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Sugar-Starvation-Induced Changes of Carbon Metabolism in Excised Maize Root Tips

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Excised maize (*Zea mays* L.) root tips were used to study the early metabolic effects of glucose (Glc) starvation. Root tips were prelabeled with [1-^{13}C]Glc so that carbohydrates and metabolic intermediates were close to steady-state labeling, but lipids and proteins were scarcely labeled. They were then incubated in a sugar-deprived medium for carbon starvation. Changes in the level of soluble sugars, the respiratory quotient, and the ^{13}C enrichment of intermediates, as measured by ^{13}C and ^{1}H nuclear magnetic resonance, were studied to detect changes in carbon fluxes through glycolysis and the tricarboxylic acid cycle. Labeling of glutamate carbons revealed two major changes in carbon input into the tricarboxylic acid cycle: (a) the phospho*enol*pyruvate carboxylase flux stopped early after the start of Glc starvation, and (b) the contribution of glycolysis as the source of acetyl-coenzyme A for respiration decreased progressively, indicating an increasing contribution of the catabolism of protein amino acids, fatty acids, or both. The enrichment of glutamate carbons gave no evidence for proteolysis in the early steps of starvation, indicating that the catabolism of proteins was delayed compared with that of fatty acids. Labeling of carbohydrates showed that sucrose turnover continues during sugar starvation, but gave no indication for any significant flux through gluconeogenesis.

In the last few years, various works have established that the supply of carbohydrates to nongreen plant cells is variable and controls a number of the cell's activities, including gene expression and basal metabolism (for review, see Koch, 1996). Similar effects of sugar starvation on metabolism have been observed in different plant materials, such as maize (*Zea mays* L.) root tips, asparagus spears, *Acer* spp., or cucumber cells, and in dark-senescing leaves. For example, the respiration rate declines because of a decrease in the demand for ATP rather than a limitation by the substrate (Brouquisse et al., 1991); the respiratory quotient declines from 1 to 0.75 (Saglio et al., 1980; Brouquisse et al., 1991); and total proteins and lipids decrease, whereas Asn accumulates (Genix et al., 1990; Brouquisse et al., 1992, and refs. therein), indicating that proteins and lipids replace carbohydrates as respiratory substrates.

Various enzymatic activities change in a coordinated way. Those linked with carbohydrate metabolism decrease, whereas proteolytic activities (James et al., 1993) and both the peroxisomal (De Bellis et al., 1990; Dieuaide et al., 1992) and mitochondrial (Dieuaide et al., 1993) β-oxidation activities increase. Salvage pathways are also induced, at least transiently, by sugar starvation. For example, increases in the enzyme activity or transcript level of Asn synthetase have been observed in all plant systems examined so far (Brouquisse et al., 1992; Davies and King, 1993; Chevalier et al., 1996a), with the function of this salvage pathway being to retain amino nitrogen lost after amino acid degradation. The activities of or transcripts coding for malate synthase and isocitrate lyase, the two enzymes necessary for the glyoxylate cycle, have been found to be increased in a number of sugar-starved plant tissues (Kudielka and Theimer, 1983; Graham et al., 1994). This pathway is anaplerotic, i.e. it converts acetyl units to the four carbon organic acid succinate, thus allowing net biosyntheses from intermediates of the TCAC. In some cases, such as in senescing leaves, it appears to be linked with gluconeogenesis (Gut and Matile, 1988), but in detached leaves or in protoplasts, its function may be to provide four carbon precursors for amino acid synthesis (Graham et al., 1994; Kim and Smith 1994). In maize root tips only malate synthase was detected after starvation, and it was suggested that the role of this enzyme might be limited to the salvage of glyoxylate resulting from Gly catabolism (Dieuaide et al., 1992).

Although the changes induced upon sugar starvation ultimately lead to cell disorganization and death, they appear to be essential for survival when the sugar supply stops (Brouquisse et al., 1992). In maize root tips major changes at both the metabolic (respiration, metabolite levels) and gene expression (Chevalier et al., 1995, 1996b) levels occur within the first hours after transfer to starvation conditions, long before carbohydrates are exhausted. In sink tissues or cells, gene expression has been shown to be under metabolic control either by carbohydrates themselves (Roitsch et al., 1995) or by hexose phosphates (Graham et al., 1994). As previously suggested, Pi, amino acids, ammonium, nucleotides (Brouquisse et al., 1992), and other metabolites that have levels that change during starvation, such as those linked with mitochondrial respiration (Aubert et al., 1996), are potential secondary signals for the

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Abbreviation: TCAC, tricarboxylic acid cycle.

induction of different pathways during starvation. In mammals, the three-carbon glycolytic intermediates and the nongluconeogenic amino acid Leu are involved in the regulation of the synthesis and secretion of insulin (German, 1993). It is therefore essential to correlate changes in gene expression with those in metabolism to understand how the acclimation response to carbon starvation unfolds.

The present work attempts to identify changes in intermediary metabolism that occur in the first stages of sugar starvation. It is based on a recent study of intermediary metabolism in Glc-fed excised maize root tips (Dieuaide-Noubhani et al., 1995), which allowed the identification and quantification of various metabolic fluxes, including those through PEP carboxylase, the pentose phosphate pathway, and a number of futile cycles. In our work, the excised root tips were prelabeled with [1-^{13}C]Glc and submitted to sugar starvation, and then labeling of intermediates was determined. The results indicate that a number of changes occur, including a cessation of the PEP carboxylase flux and a progressive replacement of sugars by lipids and/or proteins as the source of acetyl-CoA entering the TCAC. Two metabolically distinct steps were distinguished in the early phase of starvation, with lipolysis starting before proteolysis.

