Enhancement of Glucose Utilization in Provision of Carbon Skeletons for Ammonium Assimilation in Wheat Roots

Koga, Nobuhisa
Department of Agricultural Chemistry, Division of Agriculture, Kyushu University Graduate School

Ikeda, Motoki
Laboratory of Plant Nutrition, Division of Soil Science and Plant Production, Department of Plant Resources, Faculty of Agriculture, Kyushu University

古賀, 伸久
九州大学大学院農学研究院

https://doi.org/10.5109/24351

出版情報：九州大学大学院農学研究院紀要. 45 (1), pp.7-13, 2000-11. 九州大学農学部
バージョン：
権利関係：

J. Fac. Agr., Kyushu Univ., **45** (1), 7–13 (2000)

Enhancement of Glucose Utilization in Provision of Carbon Skeletons for Ammonium Assimilation in Wheat Roots

Nobuhisa Koga* and Motoki Ikeda**

Laboratory of Plant Nutrition, Division of Soil Science and Plant Production,
Department of Plant Resources, Faculty of Agriculture,
Kyushu University, Fukuoka 812–8581, Japan
(*Received April 13, 2000 and accepted August 18, 2000*)

In providing carbon skeletons to be expended for amide synthesis during ammonium assimilation, glucose utilization in roots was studied. The roots of young wheat plants grown without nitrogen for 3 d and grown with 4 mM NO_3^- or NH_4^+ for 1 d were fed with ^{14}C–glucose for 3 h in the presence of NO_3^- or NH_4^+, and the distribution of ^{14}C–metabolites within the plants was examined. The NH_4^+ supply changed the distribution of ^{14}C to a greater extent than the NO_3^- supply. In roots grown with NH_4^+ for 1 d, the incorporation of ^{14}C into 80% ethanol–soluble materials in roots increased, and the ratio of ^{14}C in basic metabolites to ^{14}C in acidic metabolites in the fraction was high. The concentration and ^{14}C–labeling of citrate and malate in roots were reduced by the prolonged supply of NH_4^+. In contrast, the asparagine concentration conspicuously increased, and asparagine was heavily labeled in roots of NH_4^+–grown plants. When roots were treated with methionine sulfoximine, however, ^{14}C–labeling of basic metabolites did not increase despite the supply of NH_4^+. These results indicate that the supply of NH_4^+ has a stimulatory effect on the degradation of glucose in wheat roots to provide carbon skeletons necessary for amide synthesis during NH_4^+ assimilation.

INTRODUCTION

Ammonium is one of inorganic nitrogen nutrients available for plants. When large amounts of NH_4^+ are supplied, however, plants show depressed growth due to the cytotoxicity of ammonium (Engels and Marschner 1995). In order for plants to keep the intracellular concentration of NH_4^+ low, NH_4^+ taken up by roots has to be rapidly assimilated into amide compounds such as glutamine and asparagine within roots (Sechley *et al.* 1992; Oaks 1994). The synthesis of glutamine and asparagine requires the supply of 2–oxoglutarate and oxaloacetate, respectively. Thus, continuous provision of these organic acids is required when NH_4^+ assimilates are accumulated by roots and/or exported to shoots during NH_4^+ nutrition. In this process, a sizable portion of the products by dark carbon fixation is utilized as carbon skeletons of the amide compounds (Sechley *et al.* 1992; Huppe and Turpin 1994). In fact, it was already reported that dark carbon fixation in roots was enhanced by the supply of NH_4^+, and its products were utilized for the synthesis of NH_4^+ assimilates (Ikeda *et al.* 1992; Cramer *et al.* 1993; Koga and Ikeda 2000). In higher plants, phospho*enol*pyruvate (PEP) is one of substrates for the reaction

* Department of Agricultural Chemistry, Division of Agriculture, Kyushu University Graduate School
Present address: Upland Agriculture Research Center, Hokkaido National Agricultural Experiment Station, Memuro, Hokkaido, 082–0071 Japan
** Corresponding author (E-mail: ikedam@agr.kyushu-u.ac.jp)

of dark carbon fixation (Huppe and Turpin 1994). The accelerated production of PEP is therefore essential to support the enhanced dark carbon fixation for NH_4^+ assimilation in roots. PEP is provided by the glycolytic degradation of sugars translocated from shoots (Huppe and Turpin 1994). Here, we investigated glucose metabolism within roots when NO_3^- or NH_4^+ was supplied as the nitrogen source, from a viewpoint of replenishment of carbon skeletons expended for NH_4^+ assimilation.

