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Effect of Glucose on the Induction of Nitrate Reductase in Corn Roots[1]

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MUHAMMAD ASLAM AND ANN OAKS[2]
Department of Biology, McMaster University, Hamilton, Ontario, Canada L8S 4K1

ABSTRACT

In *Zea mays* L., addition of glucose to the induction medium has no effect on the induction of nitrate reductase during the initial 3 hours either in root tips (0–10 mm) or mature root sections (25–35 mm). With longer times, higher levels of enzyme activity are recovered from both root segments when glucose is present in the incubation medium. The induction in root tips is saturated by 10 mM NO_3^-. Higher concentrations of NO_3^- are required for saturation in mature root sections. The response to glucose is seen over a wide range of external NO_3^- concentrations.

Nitrate reductase activity is lost rapidly when nitrate is withdrawn from the induction medium. Additions of glucose do not prevent this loss. Additions of glucose have no effect on total uptake of NO_3^- by the root segments but they increase the anaerobic NO_2^- production in both root tips and mature root segments. This latter measurement is considered to be an estimate of an active NO_3^- pool in the cytoplasm. Thus the results show that glucose alters the distribution of NO_3^- within the root sections. This may be an important factor in controlling the *in vivo* stability of the enzyme or its rate of synthesis.

In cereals, carbohydrates represent up to 80% of the materials transported to the embryo from the endosperm (5, 17). These sugars are a major source of energy and building blocks for biosynthetic reactions. The endosperm is also a rich source of amino acids which may be incorporated directly into embryo protein (8, 17, 19). These transported amino acids also inhibit the biosynthesis of certain amino acids in the growing regions of the embryo (15, 16), and thus could limit the contribution of transported sugars to biosynthetic reactions. Little is known about regulation of specific metabolic pathways which might be modulated by soluble carbohydrates. In corn root tips, increased incorporation of acetate-carbon into protein amino acids is seen with additions of glucose or sucrose (20). This effect is not seen in mature root sections (18). In order to understand the effect of glucose on protein synthesis more precisely, we decided to investigate its influence on a particular enzyme system, nitrate reductase.

MATERIALS AND METHODS

Growth and Induction Conditions. Seeds of *Zea mays* L. (var. W64A × 182E, Warwick Seed Company of Blenheim, Ontario) were washed two times with distilled H_2O to remove excess fungicide. They were then planted on NO_3^--free 0.9% agar which contained one-tenth strength Hoagland solution and an additional supplement of 0.08 μM molybdenum (22, 26). The seedlings were grown for 64 hr at 26 C in the dark before transfer to the induction medium. The induction medium contained the appropriate concentration of NO_3^- in one-tenth strength, Mo-supplemented Hoagland solution. The seedlings were supported with a plastic matting in 250 ml of medium which was aerated throughout the experimental time (25). At appropriate time intervals, the root tips (0- to 10-mm sections) and mature root sections (25–35 mm from the root tip) were harvested, frozen immediately in liquid nitrogen, and stored at −20 C until extraction. The enzyme in root tip sections was stable and hence these sections were stored overnight at −20 C before extraction and assay. Mature root sections were assayed immediately after the liquid nitrogen treatment since there was some loss of nitrate reductase activity with overnight storage at −20 C.

Fifty root sections were used per treatment. When glucose was used in the induction medium, it was added to the solution 2 hr before the addition of NO_3^-. For turnover studies, the enzyme was induced for 2.5 hr in the appropriate medium before transfer to NO_3^--free solutions.

Preparation of Cell-free Extracts and Assay of Nitrate Reductase. The root sections were weighed and then homogenized with mortar and pestle in 4 volumes of 0.1 M HEPES buffer (pH 7.4), containing 0.5 mM EDTA and 5 mM cysteine. The extracts were centrifuged at 30,000*g* for 15 min. Inclusion of BSA or casein in the extraction medium did not improve the recovery of nitrate reductase activity.

