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Transient Accumulation of Nitrite Reductase mRNA in Maize following the Addition of Nitrate

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ABSTRACT

Expression of the gene coding for nitrite reductase (NiR) is induced upon the addition of nitrate. We have analyzed this induction process in hydroponically grown maize (*Zea mays* L.) seedlings where the level of nitrate in the medium can be easily manipulated. There is a rapid induction of NiR mRNA upon addition of nitrate, increasing first in the roots and then in the leaves. The rapidity of the response depends on the nitrate concentration and the growth medium. However, the general pattern of expression is the same: the mRNA level increases, reaches a maximum, and then decreases, despite the fact that the nitrate concentration in the medium remains constant. This decline in mRNA level can be quite rapid, particularly in root tissue. If the nitrate is given as a pulse, the mRNA levels decrease even more rapidly. It is clear that the NiR mRNA is short-lived, with a half-life in the roots of less than 30 minutes. The NiR protein level, on the other hand, increases gradually somewhat after the increase in mRNA and remains at high levels at least for 24 hours after the addition of nitrate.

Nitrogen is a major limiting nutrient for plants and is generally available in the form of nitrate (12). However, the amount of nitrate available during the growing season depends upon a number of environmental factors including rainfall and soil composition as well as the timing of fertilizer applications. The nitrate uptake and assimilatory proteins are required only when nitrate is available, and plants regulate the expression of these genes. However, the regulatory mechanisms controlling their expression is at present poorly understood.

The genetics of nitrate utilization has been studied extensively in the filamentous fungi *Aspergillus nidulans* and *Neurospora crassa*. Numerous mutants have been isolated that exhibit changes in the structural genes whose products are involved in nitrate uptake and assimilation (4, 8). Furthermore, mutations in genes whose products regulate the expression of the structural genes have also been noted (ref. 2 and references therein). There are two regulatory genes: the first is a gene involved in the activation of the uptake and assimilatory genes upon the addition of nitrate to the medium; the second regulatory gene is involved in catabolite repression, preventing the expression of the structural genes in the presence of a reduced nitrogen source such as ammonia (1).

In higher plants, it has been shown that nitrate induces the synthesis of nitrate reductase (NR)[3] and nitrite reductase (NiR) proteins (see Guerrero *et al.* [10] for a review). With the cloning of these genes, it has been possible to demonstrate that the mRNA levels for these genes increase considerably upon the addition of nitrate (3, 5, 7, 9, 16). The rate with which nitrate is taken into root tissue has also been shown to be increased in the presence of nitrate (13). This induction by nitrate of the nitrate utilization pathway bears a striking similarity to that seen with the better characterized filamentous fungi, with the notable exception that catabolite repression by reduced nitrogenous compounds does not appear to occur in plants (10).

In this paper, we utilize a maize NiR cDNA clone (16) as a probe to analyze the regulation by nitrate of NiR gene expression in hydroponically grown maize seedlings. Upon addition of nitrate to the medium, a rapid induction in the NiR mRNA levels is seen. The rapidity of the response depends upon the concentration of nitrate as well as the growth conditions. After the initial induction, there is a fairly rapid decrease in the NiR mRNA level, even though the nitrate concentration in the medium remains constant, indicating that several modes of regulation are involved.

MATERIALS AND METHODS

Plant Growth Conditions

Maize seeds (*Zea mays* Funk inbred 6N603) were germinated in distilled-water-soaked germination paper in the dark for 3 to 4 d. Seedlings having roots approximately 4 cm long were inserted into slits cut into packing foam (Fidelity Products Co.). The seedlings were grown hydroponically by floating the foam pads on the surface of the media. Two salt media were used: (a) minimal medium, 10 mM Mes (pH 5.5), 5 mM $CaSO_4$; (b) complete medium (minus nitrogen), 5 mM KH_2PO_4, 1 mM $CaCl_2$, 1 mM $MgSO_4$, 1.25 mM K_2SO_4, 0.8 mL/L micronutrients (90 mM $MnCl_2$, 5 mM H_3BO_3, 0.78 mM $ZnSO_4$, 0.32 mM $CuSO_4$, 0.11 mM Na_2MoO_4), 1.6 mL/L iron-EDTA complex (12 g of Na_2EDTA, 8.7 g of $FeCl_2$ per liter). The plants were grown until they were 10 to 12 cm tall prior to induction with nitrate. Circulation was provided by a submersible pump (Little Giant Series One). The plants were grown under a 16-h light/8-h dark regime in the greenhouse. After nitrate induction, the nitrate in the medium was meas-

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[3] Abbreviations: NR, nitrate reductase; NiR, nitrite reductase.

ured using a Corning nitrate electrode to ensure that it remained constant throughout the experiment.

