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Quantitative Intercellular Localization of NADH-Dependent Glutamate Synthase Protein in Different Types of Root Cells in Rice Plants[1]

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The quantitative analysis with immunogold-electron microscopy using a single-affinity-purified anti-NADH-glutamate synthase (GOGAT) immunoglobulin G (IgG) as the primary antibody showed that the NADH-GOGAT protein was present in various forms of plastids in the cells of the epidermis and exodermis, in the cortex parenchyma, and in the vascular parenchyma of root tips (<10 mm) of rice (*Oryza sativa*) seedlings supplied with 1 mM NH_4^+ for 24 h. The values of the mean immunolabeling density of plastids were almost equal among these different cell types in the roots. However, the number of plastids per individual cell type was not identical, and some parts of the cells in the epidermis and exodermis contained large numbers of plastids that were heavily immunolabeled. Although there was an indication of labeling in the mitochondria using the single-affinity-purified anti-NADH-GOGAT IgG, this was not confirmed when a twice-affinity-purified IgG was used, indicating an exclusively plastidial location of the NADH-GOGAT protein in rice roots. These results, together with previous work from our laboratory (K. Ishiyama, T. Hayakawa, and T. Yamaya [1998] Planta 204: 288–294), suggest that the assimilation of exogeneously supplied NH_4^+ ions is primarily via the cytosolic glutamine synthetase/plastidial NADH-GOGAT cycle in specific regions of the epidermis and exodermis in rice roots. We also discuss the role of the NADH-GOGAT protein in vascular parenchyma cells.

The GS (EC 6.3.1.2)/GOGAT cycle is a major pathway in the assimilation of NH_4^+ under normal metabolic conditions in higher plants (Lea et al., 1990; Sechley et al., 1992). GOGAT catalyzes the transfer of the amide group of Gln formed by the reaction of GS onto 2-oxoglutarate to yield two molecules of Glu. One of the Glu molecules can be utilized as a substrate for the synthesis of Gln via the GS reaction, and the other can be used for further metabolic reactions. At least two molecular species of GOGAT exist in higher plants, one requiring NADH as a reductant (NADH-GOGAT; EC 1.4.1.14) and the other requiring the reduced form of Fd (Fd-GOGAT; EC 1.4.7.1) (Lea et al., 1990; Sechley et al., 1992).

Fd-GOGAT is the major form of GOGAT in green tissues, and this enzyme is well characterized. Full-length cDNAs encoding Fd-GOGAT have been isolated from leaves of maize (Sakakibara et al., 1991) and Arabidopsis (Suzuki and Rothstein, 1997). The enzyme has also been located in the chloroplast stroma of tomato (Botella et al., 1988) and maize (Becker et al., 1993). Its major role in green leaves is the reassimilation of NH_4^+ released from the photorespiratory pathway, which is supported by analysis of mutants lacking Fd-GOGAT in both Arabidopsis (Somerville and Ogren, 1980) and barley (Kendall et al., 1986). In roots Fd-GOGAT is probably localized in plastids (Suzuki et al., 1981) and is thought to be involved in the assimilation of NH_4^+ ions formed from the primary assimilation of nitrate (Redinbaugh and Campbell, 1993). In contrast, much less attention has been given to the molecular and physiological characteristics of NADH-GOGAT in higher plants. One exception is for the enzyme in root nodules of the legume species alfalfa (Temple et al., 1998), for which complete structures of the gene and cDNA for NADH-GOGAT have been reported (Gregerson et al., 1993; Vance et al., 1995). In both alfalfa and bean, expression of NADH-GOGAT genes appears to be developmentally regulated in response to the formation of functional root nodules (Chen and Cullimore, 1988; Anderson et al., 1989; Gregerson et al., 1993; Vance et al., 1995).

In rice (*Oryza sativa*), a nonlegume species, we have shown previously that the content and activity of NADH-GOGAT protein are high in the unexpanded, nongreen leaf blade (Yamaya et al., 1992) and in the spikelets during the early stages of ripening (Hayakawa et al., 1993). The NADH-GOGAT protein is localized in specific cell types in developing tissues, such as the metaparenchyma and mestome-sheath cells of the vascular bundles of the de-

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Abbreviation: GS, Gln synthetase.

veloping leaves, and in vascular parenchyma cells, the nucellar projection, and the nucellar epidermis of young grains of rice (Hayakawa et al., 1994). These results suggest that in young rice leaves and in grains at the early stage of ripening the apparent function of NADH-GOGAT is in the remobilization of Gln, which has been exported via the phloem and xylem from senescencing tissues and roots.

