Soluble Sugars, Respiration, and Energy Charge during Aging of Excised Maize Root Tips

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ABSTRACT

Oxygen uptake and energy charge were monitored during aging of excised maize root tips and related to the soluble sugar content and exogenous sugar supply.

Oxygen uptake declined immediately after excision to 50 to 30% of its initial value after 8 and 24 hours of aging at 25 C. There was also a sharp decline of the total sugar content (glucose, fructose, and sucrose). Starch content was very low at the time of excision and almost negligible 5 hours later. During the same period, the respiratory quotient declined from 1 to 0.75 and then remained stable.

The addition of exogenous sugars induced a rapid rise of the respiratory rate which stabilized at a level correlated to the external sugar concentration. Addition of 0.2 molar glucose was necessary to restore the respiratory rate to the initial, also the maximum, level. These results indicate that metabolic activity of root tips is highly reliant on sugar import and carbohydrate reserves at the time of excision cannot compensate for the cessation of import. The control of respiration by substrate supply is in good agreement with the failure for dinitrophenol to stimulate oxygen uptake in aged sugar-depleted root tips.

The energy charge remained constant at about 0.9, irrespective of the presence or absence of glucose and in spite of a large decline of respiratory activity in aged, sugar-depleted tissues.

In normoxia as defined by Pradet and Bomsel (16), the respiratory rate of tissues is not limited by the O2 partial pressure. Under such conditions, most of the biological energy provided to the cells comes from sugar oxidation through respiratory pathways. The sugar supply of non-photosynthetic tissues varies depending upon factors which affect the efficiency of carbon fixation by leaves and transport of the recently synthesized carbohydrates (5, 7, 13, 21).

The question arises as to how metabolism adjusts to such fluctuation. Stimulation of root respiratory rate by light after darkness has been reported previously (6, 9). Hatrick and Bowling (10), using sunflower and barley, reported evidence for a complete dependence of root respiration on the rate of assimilate translocation from the shoot, suggesting that roots of young herbaceous plants have no reserves which might reduce the effect of fluctuation in the rate of translocation of photosynthetic sugars.

The present study is part of an effort to understand the factors which limit and control the metabolic activity of roots. Our approach was to relate O2 uptake, taken as a measure of the metabolic activity, to soluble sugar and adenine nucleotide content of the tissues at various times after excision. The data reported demonstrate the influence of exogenous sugars on the metabolic activity of root tips. The significance of energy charge as parameter of normoxic cellular metabolism is discussed.

MATERIALS AND METHODS

Primary root tips, 0.5 cm, were cut from maize seedlings (Zea mays L. INRA 402) germinated for 3 days at 25 C between sheets of filter paper soaked with 2 mM CaCl2.

Determinations of Gas Exchange. Measurements of the rate of O2 uptake and CO2 release by excised root tips were performed in a Warburg respirometer (22).

Thirty root tips were transferred after excision to a 15-ml Warburg flask containing either 2 ml of diluted culture medium (see below) or a gaseous environment maintained saturated with H2O by placing a soaked accordion filter paper in the central well. In this latter case, normal air was used as a gas phase. When tissues were immersed, the flask was flushed with pure O2 (0.5 min, 150 ml/min) and incubated with shaking at 25 C. After a 15-min equilibration time, readings were taken every 30 min.

The diluted culture medium contained 0.7 mM KNO3, 0.65 mM Ca(NO3)2, 0.25 mM KH2PO4, 0.16 mM MgSO4, 0.75 mM NH4NO3, 50 mM NaCl, and the following micronutrients: 6 \( \mu \)M H3BO3, 2.25 \( \mu \)M MnSO4, 1 \( \mu \)M ZnSO4, 25 \( \mu \)M CuSO4, 10 \( \mu \)M (NH4)2MoO4, and 20 \( \mu \)M FeEDTA. The pH of the solution was adjusted to 6.0.

The effects of sugars and/or DNP were studied by addition to the diluted culture medium. In some experiments, noted in the text, a mixture of antibiotics was used to prevent contamination during long incubation times with sugars. Final concentrations were: tetracyclin-HCl (0.1 mg/ml); Nystatin (to saturation); penicillin G (400 units/ml). In most experiments, the use of antibiotics was avoided.

Three flasks were used for each determination and the variations of the atmospheric pressure were corrected by use of three manometers. At the end of each experiment, the root tips were removed and the presence of bacterial or fungal contamination was checked by measuring the O2 uptake of the incubation medium itself. In all experiments reported here, there was no significant contamination.

Tissue Fixation and Nucleotide Extraction. After various incubation times, the root tips were put in 50-ml beakers and rapidly frozen by addition of cold diethyl-ether (-100 C). The samples were stored at -25 C until assayed.