Protein Extraction and Protein Blot Analysis

Protein was extracted from tissue that had been frozen in liquid N_2 at various times after addition of nitrate. Six to ten g of tissue were ground in a Waring blender with 40 mL of buffer (10 mM Tris [pH 8.0], 100 mM NaCl, 2 g/L Tris base [ref. 17]). Cold acetone was added to the homogenate to a final concentration of 35% (v/v). After stirring for 15 min, the mixture was centrifuged at 7000*g* for 30 min. Acetone was added to the supernatant to a final concentration of 70% (v/v), and the solution was stirred for 15 min and centrifuged at 2500*g* for 5 min. The pellet was resuspended in 100 mM Tris (pH 8.0), 1 mM EDTA and was frozen at −20°C.

The protein (50 μg/lane) was subjected to electrophoresis using a 7.5% SDS-polyacrylamide gel. The proteins were then blotted onto an Immobilon membrane (Millipore) using an American Bionuclear Blotting Apparatus. The membrane was blocked with 2% Tween 20, 30 mM Tris (pH 10.2), 150 mM NaCl for 10 min. Rabbit anti-NiR antiserum was added at a 1/1000 dilution in wash buffer and incubated overnight on a rocking platform. The membrane was washed four times in 0.05% Tween, 30 mM Tris (pH 10.2), 150 mM NaCl. Goat anti-rabbit IgG alkaline phosphatase conjugate (Bio-Rad) was used as the second antibody for the detection of the NiR-antibody complex.

Isolation of Partially Purified Maize NiR

Maize NiR was partially purified from 2-week-old seedling leaves following the procedure of Lancaster *et al.* (17). The procedure involved homogenization, acetone extraction and dialysis, DE52 ion-exchange chromatography, ammonium sulfate fractionation, a second DE52 ion-exchange column, DEAE Sephadex A-50 column chromatography, and a final ammonium sulfate fractionation. This procedure, although not yielding pure NiR, as it does in spinach, resulted in a highly enriched fraction that cross-reacts with spinach NiR polyclonal antibody.

RNA Isolation and RNA Blot Analysis

Plants were harvested, fast-frozen in liquid N_2, and stored at −20°C. Five grams of either leaves or roots were added to 10 mL of extraction buffer (50 mM Tris-HCl [pH 8.0], 4% sodium *p*-aminosalicylate, 1% sodium 1,5-naphthalenedisulfonate) and 10 mL of buffer-saturated phenol. The mixture was homogenized with a Brinkman Polytron and then shaken for 20 min at 300 rpm on a New Brunswick G-33 gyratory shaker. After the addition of 10 mL of chloroform, the mixture was shaken for an additional 10 min prior to centrifugation at 7000 rpm in an SS34 rotor (Sorvall). The aqueous phase was reextracted with 10 mL of chloroform and made 2 M with LiCl. After overnight precipitation at 4°C, the RNA was sedimented in an SW41 rotor (Beckman) at 25 K for 2 h. The RNA was resuspended in 1% SDS, 5 mM EDTA, 20 mM NaOAc, 40 mM Tris-HCl (pH 7.5) and was precipitated with ethanol. The resulting pellet was redissolved in dH_2O, reprecipitated in ethanol, and finally redissolved in dH_2O and stored at −90°C.

For RNA blot hybridization, 20 μg of leaf or 10 μg of root total cellular RNA was subjected to electrophoresis through a 1.2% agarose, 2.2 M formaldehyde gel, and the RNA was blotted onto nitrocellulose. The probe used for the hybridization was a NiR cDNA insert from the plasmid pCIB801 (16). Filters were allowed to decay and then rehybridized with nick-translated soybean actin gene (19). The amount of NiR mRNA was determined by scanning the autoradiograms from at least two different exposures with a Biomed Instruments scanning densitometer.