Our previous studies also showed that the transcripts and protein for NADH-GOGAT in whole roots or root tips of rice plants accumulate markedly within 12 h of supplying as little as 50 μM NH_4^+ (Yamaya et al., 1995; Hirose et al., 1997). Similar responses in the expression of NADH-GOGAT mRNA have been seen in rice cell cultures (Hayakawa et al., 1990; Watanabe et al., 1996). In roots NADH-GOGAT protein is detected in the cells of the central cylinder, the cortex, and the apical meristem in the absence of an exogenous NH_4^+ supply, but the protein is markedly accumulated in two cell layers of the root surface, the epidermis and exodermis, when the roots are supplied with 1 mM NH_4^+ for 24 h (Ishiyama et al., 1998). These results suggest that NADH-GOGAT is important for the primary assimilation of NH_4^+ ions and for the cytosolic GS reaction in the two cell layers of the root surface in rice plants.

The intracellular localization of NADH-GOGAT protein in higher plants has been the subject of some debate. Cellular and subcellular fractionation studies using density-gradient centrifugation methods have shown that most NADH-GOGAT activity appears to be in the plastids in roots (Emes and Fowler, 1979; Suzuki et al., 1981; Emes and England, 1986), in shoots (Matoh and Takahashi, 1981), and in root nodules (Chen and Cullimore, 1989). On the other hand, Hecht et al. (1988) suggested that NADH-GOGAT is located in the cytosol in cotyledons of mustard seedlings. It is crucial to resolve both the inter- and intracellular localization of NADH-GOGAT protein in plant tissue to establish the role and regulation of this enzyme in higher-plant nitrogen metabolism.

We used immunogold-labeling electron microscopy to investigate the quantitative inter- and intracellular localization of NADH-GOGAT protein in various tissues of root tips of rice seedlings supplied with 1 mM NH_4^+ for 24 h. Protein was detected using a monospecific anti-NADH-GOGAT antibody (Hayakawa et al., 1992). The results clearly show that NADH-GOGAT is located in the plastids of cells in the epidermis and exodermis, the cortical parenchyma, and the vascular parenchyma of rice roots. In addition, the density of immunogold labeling of NADH-GOGAT was approximately the same in all of the plastids in these different cells, indicating that the capacity for NH_4^+ assimilation is the same in all plastids. However, the specific localization within the outer cell layers appears to be due to the presence of large numbers of plastids in these cells. To our knowledge, this is the first study to use immunological methods to determine directly the inter- and intracellular localization of NADH-GOGAT protein in higher plants.

MATERIALS AND METHODS

Plant Growth

Rice (*Oryza sativa* L. cv Sasanishiki) seedlings were grown hydroponically, as described previously (Ishiyama et al., 1998). Seeds were soaked in distilled water at 30°C for 1 d, and approximately 40 germinated seeds were transferred to a nylon net floating on tap water that had been adjusted to pH 5.5 in a 12-L plastic container. They were grown in tap water up to d 26 in a greenhouse. On d 26, when the nutrition of the endosperm had thoroughly been utilized, the seedlings were transferred to a quarter-strength basal nutrient solution containing 1 mM NH_4Cl and then grown for a further 24 h.

Electron Microscopy Preparation

Electron microscopy preparation was performed according to protocols described previously (Peat and Tobin, 1996). Crown roots were harvested from 10 randomly selected seedlings, and the region from the tips to 10 mm of the roots were cut into 1-mm-thick transverse sections. The sections were fixed overnight at 4°C in 4% (w/v) paraformaldehyde and 0.5% (v/v) glutaraldehyde in 100 mM sodium cacodylate-HCl, pH 7.4. Sections were rinsed thoroughly with 100 mM sodium cacodylate-HCl, pH 7.4, dehydrated in an ethanol series, and then infiltrated with London White Resin at less than 4°C. Polymerization was initiated at −20°C under UV light in a UV polymerizer (model TUV200, Dosaka EM, Kyoto, Japan) using 0.5% (w/v) benzoin methyl ether (Sigma) and kept for 24 h. Silver-gold ultrathin sections were cut using an ultramicrotome (Reichert, Vienna, Austria) and placed onto Formvar (TAAB Laboratories, Aldermaston, UK) and carbon-coated nickel grids.

Monospecific Antibody

Rabbit polyclonal IgG raised against NADH-GOGAT purified from rice cell cultures (Hayakawa et al., 1992) was further purified with the antigen, as described previously (Yamaya et al., 1992). The anti-NADH-GOGAT IgG that had been affinity-purified either once or twice was used for all experiments.

