Photooxidative damage to plastids affects the abundance of nitrate-reductase mRNA in mustard cotyledons

C. Schuster and H. Mohr*

Biologisches Institut II der Universität, Schänzlestrasse 1, D-7800 Freiburg i.Br., Federal Republic of Germany

Abstract. It was found previously that in the mustard (Sinapis alba L.) seedling (Schuster et al. 1989, Planta 177, 74–83) the action of nitrate and phytochrome on the appearance of cytosolic nitrate reductase (NR) is abolished if the plastids are damaged by photooxidation. In the present study this finding has been corroborated by the following results: (i) the appearance and disappearance of NR activity are strictly correlated with the appearance and disappearance of immunoresponsive NR protein; (ii) the appearance of NR correlates with the appearance of translatable NR mRNA; (iii) photo-destruction of the plastids strongly reduces the level of NR mRNA. It is concluded that the dependence of the NR level on the state of the plastids can be detected at the level of its mRNA and is not attributable to an inactivation of the enzyme.

Key words: Nitrate-reductase mRNA – Photooxidation of plastids – Plastidic factor – Sinapis (nitrate-reductase mRNA)

Introduction

In the cotyledons of the mustard seedling, two isoforms of nitrate reductase (NR) are induced in a synergistic manner by nitrate and light, operating via phytochrome (Schuster et al. 1989). The appearance of NR activity in squash and barley was recently found to be correlated with an increase in the level of RNA which hybridizes to NR cDNA (Rajasekhar et al. 1988; Melzer et al. 1989). Oaks et al. (1988) obtained immunological evidence that an inactive NR-protein precursor is induced in corn seedlings by extremely low levels of NO₃⁻ or by some other unidentified factor, and that higher levels of NO₃⁻ are necessary for converting the inactive NR-cross-reacting protein to a form of the enzyme capable of reducing NO₃⁻ to NO₂⁻.

A ‘plastidic factor’ has previously been postulated to be obligatorily involved in the transcriptional control of nuclear genes encoding for proteins destined for the chloroplast (see Oelmüller 1989, for a review). Photooxidative damage of the plastid is thought to destroy the ability of the organelle to produce this signal. If the plastids are damaged by photooxidation, the action of nitrate and phytochrome on the appearance of cytosolic NR is also abolished (Schuster et al. 1989). It appears that the plant cell regulates the appearance of nitrate-induced NR as if it were a plastidic protein.

In the present study we addressed three questions: (i) Is the appearance of NR activity in cotyledons of the mustard seedling attributable solely to the de-novo synthesis of enzyme protein or does activation of an inactive, NR-cross-reacting precursor protein play a part? (ii) Does the appearance of NR correlate with the appearance of NR mRNA? (iii) Is the decrease in the level of NR following a photooxidative treatment of the plastids correlated with a decrease in the NR-mRNA level?

Material and methods

Seeds of white mustard (Sinapis alba L., harvest 1984) were produced by a commercial grower from our original seed stock (Mohr 1957). They were selected and grown at 25 ± 0.5°C as described previously (Mohr 1966). Treatment of the seedlings with Norflurazon [NF; SAN 9789, 4-chloro-5-(methylamino)-2-(c₆,₇,₇-trifluorom-tolyl)-3-(2H-pyridazinone)] was carried out as described previously (Frosch et al. 1979). At a concentration of 1 · 10⁻⁵ M, Norflurazon inhibits synthesis of coloured carotenoids almost completely without affecting morphogenesis of the seedlings (Reiß et al. 1983).

Nitrate reductase was induced by supplying 15 mM KNO₃ from sowing onwards.

* To whom correspondence should be addressed

Abbreviations: NR = nitrate reductase (EC 1.6.6.1)
Crude extracts of NR were prepared and assayed as described previously (Schuster et al. 1987).

The rabbit antiserum to pure NR from maize leaves that was used in the present study was a gift from Dr. Ann Oaks (University of Guelph, Guelph, Ontario, Canada). The antiserum was prepared by Dr. M. Poulle (University of Guelph).

Immunotitration was done in principle as described by Brödenfeldt and Mohr (1986).

For in-vivo labelling of total protein, seedlings were grown in continuous far-red light for 54 h, either kept on water or on nitrate solution (15 mM KNO₃) from sowing onwards. Twenty seedlings, after excision of the taproot, were placed for 18 h in special containers (Jakobs and Mohr 1966) supplied with 5 ml of the above solutions (water or nitrate solution) containing 2.96 MBq of [³⁵S]methionine.

For in-vitro translation, total RNA was isolated as described by Link (1982) and 30 µg of the RNA used to prime an in-vitro translation system (rabbit reticulocyte system, amino-acid depleted; Amersham, Braunschweig, FRG) in the presence of [³⁵S]methionine and [³⁵S]cysteine (18.5-25.9 kBq·µl⁻¹). For details, see Oelmüller and Mohr (1986).

For analysis, [³⁵S]-labelled proteins from the crude extracts and translation products were subjected to immunoprecipitation with NR antiserum in principle as described by Oelmüller and Mohr (1986). Precipitation of the antigen-antibody complex was achieved by using Protein A-Sepharose instead of Staphylococcus aureus cells. Labelled products were analysed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and fluorography (Bonner and Laskey 1974).

The NR protein was concentrated by chromatography as described previously (Schuster et al. 1989).

Statistics. The results obtained by immunotitration are based on two to four independent experiments. The enzyme assays for every immunotitration curve were performed in duplicate. The in-vitro translation data were confirmed in three independent experiments.

Results

Immunotitration. Using established immunotitration techniques (Brödenfeldt and Mohr 1986) we have found that during the experimental period the NR activity of an extract is strictly proportional to the amount of immunoresponsive material (Fig. 1). The dashed lines in the figure show that the amount of antiserum required to reduce enzyme activity by a certain percentage (25, 40, 50%) is directly proportional to the original enzyme activity. This means that the enzyme activity of an extract – without antiserum – is proportional to the amount of immunoresponsive material in that extract (for details, see Brödenfeldt and Mohr 1986). We may conclude that the immunoresponsive activity per unit of active enzyme does not change with time. Any increase in enzyme activity can be attributed to synthesis de novo of enzyme protein, and any decrease in enzyme activity to a loss of immunoresponsive enzyme protein.

Specificity of the antiserum. In an extract from mustard cotyledons the antiserum directed against maize NR recognizes a single 105-kilodalton (kDa) band (Fig. 2, right lane) which agrees with the molecular weight of the NR subunit from maize under denaturing conditions (Commère et al. 1986). In the absence of nitrate only traces of label can be detected (Fig. 2, left lane). Corresponding results were obtained in previous measure-