MATERIALS AND METHODS

Chemicals

Analytical-grade mineral salts were purchased from Merck (Darmstadt, Germany), [1-^{14}C]Glc (1.856 GBq/mmol) was obtained from Dositech (Paris, France), and [1-^{13}C]Glc (99% enrichment) came from Commissariat à l'Energie Atomique (Gif-sur-Yvette, France). Protease from *Streptomyces griseus* (pronase) was from Sigma.

Plant Materials and Treatments

Maize (*Zea mays* L. cv DEA; Pioneer France, Maïs, France) seeds were germinated for 3 d in the dark at 25°C as described by Brouquisse et al. (1991). The 3- mm tips of primary roots were excised and treated in three steps differing by Glc supply. The first two steps of prestarvation and Glc feeding were essentially as described by Dieuaide-Noubhani et al. (1995). Briefly, prestarvation was for 4 h in a medium containing various minerals but no sugars (see Brouquisse et al., 1991) called medium A. Glc refeeding was by transfer to the same medium supplemented with either labeled or unlabeled 200 mM Glc for different periods of time. The third step of treatment was starvation for up to 20 h by incubation in medium A. All liquid media (approximately 50 μL/tip) were bubbled with a N_2/O_2 mixture (50:50, v/v), at a temperature of 25°C. At the end of step II (incubation in the presence of Glc), before either analysis or further treatment, the root tips were extensively washed with water to eliminate extracellular Glc, and were trimmed to 3 mm when further growth had occurred.

Respiratory quotients were measured in 15-mL Warburg vials as described by Brouquisse et al. (1991), with 30 maize root tips per sample, using the direct method for CO_2 (Umbreit et al., 1964).

Analysis of Metabolites

The extraction of soluble components was performed using boiling aqueous solutions of ethanol as described previously (Salon et al., 1988). Each extract was evaporated to dryness (Speed-Vac, Savant Instruments, Farmingdale, NY), dissolved in 1 or 2 mL of water, and used for the preparation of lipids and water-soluble compounds. Lipids were extracted and saponified, and the fatty acids were extracted as described (Dawson, 1974). The amino acid and sugar fractions were separated by ion-exchange chromatography (Salon et al., 1988). HPLC analysis of sugars was as described (Dieuaide-Noubhani et al., 1995). Purified Glu and the noncarboxylic amino acid fraction, which contains Ala, were prepared from the amino acid fraction as described (Dieuaide et al., 1995), and used for the NMR analysis (see below). The residue of ethanolic extraction was used for the extraction of amino acids incorporated in proteins (referred to as protein amino acids below), according to the work of Dickson (1979). It was incubated with 3.2 units of pronase for 24 h at 30°C in 50 mM Tris, pH 7.4 (1 mL/30 tips). The radioactivity of the supernatant, corresponding to hydrolyzed proteins, was determined by scintillation counting.

NMR Spectroscopy

For NMR determinations of enrichments, 1500 prestarved maize root tips (about 3 g) were incubated in 75 mL of medium A supplemented with 200 mM [1-^{13}C]Glc for 4 h. They were divided into three 1-g samples; one sample was washed immediately with abundant ice-cold water to eliminate exogenous Glc and frozen in liquid N_2; the other two were washed with water at 25°C and incubated in medium A (deprived of Glc) for 6 and 16 h. Metabolites were extracted as described above, and the three fractions containing total soluble sugars, the noncarboxylic amino acids, and purified Glu were evaporated to dryness under vacuum (Speed-Vac) and dissolved in 350 μL of ^2H_2O. ^{13}C NMR spectra and ^{1}H NMR spectra were obtained at 100.6 and 400 MHz, respectively, with a spectrometer (AM 400, Bruker, Wissembourg, France) as described previously (Dieuaide-Noubhani et al., 1995).

The absolute ^{13}C enrichments of Glu carbons C-2, C-3, and C-4, of Ala C-3, of α and β Glc C-1, and of Suc glucosyl C-1 were determined from the ^{1}H NMR spectra as the ratio of the area of the satellites (^{13}C-^{1}H coupling) to the total area of the multiplet. These determinations were in agreement with the relative enrichments determined from the ^{13}C spectra by comparing the peak areas with those of the same resonances from nonenriched Glu, the latter being equally labeled at all carbons by the 1.1% natural abundance. The relative enrichment of Glu carbon C-2 in Figure 5D was determined from the peak area in the ^{13}C spectrum of Glu and from the absolute enrichment of the carbons C-3 and C-4. The same method was used to determine the enrichments of the Suc and Glc carbons C-2 to C-6 from the

absolute enrichment of their respective C-1 (see Dieuaide-Noubhani et al., 1995).

RESULTS

Preloading with 200 mM [1-^{13}C]Glc for 4 h Strongly Labels Metabolic Intermediates but Scarcely Labels Cellular Lipids and Proteins

The labeling of metabolic intermediates was studied during a starvation treatment in maize root tips that had been labeled by feeding with [1-^{13}C]Glc after a prestarvation period of 4 h, as previously described (Dieuaide-Noubhani et al., 1995). To detect changes in metabolism induced by sugar starvation, it was essential that (a) carbon starvation remain inducible in root tips preincubated with labeled Glc, and (b) sufficient labeling of carbohydrates and metabolic intermediates occur with a low labeling of proteins and/or lipids so that the substitution of carbohydrates by lipids and proteins as respiratory substrates could be detected. A duration of 4 h for the labeling step and of 6 and 16 h for the starvation times was defined from the following experiments, in which the labeling of different classes of metabolites and the level of endogenous carbohydrates were studied (Figs. 1 and 2).

