Plant Physiol. (1995) 108: 1487–1494

Early Iron Deficiency Stress Response in Leaves of Sugar Beet[1]

Thomas L. Winder and John N. Nishio*

Department of Botany, University of Wyoming, Laramie, Wyoming 82071–3165

Iron nutrient deficiency was investigated in leaves of hydroponically grown sugar beets (*Beta vulgaris*) to determine how ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) gene expression is affected when thylakoid components of photosynthesis are diminished. Rubisco polypeptide content was reduced by 60% in severely iron-stressed leaves, and the reduction was directly correlated to chlorophyll content. The concentration of Rubisco protein in iron-stressed leaves was found to be regulated by availability of mRNAs, and CO_2 fixation by Rubisco was reduced from 45 μmol CO_2 m^{-2} s^{-1} in extracts from iron-sufficient leaves to 20 μmol CO_2 m^{-2} s^{-1} in extracts from severely stressed leaves. The rate of CO_2 fixation was directly correlated to leaf chlorophyll content. Rubisco in iron-sufficient control leaves was 59% activated, whereas in severely stressed leaves grown under the same light, Rubisco was 43% activated. RNA synthesis was reduced by about 50% in iron-deficient leaves, but 16S and 25S rRNA and ctDNA were essentially unaffected by iron stress.

The role of Fe in plant growth and metabolism is well known (Price, 1968), and Fe deficiency symptoms have long been recognized (Gris, 1844). Fe deficiency leads to crop productivity losses when plants are unable to reduce insoluble Fe(III) oxides in the soil to Fe(II) (Guerinot and Yi, 1994). Early research into the cause of reduced plant productivity in Fe-deficient plants focused on the effects on Chl and protein metabolism. Studies of tobacco, maize, and barley found that Fe deficiency caused reductions in chloroplast protein, whereas nonchloroplast protein was less affected (Shetty and Miller, 1966; Pushnik and Miller, 1982). Chloroplast rRNA, but not cytoplasmic rRNA, was found to be reduced in Fe-deficient maize (Lin and Stocking, 1978) and pea (Spiller et al., 1987), and derangement of polyribosomes into monosomes was reported to occur in Fe-deficient maize (Lin and Stocking, 1978).

Much is known about how Fe nutrient deficiency affects photosynthesis. For example, Fe stress alters chloroplast ultrastructure (Spiller and Terry, 1980) and protein and lipid composition of thylakoid membranes (Nishio et al., 1985); it reduces electron transport capacity in thylakoids (Spiller and Terry, 1980); and it diminishes noncyclic ATP formation (Terry, 1980) and leaf ATP levels (Arulanantham et al., 1990). Although many parameters of photosynthetic performance are influenced by Fe stress, Fe deficiency does not affect leaf expansion, numbers of cells and chloroplasts per leaf area, and light absorption (Terry, 1980). Fe stress also does not affect the activity of such key photosynthetic enzymes as malic enzyme, PEP carboxylase, glyceraldehyde-3-phosphate dehydrogenase, and Fru-1,6-bisphosphatase (Stocking, 1975; Taylor et al., 1982).

The nutritional status of plants and plant cells has been shown to alter the expression and in vivo activities of several photosynthetic enzymes including Rubisco (Rowland-Bamford et al., 1991; Krapp et al., 1993; Makino et al., 1994), but whether Rubisco co-limits photosynthesis in Fe-deficient leaves is somewhat controversial. Activity of Rubisco in Fe-deficient sugar beets (*Beta vulgaris* L.) was reduced, but the extent that the activity was affected varied widely, and the changes were generally not to the same extent as other photosynthetic parameters (Terry, 1980; Taylor and Terry, 1984, 1986; Arulanantham et al., 1990). Diminished phosphoribulokinase activity and reduced RuBP pool size has also been suggested as limiting photosynthesis under Fe deficiency (Arulanantham et al., 1990), although increased phosphoribulokinase activity has also been reported (Taylor et al., 1982).

An effective way to study events during the early Fe stress response is to use a system in which Fe deficiency symptoms occur rapidly, but plant growth and development are otherwise unaffected (Terry, 1980). Healthy 3- to 4-week-old hydroponically grown sugar beet plants become chlorotic after 4 d in Fe-deficient media, and severe chlorosis occurs after 8 d (Terry, 1980). Because proteins of the photosynthetic apparatus are the first proteins affected by Fe deficiency stress, this system can be used to study how genes are regulated and coordinated when photochemical capacity is diminished.

The goals of the present research were to investigate how protein synthesis is regulated during the onset of Fe deficiency and to reexamine the effect of Fe deficiency on Rubisco. Previous reports of the influence of Fe deficiency on Rubisco activity were equivocal; therefore, to corroborate our data, we also measured *rbc*L and *rbc*S gene expression under conditions of limited photochemical capacity as induced by Fe deficiency stress.

[1] Supported in part by U.S. Department of Agriculture Competitive Research Grant Nos. 91–37100–6672 and 93–37100–8855 and National Science Foundation Experimental Program to Stimulate Competitive Research.

* Corresponding author; e-mail nishio@uwyo.edu; fax 1–307–766–2851.

Abbreviations: IRE, iron-responsive element; LSU, large subunit polypeptide of Rubisco; *rbc*L, gene for the large subunit of Rubisco; *rbc*S, gene for the small subunit of Rubisco; RuBP, ribulose-1,5-bisphosphate; SSU; small subunit polypeptide of Rubisco.