RESULTS

Induction of NiR Protein by Nitrate in Different Salt Media

Maize seedlings were grown hydroponically for 8 d postgermination in four different media. These were: (a) a minimal medium containing only Mes buffer and 5 mM $CaSO_4$; (b) a complete medium minus nitrogen; (c) a complete medium minus nitrogen and sulfur; (d) a complete medium minus nitrogen and iron. The root morphology of the plants grown in the minimal medium was different than that of seedlings grown in the more complete media. Roots were considerably longer with less lateral branching. Roots were collected from at least 10 plants and the remaining plants induced with 20 mM nitrate. Root samples were collected at 6 and 24 h after induction. Protein was extracted from the roots, separated via SDS-PAGE, and analyzed for the presence of NiR by reaction to anti-NiR serum as described in "Materials and Methods." As can be seen in Figure 1, there is little difference in the induction of the NiR protein in the different media, despite the difference in root morphology. Furthermore, there was no discernible difference in the expression of the protein when different cations (K^+, Ca^{2+}, NH_4^+) were used as the counterion during the induction process (data not shown). Therefore, to simplify the medium used, plants were grown in either the minimal medium or the complete medium (minus N) for the analysis of nitrate regulation. As will be seen below, although there are differences in the timing of the nitrate-induced

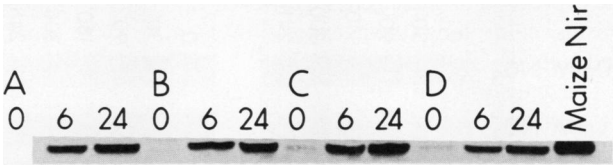

Figure 1. Induction of NiR protein by nitrate in roots of maize seedlings grown in different media. Hydroponically grown maize seedlings were grown in four different media: A, minimal medium; B, complete medium minus nitrogen; C, complete medium minus nitrogen and sulfur; D, complete medium minus nitrogen and iron. Roots were harvested 9 d, postgermination and the remaining plants induced with 20 mM nitrate. Further root samples were harvested 6 and 24 h after the addition of nitrate. Protein was extracted from the roots as described in "Materials and Methods" and 50 μg of protein per lane was subjected to electrophoresis on a 7.5% SDS-polyacrylamide gel. The protein was blotted onto Immobilon filter paper and the NiR protein detected with anti-NiR serum as described in "Materials and Methods."

response depending on what medium is used, the general regulatory pattern is the same.

Induction by Nitrate of NiR mRNA

Maize seedlings grown in the minimal salt medium were induced with varying concentrations of nitrate. Plants were harvested at different times and total cellular RNA was isolated from roots and from leaves. The NiR mRNA was detected by RNA blot hybridization using nick-translated NiR cDNA as a probe. The results are shown in Figure 2. There is a rapid induction in the NiR mRNA levels after the addition of nitrate. Induction occurs first in the roots and then in the leaves, perhaps reflecting the time at which inducing concentrations of nitrate become available to the different organs. In plants induced with 20 mM nitrate, the NiR mRNA increases at least 100-fold in the roots after 30 min, whereas in leaf tissue there is approximately a 50-fold increase after 90 min (measured by scanning densitometry as described in "Materials and Methods"). In general, the lower the nitrate concentration, the longer it takes to reach maximal levels of NiR mRNA. In the leaves there always appeared to be a low but detectable constitutive level of NiR mRNA. Since there are at least two NiR genes per maize haploid genome (16), the constitutive expression detected might be due to the transcription of a different NiR gene from that exhibiting induced expression.