Nitrate reductase was assayed by following the conversion of NO_3^- to NO_2^- according to the method of Hewitt and Nicholas (11). The assay mixture contained 16 μmoles of potassium phosphate buffer (pH 7), 0.564 μmoles of NADH, 20 μmoles of KNO_3, 130 μmoles of HEPES buffer (pH 7), and extract in a final volume of 1.5 ml (25, 26). The reaction temperature was 28 C. The reaction was stopped by the addition of sufficient amounts of alcohol dehydrogenase (50 μg) and acetaldehyde (2 mg) to completely oxidize remaining NADH in 2 to 3 min. Nitrite was determined by adding 1 ml of 1.0% (w/v) sulfanilamide in N HCl and 1 ml of 0.2% (w/v) N-(1-naphthyl)ethylenediamine dihydrochloride. The absorbance was read at 540 nm after 15 min. Nitrate reductase activity was calculated as nmoles of NO_2^- formed/mg of protein·hr.

***In Vivo* Assay of Nitrate Reductase.** The *in vivo* assay method described by Ferrari and Varner (6) was used. The assay mixture contained 0.1 M potassium phosphate buffer (pH 7.5), 0.1 M KNO_3, and 5% (v/v) 1-propanol in a total volume of 1.7 ml. Thirty root sections were used per treatment and were incubated at 28 C under anaerobic conditions. The NO_2^- production was linear from 15 to 90 min.

Nitrate and Protein Determinations. Total NO_3^- was measured in the extracts after enzymic reduction to NO_2^-. NO_2^- values

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[2] To whom reprint requests should be sent.