MATERIALS AND METHODS

Growth Conditions

Sugar beet (*Beta vulgaris* L. cv F58–554H1) cv NB1xNB4 was grown hydroponically in half-strength modified Hoagland solution (Hoagland and Arnon, 1950). The final concentration of Fe, as Fe·EDTA, was 15 μM. Plants were maintained at 550 μmol m^{-2} s^{-1} PPFD on a 16-h photoperiod at 24°C and 20°C at night; RH was 70%. Under this temperature regime growth media were not exposed to less than 23.5°C at any time of the day or night (S.A. Campbell and J.N. Nishio, unpublished data). Approximately 2 weeks after seedlings were initiated in hydroponics, plants of similar size were transferred to Fe-deficient media to initiate chlorosis. The pH of Fe-free media was maintained near 8 by adding 5 mM $NaHCO_3$ (S.A. Campbell and J.N. Nishio, unpublished data). When minus-Fe treatments were started, plants of the same age and size were also transferred into fresh complete media for use as controls. Chlorosis was induced in 4 to 5 d, and severe chlorosis (<15 nmol Chl cm^{-2}) occurred about 8 d after transfer to Fe-free media; necrosis was not visible until the leaves were below 5 nmol Chl cm^{-2}). Three days after the plants were moved to Fe-deficient media, leaves were harvested for measurements 2 to 4 h after the start of the photoperiod. Immediately after harvesting, leaves were placed on ice, and assays were performed within 30 min. Young leaves of approximately 250 cm^{-2} in size were used for all experiments. To ensure no nutrients other than Fe were limiting during the experimental period, boosts of one-quarter-strength modified Hoagland were supplied to control and Fe-deficient plants every other day during the experimental period.

In Vivo Translation

Leaf discs (0.5 cm^2) were labeled in 100 μL of 20 mM Hepes-KOH, pH 7.8, 0.4 mM EDTA, pH 8.0, 0.4 mM $MgCl_2$, 0.4 mM $MnCl_2$, 50 mM $NaHCO_3$, 0.04% Triton X-100, 2.5 mM amino acids (minus Met and Cys), and 100 μCi of L-[^{35}S]Met and L-[^{35}S]Cys (Tran^{35}S-Label, 1174 Ci/mmol; ICN) for 1 h with a PPFD of 500 μmol m^{-2} s^{-1}. The discs were vacuum infiltrated at the onset of labeling and again after 30 min. Each disc was then homogenized in 75 μL of buffer A (10 mM Tris-HCl, pH 8.0, 10 mM EDTA, pH 8.0, 5 mM aminocaproic acid, 2 mM benzamidine, 1% β-mercaptoethanol, and 0.5 mM PMSF) in an Eppendorf tube using a micropestle. After homogenization, samples were centrifuged at 12,000*g* to make soluble (supernatant) and membrane (pellet) fractions. Incorporation of labeled amino acids in the two fractions was determined by TCA precipitation and liquid scintillation spectroscopy.

To determine relative incorporation into LSU and SSU, samples were separated on 14% polyacrylamide gels and stained with Coomassie brilliant blue. LSU and SSU bands were cut from the gels and homogenized in 200 μL of buffer A with a micropestle. The homogenate was transferred to scintillation vials, and ^{35}S incorporation was determined by liquid scintillation spectroscopy.

Rubisco Content

Rubisco LSU and SSU polypeptide content in chlorotic and Fe-sufficient control leaves was determined by SDS-PAGE and densitometry. Part of the sample prepared to measure Rubisco activity was diluted 2:1 with SDS-PAGE sample buffer (100 mM Tris-HCl, pH 6.8, 6% SDS, 30% Suc, 0.05% bromphenol blue, and 0.5% β-mercaptoethanol) and heated at 95°C for 1 min. Samples were separated on 1-mm-thick 14% polyacrylamide gels (Laemmli, 1970), and the gels were stained with Coomassie brilliant blue. A_{595} of LSU and SSU polypeptides was determined with a Beckman DU1000 spectrophotometer fitted with a Gilford Instruments (Oberlin, OH) scanning attachment and a Hewlett-Packard integrator.

In Vitro Rubisco Assay

RuBP carboxylation was assayed by measuring NADH oxidation according to the method of Usada (1984), except DTE was replaced with 5 mM DTT and KCl was omitted. Extracts were prepared by grinding leaf tissue (10.5 cm^2) in 3 mL of ice-cold homogenization buffer (100 mM Hepes-KOH, pH 7.5, 0.5 mM EDTA, pH 8.0, 10 mM potassium acetate, 5 mM DTT, 20 mM β-mercaptoethanol, 5% [v/v] glycerol, 1% [w/v] PVP, 0.05% [w/v] Triton X-100, and 0.5 mM PMSF) in a prechilled mortar and pestle. Debris was pelleted by a 4-min centrifugation at 1000*g*, and 15 μL of the supernatant was used per assay. Assay mixtures contained 100 mM Hepes-KOH, pH 7.8, 10 mM $MgCl_2$, 1 mM EDTA, pH 8.0, 5 mM DTT, 10 mM $NaHCO_3$, 1 mM ATP, 5 mM phosphocreatine, 0.2 mM NADH, 4 units of glyceraldehyde-3-phosphate dehydrogenase, 4 units of 3-phosphoglycerate phosphokinase, and 2 units of creatine phosphokinase. The assays were initiated by adding 1 mM RuBP. Total extractable activity was determined after aliquots of extract were incubated at room temperature for 5 min with 10 mM $MgCl_2$ and 10 mM $NaHCO_3$.