MATERIALS AND METHODS

Plant growth

Seeds of wheat (*Triticum aestivum* L. cv. Saitama 27) were germinated on wet filter paper. The plants were cultivated on washed sand for several days and then grown by hydroponic culture with continuous aeration in a phytotron (20 °C, 70% relative humidity) of Biotron Institute Kyushu University. One-eighth strength modified Hoagland solution (pH6) containing 2 mM $NaNO_3$ was supplied as a nutrient solution and renewed every other day (Koga and Ikeda 1997).

Growth with NO_3^- or NH_4^+ and MSX treatment

Three-week-old plants were supplied with nitrogen-free one-eighth strength nutrient solution for 3 d. To prepare plants grown with NO_3^- or NH_4^+ for 1 d, one-quarter strength nutrient solution containing either 4 mM $NaNO_3$ or 2 mM $(NH_4)_2SO_4$ was supplied for 1 d, following the nitrogen-free treatment. In plants treated with methionine sulfoximine (MSX), MSX was supplemented to the nitrogen-free nutrient solution at a final concentration of 100 μM for 3 h prior to the supply of NH_4^+ (Oaks *et al.* 1998).

^{14}C-glucose feeding

The plants grown without nitrogen for 3 d and those with NO_3^- or NH_4^+ for 1 d were acclimated to darkness for 1 h at 20 °C in a growth chamber. Subsequently, the plants were placed in a wide-mouth bottle containing 100 mL of ^{14}C-glucose solution and supported with a rubber stopper sealed with moist flour wad. The ^{14}C-glucose solution was one-quarter strength nutrient solution containing 0.1 mM ^{14}C-glucose (14.8 MBq $mmol^{-1}$, D-[U-^{14}C] glucose, Amersham Life Science, UK) and 4 mM $NaNO_3$ or 2 mM $(NH_4)_2SO_4$. A small vial was hung in the bottle. A filter paper segment moistened with 10% (w/v) NaOH was placed in the vial to recover $^{14}CO_2$ released from roots. The plants were incubated for 3 h with shaking (100 rpm) at 20 °C in a dark growth chamber. The feeding experiment was carried out in duplicate. Following ^{14}C-glucose feeding, the roots were rinsed three times with deionized water. The plants were separated into shoots and roots. These parts were chopped, weighed and kept frozen at -70 °C until extraction.

Extraction, ion-exchange fractionation and radioactivity measurements

Shoots were hydrolyzed with 6 N HCl for 6 h at 80 °C, and the radioactivity of the hydrolyzates was determined. Roots were extracted three times with 80% (v/v) ethanol at 80 °C. The radioactivity of the extracts was measured as 80% ethanol-soluble materials. The extracts were further fractionated using Dowex 50 W (H^+ form) and Dowex 1 × 8 (CH_3COO^- form) columns connected in series. Neutral metabolites were eluted from

the columns with deionized water. After the columns were disconnected, basic and acidic metabolites were eluted from Dowex 50 W and Dowex 1×8 columns with 2 M NH_4OH and 8 M HCOOH, respectively. The radioactivity of the eluates was measured. The root residues were dried at 50 °C overnight and immersed in a scintillation cocktail to measure the radioactivity in 80% ethanol–insoluble materials. The filter paper segments that absorbed $^{14}CO_2$ were dried at 50 °C for 6 h, and the radioactivity in each segment was measured in a scintillation cocktail. Radioactivity was determined with a liquid scintillation counter (LSC–500 model, Aloka, Japan).

Quantification and radioactivity measurements of individual organic acids and amino acids

Major organic acids in acidic fractions were separated using HPLC (PV980 model pump, Jasco Corporation, Japan) with a Shim–pack SCR–102 H column (Shimadzu, Japan), detected at 210 nm and collected into counting vials for measurements of ^{14}C. Major amino acids in basic fractions were quantitated as described by Yamaya and Matsumoto (1988). For analysis of ^{14}C–amino acids, the sample was separated on a silica gel plate (Silica gel 60, 20×20 cm, Merck, Germany). The plate was developed with phenol–water (4:1 [v/v]) solvent in the first dimension and done with 1–butanol–acetic acid–water (4:1:1 [v/v/v]) solvent in the second dimension. The spots corresponding to individual amino acids were visualized by spraying an *o*–phthalaldehyde solution. The solution contained 80 mg of *o*–phthalaldehyde, 1 mL of ethanol and 0.2 mL of 2–mercaptoethanol in 100 mL of 0.4 M sodium borate buffer (pH 9). Silica gel on each spot was scraped and collected into a counting vial by suction. The radioactivity was determined with the liquid scintillation counter.