Immunoblotting

The preparation of the crude protein fraction from rice roots was performed according to the protocol of Ishiyama et al. (1998). This fraction was separated by SDS-PAGE (7% [w/v]) according to the method of Laemmli (1970) and immunobloted with affinity-purified anti-NADH-GOGAT IgG, as described previously (Hayakawa et al., 1994). The protein content was determined by the method of Bradford (1976) using BSA as the standard.

Immunolabeling

Immunolabeling was performed according to protocols described previously (Peat and Tobin, 1996). To prevent

nonspecific binding of the antibody, grids were floated on 0.5 M NH_4Cl for 1 h and on blocking buffer (3% [w/v] NaCl, 1% [w/v] globulin-free BSA, and 0.2% [v/v] Tween-20 in 10 mM sodium phosphate, pH 7.3) for 1 h. The grids were then incubated with anti-NADH-GOGAT IgG, which was diluted 1:2 in the blocking buffer overnight at 4°C. After the reaction grids were washed thoroughly in the blocking buffer and then incubated with the secondary antibody (goat anti-rabbit, 15-nm gold conjugate, Biocell, Cardiff, UK), and diluted 1:30 in the blocking buffer overnight at 4°C. After the grids were washed again in blocking buffer, they were rinsed thoroughly in double-distilled water. Control sections were incubated with the same dilution of anti-NADH-GOGAT IgG that had been preabsorbed with excess amounts of purified NADH-GOGAT, as described previously (Hayakawa et al., 1994). After the immunolabeling, grids were stained with 2% (w/v) uranyl acetate and 0.3% (w/v) lead citrate. The gold particles were observed using a transmission electron microscope (model 301, Philips, Cambridge, UK) at 60 kV.

Quantification of Immunogold Labeling

Random micrographs (six to nine for each cell type) were taken at a magnification of ×34,000. Immunogold label was quantified by counting gold particles per unit of area of the cell compartment using computerized image analysis (AnalySIS Software, Soft-Imaging Software GmbH, Munster, Germany) to give final mean values of gold label per square micrometer. The software analyzed captured images from electron microscope negatives illuminated on a light box and calibrated to give a figure of pixels per square micrometer, after which an instant calculation of the area of any object traced on the screen could be obtained. Gold particles were counted by eye, as for the manual method (Peat and Tobin, 1996).

RESULTS

Specific Cross-Reactivity of Affinity-Purified Antibodies

The cross-reactivity of the antibody applied to immunogold electron microscopy analysis must be more highly specific to the target antigens than that of the antibody applied to other immunocytological methods. Our single-affinity-purified anti-NADH-GOGAT IgG cross-reacted monospecifically with denatured NADH-GOGAT in extracts prepared from leaves (Yamaya et al., 1992) and spikelets (Hayakawa et al., 1993) of rice plants when denatured proteins were separated by SDS-PAGE. The antibody also cross-reacted monospecifically with the native form of NADH-GOGAT purified from cultured rice cells when the purified protein was separated by nondenaturing PAGE (Hayakawa et al., 1994).

In extracts prepared from rice roots, the single-affinity-purified anti-NADH-GOGAT IgG cross-reacted specifically with a single polypeptide of approximately 200 kD following SDS-PAGE and immunobloting (Fig. 1A), which corresponds to the size of NADH-GOGAT (Hayakawa et al., 1992). In the case of the double-affinity-purified anti-NADH-GOGAT IgG, this was also monospecific for NADH-GOGAT, although the titer was slightly reduced (data not shown). Preabsorption of the anti-NADH-GOGAT IgG with excess amounts of purified NADH-GOGAT resulted in complete removal of cross-reactivity of the IgG on the immunoblot (Fig. 1B). This observation is an essential control for judging the meaning of the results obtained in the following immunolabeling experiments.

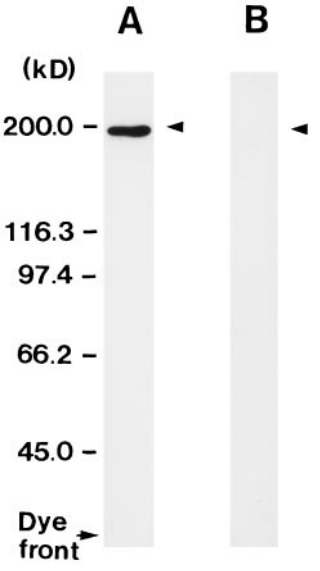

Figure 1. Western blot showing the cross-reactivity of single-affinity-purified anti-NADH-GOGAT IgG to NADH-GOGAT protein of rice roots. A, Immunoblot of a crude extract from rice roots (10 μg) labeled with single-affinity-purified anti-NADH-GOGAT IgG. B, Same as A except that the NADH-GOGAT IgG was preabsorbed with an excess amount of the NADH-GOGAT protein purified from cultured rice cells prior to immunolabeling. The position and sizes (in kD) of protein markers are indicated at the left.