Labeling of Fatty Acids and Proteins

Maize root tips were prestarved for 4 h and then incubated with [1-^{14}C]Glc for up to 10 h. Because the root tips grew, they were trimmed to 3 mm at each sampling time during incubation on Glc. Free amino acids, fatty acids, and protein amino acids were extracted as described in "Materials and Methods" from the 3-mm tips, and incorporated radioactivity was measured (Fig. 1). It was previously known that (a) Suc and Fru reach steady-state labeling within 2 h, and (b) Ala, Glu, and Asp are labeled without any lag phase, whereas a 2-h lag is observed for Asn and Gln (Dieuaide-Noubhani et al., 1995). The labeling of fatty acids and protein amino acids lagged for 4 h but then increased rapidly until 10 h. At that time, the labeling of proteins and lipids would be expected to hamper the detection of a carbon flux from fatty acids and proteins into the TCAC. After 4 h of incubation, however, fatty acids and proteins were weakly labeled, whereas the enrichments of free amino acids and Glc were about 80% of those observed at steady state (Dieuaide-Noubhani et al., 1995). Thus, 4 h was chosen as the incubation time in the presence of Glc.

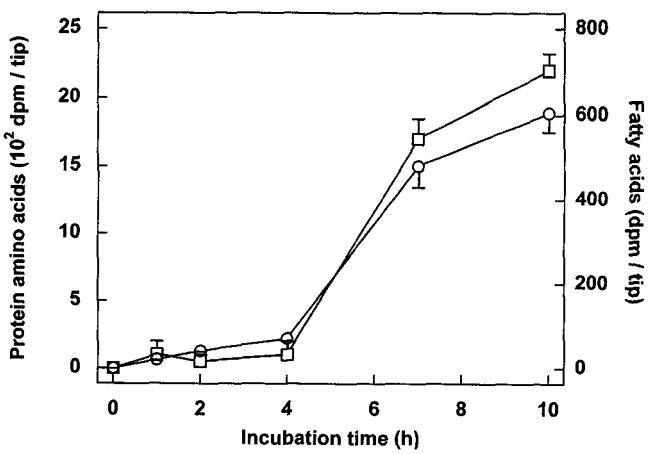

Figure 1. Labeling of protein amino acids (○) and fatty acids (□) during incubation of prestarved maize root tips with [1-^{14}C]Glc. Prestarved maize root tips were incubated with 200 mM [1-^{14}C]Glc (23 dpm nmol^{-1}). Proteins and fatty acids were extracted as described in "Materials and Methods" and their total radioactivity was determined. Data are the means of determinations on four samples from two independent experiments. Error bars show ± SD.

Changes in Soluble Sugars

Figure 2 shows the changes in Glc, Fru, and Suc contents of root tips during the three steps of prestarvation, refeeding with 200 mM Glc, and starvation for up to 20 h. After 4 h of Glc refeeding the Glc level was slightly higher, and the levels of Suc and Fru were about 40% higher than the level at time 0. When maize root tips were Glc starved after refeeding, the level of all three sugars decreased rapidly. After 8 h of starvation Fru was at the detection limit (1 nmol/tip) and Suc remained constant near 5 nmol/tip. Glc decreased more slowly and reached a plateau at 25 nmol/tip, corresponding to 20% of the initial level, after 12 h of starvation. These results indicate that in spite of being slightly overloaded with carbohydrates, the root tips became starved of carbohydrates in a way similar to tips starved immediately after excision (Fig. 2, inset) (Saglio and Pradet, 1980; Brouquisse et al., 1991). The observation that carbohydrates continued to accumulate when phase 2 was prolonged confirms that the decrease in carbohydrates observed in step 3 corresponds to sugar starvation rather than to senescence of the tissues.

Respiratory Quotient

The respiratory quotient was also measured during the starvation treatment (Fig. 3). It was found to remain close to 1 in the first 4 h of incubation in the Glc-deprived medium, and to decrease to 0.7 in the subsequent 4 h. This suggests that lipids and proteins are progressively substituted for carbohydrates as respiratory substrates between 4 and 8 h of Glc starvation. By contrast, the respiratory quotient decreases with no delay when root tips are submitted to Glc starvation immediately after excision (Saglio and Pradet, 1980). The reason for the delay observed here is not clear, but may be overloading with sugars or metabolic slowing induced by the prestarvation treatment.

