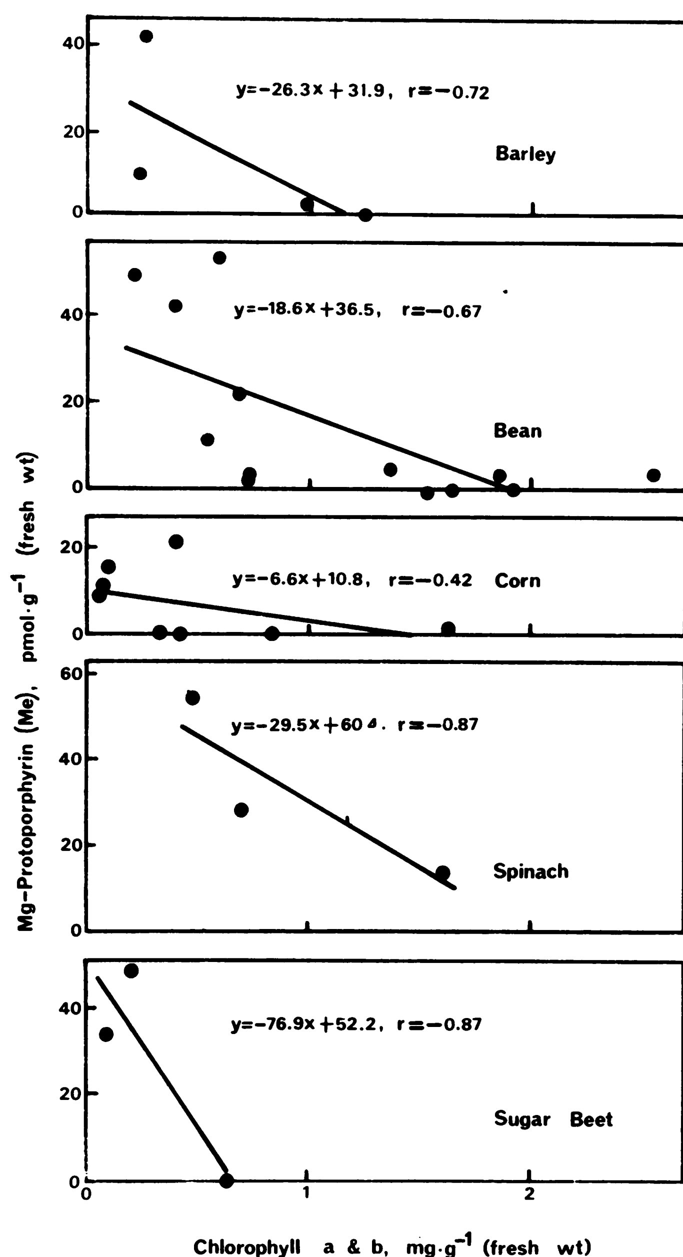

FIG. 1. Mg-Proto(Me) and Chl accumulation during Fe stress. One-week-old barley, bean, corn, spinach, or sugarbeet seedlings were grown hydroponically for a maximum of 11 days in Fe-deficient or Fe-sufficient half-strength Hoagland solution.

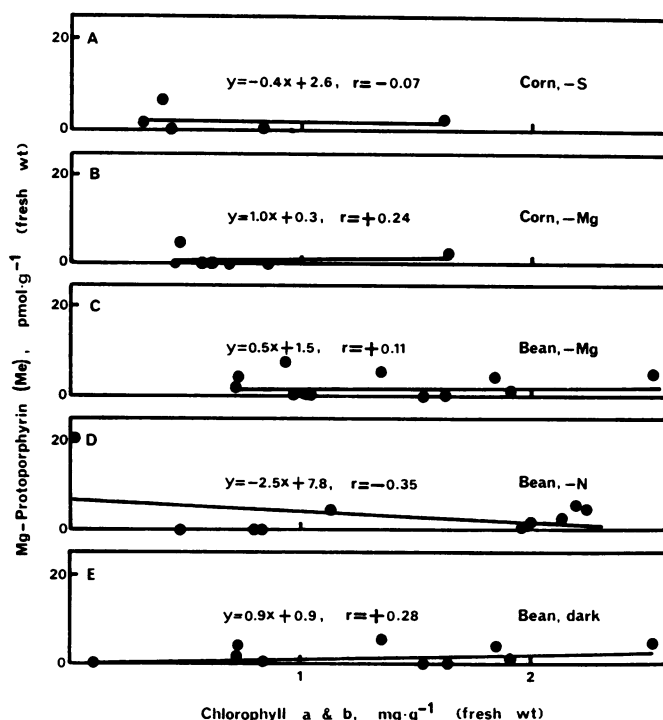

FIG. 2. Mg-Proto(Me) and Chl accumulation during various stress conditions. A to D, 1-week-old corn or bean seedlings were grown hydroponically for a maximum of 11 days in half-strength Hoagland solution deficient or sufficient in S, Mg, or N. E, 1-week-old bean seedlings were grown in half-strength Hoagland solution up to 11 days in the dark.