From the results shown in Figure 2, it is clear that the level of NiR mRNA decreased rapidly after a maximum RNA level was reached. This occurred despite the fact that the NO_3^- level in the medium in all the experiments remained constant throughout the course of the experiment, as measured using a nitrate electrode (see "Materials and Methods"). From the data in Figure 2, it appears that the more rapid the induction in the NiR mRNA levels, the more rapid the subsequent decline in these levels. In plants induced with 20 mM nitrate, the root NiR mRNA decreased 10-fold (measured by scanning densitometry as described in "Materials and Methods") between 30 and 180 min after induction, whereas in leaves there was a slower fourfold decline between 90 and 300 min. When the blots were reprobed with the soybean actin gene (19), a constant actin mRNA level was seen, indicating that the same amount of RNA was loaded in each lane (data not shown).

Figure 3 presents the RNA gel blot analysis of NiR mRNA from maize seedlings grown in the more complete salt medium sampled at various times after induction with 20 mM nitrate. The general pattern in the induction of expression of the NiR gene is the same as was found for plants grown in the minimal salts. The major difference is in the rapidity of the decline of message. Seedlings grown in minimal salts and induced with 20 mM nitrate reach maximum levels of NiR mRNA at 30 min, followed by a rapid decline. The pattern of expression seen in Figure 3 is almost identical to that seen for plants grown in minimal salts induced with 1 mM nitrate (see Fig. 2). We have not yet determined the cause for this difference in sensitivity to nitrate concentration.

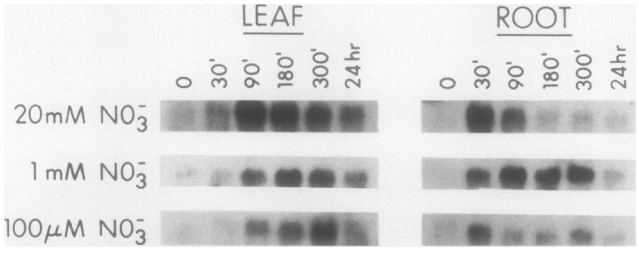

Figure 2. Induction of NiR mRNA in plants grown in minimal medium upon the addition of different concentrations of nitrate. Maize seedlings were grown hydroponically for 9 to 10 d postgermination. Immediately after harvesting the zero time sample, nitrate was added to the medium to a final concentration of either 20 mM, 1 mM, or 100 μM. Leaves and roots were collected at the indicated times after nitrate addition and quickly frozen in liquid nitrogen. Total cellular RNA was isolated from the leaves and roots, and 20 μg of leaf RNA or 10 μg of root RNA was subjected to electrophoresis on a 2.2 M formaldehyde, 1.2% agarose gel. The RNA was blotted onto nitrocellulose and then hybridized with nick-translated NiR cDNA isolated from pCIB801 (16). The NiR mRNA was detected by autoradiography.

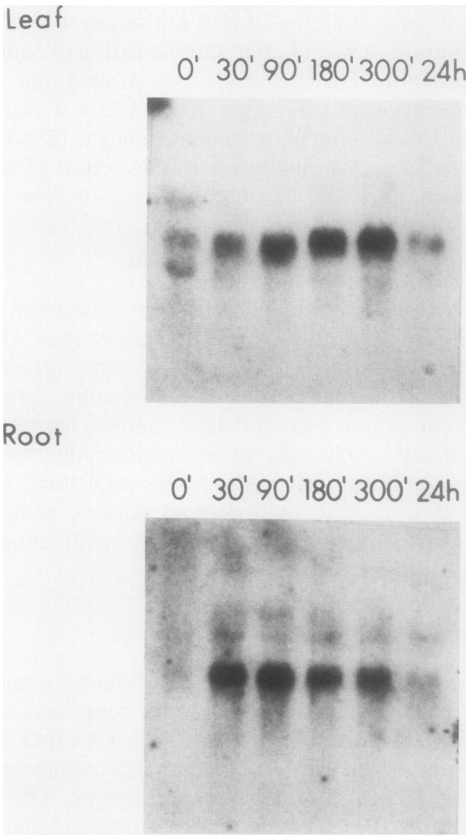

Figure 3. Induction of NiR mRNA by nitrate in plants grown in the complete salts. Maize seedlings were grown hydroponically for 9 d postgermination in the complete salts medium minus nitrogen. A sample of plants was harvested just prior to the addition of 20 mM nitrate. After the addition of nitrate, plants were harvested at the times shown and the leaves and roots quick-frozen in liquid nitrogen. Total cellular RNA was isolated, and either 20 μg of leaf RNA or 10 μg of root RNA was subjected to electrophoresis on a 2.2 M formaldehyde, 1.2% agarose gel. The RNA was blotted onto nitrocellulose and the NiR mRNA detected using the nick-translated NiR cDNA fragment isolated from pCIB801 as the probe.