Carbohydrate Labeling Reveals Continued Suc Turnover and the Absence of Gluconeogenesis in the Early Steps of Glc Starvation

The ^{1}H NMR spectra of the soluble carbohydrate fraction obtained from maize root tips incubated for 4 h with [1-^{13}C]Glc is shown in Figure 4A, and those after starvation

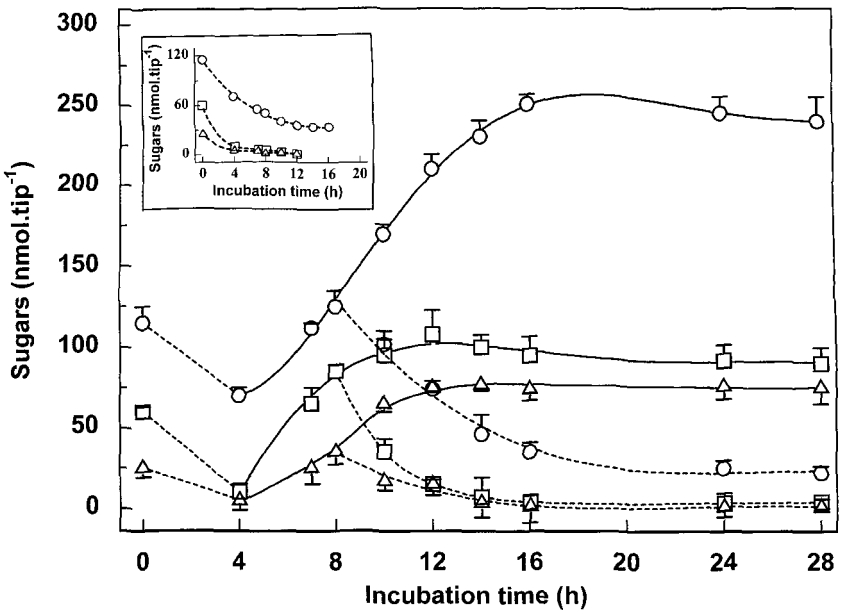

Figure 2. Changes in Glc (○), Fru (△), and Suc (□) in excised maize root tips. Excised maize root tips were successively prestarved for 4 h in mineral medium A, as described in "Materials and Methods," and transferred to the same medium supplemented with 200 mM Glc for 4 h. After 4 h of incubation with Glc, some roots were transferred again to medium A for Glc starvation. Sugars were extracted and measured as described in "Materials and Methods." Inset, Maize root tips were Glc starved immediately after excision. Continuous and dotted lines correspond to treatment with and without Glc, respectively. The data (nanomoles of each carbohydrate) are the mean of determinations on four samples (30 root tips per sample) from two independent experiments. Error bars show ± SD.

are shown in Figure 5, A and B. The changes in enrichments of carbon atoms determined from such spectra are shown in Figure 6A.

After 4 h of labeling the enrichment in Glc C-1 of 85% was similar to that observed at steady state (Dieuaide-Noubhani et al.,1995), but the enrichment in Glc C-6 was only 4.1%. Similar results were observed for the glucosyl moiety of Suc (data not shown). The randomization of C-1 and C-6 of the hexose phosphates results from both the resynthesis of hexose phosphates from triose phosphates (reversibility of glycolysis) and the transaldolase reaction (Dieuaide-Noubhani et al., 1995).

During starvation, the labeling pattern of Glc was modified in that the randomization between C-1 and C-6 increased, and the total enrichment of the molecule decreased (Fig. 6). However, no change occurred at the level of carbons C-2 to C-5, which remained unlabeled (Fig. 5, A and B). Two major features can be deduced from these data. First, the appearance of increased randomization in free Glc indicates that the cycle of synthesis and hydrolysis of Suc continues beyond the first 6 h of starvation; however, whether this rate is as high as in Glc-fed tissues cannot be established. Second, the absence of label in the carbohydrate carbons C-2 to C-5 indicates the absence of gluconeogenesis. This was already suggested from the absence of any detectable isocitrate lyase activity after 24 h of starvation (Dieuaide et al., 1992). The total enrichment of soluble carbohydrates decreased strongly during starvation (Fig. 6); in free Glc the sum of the C-1 and C-6 enrichments decreased from 89 ± 2 to 58 ± 6 (n = 3) between time 0 and 16 h of starvation, respectively. The interpretation of this decrease is not straightforward because it may result from different pathways. A first possibility is the continued operation of the pentose phosphate pathway, although perhaps with a slower rate (see below). Another possibility involves the turnover of cell wall polysaccharides such as β-glucans (Gibeaut and Carpita, 1991), which, like proteins and lipids, may be weakly labeled after 4 h. A third possibility is the resynthesis of hexose phosphates from triose phosphates, which may incorporate unlabeled glycerol phosphate from phospholipids.

The Labeling of Amino Acids Reveals Dramatic Changes in Carbon Inputs into the TCAC during the Early Steps of Glc Starvation

The labeling of Ala is usually found to reflect the labeling of pyruvate, which is essentially the product of glycolysis, but may also arise from the catabolism of some of the

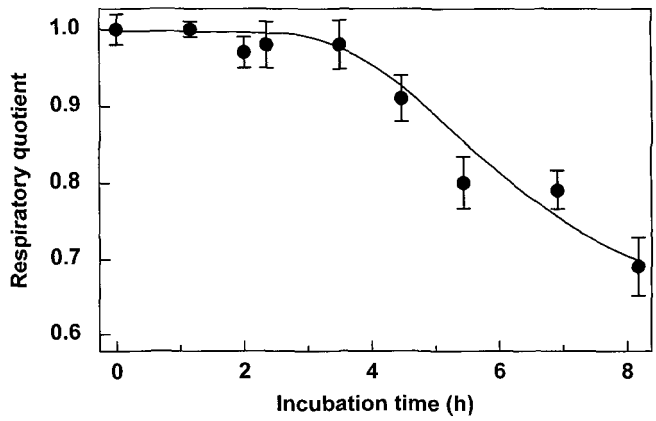

Figure 3. Respiratory quotient of maize root tips during carbon starvation. Maize root tips that had been treated by prestarvation (4 h) and Glc refeeding (4 h of incubation in the presence of 200 mM Glc) were transferred for Glc starvation in Warburg vials (30 tips per vial, in 1.8 mL of medium A) and their respiratory quotient (respiratory quotient = rate of CO_2 evolution/rate of O_2 consumption) was determined manometrically. Data are the means of three measurements. Error bars show ± SD.