In Vivo Transcription

[^{32}P]UTP incorporation into RNA was used to measure overall transcription activity. Leaf discs were labeled with UTP essentially as with Met except, in place of amino acids, the labeling cocktail contained 150 μM ATP, CTP, and GTP, 50 μM cold UTP, and 10 μCi of [^{32}P]UTP (3000 Ci/mmol). Discs were labeled for 30 min in light and then homogenized and extracted with phenol. Incorporation was determined with DE-81 cellulose paper and liquid scintillation spectroscopy.

mRNA and rRNA Quantification

Steady-state levels of *rbc*S and *rbc*L mRNA and 16S and 25S rRNA were measured in total RNA using gene-specific probes. RNA was extracted from 0.5-cm^2 leaf discs by homogenizing in 100 μL of 50 mM Tris-HCl, pH 8.0, 15 mM EDTA, pH 8.0, 200 mM NaCl, 2% SDS, 2% β-mercaptoethanol, and 10 mM DTT in an Eppendorf tube with a micropestle. Samples were extracted with phenol/chloroform and the aqueous phases were precipitated first with etha-

nol and then with 0.25 volumes of 10 M LiCl. RNA was denatured and applied to nitrocellulose membrane on a dot-blot manifold (Sambrook et al., 1989). RNA isolated as described above was also separated on formaldehyde-containing agarose gels (Sambrook et al., 1989) for RNA gel blots.

*rbc*L mRNA was quantified with the tobacco *rbc*L gene kindly provided by Dr. M. Sugiura (Nagoya University, Nagoya, Japan). The *rbc*L-specific 1.35-kb *Bam*HI fragment from pTB29 was gel purified and labeled with [α-^{32}P]dCTP (NEN, 3000 Ci/mmol) using a multiprime DNA-labeling system (Amersham). Hybridizations were done in 50% formamide at 42°C (Sambrook et al., 1989), and washes were done in 1.0× SSC plus 0.1% SDS at 60°C. *rbc*S mRNA was quantified with the oligonucleotide 5'-TTCCA-CATIGTCCAA/GTA-3', corresponding to the conserved amino acids YWTMWK in SSU polypeptides (Goldschmidt-Clermont and Rahire, 1986). The oligonucleotide was labeled with [γ-^{32}P]ATP (ICN, 7000 Ci/mmol) and polynucleotide kinase. Hybridization of the oligonucleotide to RNA dot blots was done at 40°C, and washes were done in 2× SSC plus 0.1% SDS at 40°C. The *rbc*S-specific oligonucleotide was also tested by hybridization to cloned pea *rbc*S (kindly provided by Dr. H. Bohnert, University of Arizona, Tucson).

16S rRNA was measured with the barley 16S rRNA gene kindly provided by Dr. John Mullet (Texas A&M University, College Station), and 25S rRNA was measured with wheat 25S rRNA sequence from pTA71 (Gerlach and Bedbrook, 1979). Gel-purified rRNA-specific probes were labeled as described for the *rbc*L probe. Hybridizations of rRNA dot and northern blots were done in 50% formamide at 42°C, and blots were washed in 0.1× SSC plus 0.1% SDS at 65°C. After RNA dot blots were exposed to film, the dots were cut from the membrane and counted by liquid scintillation spectroscopy.

ctDNA Quantification

Relative ctDNA copy number was determined with the tobacco *rbc*L gene. Total DNA was extracted from leaf discs using standard phenol/chloroform extraction and ethanol precipitation procedures (Sambrook et al., 1989). DNA was treated with RNase and then denatured and applied to nitrocellulose dot blots. Hybridizations were done in 50% formamide at 42°C and the blots were washed in 1× SSC plus 0.1% SDS at 60°C. The dots were counted by liquid scintillation spectroscopy.

Chl and Protein Determinations

Chl was determined in 95% ethanol (Lichtenthaler, 1987), and protein was determined by the modified Lowry assay (Markwell et al., 1981).

RESULTS

Protein Synthesis and Fe Stress

In vivo synthesis of leaf soluble and membrane proteins was significantly affected by Fe deficiency. Incorporation of [^{35}S]Met into soluble proteins in stressed leaves was reduced about 60% compared with Fe-sufficient leaves, and incorporation into the membrane fraction was reduced nearly 80% (Fig. 1). Incorporation of [^{35}S]Met into LSU and SSU in Fe-deficient leaves followed the same pattern as synthesis of total leaf protein (Fig. 2). Translation of Rubisco subunits (measured by liquid scintillation counting of Coomassie blue-stained bands cut from gels) was reduced to 40% of the control rates in moderately chlorotic leaves and to 20% of control rates in chlorotic stressed leaves.

We investigated the possibility that Fe resupply to Fe-deficient leaf tissue might alleviate the Fe stress-induced lesion in translation. Chelated Fe was vacuum infiltrated into leaf discs during in vivo translation experiments. We found no increase in efficiency of [^{35}S]Met incorporation in stressed leaves when Fe was resupplied, compared to stressed leaves without added Fe (data not shown). Apparently, Fe deficiency does not alter the translational apparatus but affects protein synthesis at some other level.