Induction by Nitrate of the NiR Protein

The pattern of induction of NiR protein levels is shown in Figure 4. In the experiment shown here, the material harvested was the same as was used for the analysis of the NiR mRNA (Figs. 2 and 3). NiR protein is present in leaves prior to the addition of nitrate (Fig. 4). This is consistent with the finding from the analysis of the NiR mRNA, where a small but reproducible level of constitutively synthesized mRNA was detectable (Figs. 2 and 3). After the addition of nitrate, the amount of NiR protein increases, but not as dramatically as does the mRNA. This increase occurs at an earlier time in plants grown on minimal salts (Fig. 4, panel A) than in plants grown in the more complete medium (Fig. 4, panel B), which correlates well with the rapidity of the mRNA response in these plants. In roots NiR protein is not detectable prior to the addition of nitrate and consequently there is a large induction in the accumulation of this protein. As in the leaves, this increase is most rapid in plants grown in minimal salts (Fig. 4, panel A). The synthesis of the NiR protein lags behind the synthesis of the NiR mRNA.

Reinduction of the NiR Gene

Seedlings grown in the minimal salt medium were induced with 20 mM nitrate for 90 min, after which time the nitrate was removed from the medium for 24 h. The seedlings were then reinduced with 20 mM nitrate. Root and leaf samples were collected at the times indicates in Figure 5, RNA was extracted, and the NiR mRNA was detected by RNA blot hybridization (Fig. 5). There are two features of these results worthy of note. The first is that the levels of mRNA decrease slightly more rapidly upon the removal of nitrate than in its presence (see Fig. 2 to compare). The synthesis of the mRNA presumably stops earlier under these conditions. However, it is clear that the mRNA stability is very low, having a half-life of less than 30 min in roots and 40 min in leaves, based on densitometric scanning of the autoradiograms. The second point is that reinduction of this gene does not lead to as large a synthesis of the NiR mRNA as the primary induction.

These experiments were repeated for plants grown in the complete salts medium and the results are shown in Figure 6.

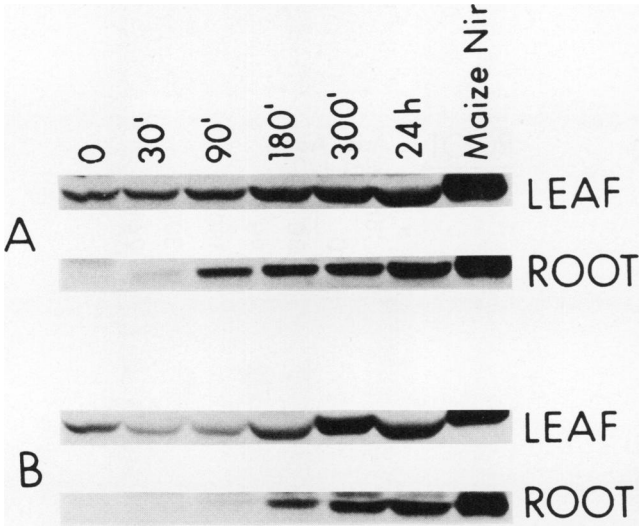

Figure 4. Induction of NiR protein by the addition of nitrate. Seedlings grown for 10 d postgermination were induced with 20 mM nitrate. Roots and leaves were harvested either just prior to the addition of nitrate or after its addition at the times indicates. Protein was extracted as described in "Materials and Methods" and 50 μg was subjected to electrophoresis on 7.5% SDS-PAGE. The protein was blotted onto Immobilon filter paper and the NiR polypeptide detected by its reaction with anti-NiR serum as described in "Materials and Methods." A, Plants grown in minimal salts medium; B, plants grown in complete salts minus nitrogen medium. Maize NiR, partially purified maize NiR protein.