RESULTS

The distribution of ^{14}C in released $^{14}CO_2$ and three fractions of wheat plants grown with NO_3^- or NH_4^+ was shown in Fig. 1. The growth period with NO_3^- hardly affected the distribution of ^{14}C whereas the growth period with NH_4^+ obviously altered it. In plants grown with NH_4^+ for 1 d, the partitioning of ^{14}C to 80% ethanol–soluble materials in roots was larger, as compared to the plants grown without nitrogen for 3 d. In roots where NH_4^+ assimilation was inhibited by MSX, the incorporation of ^{14}C into the soluble materials did not increase although a considerable amount of NH_4^+ was found in the roots. Neither NH_4^+ supply nor NO_3^- supply affected $^{14}CO_2$ release from the roots.

The ratio of basic ^{14}C–metabolites to acidic ^{14}C–metabolites in roots grown with NO_3^- and NH_4^+ for 1 d was 2.13 and 3.32, respectively and was higher than that in roots grown with no nitrogen for 3 d (Table 1). In particular, ^{14}C–glucose was sizably converted to basic ^{14}C–metabolites in roots grown with NH_4^+ for 1 d. In roots treated with MSX prior to the NH_4^+ supply, the incorporation of ^{14}C into basic metabolites was reduced, in contrast to the roots grown in NO_3^- and NH_4^+ media without MSX. The supply of NO_3^- for a longer period of time moderately lowered the concentrations of citrate and malate in roots, while the prolonged supply of NH_4^+ markedly decreased the concentrations of these organic acids (Table 2). Similar to the concentrations of organic acids, the radioactivity in citrate and malate produced from ^{14}C–glucose was obviously decreased by the prolonged supply

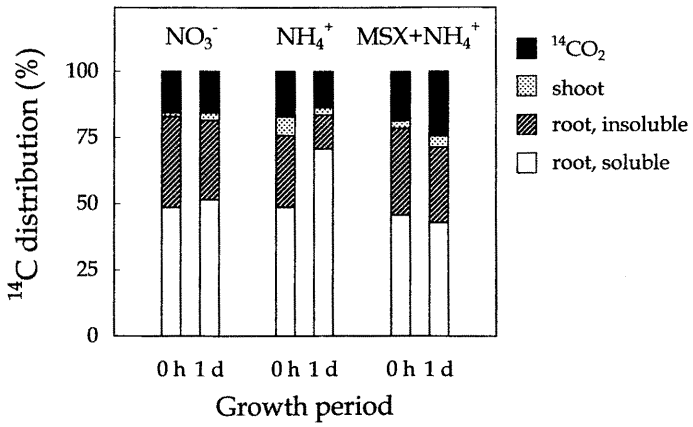

Fig. 1. Effects of nitrogen sources and methionine sulfoximine on the metabolism of ^{14}C–glucose in the roots of wheat plants. Before ^{14}C–glucose feeding, some plants did not receive nitrogen (0 h), and other plants were grown with NO_3^- or NH_4^+ for 1 d (1 d). MSX was supplemented for 3 h prior to the supply of NH_4^+. ^{14}C–glucose was fed to all plants for 3 h in the presence of NO_3^- or NH_4^+. The data are means of two independent plants.

Table 1. Effects of nitrogen sources and methionine sulfoximine on the distribution of ^{14}C from ^{14}C–glucose in 80% ethanol–soluble materials in wheat roots.

Nitrogen treatment	Growth period	Neutral	Acidic (A)	Basic (B)	(B)/(A)
		% of 80% ethanol–soluble materials			
NO_3^-	0 h	36.0	32.9	31.1	0.95
	1 d	32.0	21.7	46.3	2.13
NH_4^+	0 h	19.6	36.6	43.8	1.20
	1 d	25.3	17.3	57.4	3.32
MSX+NH_4^+	0 h	24.4	53.7	21.9	0.41
	1 d	34.9	37.5	27.6	0.74

Wheat plants received no nitrogen (0 h) and those grown with NO_3^- or NH_4^+ for 1 d (1 d) were used in the experiment. ^{14}C–glucose was fed to all plants for 3 h in the presence of NO_3^- or NH_4^+. MSX was supplemented for 3 h prior to the NH_4^+ supply. The data are means of two independent plants.

of NH_4^+. When wheat plants treated with MSX was grown with NH_4^+, the roots contained citrate and malate at higher levels than the roots grown in NO_3^- and NH_4^+ media without MSX. A considerable amount of succinate was contained in the roots, but its radioactivity was much lower than that of citrate and malate independent of the nitrogen source. The concentration of fumarate was negligible, and its radioactivity was undetectable.