Leaf Protein Content and Fe Stress

Rubisco LSU and SSU polypeptides decreased with decreasing Chl content when measured on a per area basis (Fig. 3A). The enzyme subunit concentrations were as low as 40% of control levels in severely stressed leaves, and

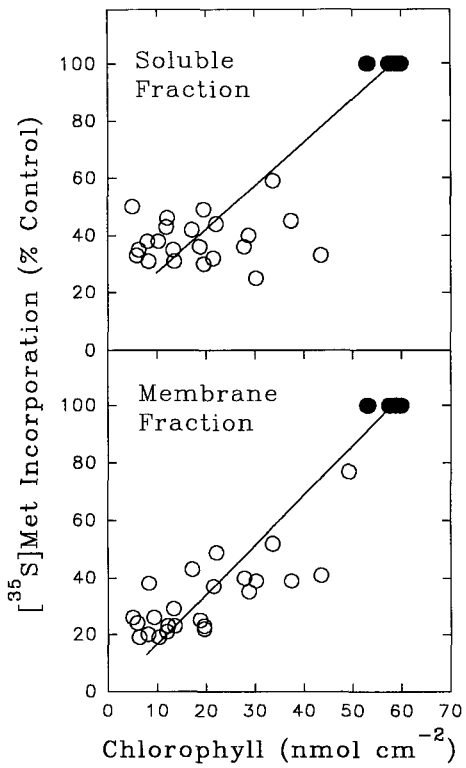

Figure 1. Effect of Fe deficiency on protein synthesis in sugar beet leaves. In vivo [^{35}S]Met incorporation was measured in Fe-deficient (○) and Fe-sufficient control (●) leaves by TCA precipitation and liquid scintillation spectroscopy. r^2 = 0.851 and 0.931 for soluble and membrane fractions, respectively.

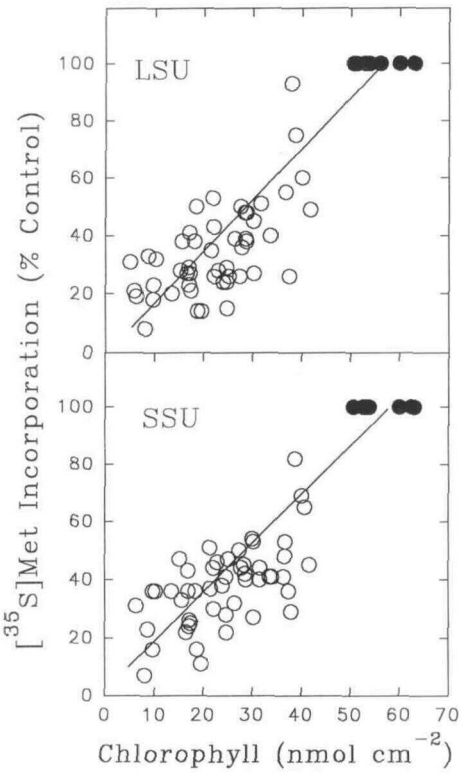

Figure 2. Effect of Fe deficiency on Rubisco protein synthesis. [^{35}S]Met incorporation into LSU and SSU by Fe-deficient (○) and Fe-sufficient (●) plants. Incorporation was measured by liquid scintillation spectroscopy of LSU and SSU bands excised from Coomassie blue-stained polyacrylamide gels. r^2 = 0.859 for LSU translation and 0.860 for SSU translation.

abundance of the polypeptides was linearly correlated to Chl content (r^2 = 0.931 for LSU and 0.930 for SSU; Fig. 3B). Total leaf protein content per unit area, however, was only slightly changed by Fe deficiency; even in severely stressed leaves, soluble and total membrane fractions were reduced by only about 20% (Fig. 4). Because Rubisco can account for 20 to 25% of leaf protein (Evans, 1989) and 50% of the chloroplast soluble protein (Ellis, 1979), the approximately 20% reduction in leaf soluble protein in Fe-stressed leaves is likely caused mostly by the reduction in Rubisco protein. Similarly, a large decline in thylakoid membrane proteins with Fe deficiency stress (Nishio et al., 1985) probably also explains most of the loss of total membrane proteins.

RuBP Carboxylase Activity and Fe Stress

Consistent with the loss of Rubisco protein, the rate of fully activated CO_2 fixation by RuBP carboxylase was reduced more than 50% in severely Fe-deficient leaves. The rate of CO_2 fixation measured in extracts from control leaves was 45 μmol CO_2 m^{-2} s^{-1}, whereas from moderately and severely stressed leaves the rates were 35 and 20 μmol CO_2 m^{-2} s^{-1}, respectively (Fig. 5). CO_2 fixation and Chl content were linearly correlated (r^2 = 0.92), even in severely chlorotic leaves. When the rate of RuBP carboxylase activity was measured as a percentage of fully activated carboxylase, the in vivo rate was less in stressed leaves (43% activated in vivo) compared with control leaves (59% activated in vivo).

ctDNA Content and Fe Stress

ctDNA content was quantified to discriminate between reduced transcriptional activity and apparent reduced transcription due to limitation of DNA template. No differences in ctDNA content per unit leaf area were found between Fe-sufficient leaves and Fe-deficient leaves. Thus, it appears that Fe stress does not alter chloroplast gene expression by reducing the number of chloroplast genomes per unit area.