Five or 10 frozen root tips then were transferred to a 5-ml conical glass homogenizer and homogenized with 100 \( \mu \)l 0.6 M trichloroacetic acid in diethyl-ether maintained at -20 C (23). The following operations were carried out at 0 to 2 C. Tissue was homogenized in 2 \( \times \) 250 \( \mu \)l and 1 \( \times \) 500 \( \mu \)l aqueous 0.6 M trichloroacetic acid. The homogenate was transferred to a centrifuge tube. The homogenizer was rinsed three times with 1 ml 0.6 M diethyl-ether. The suspension was placed on ice for more than 2 hours.

Abbreviation: DNP: dinitrophenol.
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m trichloroacetic acid, and the extracts pooled together, and centrifuged at 20 000g for 10 min. Trichloroacetic acid was removed by three extractions with 3 volumes of diethyl-ether. Remaining traces of ether were eliminated by air bubbling and the pH was adjusted to neutrality by diluted NaOH. The nucleotides were assayed by the bioluminescence method (20) using the Pico ATP biophotometer (Sté Jobin et Yvon, France). ADP and AMP were converted to ATP according to Pradet (14) as modified by Saglio et al. (19). Internal standardization was used in all ATP determinations.

Extraction of Sugars from Plant Material. Soluble sugars (sucrose, glucose, and fructose) were extracted either with nucleotides as described above or by boiling ethanol (80% [v/v] in H2O). Both methods gave similar results.

After nucleotides had been determined, the remaining extract was freeze-dried and resuspended in 0.1 M Na-acetate (pH 4.6) (1 to 2 ml for 10 root tips). The samples were kept frozen until assayed.

Ethanol extraction involved samples of 10 tips extracted three times for 5 min each time in 5 ml boiling 80% alcohol. The extract then was dried in a rotary evaporimeter resuspended in 2 ml 100 mM acetate buffer (pH 4.6) and kept frozen until assay.

Starch extraction was adapted from Dekker and Richards (4). Fifty root tips were homogenized with 500 μl 0.5 M NaOH in a 5-ml conical glass homogenizer, allowed to stand for 1 h at room temperature, and neutralized with 1 ml 0.5 N acetic acid. After clarification by centrifugation, the samples were stored frozen until assayed.

Determination of Sugars. The soluble sugars (glucose, fructose, and sucrose) contained in 100 μl root tip extract were determined successively by a specific enzymic method adapted from Bernt and Bergmeyer (2) and Bergmeyer and Bernt (1) to 1 ml spectrophotometric cuvettes. An internal standardization (0.5 μmol sucrose) was included in each determination which was done in triplicate.

For starch determination, 100 μl amyloligosidase (1 mg/ml in 0.1 M acetate buffer (pH 4.6) was added to 100 μl starch solution and kept at 30°C for 1 h. Glucose produced from starch then was analyzed as above and determined by difference with the free glucose in the sample. Soluble starch (BDH) was purified free of soluble sugars by three extractions with boiling 80% (v/v) ethanol and then dried at 50°C overnight under vacuum. In each starch determination, 25 μg of this preparation was included as an internal standard.

RESULTS

Decline of Respiration Rate after Excision in Maize Root Tips. We investigated the effect of aging after excision on the rate of O2 uptake by maize root tips using time as a variable (Fig. 1). The rate of O2 uptake began to decline almost immediately after excision and reached about 50 and 30% of its initial value after 8 and 24 h of incubation at 25°C, respectively. The patterns of decline were parallel in root tips incubated in a H2O-saturated gaseous environment and in liquid culture medium.

In the plant material used in this study, it was found (F. Bruzau, preliminary results) that respiration was limited in a gaseous environment when O2 concentration became lower than 13%. Above this critical oxygen pressure as defined by Berry and Norris (3), the rate of O2 uptake remained constant. In liquid medium, the critical O2 pressure increased by up to 30% even under vigorous shaking, as reported also for other tissues (12). Hence, we used high O2 partial pressures to maintain immersed tissues well above the critical O2 pressure. Then limitation of respiration cannot be explained by a lack of O2.

The RQ (RQ = CO2 output/O2 input) of maize root tips was equal to 1 just after excision (Fig. 2). Then it declined, stabilizing after 4 h around values close to 0.75.