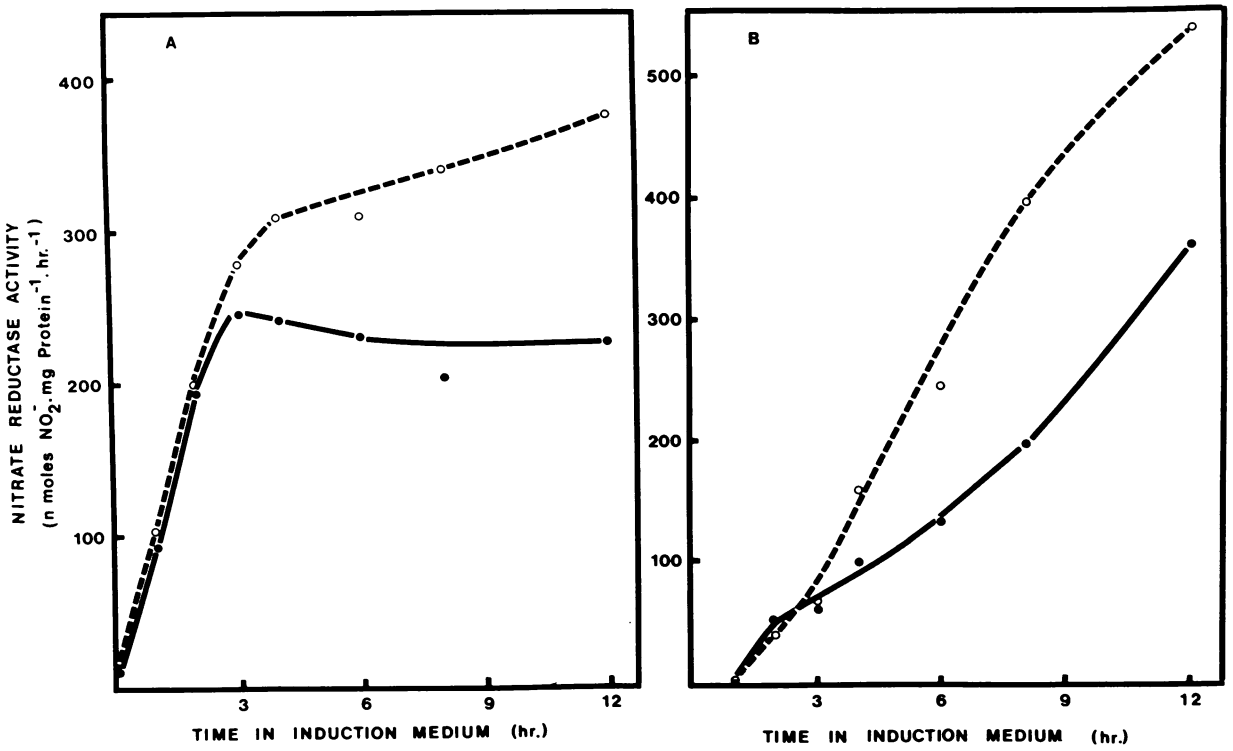

FIG. 1. Kinetics of nitrate reductase induction in the presence and absence of 0.1 M glucose. Seedlings were grown on minus-nitrate agar for 64 hr at 26 C and then transferred to solutions containing 0 or 0.1 M glucose in one-tenth strength Hoagland solution lacking NO_3^-. After 2 hr, 5 mM KNO_3 was added to start the induction. At the designated times, the 10-mm root tips (A) and 25- to 35-mm mature root sections (B) were harvested and frozen immediately in liquid nitrogen. Minus glucose (●); plus glucose (○).

were calculated from a standard curve prepared on the day of the assay. The source of the nitrate reductase was a partially purified preparation of the enzyme from 7-day-old corn leaves.

The method described by Ferrari *et al.* (7) was used to measure the metabolic NO_3^- pool. Thirty root segments were used for this determination. After induction of the enzyme for appropriate time intervals, the roots were washed with distilled H_2O, excised, and placed in test tubes which contained 1.7 ml of 0.1 M potassium phosphate buffer (pH 7.5). The contents of the tubes were then stoppered and incubated at 28 C for the required time. The production of NO_2^- was stopped by adding 1 ml each of sulfanilamide and N-(1-naphthyl)ethylenediamine dihydrochloride. The color development was measured as described previously. The amount of NO_2^- produced under anaerobiosis was designated as the metabolic NO_3^- pool (7).

Soluble protein was determined by the method of Lowry *et al.* (14) after precipitation with 10% trichloroacetic acid. BSA, fraction V, was used as the protein standard.

RESULTS

Effect of Glucose on Induction of Nitrate Reductase. The results in Figure 1A show that in root tips the nitrate reductase activity increased during the first 3 hr of NO_3^- treatment and then tended to level off. The initial increase in nitrate reductase activity is not influenced when glucose is included in the induction medium. With glucose in the induction medium, the activity continued to increase at the initial rate for about 4 hr and no clear-cut plateau was achieved during the 12-hr experimental time. In mature root sections, very little nitrate reductase activity was detected during the first hour of induction (Fig. 1B). After this time the activity increased over a 12-hr experimental time. The rate of increase was appreciably faster in the presence of glucose than in the control after the initial 3-hr period (54 units/hr as compared with 33 units/hr in the control). Glucose also enhanced the nitrate reductase activity measured with the *in vivo* assay after an induction period of 6 hr (Table I). Higgins *et al.* (12) and Radin (23) have also observed an enhancement of nitrate reductase activity by sucrose in mung bean roots and by glucose in cotton roots, respectively.

Table I. *Comparison of in Vivo and in Vitro Nitrate Reductase Activity in Corn Roots*

Roots were grown and induced as described in Figure 1. The enzyme activities were assayed after 6 hr of induction.

Treatments	Root Tips		Mature Root Sections	
	In vivo	*In vitro*	*In vivo*	*In vitro*
	nmoles NO_2^-/hr·mg protein			
−Glucose	30	155	147	127
+Glucose	61	294	252	195

In our experiments, the maximum induction in root tips was obtained with 5 mM glucose in the induction medium. Higher concentrations (0.1 M) were needed to get the maximum induction in mature root sections (Fig. 2).

