Chloroplast photooxidation affects the accumulation of cytosolic mRNAs encoding chloroplast proteins in maize

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Abstract. Maize (Zea mays L.) seedlings were grown in the presence or absence of an herbicide, norflurazon (4-chloro-5-(methylamino)-2-(x,x,x-trifluoro-m-tolyl)-pyridazinone), which prevents the accumulation of colored carotenoids. In the absence of carotenoids, plants grown in high light incur extensive photooxidative damage to their plastids, but relatively little damage elsewhere. Growth in very low light minimizes chlorophyll photooxidation and allows chloroplast development to proceed. We have previously reported that mRNA encoding light-harvesting chlorophyll a/b protein (LHCP) fails to accumulate in high-light-grown carotenoid-deficient seedlings, but accumulates normally in carotenoid-deficient seedlings grown in low light. Here we extend these results by examining the levels of translatable mRNAs encoding seven additional nuclear-encoded chloroplast proteins. When norflurazon-treated seedlings were grown in low light for 8 d and then transferred to high light for 24 h, three cytosolic mRNAs (plastocyanin, Rieske Fe-S protein, and the 33-kdalton (kDa) subunit of the photosystem II O2-evolving complex) decreased to less than 1% the amount found in untreated seedlings. Two other mRNAs (NADP malic enzyme, EC 1.1.1.40, and the 23-kDa subunit of the photosystem II O2-evolving complex) decreased significantly but not to levels as low as the first three. Levels of translatable mRNA for two other chloroplast proteins (pyruvate orthophosphate dikinase, EC 2.7.9.1, and ferredoxin NADP oxidoreductase, EC 1.18.1.2) were not reduced in nonflurazon-treated seedlings after 24 h in high light, but did not show the normal light-induced increase found in untreated plants. Photooxidative damage in the chloroplast thus affects the accumulation of a number of cytosolic mRNAs encoding proteins destined for the chloroplast.

Key words: Chloroplast proteins - Chloroplast (photooxidation) mRNA (chloroplast proteins) - Nucleus-chloroplast interactions - Photooxidation (chloroplasts) - Zea (mRNA accumulation).

Introduction

When carotenoid-deficient seedlings are grown in the light, photooxidative destruction of many chloroplast components results as a consequence of the absence of photoprotective carotenoids. Chlorophyll is photooxidized (Griffiths et al. 1955; Anderson and Robertson 1960; Frosch et al. 1979) and there is little or no development of internal chloroplast membranes (Bachmann et al. 1967 and many others; Frosch et al. 1979). Plastid ribosomes and RNAs fail to accumulate (Reiss et al. 1983; Mayfield et al. 1986b), as do plastid proteins (Reiss et al. 1983; Harpster et al. 1984). Enzyme activities in other subcellular compartments are not affected (Blume and McClure 1980), and cytosolic ribosomes are still present (Reiss et al. 1983) as well as most cytosolic mRNAs (Mayfield and Taylor 1984). Damage appears to be confined solely to the plastid and peroxisomal compartments (Feierabend and Kemmerich 1983). Morphogene-
sis is also normal in albino plants (Jabben and Deitzer 1979; Froesch et al. 1979; Gorton and Briggs 1980; Reiss et al. 1983), including the pho-
tochrome-mediated suppression of mesocotyl elon-
gation.

We have previously shown that another effect
of a carotenoid deficiency is to block the accumula-
tion of cytosolic mRNA encoding a major chloro-
plast thylakoid protein, the light-harvesting chloro-
phyll a/b protein of photosystem II (LHCP) (Mayfield and Taylor 1984). Loss of LHCP-
mRNA accumulation has been seen both in nucle-
ar mutants with carotenoid deficiencies (Mayfield
and Taylor 1984; Batschauer et al. 1986) and in
seeds made carotenoid-deficient by application
of the herbicide norflurazon (Mayfield and Taylor
1984; Batschauer et al. 1986; Oelmiiller and Mohr
1986). The absence of carotenoids per se is not
responsible for the failure to accumulate LHCP
mRNA. Carotenoid-deficient plants grown under
(low) white light or far-red light, conditions which
minimize photooxidative damage to the chloro-
plast, accumulate normal levels of LHCP mRNA
(Taylor et al. 1984; Batschauer et al. 1986). When
low-light-grown plants are shifted to high light,
chlorophyll is rapidly photooxidized and LHCP
mRNA rapidly disappears (Taylor et al. 1984).

An important question raised by these findings
is whether the accumulation of all cytosolic
mRNAs encoding proteins destined for the chloro-
plast is affected by a carotenoid deficiency. We
have addressed this question by measuring the rela-
tive amounts of seven cytosolic mRNAs, in addi-
tion to LHCP and SSu, which all code for chloro-
plast proteins. Because we do not have cloned
DNA probes with which to measure these mRNAs
directly, we have made estimations by immunopre-
cipitation of their in-vitro translation products.