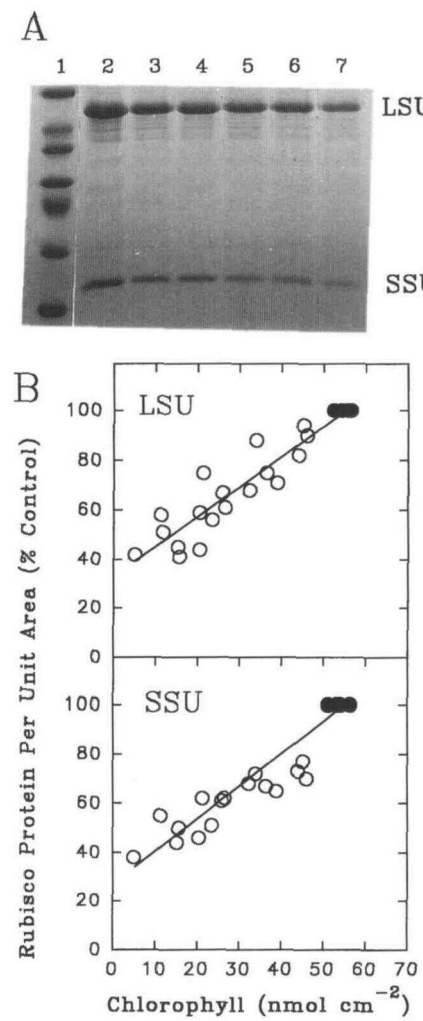

Figure 3. Rubisco protein per unit leaf area in Fe-deficient and Fe-sufficient control plants. A, SDS-PAGE of polypeptides in the soluble fraction of Fe-stressed and Fe-sufficient plants. Chl *a* plus *b* in the control leaf (lane 2) was 53 nmol cm^{-2}, and in Fe-deficient leaves Chl *a* plus *b* was 39, 30, 20, 14, and 6 nmol cm^{-2} (lanes 3–7, respectively). Molecular mass markers (lane 1) are 66, 45, 36, 29, 24, 20, and 14 kD. B, Densitometric measurements of LSU and SSU protein content in Fe-deficient (○) and Fe-sufficient (●) plants. r^2 = 0.931 for LSU and 0.930 for SSU protein.

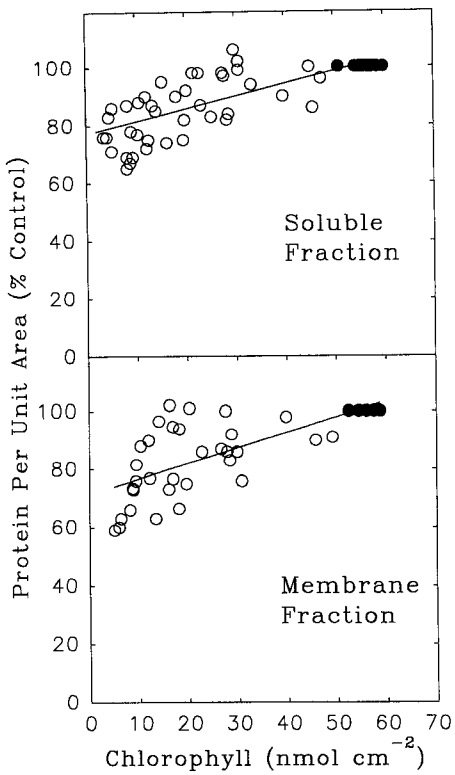

Figure 4. Effect of Fe deficiency on protein content in sugar beet leaves. Leaf protein from Fe-deficient (○) and Fe-sufficient control (●) plants was fractionated into soluble and membrane fractions. r^2 = 0.74 and 0.66 for soluble and membrane fractions, respectively.

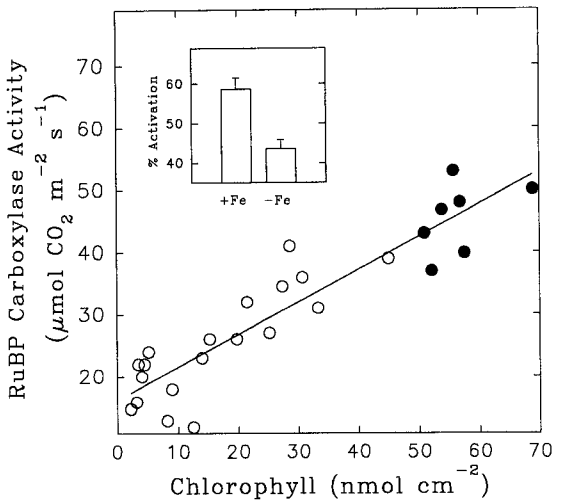

Figure 5. Rubisco activity in Fe-deficient (○) and Fe-sufficient control (●) plants. Total extractable activity in leaf extracts was determined after samples were activated in vitro with Mg^{2+} and CO_2. r^2 = 0.920. The inset shows the relationship of in vivo activation of RuBP carboxylase to Fe stress. In control leaves (45–60 nmol Chl cm^{-2}) Rubisco was 58.7 ± 2.9% activated, and in Fe-deficient leaves (6–12.5 nmol Chl cm^{-2}) Rubisco was 43.4 ± 2.4% activated. n = 3 for both control and Fe-deficient leaves.

Transcription and Fe Stress

To determine whether mRNA synthesis is a controlling factor for the general decline observed in protein synthesis, transcriptional activity was measured in Fe-sufficient and Fe-deficient leaves (Fig. 6). Total in vivo incorporation of [^{32}P]UTP was reduced by about 50% in severely stressed leaves compared to Fe-sufficient control leaves, suggesting that availability of mRNAs contributes to diminished protein synthesis.

rRNA Content and Fe Stress

When RNA from Fe-sufficient and Fe-deficient leaves was loaded onto a denaturing gel on an equal leaf area basis, blotted to nitrocellulose, and probed with 16S and 25S rRNA probes, no significant loss of either rRNA was detected. Dot-blot hybridizations and scintillation counting also showed that there was no significant change in 16S rRNA and 25S rRNA in Fe-deficient leaves. This finding is in contrast to previous reports in which plants were exposed to Fe deficiency stress for longer periods (Lin and Stocking, 1978; Spiller et al., 1987).

*rbc*L and *rbc*S mRNA Abundance and Fe Stress

Although total cellular RNA content (as determined by rRNA abundance) was only slightly affected by Fe deficiency, hybridizable mRNA for LSU and SSU polypeptides was linearly correlated to Chl content in Fe-stressed leaves (Fig. 7; r^2 = 0.922 for *rbc*L and 0.796 for *rbc*S). Severely chlorotic leaves contained 40% of control levels of *rbc*L and *rbc*S mRNA, a change consistent with changes in carboxylase activity and protein levels.