When the roots were treated with various concentrations of NO_3^- for a period of 6 hr, the roots (tip and mature sections) had higher levels of nitrate reductase activity when glucose was present (Fig. 3, A and B). The effect of glucose was more pronounced in root tip than in mature root sections.

Effect of Glucose on Turnover of Nitrate Reductase. Nitrate reductase activity is lost rapidly when NO_3^- is withdrawn from the induction medium (22). To investigate whether glucose would affect the decay of enzyme activity, the roots were induced for 2.5 hr with 5 mM NO_3^- with and without 0.1 M glucose. The roots

were then transferred to treatment solutions lacking NO_3^-. There was a rapid loss of nitrate reductase activity in both root tip and mature root sections (Fig. 4, A and B). Glucose had little influence in preventing the loss in root tip sections but appeared to have a protective effect in mature root sections. When the synthesis of the active enzyme was stopped by the inclusion of tungstate in the treatment solutions (10) the loss of enzyme activity was even more pronounced. In this case, glucose did not prevent the normal loss of nitrate reductase activity in the mature root sections.

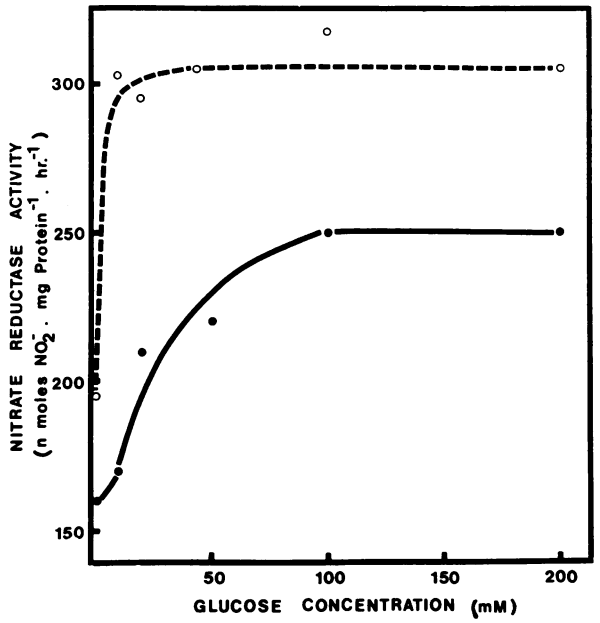

FIG. 2. Effect of glucose concentration on nitrate reductase induction. Seedlings were grown and induced as in Figure except that the induction solutions contained the required concentration of glucose. The enzymic activities were assayed after 6 hr of induction. Root tips (○); mature root sections (●).

Effect of Glucose on Anaerobic NO_2^- Production. From the foregoing experiments it is apparent that glucose does not necessarily have a direct effect on the rate of synthesis or the rate of turnover of nitrate reductase in maize roots. It could alter the availability of NO_3^- within the cell. Heimer and Filner (9) presented evidence for more than one NO_3^- pool in suspension cultures of tobacco cells and suggested that one pool, the metabolic pool, was responsible for the induction of nitrate reductase. More recently Ferrari *et al.* (7) developed a method for measuring the metabolic pool of NO_3^-. We have used this method to compare levels of total and metabolic pools of NO_3^- in corn root sections. In our system, the roots were aollwed to absorb NO_3^- for 6 hr. They were then harvested and the anaerobic NO_2^- production was followed as described in the methods. Nitrite production increased with time in both root tip and mature root sections for 60 min (Fig. 5, A and B). At this time the total NO_2^- produced by the root tip sections was 77 nmoles/g and by the mature root sections was 66 nmoles/g. When more NO_3^- was added at 60 min, NO_2^- production resumed in the root tip, but not in the mature root sections. The total accumulation of NO_2^- in 60 min in the glucose-fed root tip sections was 132 nmoles/g and in the mature root sections, 86 nmoles/g. In each case, glucose treatments enhanced the metabolic NO_3^- pool. As with the enzyme induction (Figs. 1 and 3), the glucose effect was more pronounced in the root tip sections.