Materials and methods

Plant materials and growth conditions. Zea mays L. seeds (car-
yopses; inbred B73; gift of Pioneer Hi-bred International, John-
ston, la., USA) were imbibed for several hours in either water
or 5 × 10\(^{-3}\) M norflurazon (Sandoz 1979); 4-chloro-5-(methyla-
mino)-2-(a,a,a-trifluoro-m-tolyl)-3H(pyrindazinone), planted
on soil, covered with 2 cm of vermiculite, and placed in a
growth chamber. The seedlings were grown for 8 d under low-
photon-fluence-rate light (12 h light from incandescent lamps,
0.011 \(\mu\)mol photon m\(^{-2}\) s\(^{-1}\), 30°C; 12 h dark periods, 23°C)
and were watered either with water or 5 × 10\(^{-3}\) M norflurazon.
After 8 d of growth, half of the seedlings were harvested into
liquid nitrogen; the remaining seedlings were shifted into high-
fluence-rate light (96 \(\mu\)mol photon m\(^{-2}\) s\(^{-1}\) from fluorescent
cool white; Westinghouse, Danvers, Mass., USA) and incan-
descent lamps) and harvested 24 h later. The seedlings were
shifted into high light several hours into the daily light period
and maintained on the same 12-h photoperiod throughout.
The green leaf tips of norflurazon-treated plants were discarded (ap-
proximately the uppermost 0.5-cm sections); an equivalent
section was also discarded from untreated plants.

Isolation of RNA. The RNA was isolated as described by
Schmidt et al. (1981) except that seedlings were pulverized in
a coffee grinder in the presence of solid CO\(_2\) and resuspended
in 0.5 M guanidium thiocyanate, 10 mM ethylenediaminetetraaci-
ic acid (EDTA), 20 mM 2-amino-2-(hydroxymethyl)-1,3-pro-
panediol (Tris)-Cl, pH 7.5, 100 mM 2-mercaptoethanol, and
16 mM diethyliothiocarbamate. Polyadenylated RNA (poly-
(A)RNA) was enriched by chromatography over oligothymi-
dylic acid-cellulose (Aviv and Leder 1972).

In-vitro translation-immunoprecipitation. In-vitro transla-
tions were performed for 1 h at 30°C with nuclease-treated rabbit
reticulocyte lysate (Pelham and Jackson 1976) (Amersham, Ar-
lington Heights, Ill., USA) in 12-µl reaction mixtures containing
0.5 µg of poly(A)RNA and 10\(^{5}\) Bq of \(^{15}\)S methionine (Amers-
ham; > 3·10\(^{15}\) Bq/mmol). The relative amount of total incor-
porated counts was determined for each set of in-vitro transla-
tions by counting aliquots spotted onto DE81 filters (Whatman,
Fisher Scientific, Santa Clara, Cal., USA) and washed in 0.5 M
Na\(_2\)HPO\(_4\).

Immunoprecipitation of in-vitro translation products was
performed using formalin-fixed Staphylococcus aureus cells to
adsorb antigen-antibody complexes (Kessler 1981). Non-speci-
fically adsorbed in-vitro products were removed by pre-incubat-
ing with S. aureus cells for 15 min at room temperature in
25 mM Tris-Cl, pH 7.4, 10 mM EDTA, 350 mM NaCl, 0.15% Triton
X-100. The cleared supernatant was incubated with anti-
serum for at least 4 h at 4°C. One-half volume of washed 10% (v/v)
S. aureus cells were pelleted in an Eppendorf (Fisher Scien-
tific) microfuge. The pellet was washed two times in 25 mM
Tris-Cl, pH 7.4, 10 mM EDTA, 700 mM NaCl, 0.15% Triton
X-100 (octylphenoxypolyethoxylethanol) and one time in
10 mM Tris-Cl, pH 7.4. In some cases the non-adsorbed in-
vitro translation products in the supernatant were reacted se-
quentially with a second antiserum. The pellet was resuspended
in 30 µl of 10% glycerol, 5% 2-mercaptoethanol, 2.5% sodium
dodecyl sulfate (SDS), 60 mM Tris-Cl (pH 6.8), bromphenol
blue. Antigen was released from the S. aureus cells by boiling
the sample for 4 min. The S. aureus cells were removed by a
brief centrifugation and the supernatants were analyzed on 10% polyacrylamide gels using the discontinuous gel system of
Laemmli (1970). Supernatants from the immunoprecipitations
were loaded onto the gel so that the volumes loaded represented
an equal number of total counts from each in-vitro translation.
Detection of the radiolabeled products was performed by fluor-
ography in the presence of En3Hance (New England Nuclear,
Boston, Mass., USA). Immunoprecipitation products were