Structural Observations

For the immunogold electron microscopy analysis, data were collected from three tissue types 10 mm distal to the tip of the rice root that had been supplied with 1 mM NH_4^+ for 24 h. This central cylinder tissue comprised the xylem, phloem, vascular parenchyma, and pericycle, which is bound by the endodermis (Fig. 2, A and C); the surrounding cortex, which consists of several layers of cortical parenchyma cells (Fig. 2, A and B); and two cell layers of the root surface, which consists of the epidermis and exodermis (Fig. 2, A and B).

Although in a previous study it was difficult to achieve good structural preservation for the single, outer layer of epidermis cells of barley roots (Peat and Tobin, 1996), we were successful in preserving these cells of rice roots in the present study (Fig. 2B). Some portions of the cells of the epidermis and exodermis contain large numbers of plastids (Fig. 3B). Cells of the cortical parenchyma are highly vacuolate, with only a thin layer of cytoplasm (Figs. 2B and 3C), whereas vascular parenchyma cells of the central cylinder often appear to have a greater area of cytoplasm

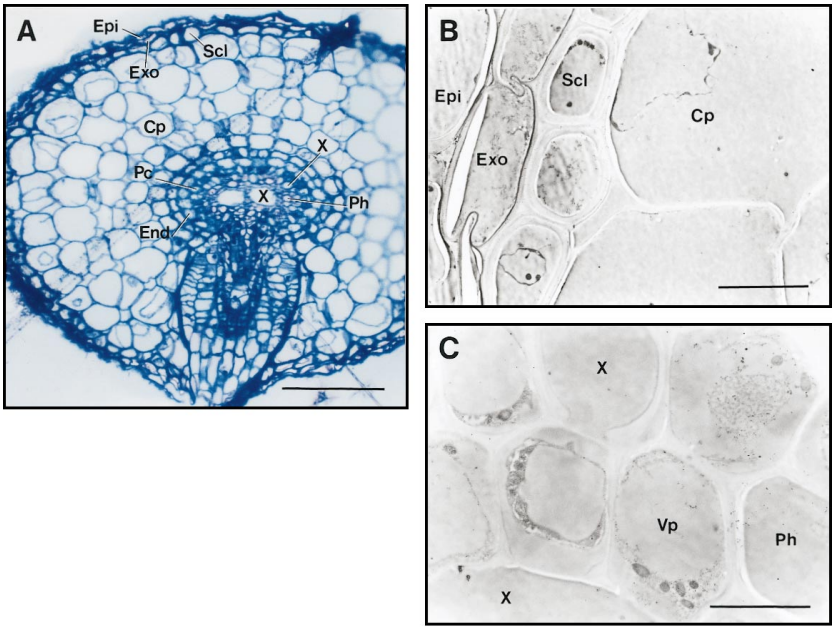

Figure 2. Rice root morphology in cross-section. Tissue was from a seedling grown with 1 mM NH_4^+ for 24 h and is representative of that sampled from the tip (<10 mm) and used for immunogold studies. A, Light microscopy analysis of a transverse section of the root. Contrast was obtained with 0.1% Toluidine blue. Bar = 100 μm. B, Transmission electron microscopy analysis of the epidermis, exodermis, sclerenchyma, and cortex. Bar = 5 μm. C, Transmission electron microscopy analysis of the central cylinder. Bar = 5 μm. Epi, Epidermis; Exo, exodermis; Scl, sclerenchyma; Cp, cortex parenchyma; Pc, pericycle; End, endodermis; X, xylem; Ph, phloem; Vp, vascular parenchyma.

containing some microbodies (Fig. 2C) in the transverse section.

Quantification of Immunogold Labeling for the NADH-GOGAT Protein in Subcellular Organelles

Immunolabeling with the single-affinity-purified anti-NADH-GOGAT IgG appeared to be specific for plastids, with gold label apparent on plastids in cells of the epidermis and exodermis (Fig. 3, A and B), cortical parenchyma (Fig. 3C), and vascular parenchyma (Fig. 3D). With the exception of the mitochondria (see below), background labeling of other tissues or other cellular compartments such as the nucleus, cytosol, vacuole, and cell wall was extremely low or negligible. The control sections, which were labeled with anti-NADH-GOGAT IgG that had been preabsorbed with excess amounts of purified NADH-GOGAT, showed very little immunogold labeling (Fig. 3E). Quantification of the labeling on these sections resulted in a mean immunolabeling density of plastids in the three tissue types (the epidermis and exodermis, the cortex, and the central cylinder) that was approximately 10^1- to 10^2-fold higher than that of the cell wall, cytosol, vacuole, and air space (Table I). Although labeling on the mitochondria was lower than that of the plastids, it was somewhat higher than that of the other subcellular compartments. The values of mean immunolabeling density of plastids in the epidermis and exodermis, cortical parenchyma, and vascular parenchyma were almost equal: 155.7, 158.0, and 141.2 gold particles μm^{-2}, respectively.