Kinetics of NO_3^- Accumulation. The results in Tables II and III show that total NO_3^- accumulation in root tip and mature root sections was the same in glucose-treated and control root sections. The metabolic NO_3^- pool, on the other hand, was enhanced by glucose additions. Increasing levels of exogenous NO_3^- result in higher levels of the total and metabolic NO_3^- (Table II). In a kinetic study, the glucose effect on the metabolic NO_3^- pool is clearly apparent 2 hr after the addition of NO_3^- to root tip sections (Table III). The development of the metabolic NO_3^- pool

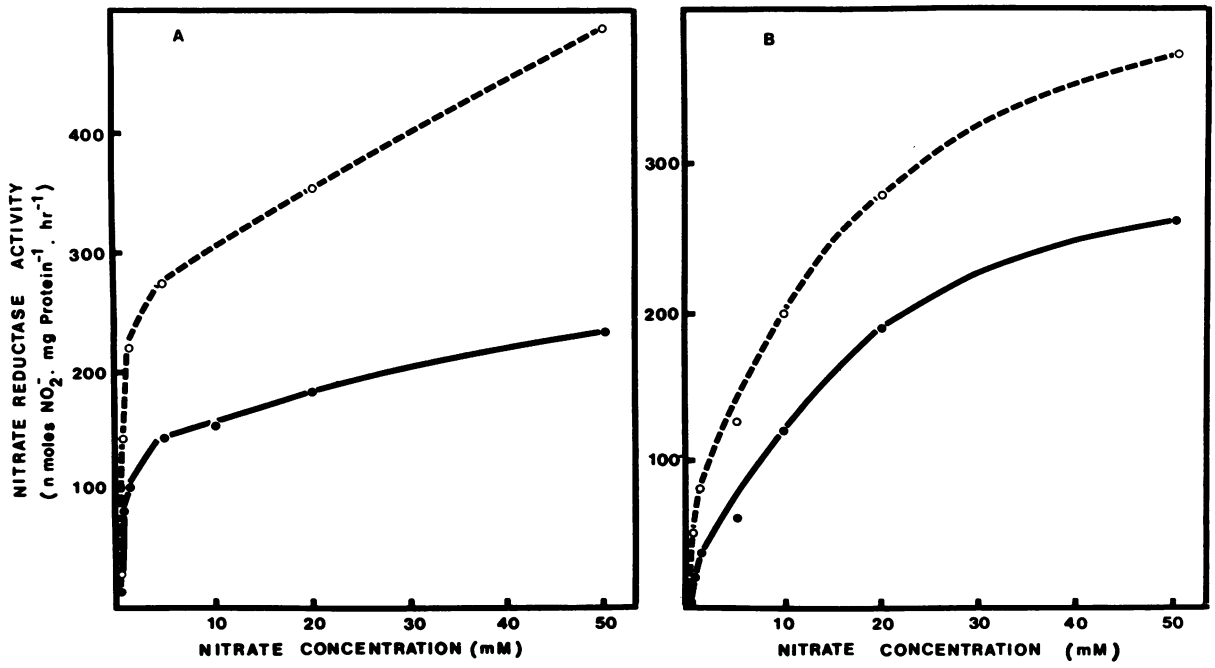

FIG. 3. Effect of nitrate concentration on the induction of nitrate reductase in the presence (○) and absence (●) of 0.1 M glucose. Seedlings were grown and induced as described in Figure 1. The enzymic activities were assayed after 6 hr of induction. A: Root tips; B: Mature root sections.