Table I. *Chl Deficiency and Mg-Proto(Me) Accumulation under Anaerobiosis*

P. pectinatus and *P. nodosus* were grown for 14 days in one-eighth strength Hoagland solution with either air or N_2, under 3 to 5 $\mu E\ m^{-2}\ s^{-1}$ PAR.

	Air		N_2	
	Mg-Proto(Me)	Chl *a* + *b*	Mg-Proto(Me)	Chl *a* + *b*
	nmol g^{-1} *fresh wt*			
P. pectinatus	0.22	359	0.71	25
P. nodosus	0.02	294	0.21	111

aerobically with or without ALA (Table IV). Pchlide accumulation was low unless both ALA and O_2 were present during the incubation. During the anaerobic treatment there was an accumulation of Mg-Proto(Me) which increased significantly if the tissue was treated with ALA.

DISCUSSION

Although the actual amounts of Mg-Proto(Me) accumulated under our experimental conditions are considerably smaller, our observations with plants grown on Fe-deficient cultures agree with the reports of other workers who have used plant tissue segments incubated with Fe-chelating agents. Chelators such as α,α'-DP, *o*-phenanthroline, and 8-hydroxyquinoline are thought to produce *in vivo* Fe deficiency, resulting in a shortage of free heme and heme compounds, particularly in growing tissues and in cells undergoing rapid heme turnover. Using such reagents, Granick (10), Duggan and Gassman (7), Ryberg and Sundquist (17), and Vlcek and Gassman (21) observed the accumulation of Mg-Proto Me and other intermediates of the Mg-branch of the tetrapyrrole pathway, instead of Chl, in higher plant tissues.

The accumulation of Mg-Proto(Me) by Fe-deficient tissues is consistent with the loosening of the postulated Heme-feedback control of ALA synthesis when Fe is in short supply (5); and the accumulation of Mg-Proto(Me) under anaerobic conditions, is consistent with the observed *in vitro* aerobic oxidation of Mg-Proto(Me) (5). However, there are other experimental observations that are not easily explained on these bases, but point rather to the presence of an Fe, O_2-requiring step between Mg-Proto Me and Pchlide, as postulated by Jones (12).

Fe-deficient tissue incubated with ALA accumulated less Pchlide than the Fe-sufficient controls, but accumulated large amounts of Mg-Proto(Me) (Table II, Fig. 3). Likewise, tissue treated with ALA under anaerobic conditions accumulated less Pchlide than the aerobic controls, but accumulated some Mg-Proto(Me), while no detectable Mg-Proto(Me) accumulated in the

Table II. *Accumulation of Mg Proto(Me), Pchlide, and Chl by Fe-Sufficient and Fe-Stressed Corn Leaf Tissue*

Leaf tissue from corn plants grown hydroponically in Fe-deficient or Fe-sufficient media was incubated with or without 10 mM ALA for 20 h in the dark or in the light: 40 μE m^{-2} s^{-1} PAR (General Electric F40WW fluorescent tubes) or 3 μE m^{-2} s^{-1} PAR (Ken-rad 75 Watt incandescent bulbs).

Experiment No. and Conditions	+Fe (Control)			−Fe (Iron-Stressed)		
	Mg-Proto(Me)	Pchlide	Chl $a + b$	Mg-Proto(Me)	Pchlide	Chl $a + b$
	nmol g^{-1} fresh wt					
Dark incubation						
1, − ALA	0.01	0.77	1016	0.63	0.54	152
2, − ALA	0.05	0.96	838	0.01	0.22	228
1, + ALA	2.58	44.64	712	19.86	16.76	138
2, + ALA	0.89	15.32	706	19.55	16.54	94
Light incubation						
3, + ALA (40 μE m^{-2}s^{-1})	0.16	0.96	1070	0.11	0.55	90
4, + ALA (3 μE m^{-2}s^{-1})	2.44	27.26	1908	4.84	6.48	93
4, + ALA (dark control)	10.06	28.79	2239	21.65	11.62	139