Several different morphological forms of plastids were observed. Some of the plastids had well-defined starch grains (Fig. 4B), whereas others contained either globular, membranous regions (Fig. 4D) or a single, internal lamella (Fig. 4C). Another group had no discernible internal organization or starch grains (Fig. 4A). Some cells in the epidermis and exodermis contained large numbers of plastids that were all heavily stained with gold particles (Fig. 3B), whereas cortical parenchyma cells contained fewer numbers of plastids (Fig. 3C). Although there are insufficient data to allow for statistically valid comparisons to be made, these observations indicate that there are different populations of plastids within the root tip (<10 mm) of the rice plants supplied with 1 mM NH_4^+ for 24 h. Peat and Tobin (1996) also reported the presence of different populations of plastids in barley roots, and these occurred in a range of different cell types. They also suggested that accumulation of plastidial GS protein differed between different forms of plastids. Our preliminary observations are that all plastids were labeled with the NADH-GOGAT antibody but that some regions of the epidermis and exodermis contained higher numbers of plastids.

To determine whether the labeling on the mitochondria was due to the presence of NADH-GOGAT protein in this organelle or to some artifact of the technique, we prepared a further purification of the IgG by affinity purifying it twice against pure NADH-GOGAT protein. Using this antibody we could not detect any immunolabeling of the mitochondria, although the immunolabeling of plastids was slightly decreased (Fig. 5, A and B, are representative micrographs of twice-affinity-purified anti-NADH-GOGAT-labeled sections). We conclude from these results that NADH-GOGAT protein is specifically located in the plastids of rice roots.

DISCUSSION

Even though molecular and physiological properties of NADH-GOGAT have been studied recently in alfalfa nodules (Anderson et al., 1989; Gregerson et al., 1993; Vance et al., 1995) and rice plants (Hayakawa et al., 1992, 1993, 1994; Yamaya et al., 1992, 1995; Hirose et al., 1997; Ishiyama et al., 1998), the inter- and intracellular localization of NADH-GOGAT is still not fully understood. The current paper is