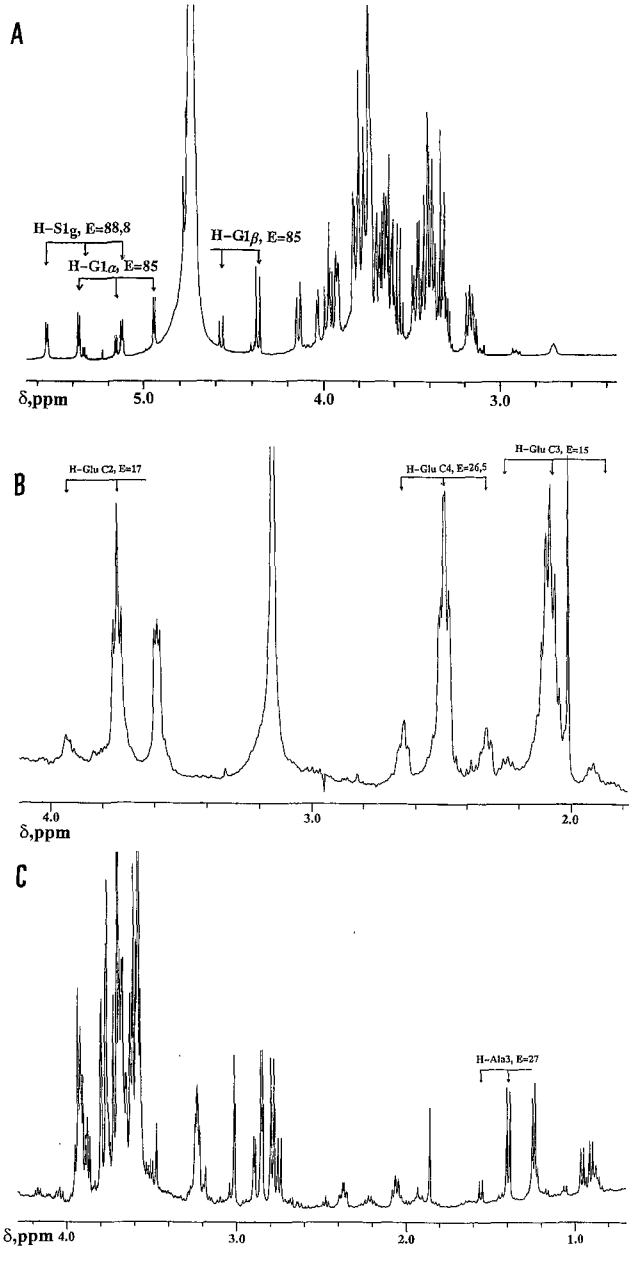

Figure 4. ^{1}H-NMR analysis of metabolites from maize root tips (about 1 g) pretreated and labeled for 4 h with [1-^{13}C]Glc as described in "Materials and Methods." Spectra of the neutral fraction (A), purified Glu (B), and noncarboxylic amino acid fraction (C) represent the accumulation of 128, 500, and 128 scans, respectively. Peak assignments: G1α, G1β, and S1$_g$, resonances of C-1 of α-Glc, β-Glc, and the glucosyl moiety of Suc, respectively; Ala3, resonance of carbon 3 of Ala.

protein amino acids. It gives rise to acetyl-CoA, which enters the TCAC at the citrate synthase step.

Monitoring Glu is essential in the analysis of carbon inputs into the TCAC, because its C-4:C-5 moiety is composed of the acetyl units incorporated at the citrate synthase step, and its C-2:C-3 moiety is the C-3:C-2 moiety of oxaloacetate. These two carbons are labeled from Glu C-4 through the TCAC; thus, in the absence of anaplerotic input into the TCAC, their specific enrichment at steady state is equal to that of C-4. Oxaloacetate is also the product of anaplerotic pathways including PEP carboxylase, the glyoxylate cycle, and the catabolism of different amino acids. Any anaplerotic flux from unlabeled precursors would dilute Glu C-2 and C-3 relative to C-4 (Malloy et al., 1987; Salon et al., 1988; Dieuaide-Noubhani et al., 1995).

The ^{1}H NMR spectra of amino acids obtained from maize root tips incubated for 4 h with [1-^{13}C]Glc are shown in Figure 4, B (purified Glu) and C (noncarboxylic amino acids). The ^{1}H NMR spectra of purified Glu obtained after starvation are shown in Figure 5, C and D. The changes in enrichment of these carbon atoms during starvation are shown in Figure 6, B and C.

After 4 h of labeling, Ala and Glu had not reached steady-state enrichment, but the carbons C-4 of Glu and C-3 of Ala were equally labeled (Fig. 6), although they had not reached steady state (Dieuaide-Noubhani et al., 1995). This shows directly that in Glc-fed root tips carbohydrates are the only source of acetyl-CoA entering the TCAC. The labeling of carbons C-2 and C-3 of Glu was less than that of C-4, resulting most probably from the PEP carboxylase activity (Dieuaide-Noubhani et al., 1995), although the delay in labeling carbons C-2 and C-3 from C-4 may also play a part in the present pre-steady-state condition.