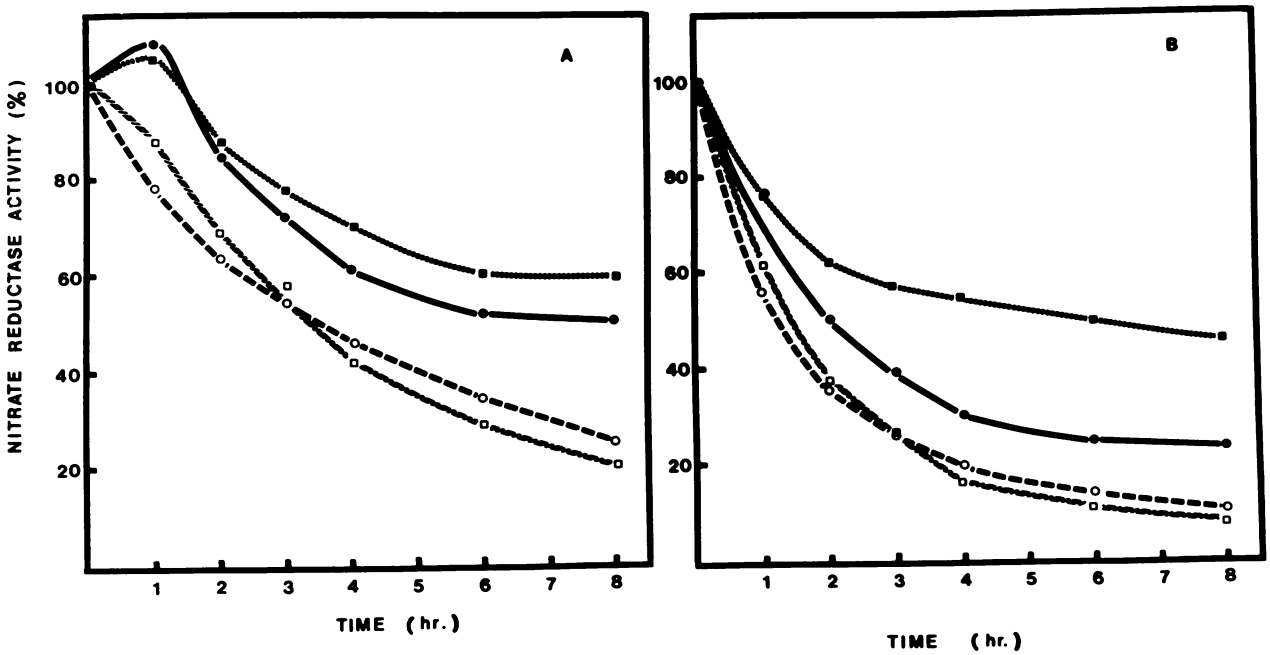

FIG. 4. Decay kinetics of nitrate reductase in the presence and absence of 0.1 M glucose. Seedlings were grown and induced as described in Figure 1. After 2.5 hr of induction, the seedlings were transferred to the treatment solutions lacking nitrate. The loss of enzyme activity was followed over a period of 8 hr. For the root tips (A), 100% activity is 230 nmoles of NO_2^- formed (mg protein·hr)$^{-1}$; for the mature root sections (B), it is 95 nmoles. Minus glucose (□, ●); plus 0.1 M glucose (■, ○); with the open symbols 100 μM tungstate was included in the medium.