Table 2. Effects of nitrogen sources and methionine sulfoximine on the concentrations and radioactivity of major organic acids in roots of wheat plants.

Nitrogen treatment	Growth period	Concentration				Radioactivity			
		Citrate	Malate	Succinate	Fumarate	Citrate	Malate	Succinate	Fumarate
		μmol g^{-1} FW				Bq g^{-1} FW			
NO_3^-	0 h	4.4	13.8	5.5	0.07	156	387	44	N.D.
	1 d	3.2	6.2	6.2	0.05	80	127	17	N.D.
NH_4^+	0 h	4.6	9.5	4.8	0.07	116	199	28	N.D.
	1 d	1.5	1.7	2.6	0.02	33	126	14	N.D.
MSX+NH_4^+	0 h	5.2	18.6	6.8	0.07	220	598	65	N.D.
	1 d	5.1	19.0	9.2	0.10	120	430	43	N.D.

N.D. : not detected

Table 3. Effects of nitrogen sources and methionine sulfoximine on the concentrations and radioactivity of major amino acids in roots of wheat plants.

Nitrogen treatment	Growth period	Concentration				Radioactivity				
		Asp	Glu	Asn	Gln	Asp	Glu	Asn	Gln	Ala
		μmol g^{-1} FW				Bq g^{-1} FW				
NO_3^-	0 h	0.20	0.26	0.52	0.06	96	250	47	50	112
	1 d	0.27	0.33	1.11	0.12	277	505	277	72	233
NH_4^+	0 h	0.19	0.23	0.50	0.26	75	149	59	389	261
	1 d	0.33	0.57	5.66	0.66	171	307	2582	231	323

The concentrations of aspartate, glutamate, asparagine and glutamine in roots increased when NO_3^- or NH_4^+ was supplied for 1 d (Table 3). Above all, a remarkably high concentration of asparagine was contained in wheat roots grown with NH_4^+ for 1 d. When NO_3^- was supplied, glutamate was more labeled with ^{14}C than glutamine and asparagine. In contrast, the radioactivity in asparagine conspicuously increased in the roots when NH_4^+ was supplied for 1 d. The ^{14}C-labeling of asparagine in roots grown with NH_4^+ for 1 d was approximately 44-fold greater than that in roots grown in nitrogen-free media. The radioactivity in glutamine was decreased by the prolonged supply of NH_4^+. The labeling of alanine was heavier when the plants were grown with NH_4^+ than when grown with NO_3^- for 1 d.

DISCUSSION

It is considered that carbon skeletons are necessary for imperative assimilation of NH_4^+ in roots when the roots take up large amounts of NH_4^+. In this study, we investigated glucose utilization in wheat roots assimilating NH_4^+ because it was considered that

provision of carbon skeletons was ultimately dependent upon carbohydrates translocating from shoots.

In case of NO_3^-–grown plants, the growth period did not affect the distribution of ^{14}C in the plants (Fig. 1). In roots of NH_4^+–grown plants, on the other hand, the incorporation of ^{14}C into 80% ethanol–soluble materials, especially into basic metabolites, increased to a greater extent than the roots of NO_3^-–grown plants (Fig. 1 and Table 1). This shows that the assimilation of NO_3^-, if any, is not so great as the assimilation of NH_4^+ in wheat roots. However, effects of NH_4^+ on the ^{14}C distribution disappeared in roots where the primary assimilation of NH_4^+ was inhibited by the action of MSX. In response to the NH_4^+ suply, therefore, a greater portion of carbon originating from glucose is utilized for the synthesis of basic metabolites for NH_4^+ assimilation in roots.

Asparagine, one of NH_4^+ assimilates in plants, considerably accumulated and was strongly labeled with ^{14}C from ^{14}C–glucose in wheat roots grown with NH_4^+ for 1 d (Table 3). Glutamine, a predominant form for storage and export of nitrogenous compounds in many kinds of plants (Lea 1993), did not accumulate and was not labeled as much as asparagine in the roots. In case of wheat roots, more carbon skeletons originating from glucose were utilized for asparagine biosynthesis. In addition, no clear difference in the release of $^{14}CO_2$ from roots was observed (Fig. 1), suggesting that the products in glucose degradation were converted to aspartate and subsequently asparagine rather than were oxidized through the tricarboxylic acid cycle in wheat roots in the course of NH_4^+ assimilation.