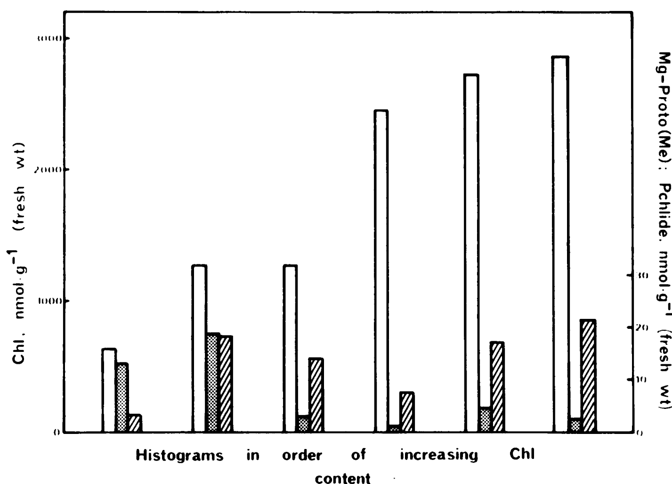

FIG. 3. Mg-Proto(Me) (▩), Pchlide (▨), and Chl (□), accumulation by bean leaf tissue grown hydroponically in Fe-deficient or Fe-sufficient half-strength Hoagland solution. Leaf segments were then incubated with 10 mM ALA in one-eighth strength Hoagland solution, +Fe or −Fe, for 20 h in the dark. Histograms are arranged in order of increasing Chl content.

Table III. *Accumulation of Mg-Proto(Me), Pchlide, and Chl by P. nodosus Grown and Fed with ALA under Aerobic or Anaerobic Conditions*

P. nodosus was grown in air or N_2 for 7 days and incubated with or without ALA for 20 h in the dark under the same gas phase.

Treatment	Air			N_2		
	Mg-Proto-(Me)	Pchlide	Chl $a + b$	Mg-Proto-(Me)	Pchlide	Chl $a + b$
	nmol g^{-1} fresh wt					
− ALA	0.07	0.98	337	1.32	0.98	14
+ ALA	0.00	70.03	384	1.86	5.89	24

aerobic controls (Table IV).

Duggan and Gassman (7), working with bean leaves observed that, as their treatment with α,α'-DP became more severe, the ratio of Mg-Proto(Me) to Pchlide increased. We have noticed the same shift to a higher Mg-Proto(Me)/Pchlide ratio with bean and corn plants grown in culture solution, as the Fe deficiency chlorosis became progressively more pronounced (Fig. 3, Table II).

Vlcek and Gassman (21) have shown that the Mg-Proto(Me)

Table IV. *Accumulation of Mg-Proto(Me), Pchlide, and Chl by Aerobic P. nodosus Tissue Fed with ALA under Air or N_2*

P. nodosus was grown in air for 4 days and incubated with or without 10 mM ALA for 20 h in the dark, under either air or N_2.

Incubation Conditions	Chl $a + b$	Mg-Proto(Me)	Pchlide
		nmol g^{-1} fresh wt	
N_2, − ALA	330	29	386
N_2, + ALA	310	42	1,040
Air, − ALA	360	0.0	351
Air, + ALA	267	0.0	13,900

that is accumulated in bean leaves incubated overnight with α,α'-DP, is converted to Pchlide during a subsequent 6-h incubation with $FeCl_2$, $ZnCl_2$, or $CoCl_2$. This conversion requires O_2, but is not inhibited by poisons of mitochondrial respiration or by mitochondrial uncouplers. Presumably, Zn^{2+} and Co^{2+} displace the endogenous Fe^{2+} from its α,α'-DP binding, and thus reactivate an Fe-requiring oxygenase. On the other hand, in their system, the conversion of the accumulated Mg-Proto(Me) to Pchlide appears to require the synthesis of new protein, because added metal salts have little effect in the presence of cycloheximide. This suggests that Zn and Co displace the endogenous Fe from its α,α'-DP binding and make it available for incorporation into an Fe-requiring oxygenase synthesized *de novo* during the 6-h incubation.

In our plants grown under Fe or O_2 deficiency, the accumulation of Mg-Proto(Me), though highly significant, was considerably less than the Chl deficit. We propose two reasons for this lack of stoichiometry:

(a) Mg-Proto(Me) tends to inhibit the formation of ALA and thus to stop its own synthesis. *In vivo*, the intraplastidic concentrations of Mg-Proto(Me) could reach levels that are sufficiently high to inhibit ALA synthesis (5).

(b) Mg-Proto(Me) is easily photo-destroyed, even at moderate light intensities, presumably because the Mg-Proto(Me) accumulated under these conditions is not stabilized by a normal membrane. Vastly reduced thylakoid systems have been demonstrated in the chloroplasts of Fe-deficient (13, 18) and anaerobic (19) tissue.