DISCUSSION

The major consequence of Fe deficiency on Rubisco was to down-regulate gene expression. LSU and SSU protein and mRNA levels were reduced to about 40% of control levels in Fe-stressed sugar beet leaves, the same extent to

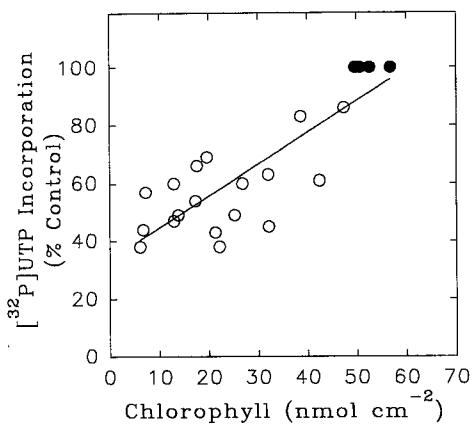

Figure 6. Effect of Fe deficiency on in vivo transcription in sugar beet leaves. [^{32}P]UTP incorporation was determined in Fe-deficient (○) and Fe-sufficient control (●) leaves after samples were filtered through DE-81 paper. r^2 = 0.83.

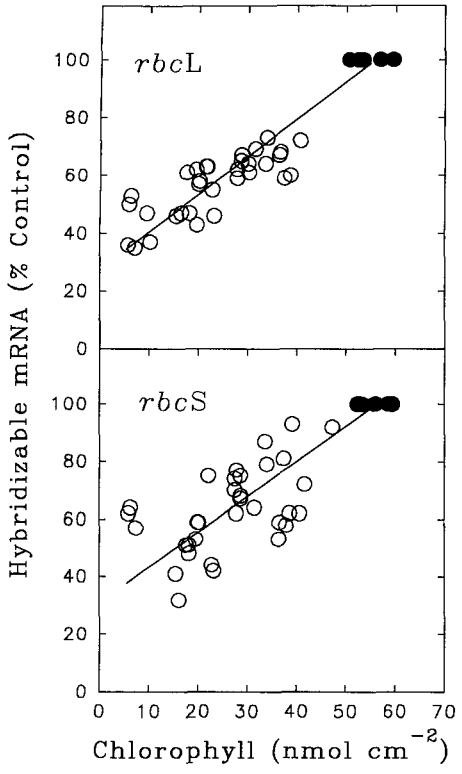

Figure 7. Effect of Fe deficiency on steady-state accumulation of mRNA for LSU and SSU. *rbc*L and *rbc*S mRNA levels were determined in Fe-deficient (○) and Fe-sufficient control (●) plants. r^2 = 0.922 for *rbc*L and 0.796 for *rbc*S mRNA.

which RuBP carboxylase activity was reduced, and protein and mRNA levels were directly correlated to Chl, even in severely stressed leaves. The loss of Rubisco protein in Fe-deficient leaves must have accounted for nearly all of the observed decline in the soluble protein fraction, since Rubisco is about 50% of the chloroplast soluble protein (Ellis, 1979). We conclude that the loss of Rubisco mRNA was not a consequence of a general decline in cellular RNA, since no significant change in 16S and 25S rRNA content was found. Extended exposure to Fe deficiency in pea (Spiller et al., 1987) and maize (Lin and Stocking, 1978) causes a reduction in chloroplast ribosomes, but we found no evidence to suggest that this occurs under short-term Fe deficiency in sugar beets.

Rubisco activity was measured by activating the enzyme in vitro with Mg^{2+} and CO_2; therefore, the rates of RuBP carboxylation represent the concentration of catalytic sites per unit area. The data indicated a reduction in catalytic sites of greater than 50% from control leaves to severely chlorotic leaves. The measured rates of carboxylation are similar to measured CO_2 gas-exchange rates in sugar beet (Taylor and Terry, 1984). Terry (1980) reported that Fe deficiency had no effect on chloroplast numbers per leaf area, and we showed that Fe deficiency did not change ctDNA content per unit leaf area. Because Fe stress lowers *rbc*L mRNA content, but has no effect on ctDNA copy number, it appears that chloroplast gene expression is regulated by transcriptional and/or posttranscriptional processes during the onset of Fe deficiency.

The initial physiological response to Fe deficiency in sugar beet leaves appears to be loss of photochemical and CO_2 assimilation (i.e. CO_2 fixation by RuBP carboxylase) capacities. Fe stress diminishes photosynthetic competence by reducing the concentration of light-harvesting and electron transport components (Spiller and Terry, 1980; Terry, 1980) and by lowering RuBP carboxylation capacity through diminished Rubisco enzyme activation (Taylor and Terry, 1986; this study) and down-regulation of gene expression (this study). Based on previous studies and results presented here, it appears that Fe stress diminishes both photochemical and RuBP carboxylation capacities, but not the former capacity alone. The decrease in Rubisco activation measured (see also Taylor and Terry, 1986) was probably the result of a low [ATP]/[ADP] ratio in Fe-deficient leaves; low [ATP]/[ADP] ratios are known to inhibit Rubisco activase (Streusand and Portis, 1987). Severe Fe deficiency in sugar beet reduced noncyclic ATP formation by about 80% (Terry, 1980), and ATP levels were reduced by about 25% compared to controls (Arulanantham et al., 1990), which could mean that ATP levels in the chloroplast were reduced by as much as 50%, since about half of the cellular ATP is thought to be in the chloroplast.