The ^{14}C–labeling of asparagine was much greater in roots grown with NH_4^+ for 1 d than roots grown in nitrogen–free media and supplied with NH_4^+ for 3 h (Table 3). Similarly, the rate of dark carbon fixation in wheat roots was markedly increased by the NH_4^+ supply for more than 1 d (Koga and Ikeda 2000). These findings indicate that glycolytic degradation of glucose is closely associated with the dark carbon fixation necessary for amide synthesis in roots receiving NH_4^+. In other words, the increased supply of PEP produced from glucose is concomitant with provision of carbon skeletons for amide synthesis by enhanced dark carbon fixation although both phenomena occur gradually in response to the NH_4^+ supply. In a green alga *Selenastrum minutum* during nitrogen assimilation, the degradation of hexoses in glycolytic pathway appeared active according to an increased *in vivo* phosphofructokinase activity, one of rate–limiting enzymes in glycolysis, in combination with decreases in the concentrations of its inhibitory intermediates such as PEP and 3–phosphoglycerate (Botha and Turpin 1990; Huppe and Turpin 1994). Also, the activity of PEP carboxylase increased transcriptionally and post–translationally to stimulate the dark carbon fixation in roots in response to NH_4^+ (Koga and Ikeda 1997). Presumably, increases in these enzyme activities responsive to NH_4^+ have an important role in accelerating carbon flow from hexoses to amides to assimilate NH_4^+ in roots. Therefore, the supply of NH_4^+ is likely to stimulate the glycolytic process of hexoses to produce PEP and subsequent carboxylation of PEP to provide precursors for amide synthesis in roots. It is considered that provision of sufficient carbon skeletons for NH_4^+ assimilation in roots necessitates the cooperation of glucose degradation and dark carbon fixation enhanced by the supply of NH_4^+.

ACKNOWLEDGMENTS

This work was partly supported by a Grants-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan (no.0966067) to M. I.

REFERENCES

Botha, F. C. and D. H. Turpin 1990 Molecular, kinetic, and immunological properties of 6-phosphofructokinase from the green alga *Selenastrum minutum*. Activation during biosynthetic carbon flow. *Plant Physiol.*, **93**: 871–879

Cramer, M. D., O. A. M. Lewis and S. H. Lips 1993 Inorganic carbon fixation and metabolism in maize roots as affected by nitrate and ammonium nutrition. *Physiol. Plant.*, **89**: 632–639

Engels, C. and H. Marschner 1995 Plant uptake and utilization of nitrogen. *In* "Nitrogen Fertilization in the Environment", ed. by P. E. Bacon, Marcel Dekker, New York, pp. 41–81

Huppe, H. C. and D. H. Turpin 1994 Integration of carbon and nitrogen metabolism in plant and algal cells. *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, **45**: 577–607

Ikeda, M., K. Mizoguchi and T. Yamakawa 1992 Stimulation of dark carbon fixation in rice and tomato roots by application of ammonium nitrogen. *Soil Sci. Plant Nutr.*, **38**: 315–322

Koga, N. and M. Ikeda 1997 Responses to nitrogen sources and regulatory properties of root phospho*enol*pyruvate carboxylase. *Soil Sci. Plant Nutr.*, **43**: 643–650

Koga, N. and M. Ikeda 2000 Methionine sulfoximine suppressed the stimulation of dark carbon fixation by NH_4^+ nutrition in wheat roots. *Soil Sci. Plant Nutr.*, **46**: 393–400

Lea, P. J. 1993 Nitrogen metabolism. *In* "Plant Biochemistry and Molecular Biology", ed. by P. J. Lea and R. C. Leegood, John Wiley & Sons Ltd., London, pp. 155–180

Oaks, A. 1994 Primary nitrogen assimilation in higher plants and its regulation. *Can. J. Bot.*, **72**: 739–750

Oaks, A., S. Sivasankar and V. J. Goodfellow 1998 The specificity of methionine sulfoximine and azaserine inhibition in plant tissues. *Phytochemistry*, **49**: 355–357

Sechley, K. A., T. Yamaya and A. Oaks 1992 Compartmentation of nitrogen assimilation in higher plants. *Int. Rev. Cytol.*, **134**: 85–163

Yamaya, T. and H. Matsumoto 1988 Analysis of phenylthiocarbamyl-amino acids at pico-mole level by high performance liquid chromatography and application of plant materials. *Soil Sci. Plant Nutr.*, **34**: 297–302