Acknowledgments—We are obliged to Dr. Kevin Smith for his generous gift of Mg-Proto Me_2, to Drs. L. W. J. Anderson and L. A. Wright, Jr. for the starting material of *Potamogeton nodosus* Poir. and *Potamogeton pectinatus* L., and to H. Drever for his help in growing the corn and bean seedlings. T. P. Fuesler helped by drawing the figures and proofreading the manuscript. We are indebted to B. M. Chereskin for the Pchlide standard.

LITERATURE CITED

1. Anderson JM, NK Boardman 1964 Studies on the greening of dark grown bean leaves. Aust J Biol Sci 17: 93–101
2. Arnon DE 1949 Copper enzymes in isolated chloroplasts. Polyphenol oxidase in *Beta vulgaris*. Plant Physiol 24: 1–15
3. Beale SI, PA Castelfranco 1974 The biosynthesis of δ-aminolevulinic acid in higher plants I. Accumulation of δ-aminolevulinic acid in greening plant tissues. Plant Physiol 53: 291–296
4. Castelfranco PA, JD Weinstein, S Schwarcz, AD Pardo, BE Wezelman 1979 The Mg insertion step in chlorophyll biosynthesis. Arch Biochem Biophys 192: 592–598
5. Chereskin BM, PA Castelfranco 1981 Effects of iron and oxygen on chlorophyll biosynthesis. II. Observations on the biosynthetic pathway of isolated etiochloroplasts. Plant Physiol 68: 112–116
6. Dekock PC, K Commisiong, VC Farmer, RHE Inkson 1960 Interrelationships of catalase, peroxidase, hematin, and chlorophyll. Plant Physiol 35: 599–604
7. Duggan J, M Gassman 1974 Induction of porphyrin synthesis in etiolated bean leaves by chelators of iron. Plant Physiol 53: 206–215
8. Evans HJ 1959 The biochemical role of iron in plant metabolism. School of Forestry Bull 15, Duke University, Durham, North Carolina
9. Granick S 1959 Magnesium porphyrins formed by barley seedlings treated with δ-aminolevulinic acid. Plant Physiol 34: S-XVIII
10. Granick S 1961 Magnesium protoporphyrin monoester and protoporphyrin monomethyl ester in chlorophyll biosynthesis. J Biol Chem 236: 1168–1172
11. Hsu WP, GW Miller 1970 Coproporphyrinogenase in tobacco (*Nicotiana tabacum* L) Biochem J 117: 215–220
12. Jones OTG 1963 The production of magnesium protoporphyrin monomethyl ester by *Rhodopseudomonas spheroides*. Biochem J 86: 429–432
13. Lamprecht T 1961 Die Feinstruktur der Plastiden von *Tradescantia albiflora* (Kth) bei Eisen Mangelchlorose. Protoplasma 53: 162–199
14. Lascelles J 1959 Adaptation to form bacteriochlorophyll in *Rhodopseudomonas spheroides*: changes in activity of enzymes concerned in pyrrole synthesis. Biochem J 72: 508–518
15. Marsh HV JR, HJ Evans, G Matrone 1963 Investigations of the role of iron in chlorophyll metabolism II. Effect of iron deficiency on chlorophyll synthesis. Plant Physiol 38: 638–642
16. Rebeiz CA, M Abou Haidar, M Yaghi, PA Castelfranco 1970 Porphyrin biosynthesis in cell-free homogenates from higher plants. Plant Physiol 46: 543–549
17. Ryberg M, C Sundquist 1976 The influence of 8-hydroxyquinoline on the accumulation of porphyrins in dark-grown wheat leaves treated with δ-aminolevulinic acid. Physiol Plant 36: 356–361
18. Spiller S, N Terry 1980 Limiting factors in photosynthesis II. Iron stress diminishes photochemical capacity by reducing the number of photosynthetic units. Plant Physiol 65: 1159–1169
19. Vander Zee D, RA Kennedy 1979 Ultrastructural studies of *Echinochloa oryzicola* shoots germinated under anaerobic conditions. Plant Physiol 63: S-97
20. Vartapetian BB, IN Andreeva, N Nuritdinov 1978 Plant cells under oxygen stress. *In* DD Hook, RMM Crawford, eds, Plant Life in Anaerobic Environments. Ann Arbor Science Publ, Ann Arbor, Mich, pp 13–88
21. Vlcek LM, ML Gassman 1979 Reversal of α,α′-dipyridyl-induced porphyrin synthesis in etiolated and greening red kidney bean leaves. Plant Physiol 64: 393–397
22. Wang WY 1980 Photoactivation of chlorophyll synthesis and cytochrome oxidase activity in anaerobically germinated seedlings of *Echinochloa crusgalli* var. oryzicola. Plant Physiol 65: 451–454