Arulanantham et al. (1990) reported a 50% decline in Rubisco activity with severe Fe deficiency, but because RuBP levels were reduced by about 70%, they concluded that diminished RuBP, not Rubisco activity, limited photosynthesis in Fe-deficient leaves. This conclusion was based on calculations showing that the ratio of RuBP to Rubisco catalytic sites is lower than 2 (even in Fe-sufficient control leaves), a state that is believed to limit photosynthesis (Woodrow and Berry, 1988). The calculations, however, may be incorrect because carboxylation rates used to calculate the concentration of active sites were 2.5 to 3.7 times higher than all other reported rates for the same species under the same light regime (150 μmol CO_2 m^{-2} s^{-1} compared to 40–60 μmol CO_2 m^{-2} s^{-1} [Terry, 1980; Taylor and Terry, 1984, 1986; this report]). If more representative rates of Rubisco activity are used to recalculate RuBP:catalytic sites, then one finds that RuBP is actually in excess (about 4 mol RuBP mol^{-1} Rubisco catalytic site) in Fe-sufficient as well as in Fe-deficient leaves; thus, RuBP may not be limiting in Fe-deficient leaves. In fact, saturating RuBP levels have been measured in conditions of limited photophosphorylation, i.e. low light, in sugar beet (Kobza and Seemann, 1988) and in wheat (Perchorowicz and Jensen, 1983). Furthermore, the effect of Fe deficiency on phosphoribulokinase activity in sugar beet is equivocal. In one study (Taylor et al., 1982), phosphoribulokinase activity in Fe-deficient leaves was more than 2 times higher than in Fe-sufficient control leaves, whereas in another report (Arulanantham et al., 1990) activity in Fe-deficient leaves was 30% lower than in Fe-sufficient leaves.

The finding that Fe deficiency diminished expression of the nuclear-encoded SSU in the same manner as the chloroplast-encoded LSU demonstrated that this stress re-

sponse is not limited to chloroplast-encoded proteins. When Fe is resupplied to chlorotic plants, synthesis is induced for some chloroplast-encoded mRNAs (Laulhère and Mache, 1978), and for nuclear-encoded mRNAs for SSU, Chl *a/b*-binding proteins (Spiller et al., 1987), and ferritin (Proudhon et al., 1989), demonstrating that Fe affects transcription. The question of how Fe nutrient deficiency stress results in down-regulation of Rubisco gene expression is an interesting one. Lobréaux et al. (1993) found that ABA applied to Fe-deficient maize plantlets could partially replace Fe as the inducer of ferritin synthesis and that endogenous ABA concentrations increased 4.5-fold when Fe was resupplied. Although this work identifies a role for ABA in enabling plant cells to (presumably) avoid Fe toxicity during Fe resupply, we do not know whether ABA is involved in the stress response during the onset of Fe deficiency. Also a possibility is involvement of regulatory sequences that are directly responsive to intracellular Fe concentration. In animal cells, Fe-mediated translation of ferritin is regulated by IREs and *trans*-acting IRE-binding proteins. This type of regulation causes rapid synthesis of the Fe storage protein when unbound Fe is available in the cell. Although plant ferritins share a high degree of sequence homology to animal ferritins (Ragland et al., 1990) and perform the same function as animal ferritins (Andrews et al., 1992), IREs have not been found in plant ferritin genes (Lescure et al., 1991).

In conclusion, we demonstrated that Fe stress reduces activation of Rubisco, as well as expression of the enzyme subunits. In general, most of the decline in protein synthesis and protein content observed during the onset of Fe deficiency can probably be explained by large reductions in synthesis of photosynthetic proteins. From studies of transgenic tobacco with reduced expression of Rubisco, Stitt and Schulze (1994) concluded that plants can balance the capacity of Rubisco to match the rest of the photosynthetic machinery to avoid one-sided limitations of photosynthesis by Rubisco. We demonstrated that, in conditions of reduced photochemical capacity, sugar beets can also balance (in this case, down-regulate *rbc*L and *rbc*S mRNA availability) Rubisco to match the capacity of the rest of the photosynthetic machinery.

Received December 8, 1994; accepted April 17, 1995.

LITERATURE CITED

Andrews SC, Arioso P, Bottke W, Briat J-F, von Darl M, Harrison PM, Laulhère J-P, Levi S, Lobreaux S, Yewdall SJ (1992) Structure, function, and evolution of ferritins. J Inorgan Biochem **47:** 161–174

Arulanantham AR, Rao IM, Terry N (1990) Limiting factors in photosynthesis. VI. Regeneration of ribulose 1,5-bisphosphate limits photosynthesis at low photochemical capacity. Plant Physiol **93:** 1466–1475

Ellis RJ (1979) The most abundant protein in the world. Trends Biochem Sci **4:** 241–244

Evans JR (1989) Photosynthesis and nitrogen relationships in leaves of C_3 plants. Oecologia **78:** 9–19

Gerlach WL, Bedrook JR (1979) Cloning and characterization of ribosomal RNA genes from wheat and barley. Nucleic Acids Res **7:** 1869–1885

Goldschmidt-Clermont M, Rahire M (1986) Sequence, evolution and differential expression of the two genes encoding variant subunits of ribulose bisphosphate carboxylase/oxygenase in *Chlamydomonas reinhardtii*. J Mol Biol **191:** 421–432

Gris E (1844) Nouvelles expériences sur l'action des composés ferrugineux solubles, et de la débilite des plantes. CR Acad Sci Paris **19:** 1118–1119

Guerinot ML, Yi Y (1994) Iron: nutritious, noxious, and not readily available. Plant Physiol **104:** 815–820

Hoagland DR, Arnon DI (1950) The Water-Culture Method of Growing Plants without Soil, California Agriculture Experimental Station circular 347. College of Agriculture, University of California, Berkeley