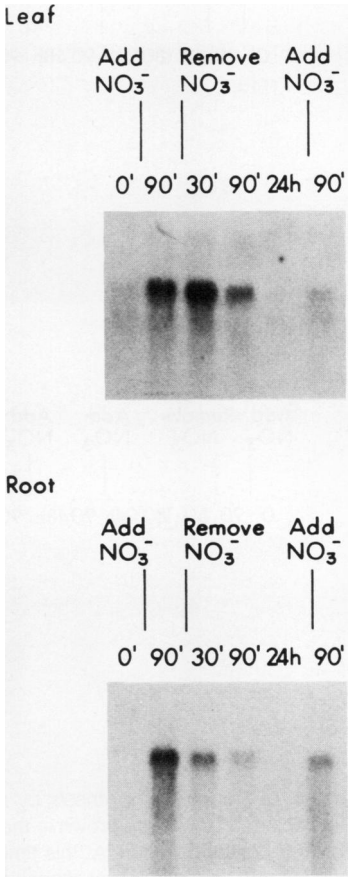

Figure 5. Reinduction of NiR mRNA by nitrate in plants grown in minimal salts. Maize seedlings were grown 9 d postgermination in the minimal salts medium. A sample was collected (0 time) and nitrate added to a final concentration of 20 mM. Another sample was collected 90 min after the addition of the nitrate and the plants were removed from the medium, rinsed in distilled water, and placed in nitrate-free medium. Samples were then collected 30 and 90 min after nitrate removal. After 24 h, another sample was collected and the remaining plants were reinduced with 20 mM nitrate. The final sample was collected 90 min after this second addition of nitrate. The leaves and roots were fast-frozen in liquid nitrogen at harvest. Total cellular RNA was isolated, and either 20 μg of leaf RNA or 10 μg of root RNA was subjected to electrophoresis on a 2.2 M formaldehyde, 1.2% agarose gel. The RNA was blotted onto nitrocellulose and hybridized with nick-translated NiR cDNA. The NiR mRNA was detected by autoradiography.

In the roots the NiR mRNA decreases rapidly following the removal of nitrate. In the leaves the decrease in NiR mRNA occurs more slowly, although this probably reflects continued synthesis of the NiR mRNA due to the continued presence of nitrate in the leaves. This is not surprising since the level of NiR mRNA in this tissue does not peak until 300 min in the presence of exogenous nitrate under these growth conditions (see Fig. 3). Reinduction of the plants 24 h later leads to the synthesis in the roots of some NiR mRNA, but not to the levels seen in the initial induction. In leaf tissues no noticeable induction occurs over the levels of mRNA still present. Nitrate was added to the plants for a third time, 48 h after the initial induction. As can be seen in Figure 6, these plants now reinduce to as high levels of NiR mRNA as in the initial induction. Therefore, whatever prevents the full reinduction of the NiR gene at 24 h either no longer has an effect at 48 h or is no longer present.