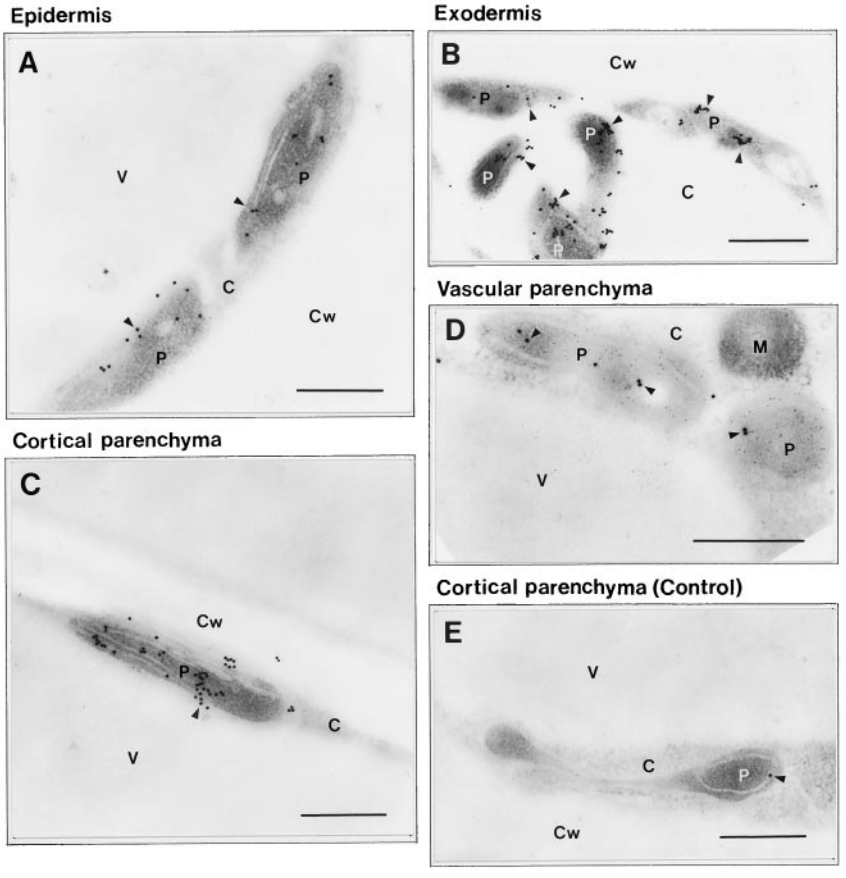

Figure 3. Immunogold labeling of NADH-GOGAT protein in rice roots with a single-affinity-purified anti-NADH-GOGAT IgG. Root tissue was from a seedling grown with 1 mM NH_4^+ for 24 h and sampled from the tip (<10 mm). A and B, Epidermis and exodermis cells. C, Cortical parenchyma cells. D, Vascular parenchyma cells incubated with single-affinity-purified anti-NADH-GOGAT IgG as the primary antibody. Note the high immunolabeling of the plastid. E, Cortical parenchyma cells from the control section incubated with anti-NADH-GOGAT IgG pretreated with an excess amount of NADH-GOGAT protein. Note the very low amount of gold label. P, Plastid; C, cytosol; Cw, cell wall; M, mitochondrion; V, vacuole. Arrowheads indicate gold label. Bars = 0.5 μm.

the first, to our knowledge, in which the intracellular localization of NADH-GOGAT in plant roots is described and it provides an estimation of the quantity of NADH-GOGAT protein among the different root cell types. Our results indicated that the immunogold-labeling density with the single-affinity-purified anti-NADH-GOGAT IgG was extremely high in the plastids of the cells of the epidermis and exodermis, the cortical parenchyma, and the vascular parenchyma in the root tip (<10 mm) of rice supplied with 1 mM NH_4^+ for 24 h. In contrast, except for the mitochondria (Table I), the labeling density was very low in other cellular compartments (Figs. 3 and 4). Labeling with a twice-affinity-purified anti-NADH-GOGAT IgG subsequently confirmed the plastidial location of the protein and eliminated the mitochondrion as a location (Fig. 5). Given that there is no other evidence of a mitochondrial location for this enzyme and that a highly purified IgG failed to detect any labeling on this organelle, the results strongly

Table I. *Quantification of immunogold labeling of rice roots with the single-affinity-purified anti-NADH-GOGAT IgG*

Rice seedlings were grown for 24 h with 1 mM NH_4^+. Six to nine micrographs of each of the three tissue types (epidermis and exodermis, cortex, and central cylinder) were taken from sections of root tip (<10 mm) incubated with single-affinity-purified anti-NADH-GOGAT IgG as the primary antibody, and analyzed as described in "Materials and Methods." Values represent the mean ± SD immunolabeling density. Numbers in parentheses are *n* values.

Cellular Compartment	Root Tissue Type		
	Epidermis/Exodermis	Cortex	Central Cylinder
	gold particles μm^{-2}		
Plastid	155.7 ± 60.21 (13)	158.0 ± 96.92 (9)	141.2 ± 37.47 (13)
Mitochondrion	108.8 (2)	N.D.[a]	49.94 ± 12.99 (12)
Cell wall	23.94 ± 10.28 (4)	12.54 ± 3.350 (5)	12.90 ± 6.569 (6)
Cytosol	9.301 ± 4.810 (4)	16.16 ± 4.801 (6)	12.76 ± 5.395 (10)
Vacuole	0.7990 ± 0.5049 (4)	0.3562 ± 0.0767 (4)	1.260 ± 0.6878 (6)
Air space	N.D.	0.3148 ± 0.4100 (4)	10.34 (2)

[a] N.D., Not detected.