In spite of the low amount of Ala present in starved maize root tips (Brouquisse et al., 1992), the enrichment of Ala C-3 at 6 and 16 h of starvation could be determined from its ^{1}H spectrum. It was found to remain close to its value before starvation (27.5 $\pm$ 1%), with only a small transient decrease to 22 $\pm$ 2% at 6 h (Fig. 6). This decrease may simply reflect the decreasing enrichment of carbohydrates. The subsequent increase to 26 $\pm$ 1.5%, which occurs in spite of the further decreasing carbohydrate enrichment, may indicate a decreasing flux through the pentose phosphate pathway.

Glu analysis by ^{1}H NMR showed that the enrichment of C-4 decreased from 26 to 17% after 6 h (Fig. 5C), and to 9% after 16 h (Fig. 5D) of Glc starvation (Fig. 6B). The enrichment of C-2 and C-3, which was close to 16% at the end of labeling, remained unchanged at 6 h of Glc starvation but then decreased similarly to C-4. The fact that carbons C-2, C-3, and C-4 were equally labeled after 6 h of starvation suggests the absence of anaplerotic flux, i.e. the cessation of the flux through PEP carboxylase and the absence of any other anaplerotic flux (see below) either from lipids (the glyoxylate cycle) or from proteolysis.

Proteolysis would simultaneously produce amino acids such as Ala, Asp, and Glu, which would have a general diluting effect on all the enriched free amino acids studied here. In addition to diluting the carbons of the acetyl unit and of Glu, proteolysis would also produce a flux of four carbon organic acids that would dilute oxaloacetate. As indicated above, the identical labeling of carbons C-2, C-3, and C-4 of Glu excludes any significant anaplerotic flux, thus implying that protein catabolism does not contribute to the supply of carbon to the TCA cycle. The gradual dilution of all glutamate carbons is explicable only by a dilution of label in acetyl CoA fed into the TCA, most