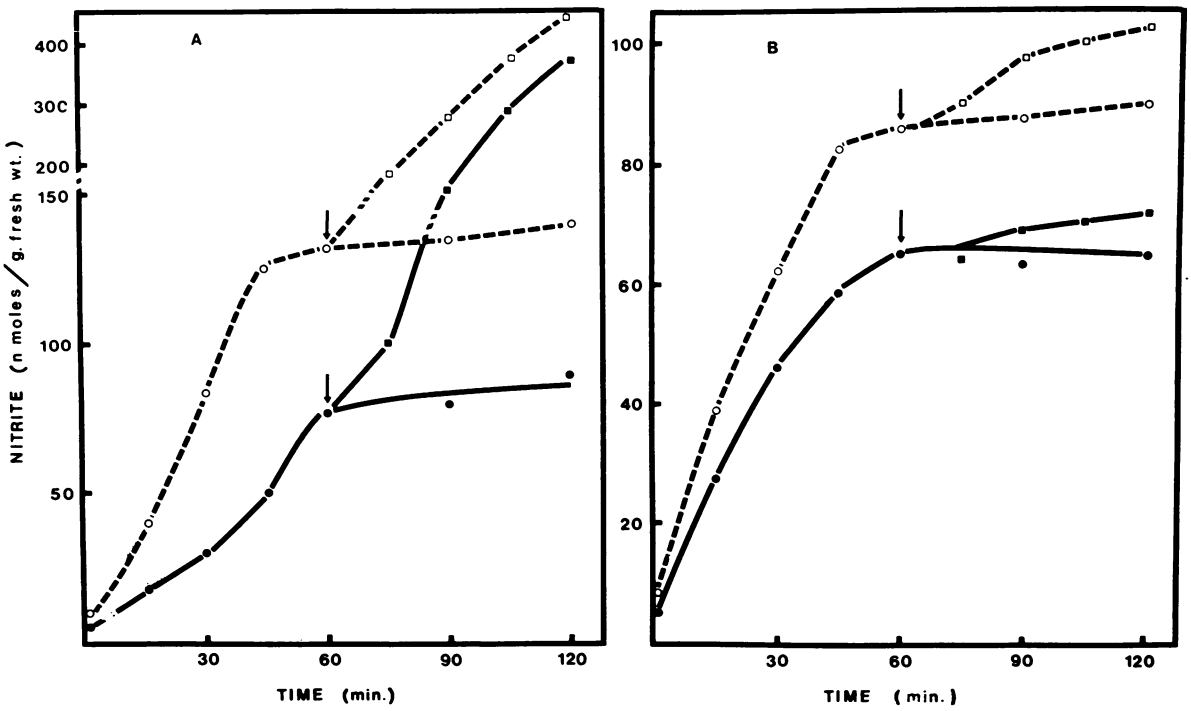

FIG. 5. Effect of glucose on anaerobic nitrite production in corn roots. Seedlings were grown and induced for 6 hr as in Figure 1. The roots were washed with distilled H_2O, excised, and anaerobic nitrite production was measured. A: Root tip sections; B: mature root sections; ●: minus glucose; ○: plus 0.1 M glucose. At 60 min, 0.1 M KNO_3 was added (■, □).

was somewhat more sluggish in the mature root sections. When the results in Table III and Figure 1 are compared it is apparent that the enlargement of the metabolic pool precedes the detection of the enhanced nitrate reductase activity in both root tip and mature root sections.

DISCUSSION

It is true that glucose could have many direct or indirect effects on protein synthesis in general and on the appearance of nitrate reductase in particular. For example, it could be a source of re-

Table II. *Effect of Glucose and Different Concentrations of Nitrate on Nitrate Accumulation in Corn Roots*

Seedlings were grown and induced as described in Figure 1. Figure 3 has the corresponding nitrate reductase activities.

	KNO_3	Root Tips		Mature Root Sections	
		−Glucose	+Glucose	−Glucose	+Glucose
	mM	*μmoles/g fresh wt*			
Total NO_3^-					
	0.5	9.1	8.1	4.5	3.5
	1	10.9	11.7	7.6	8.5
	5	16.6	14.6	10.4	9
	10	18	18.9	11.7	10.5
	20	21.6	20.7	16.4	16
	50	34.5	38	24.5	22
		nmoles/g fresh wt			
Metabolic NO_3^-					
	0.5	24	50	14	23
	1	37	75	23	41
	5	65	133	39	59
	10	93	165	47	95
	20	105	190	67	112
	50	125	265	79	122

Table III. *Effect of Glucose on Kinetics of Nitrate Accumulation in Corn Roots*

Roots were grown and induced as described in Figure 1.