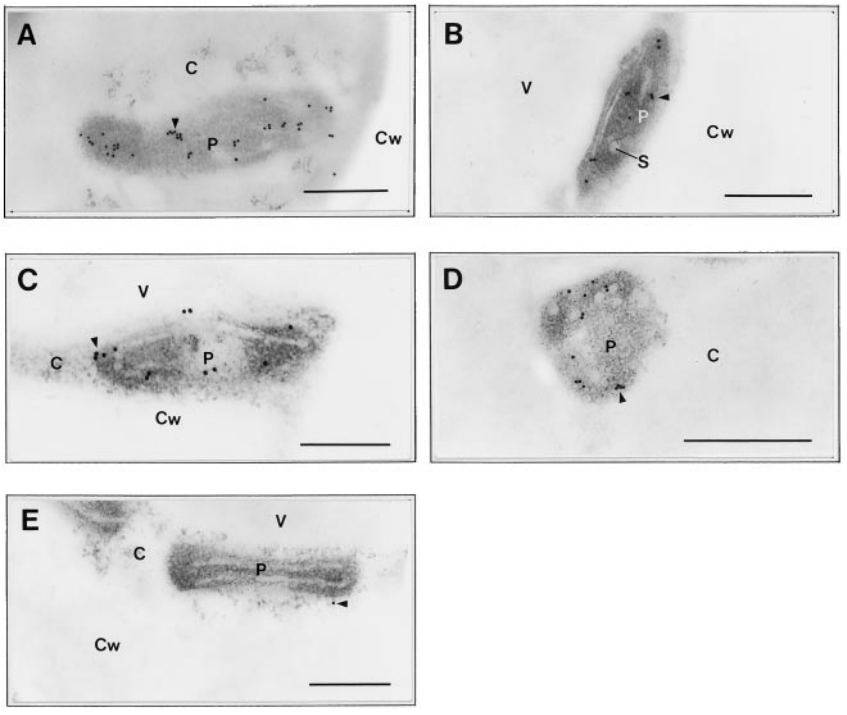

Figure 4. Immunolabeling of NADH-GOGAT protein in different populations of plastids in rice roots. Sections were labeled with single-affinity-purified anti-NADH-GOGAT IgG using tissue from a seedling grown with 1 mM NH_4^+ for 24 h and sampled from the tip (<10 mm). A and B, Epidermis; C through E, vascular parenchyma. Note the different ultrastructures of the five representative plastids shown: no defined internal structures (A), a well-defined starch grain and internal lamellae (B), a single internal lamella (C), globular, membranous structures (D), and well-developed internal lamellae (E). Sections shown in A through D were incubated with single-affinity-purified anti-NADH-GOGAT IgG as the primary antibody. The section shown in E (the control) was incubated with anti-NADH-GOGAT IgG pretreated with an excess amount of NADH-GOGAT protein. P, Plastid; C, cytosol; Cw, cell wall; S, starch grain; V, vacuole. Arrowheads indicate gold label. Bars = 0.5 μm.

suggest that NADH-GOGAT is specifically localized in the plastids of rice roots.

We recently obtained the full-length cDNA clone for rice root NADH-GOGAT (Goto et al., 1998; accession no. AB008845) and the genomic clone for the rice NADH-GOGAT gene (Goto et al., 1998; accession no. AB001916). The analyses of the deduced amino acid sequence of this cDNA and those of the N-terminal amino acid sequence of the purified NADH-GOGAT mature protein (Hayakawa et al., 1992) show that the translational product from the rice root NADH-GOGAT gene has a 99-amino acid presequence at the N-terminal region. Computer analysis for the presequence of rice root NADH-GOGAT using the PSORT program (National Institute for Basic Biology, Okazaki, Japan; Nakai and Kanehisa, 1992), which is used for predicting protein localization sites in eukaryotic cells, predicted the slight possibility that it was targeted to the plastid stroma of plant cells.

The results of our immunogold localization study strongly suggest that this presequence of rice root NADH-GOGAT contains the transit peptide for targeting to plastids. It is worthy to note that there is a short conserved sequence (GLYDP - - - - DS, where the dashes indicate the positions for which no consensus could be determined) at the C-terminal end regions of presequences observed in rice root NADH-GOGAT and alfalfa root nodule NADH-GOGAT (Gregerson et al., 1993). The highly homologous sequence to this short sequence was also observed at the C-terminal end of the presequence for *Escherichia coli* NADPH-GOGAT (Oliver et al., 1987). Because *E. coli* NADPH-GOGAT is not transported into any organelles, these conserved short regions of presequences may be important for the processing of the pre-GOGAT form to the mature protein.

Previous studies in our laboratory showed that NADH-GOGAT mRNA, protein, and activity in whole roots of rice seedlings accumulated within 12 h of the start of treatment with a low concentration of NH_4^+ (Yamaya et al., 1995; Hirose et al., 1997). This accumulation of NADH-GOGAT protein specifically occurred in the two outer cell layers of the root, the epidermis and exodermis, in the region within 10 mm from the tip in response to the supply of NH_4^+. In addition, we showed that cytosolic GS protein is also located in the epidermis and exodermis of rice roots (Ishiyama et al., 1998). Tatsumi (1982) reported that the absorption of NH_4^+ ions by rice roots occurs in both the root-tip area and the area where the secondary roots are actively developing. Morita et al. (1996) showed that there is a Casparian strip between the exodermis and cortex in rice roots, indicating that solute transport should be a symplastic process between these cell types.