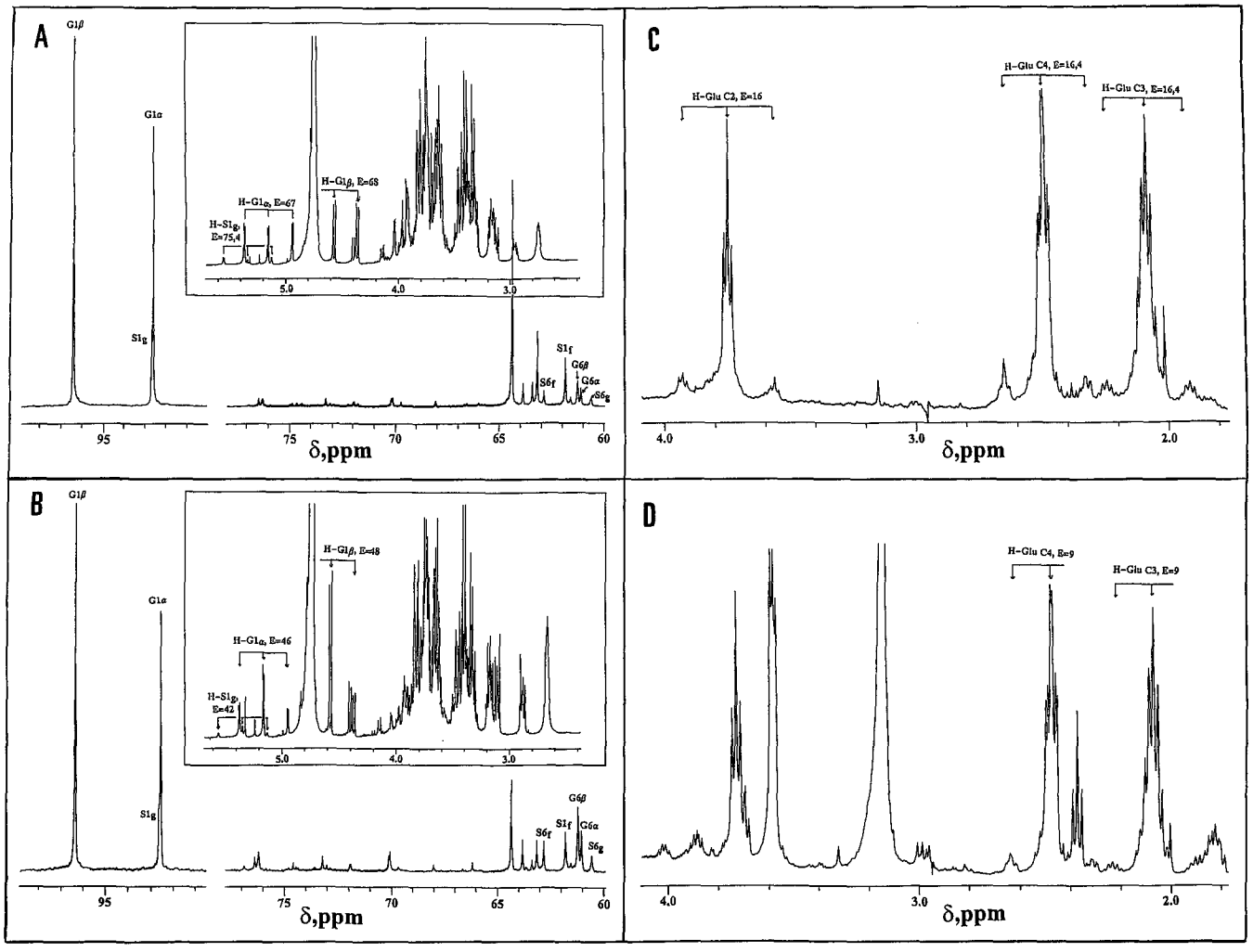

Figure 5. ^{1}H and ^{13}C NMR analysis of metabolites from maize root tips (about 1 g) submitted to sugar starvation after labeling with [1-^{13}C]Glc as described in "Materials and Methods." A and B, Proton-decoupled ^{13}C spectra and ^{1}H spectra (inset) of the sugar fraction after 6 h (A) and 16 h (B) of Glc starvation; the G1β peak of each spectrum is set to full scale. C and D, ^{1}H spectra of purified Glu after 6 h (C) and 16 h (D) of Glc starvation. The absolute enrichment of Glu carbon C-2 in spectrum D was not determined because of contaminating peaks. Peak assignments: G1α, G1β, $S1_f$, and $S1_g$, resonances of C-1 of α-Glc, β-Glc, and of the fructosyl and glucosyl moiety of Suc, respectively; G6α, G6β, $S6_f$, and $S6_g$, resonances of C-6 of α-Glc, β-Glc, and of the fructosyl and glucosyl moieties of Suc, respectively. Proton-decoupled ^{13}C spectra of the sugar fraction represent the accumulation of 400 scans; ^{1}H spectra of the sugar fraction and of purified Glu represent the accumulation of 128 and 500 scans, respectively.

probably by the increasing contribution of fatty acid β-oxidation.

DISCUSSION

The aim of this work was to analyze the effects of Glc starvation on intermediary metabolism in maize root tips. Labeling time was such that free carbohydrate labeling was close to steady state, but proteins and lipids were only slightly labeled, a condition that is necessary to allow the detection of a change of respiratory substrate from carbohydrates to either lipids or proteins. We monitored changes in the enrichment of chosen metabolites by ^{1}H and ^{13}C NMR to detect modifications in metabolic fluxes beyond those previously deduced from variations in metabolite levels (Brouquisse et al., 1991, 1992).

The Early Cessation of the PEP Carboxylase Flux Is Related to a General Decrease in Metabolism

Glc starvation induces major changes in the carbon inputs into the TCAC. The function of PEP carboxylase is to provide carbon skeletons for the net biosynthesis of compounds, such as amino acids, that are needed for root-tip growth. The interruption of this flux within 6 h of starvation corresponds to the cessation of N_2 assimilation (Brouquisse et al., 1992) and other biosynthetic activities, including cell division (Chevalier et al., 1996b; see also Hemerly

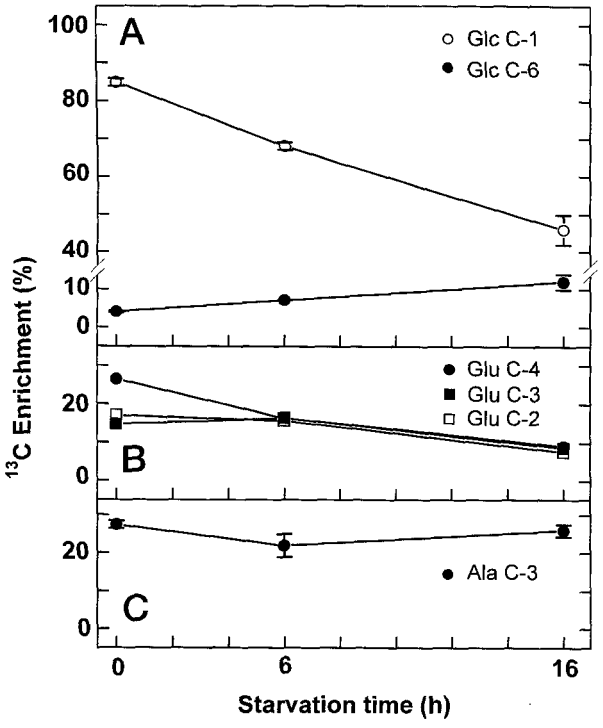

Figure 6. ^{13}C enrichments of carbohydrate and amino acid carbons after incubation of maize root tips with [1-^{13}C]Glc for 4 h (control) and after Glc starvation. ^{13}C enrichments were determined as described in "Materials and Methods" from ^{1}H and ^{13}C spectra similar to those shown in Figures 4 and 5. Results are given as mean ± SD (n = 3).

et al., 1993; Soni et al., 1995), which occur rapidly after sugar deprivation. This limitation of biosynthetic activities decreases the demand for ATP, which has been shown to lead to the decrease in respiration (Brouquisse et al., 1991), a major characteristic of sugar starvation (Saglio and Pradet, 1980; Journet et al., 1986). The progressive decline of the respiratory quotient from 1 to about 0.7 between 4 and 8 h and the decreasing enrichment of Glu C-4 in spite of the constant enrichment of Ala C-3 both indicate that the rate of glycolytic acetyl-CoA production decreases.