	Time	Root Tips		Mature Root Sections	
		−Glucose	+Glucose	−Glucose	+Glucose
	hr	*μmoles/g fresh wt*			
Total NO_3^-					
	1	6	6.8	3.1	3.6
	2	9.9	9.5	5.4	6
	3	16.6	17.1	7.7	8
	4	18	18	9.9	9.9
	6	20.2	21	15.7	16.6
	8	23	24	19.8	22
	12	24.4	25.2	26.5	25
		nmoles/g fresh wt			
Metabolic NO_3^-					
	1	21	21	9	9
	2	74	106	23	27
	3	80	160	45	56
	4	76	160	54	72
	6	74	148	72	94
	8	76	150	79	118

ducing power for the *in vivo* activity of nitrate reductase; it could supply carbon skeletons for the synthesis of protein; and it could supply energy or alter the energy charge in such a way that cell compartments or biosynthetic reactions are affected. Since the induction of nitrate reductase by NO_3^- involves the *de novo* synthesis of protein (13, 22, 24, 31), there must be a requirement for energy and amino acids. In light-grown leaves, these requirements are met by photosynthesis (1–3, 28, 29). In etiolated or carbohydrate-deficient green leaves, an exogenous supply of energy-generating compounds such as sucrose or glucose are required to obtain a maximum induction in darkness (1, 30). Travis and co-workers (28, 30) have shown that light or glucose is responsible for maintaining polyribosomes. Their results suggest that by increasing the protein synthesis machinery, light or glucose have enhanced the synthesis of nitrate reductase and presumably of other proteins. They show that energy is an important factor in the induction process.

During early seedling growth there is a rich supply of soluble carbohydrates and amino acids from the endosperm to support protein synthesis in the embryo. In fact, the rate of respiration in excised root tip sections is not altered by glucose additions (20). This observation suggests that respiratory substrates are not grossly deficient. The rate of protein synthesis is faster in the root meristem than in more mature regions of the root (4, 21). The rate of increase of a specific protein, nitrate reductase, is also faster in root tip than in mature root sections (Fig. 1). Even in this richly endowed system, additions of glucose enhance the incorporation of acetate-carbon into root tip protein (20). In this case glucose is not supplying additional carbon since it does not dilute out the contribution of [^{14}C] from acetate. It could be influencing the energy available for biosynthetic processes and hence protein synthesis. Alternatively, an altered energy balance could result in a more efficient channeling of acetate-carbon into protein.

Nitrate reductase is induced in corn roots by the addition of NO_3^- and removal of NO_3^- from the system results in an immediate loss of nitrate reductase activity (22). The present results show that additions of glucose have no effect on the initial rates of enzyme induction. After 3 to 4 hr, higher enzymic activities are observed and higher steady state levels of enzyme are achieved in root tips (Fig. 1). When synthesis of the active enzyme complex was stopped by additions of tungstate (Fig. 4), glucose did not alter the loss of enzyme activity. The results suggest that glucose does not affect the initial induction or the inactivation of the enzyme, but in some way alters the steady state level achieved.

Levels of NO_3^- itself may influence the stability of nitrate reductase. Subramanian and Sorger (27) have shown, for example, with cycloheximide-treated *Neurospora crassa*, that loss of nitrate reductase activity is reduced when NO_3^- is present in the media. We have observed that NO_3^- additions reduce the loss of nitrate reductase in tungstate-treated corn roots. Thus in corn roots also, nitrate appears to protect the enzyme from degradation *in vivo*.

Heimer and Filner (9) and Ferrari *et al.* (7) have presented results that suggest that an active or metabolic NO_3^- pool is responsive to external NO_3^- and is responsible for the induction of the enzyme. Additions of glucose to corn roots do not influence the total NO_3^- accumulation in the tissue but result in enhanced accumulations of "active NO_3^-." The increases in active NO_3^- are not observed during the initial induction process but precede the appearance of glucose-enhanced nitrate reductase activity. Our conjecture is that because of this larger metabolic pool, glucose alters either the rate of synthesis of nitrate reductase or the *in vivo* stability of the enzyme.

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