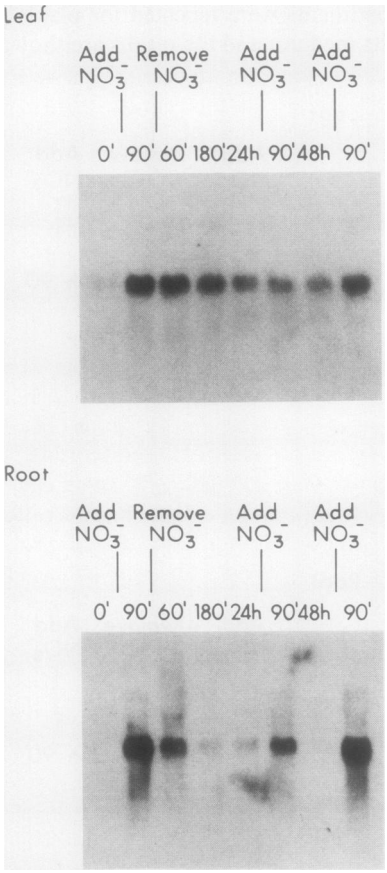

Figure 6. Reinduction of NiR mRNA synthesis by nitrate in plants grown in complete medium. Plants were grown in the complete salts minus nitrogen until 9 d postgermination. At this time a sample was collected (0 time) and nitrate added to a final concentration of 20 mM. After 90 min another sample was harvested and the plants were removed from the medium, rinsed in distilled water, and placed into nitrate-free medium. Further samples were collected 30 and 90 min after removing the plants from nitrate. After 24 h, another sample was harvested, and the plants were reinduced with nitrate added to a final concentration of 20 mM. A sample was collected 90 min later, and the plants again were removed from the medium, rinsed in distilled water, and placed in nitrate-free medium. After an additional 24 h (48 h after the initial induction), plants were harvested and the remainder reinduced again with nitrate. The final sample was collected 90 min after this addition of nitrate. The leaves and roots were quick-frozen after harvesting. Total cellular RNA was isolated and 20 μg of leaf RNA or 10 μg of root RNA subjected to electrophoresis through a 2.2 M formaldehyde, 1.2% agarose gel. The RNA was blotted onto nitrocellulose and hybridized to the nick-translated NiR cDNA. The NiR mRNA was detected by autoradiography.

Expression of NiR mRNA in the Presence of Ammonia

To test whether there is any repression by ammonia of the plant nitrate assimilatory pathway, plants were pretreated with 20 mM ammonia prior to induction with 20 mM nitrate. Total cellular RNA was extracted and analyzed for the presence of NiR mRNA by RNA blot hybridization. As shown in Figure 7, in comparison with the results in the same type of experiment minus ammonia shown in Figure 2, there is no quali-

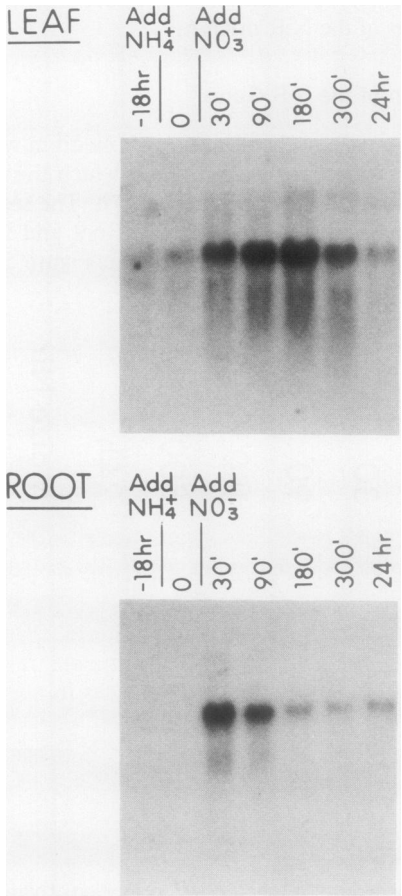

Figure 7. Induction by nitrate of NiR mRNA in seedlings pretreated with ammonia. Maize seedlings were grown in minimal salts for 9 d postgermination. Ammonium ion was then added to a final concentration of 20 mM and the plants were grown overnight. At this time, nitrate was added to a final concentration of 20 mM. −18 h = sample collected just prior to the addition of ammonia; 0 = sample just prior to the addition of nitrate; other time points are times that samples were collected after nitrate addition. RNA was isolated and RNA blot hybridization performed as described. The NiR mRNA was detected by autoradiography.

tative effect of ammonia on the expression of NiR mRNA under these conditions.

DISCUSSION

The transient induction of genes by environmental stimuli has been found in several different systems. These range from the proto-oncogene c-*fos* (15) in mammalian cells to genes induced by pathogen elicitors in plants (6, 18). The continued high level transcription of these genes in the presence of inducer could lead to wasteful synthesis of gene products and, in some cases, in their accumulation to cytotoxic levels. Additional mechanisms of regulation besides induction are therefore required to prevent the continued high level synthesis of these proteins.