In the First Hours of Glc Starvation, Fatty Acids Are the Major Respiratory Substrate

For a number of reasons, including decreased respiratory quotient, decreasing levels of fatty acids and proteins, Asn accumulation, and ammonium loss, it is now clear that in the absence of sugars plant cells use their proteins and lipids for respiration (Saglio et al., 1980; Genix et al., 1990; King et al., 1990; Brouquisse et al., 1992; Aubert et al., 1996). Because the protein mass in maize root tips is 1 order of magnitude higher than that of fatty acids (Brouquisse et al., 1991), and both decrease in parallel during sugar starvation (in percentage of initial amount; see Brouquisse et al. [1991]), proteins clearly make the major contribution to respiration during long-term sugar starvation. However, the present data show that this is not true during short-term starvation. The decreasing enrichment of Glu C-4 would be consistent with an input of unlabeled carbon from either proteins or fatty acids; however, because the enrichment of the other Glu carbons gives no evidence for an amino acid flux, the substrate for respiration must be fatty acids. This indicates that as the cells become depleted of their carbohydrates, they rely on some of their lipid constituents before mobilizing their proteins. The mechanism controlling this sequence is not known at present. The increased flux from β-oxidation between 0 and 6 h (Fig. 6, decreasing Glu C-4 enrichment) must involve an increase in the availability of substrate fatty acids. It also seems to involve an activation of β-oxidation enzymes, as suggested previously by the increased capacity for palmitic acid oxidation to CO_2 in freshly excised root tips submitted to sugar starvation for 4 h (Dieuaide et al., 1992), and by increasing β-oxidation activities up to at least 48 h (Dieuaide et al., 1993).

Does the Glyoxylate Cycle Play Any Role in Glc-Starved Maize Root Tips?

In plant cells, β-oxidation is often associated with the glyoxylate cycle: this pathway converts the acetyl units to four carbon compounds, which are anaplerotic substrates for the TCAC. In germinating seedlings and in senescing leaves, the glyoxylate cycle is associated with gluconeogenesis (Gut and Matile, 1988). The observation that glyoxylate cycle enzymes are induced, whereas PEP carboxykinase is not (Kim and Smith, 1994), suggested that in plant materials in which carbon export does not occur, the glyoxylate cycle may be used for biosyntheses other than gluconeogenesis. In a number of senescing or sugar-starved plant organs or cells, activities or mRNA amounts of malate synthase and isocitrate lyase, the two typical glyoxylate cycle enzymes, have been found to be increased (Graham et al., 1994, and refs. therein). However, in sugar-starved maize root tips the activity of malate synthase was found to increase, whereas that of isocitrate lyase remained undetectable (Dieuaide et al., 1992). This suggested that the glyoxylate cycle would not operate in this particular case. Indeed, the labeling of carbohydrates (Fig. 5, A and B) showed no detectable gluconeogenesis in the maize root tips, at least in the first hours of sugar starvation, thus clearly showing that β-oxidation is not necessarily linked with gluconeogenesis. In addition, the equal labeling of the Glu carbons C-2 to C-4 at 6 and 16 h (Fig. 6) gave no evidence for an anaplerotic carbon flux. Since during proteolysis anaplerotic substrates required for the synthesis of Asn may be supplied by the catabolism of various protein amino acids, such as Glu, the need for the glyoxylate cycle is uncertain. Clearly, the essential role of β-oxidation in sugar-starved maize root tips is to feed respiration by supplying acetyl-CoA to citrate synthase, either in the peroxisomes or in the mitochondria (Dieuaide et al., 1992, 1993). A similar situation has previously been described in

the germinating embryos of fat-storing seeds (Salon et al., 1988).

The Effect of Glc Status on the Time Course of Starvation-Related Events Indicates That Sugar Nutrition Profoundly Affects General Metabolism

When the level of amino acids was studied in maize root tips submitted to Glc starvation just after excision, significant increases in the levels of most of the free amino acids occurred between 4 and 8 h, whereas little or no change was observed in the first 4 h (Brouquisse et al., 1992). In the present work there was still no evidence of proteolysis 16 h after transferring the root tips to the starvation medium. Together with the delay in the decreases of the respiratory quotient, this indicates that the sugar starvation-related phenomena occur more slowly after the treatment of prestarvation and sugar loading than in freshly excised root tips. The delay in labeling fatty acids may be the result of a transient interruption of biosyntheses as a consequence of the prestarvation treatment. This may also apply to proteins, although the delay in that case might also correspond to the time taken to label distant intermediates. It is likely that cell division, which appears to be arrested quickly after the start of carbon starvation (Chevalier et al., 1996b), needs some time to resume. These data show that besides the cessation of the PEP carboxylase reaction and the change in respiratory substrate, sugar starvation induces a number of profound changes in cell metabolism. This interpretation is consistent with the slow recovery of the respiration rate after 5 h of starvation (Brouquisse et al., 1991).

Previous results from carbon-starved tissues or cells have shown that the capacity for ATP production remains higher than the demand (Brouquisse et al., 1991), which means that carbon starvation does not affect metabolism by limiting the supply of ATP. In addition, we found here that the cycle of synthesis and degradation of Suc was not stopped under sugar starvation. Given that cellular processes are differentially controlled by ATP supply, the least essential for the maintenance of life being the most sensitive (Buttgereit and Brand, 1995), and assuming that there would be no excess of ATP under sugar starvation, we expected that this ATP-consuming process would be stopped. Our results do not exclude the fact that the rate of Suc synthesis and degradation was decreased, but clearly show that it continues during sugar starvation. This indicates either that sparing ATP is not essential for survival, or that Suc turnover plays some essential role, even under carbon starvation.

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