Based on these results, we propose that NADH-GOGAT in the epidermis and exodermis has the function of providing the Glu required for the cytosolic GS reaction to assimilate most of the NH_4^+ ions supplied exogenously in these two cell compartments (Ishiyama et al., 1998). The present study has shown that NADH-GOGAT protein is specifically localized in the plastids of the cells of the epidermis and exodermis of root tips (Fig. 3, A and B). In addition, some portions of these cells contained many plastids (Fig. 3B); therefore, assimilation of NH_4^+ ions would mainly be catalyzed by cytosolic GS and plastidial NADH-GOGAT in these cells. Such spatial separation of two enzymes of the GS/GOGAT cycle was also suggested to occur in root nodules of French bean (Chen and Cullimore, 1989). The translocator that actively counterexchanges Gln and Glu has been demonstrated with oat chloroplasts and could be involved in the reassimilation of NH_4^+ generated

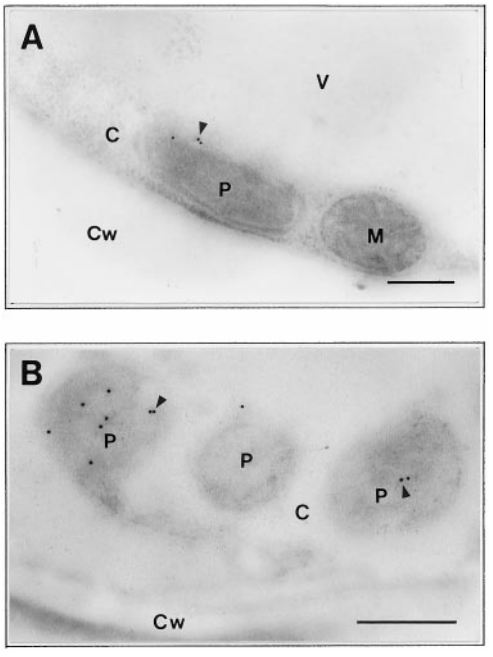

Figure 5. Immunolabeling of NADH-GOGAT protein in rice roots with double-affinity-purified anti-NADH-GOGAT IgG. Root tissue was from a seedling grown with 1 mM NH_4^+ for 24 h and sampled from the tip (<10 mm). A and B, Sections incubated with anti-NADH-GOGAT IgG, which was affinity purified twice against NADH-GOGAT protein, as the primary antibody. P, Plastid; C, cytosol; Cw, cell wall; M, mitochondrion; V, vacuole. Arrowheads indicate gold label. Bars = 0.5 μm. Note the immunolabeling in plastids, the very low immunolabeling in the cytosol, and the lack of immunolabeling in the vacuole, cell wall, and mitochondrion.

from photorespiration in leaves (Yu and Woo, 1988). We presume that a similar translocator is present in plastid membranes in the cells of the epidermis and exodermis in rice roots.

NADH-GOGAT protein was also located in the plastids of the cortical parenchyma and vascular parenchyma cells in the root tips (Fig. 3, C and D), although relatively low numbers of plastids were observed in these cells. The cortical cells of rice roots appear highly vacuolate, with only very thin regions of cytoplasm within the periphery of the cell. This is in contrast to the cortex region in barley roots, where we previously found the highest concentration of GS protein, indicating that this is the main region of NH_4^+ assimilation in this species (Peat and Tobin, 1996). In rice the area of the central cylinder could be essential for the transport of solutes from the phloem to the actively developing cells and is probably also an adaptation to growth under anaerobic conditions. Solute transport also occurs from the root surface to the xylem vessel elements. The major forms of nitrogen in both xylem sap and phloem sap of rice are Gln and Asn (Fukumorita and Chino, 1982; Hayashi and Chino, 1990). Cytosolic GS protein was detected in the central cylinder in rice (Ishiyama et al., 1998). Because the vascular parenchyma in the central cylinder is one of the most important tissues with respect to the metabolism of transported solutes, NADH-GOGAT in these cells is probably involved in the utilization of Gln transported from the shoots.

The values of mean immunolabeling density of plastids in the epidermis and exodermis, cortical parenchyma, and vascular parenchyma were almost equal (Table I), indicating that the concentration of this protein is the same in all plastids, irrespective of form or location. Because changes in both the amount of NADH-GOGAT protein and its activity in rice roots in response to NH_4^+ were almost parallel (Yamaya et al., 1995), there would appear to be no inactive form of this enzyme under these conditions. The results suggest that the mean capacities per plastid for providing Glu by the NADH-GOGAT reaction are almost equal among these different cell types. However, on a cellular basis, the epidermis and exodermis cells have been found to contain a much higher concentration of NADH-GOGAT protein than the other cells of the root (Ishiyama et al., 1998). This is accounted for by our observation that these cells also contain a higher concentration of plastids. The structure and compartmentation of root cells, as well as their protein composition, is an important factor in the control of NH_4^+ assimilation in rice.

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