The induction of NR and NiR protein synthesis upon addition of nitrate has been known for some time (ref. 10 and references therein). It has been shown for both NR in several plant species (5, 7, 9) and for NiR in spinach and maize (3, 16) that this induction occurs at the transcriptional level. The purpose of the experiments described here was to begin to understand this induction process. The NiR mRNA level is regulated in three ways. There is the initial induction of NiR mRNA synthesis caused by the addition of nitrate. This synthesis is not sustained even in the continued presence of nitrate, so a second level of regulation exists relating to the stability of the NiR message. Furthermore, an insensitivity to nitrate develops when, 90 min after the initial induction, nitrate is removed and the plants are not exposed to nitrate again for 24 h. No further induction of NiR mRNA is seen at this time. We conclude that there is a limitation of activating factor or some other type of regulatory protein involved.

Although the rapidity of the response of maize seedlings to nitrate varies depending on the growth conditions and the concentration of nitrate, the same general pattern of regulation exists. A measurable increase in NiR mRNA in response to nitrate occurs under some conditions after 5 min (data not shown) and reaches a maximum after approximately 30 min. It therefore appears that any factor that is required for induction is already present and most likely constitutively expressed. The exact nature of the inducing signal remains unknown in this system. In fungal systems, there is an activator protein which acts in *trans* to stimulate transcription of the nitrate assimilatory and uptake genes in the presence of nitrate (2, 8).

In addition to the rapid induction of NiR mRNA there is also rapid degradation of this mRNA in maize seedlings. This is most apparent in root tissue, where the level drops very quickly upon the removal of nitrate from the medium. The NiR mRNA level also drops quickly in leaves of plants grown in minimal salts, although there is continued mRNA synthesis for a short time after removing the nitrate from the medium. In plants grown in the more complete medium, the NiR mRNA level in leaves decreases more slowly upon removal of nitrate. This can probably be attributed to continued synthesis of the mRNA, since the maximal level does not occur until considerably later (300 min in the complete medium *versus* 90 min in the minimal medium). It is not possible to give an exact measurement of the stability of this mRNA without further experimentation necessary to distinguish the rate of transcription from the rate of degradation. However, it can be said that, in roots, based on the apparent abundance of the message, the half-life of the NiR mRNA is less than 30 min, whereas in leaves under some growth conditions, it is less than 40 min.

After the initial nitrate induction, under some conditions NiR mRNA synthesis decreases dramatically even in the presence of nitrate. This can be seen in the plants that are grown on constant nitrate, which over time fail to maintain the induced level of NiR mRNA. It can also be seen in the nitrate reinduction experiments. This loss of responsiveness to nitrate may be attributed to any of several causes. First, the nitrate may no longer be concentrated in the correct compartment for induction to occur. This would assume that only a portion of the total cellular nitrate is capable of inducing and furthermore is rapidly depleted but only slowly replaced. Nitrate is sequestered in the vacuole, and its accessibility for assimilation may be limited (12). Alternatively, the inducer protein might be decreased in concentration through degradation or modification during the induction process. Finally, there could be an additional regulatory protein repressing the synthesis of additional mRNA. Evidence for this last possibility has been reported by Jeter *et al.* (14). These workers isolated a barley nitrate reductase mutant in which the level of the nitrate reductase mRNA is increased over wild type, implying loss of a regulatory factor (14). Furthermore, there is some genetic evidence that the nitrate reductase gene is autoregulatory in *Aspergillus* (8). In maize, we have observed a similar regulatory pattern for nitrate reductase mRNA levels as for NiR (our unpublished results).

Nitrate addition results in an increase in NiR protein in leaves and roots of maize seedlings. The kinetics of induction parallel but lag the NiR mRNA induction. A significant difference is the stability of the protein which did not exhibit the transient pattern seen for the mRNA. Perhaps the NiR protein is involved in the resulting insensitivity to nitrate or is otherwise involved in the nitrate effects on the NiR mRNA reported here.

It is unclear whether there is regulation at the mRNA level with respect to the cell types in leaves or roots expressing the NiR gene. However, in C4 plants like maize, the NiR protein is found in mesophyll cells and not in bundle sheath cells (11). It therefore seems likely that developmental regulation of the expression of this gene exists. Whether translational or post-translational mechanisms affect the actual amount or the localization of NiR protein detected is also unclear at this time. It is important to address both of these issues in the future.

ACKNOWLEDGMENTS

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