Kobza J, Seemann JR (1988) Mechanisms for light-dependent regulation of ribulose-1,5-bisphosphate carboxylase activity and photosynthesis in intact leaves. Proc Natl Acad Sci USA **85:** 3815–3819

Krapp A, Hofmann B, Schäfer C, Stitt M (1993) Regulation of the expression of *rbc*S and other photosynthetic genes by carbohydrates: a mechanism for the 'sink regulation' of photosynthesis. Plant J **3:** 817–828

Laemmli UK (1970) Cleavage of structural proteins during assembly of the head of bacteriophage T4. Nature **227:** 680–685

Laulhère JP, Mache R (1978) Induction des synthèsis de mRNA et rRNA chlorplastique par le fer du verdissement de feuilles chlorosès. Physiol Veg **16:** 643–656

Lescure A-M, Proudhon D, Pesey H, Ragland M, Theil EC, Briat J-F (1991) Ferritin gene transcription is regulated by iron in soybean cell cultures. Proc Natl Acad Sci USA **88:** 8222–8226

Lichtenthaler HK (1987) Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. Methods Enzymol **148:** 350–382

Lin CH, Stocking CR (1978) Influence of leaf age, light, dark and iron deficiency on polyribosome levels in maize leaves. Plant Cell Physiol **19:** 461–470

Lobréaux S, Hardy T, Briat J-F (1993) Abscisic acid is involved in the iron-induced synthesis of maize ferritin. EMBO J **12:** 651–657

Makino A, Nakano H, Mae T (1994) Response of ribulose-1,5-bisphosphate carboxylase, cytochrome *f*, and sucrose synthesis enzymes in rice leaves to leaf nitrogen and their relationships to photosynthesis. Plant Physiol **105:** 173–179

Markwell MAE, Haas SM, Tolbert NE, Bieber LL (1981) Protein determination in membrane and lipoprotein samples: manual and automated procedures. Methods Enzymol **72:** 296–303

Nishio JN, Taylor SE, Terry N (1985) Changes in thylakoid galactolipids and proteins during iron nutrition-mediated chloroplast development. Plant Physiol **77:** 705–711

Perchorowicz JT, Jensen RG (1983) Photosynthesis and activation of ribulose bisphosphate carboxylase in wheat seedlings. Plant Physiol **71:** 955–960

Price CA (1968) Iron compounds and plant nutrition. Annu Rev Plant Physiol **19:** 239–248

Proudhon D, Briat J-F, Lescure A-M (1989) Iron induction of ferritin synthesis in soybean cell suspensions. Plant Physiol **90:** 586–590

Pushnik JC, Miller GW (1982) The effect of iron and light treatment on chloroplast composition and ultrastructure in iron-deficient barley leaves. J Plant Nutr **5:** 311–321

Ragland M, Briat J-F, Gagnon J, Laulhere J-P, Massenet O, Theil EC (1990) Evidence for conservation of ferritin sequence among plants and animals and for a transit peptide in soybean. J Biol Chem **265:** 18339–18344

Rowland-Bamford AJ, Baker JT, Allen LH, Bowes G (1991) Acclimation of rice to changing atmospheric carbon dioxide concentration. Plant Cell Environ **14:** 577–583

Sambrook J, Fritsch EF, Maniatas T (1989) Molecular Cloning: A Laboratory Manual, Ed 2. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY

Shetty AS, Miller GW (1966) Influence of iron chlorosis on pigment and protein metabolism in leaves of *Nicotiana tabacum* L. Plant Physiol **41:** 415–421

Spiller S, Terry N (1980) Limiting factors in photosynthesis. II. Iron stress diminishes photochemical capacity by reduc-

ing the number of photosynthetic units. Plant Physiol **65:** 121–125

Spiller SC, Kaufman LS, Thompson WF, Briggs WR (1987) Specific mRNA and rRNA levels in greening pea leaves during recovery from iron stress. Plant Physiol **84:** 409–414

Stitt M, Schulze D (1994) Does Rubisco control the rate of photosynthesis and plant growth? An exercise in molecular ecophysiology. Plant Cell Environ **17:** 465–487

Stocking CR (1975) Iron deficiency and the structure and physiology of maize chloroplasts. Plant Physiol **55:** 626–631

Streusand VJ, Portis AR Jr (1987) Rubisco activase mediates ATP-dependent activation of ribulose bisphosphate carboxylase. Plant Physiol **85:** 152–154

Taylor SE, Terry N (1984) Limiting factors in photosynthesis. V. Photochemical energy supply colimits photosynthesis at low values of intracellular CO_2 concentration. Plant Physiol **75:** 82–86

Taylor SE, Terry N (1986) Variation in photosynthetic electron transport capacity and its effect on the light modulation of ribulose bisphosphate carboxylase. Photosynth Res **8:** 249–256

Taylor SE, Terry N, Huston RP (1982) Limiting factors in photosynthesis. III. Effects of iron nutrition on the activities of three regulatory enzymes of photosynthetic carbon metabolism. Plant Physiol **70:** 1541–1543

Terry N (1980) Limiting factors in photosynthesis. I. Use of iron stress to control photochemical capacity *in vivo*. Plant Physiol **65:** 114–120

Usada H (1984) Variations in the photosynthetic rate and activity of photosynthetic enzymes in maize leaf tissue of different ages. Plant Cell Physiol **25:** 1297–1301

Woodrow IE, Berry JA (1988) Enzymatic regulation of photosynthetic CO_2 fixation in C_3 plants. Annu Rev Plant Physiol Plant Mol Biol **39:** 533–594