![Fig. 1. Total sugar content (glucose, fructose, sucrose) and O2 uptake in the absence (•) or presence (○) of added 0.2 mM glucose during aging of excised maize root tips. Antibiotics were present in the incubation media. Each point represents the mean value of three independent determinations.](image1)

![Fig. 2. Change in the RQ (RQ = CO2 output/O2 input) and O2 uptake during aging of excised maize root tips; ○, respiratory quotient; •, O2 uptake. Each point is the mean of three independent determinations. No antibiotics were added.](image2)

Control of Respiratory Rate by Soluble Sugars. Apart from O2, soluble sugars, such as glucose, fructose, or sucrose, are the main substrates for respiration. To find out if their concentrations in the tissues could account for the decline of respiration rate, these sugars were assayed in excised root tips after various times of aging at 25°C (Figs. 1 and 3). There was a sharp decline in total sugar content of the tissues immediately after excision. Glucose was the predominant sugar, followed by sucrose and fructose, in the proportion of about 4:2:1, respectively, at the time of excision. All three sugars declined in a similar fashion (Fig. 3) and 8 h after excision only glucose could be detected. Sugar concentration stabilized at about 15% the initial total sugar concentration.

Starch content of maize root tips was very low, accounting for less than 10% of the total sugar content at the time of excision. After 5 h of incubation, only trace amounts of starch could be detected (Fig. 3).

Sugars were also assayed in the liquid medium surrounding the root tips during the course of incubation. They represented less than 10% of the total sugar content of the tissues at the beginning of the incubation period and then declined rapidly to be hardly detectable 4 h later. Thus, no release occurred which might account for the decline of sugar content of the tissues.

During several hours of incubation of root tips in 0.2 mM glucose, no decline in O2 uptake occurred, the rate remaining close to that at the time of excision (Fig. 1). When 0.2 mM glucose was added 8
or 24 h after excision, the rate of O₂ uptake, which was low, increased rapidly and stabilized at the level close to the maximum (Fig. 1). Similar results were obtained when 0.2 M fructose or 0.1 M sucrose was used instead of glucose. Mannitol at the same concentration had no effect on O₂ uptake, which eliminates the increase of osmotic pressure as a possible cause accounting for this phenomenon.

To find out if the rate of respiration could be controlled by exogenous sugar supply, we carried out experiments in which maize root tips were incubated, 23 h after excision, in culture media containing a wide range of glucose concentrations (Fig. 4). The rate of O₂ uptake stabilized after 3 to 5 h, depending on experiments, at values which were directly proportional to the logarithm of the glucose concentration. Up to 0.2 M glucose was necessary to restore the maximum respiratory rate.

Effect of DNP. In order to get more information about the control of respiratory rate, we studied the effect of 10⁻³ M DNP on O₂ uptake (Fig. 5). When DNP was added to root tips supplied with 0.2 M glucose, respiration was stimulated 40% and remained steady. When DNP was added 4 h after excision to sugar-depleted root tips not supplied with exogenous glucose, it had no effect on the O₂ uptake.

Variation of Adenine Nucleotides and Energy Charge. Adenine nucleotides (ATP, ADP, and AMP) and energy charge (ATP + 0.5 ADP/ATP + ADP + AMP) were measured during the aerobic incubation of excised maize root tips and related to the rate of O₂ uptake (Fig. 6).

In the presence of 0.2 M glucose, the energy charge remained high at values close to 0.9. In the absence of sugars, it remained even higher (0.95) and was extremely stable throughout the experiment in spite of a large decrease in the rate of O₂ uptake.

The sum of nucleotides (Σ = ATP + ADP + AMP) dropped rapidly just after excision and then tended to stabilize and eventually to increase slowly in the presence of added 0.2 M glucose. In the absence of exogenous sugar supply, the initial decrease was even steeper and was followed 1 h later by a rise of the sum toward its initial value. However, the sum did not stabilize and soon declined to reach 65 and 54% of its starting value 12 and 24 h, respectively, after excision.
DISCUSSION

The results of this study show that in normoxia the respiratory rate of young excised maize root tips was highly dependent upon the soluble sugar content (glucose, fructose, sucrose) actually usable in the tissues (Figs. 1 and 4).

The immediate decline in the rate of O₂ uptake after excision indicates that no reserves present could balance the cessation in sugar translocation and maintain the respiratory metabolism at its starting level. The stabilization of the respiratory rate of sugar-depleted root tips at any level up to the maximum by an exogenous sugar supply (Fig. 4) indicates a control of respiration by the substrate. This result agrees well with the failure of DNP to stimulate O₂ uptake in root tips sugar-depleted by aging (Fig. 5). These results involving excised root tips explain the observations made on intact plants showing variations of the root respiratory rate linked to various treatments affecting either synthesis or translocation of photosynthates (6, 9).

After 8 h incubation, however, the concentration of glucose in the tissues was far from being negligible and declined only very slowly (Fig. 3). This stabilization cannot be explained by a gradual hydrolysis of starch which was nearly absent in our plant material (Fig. 3). It might be the result of the substitution of carbohydrates by other respiratory substrates or of a steady state between utilization and formation of sugars by gluconeogenesis at the expense of lipids or proteins. The decline of the respiratory quotient and its stabilization around 0.75 (Fig. 2) supports this notion. Alternatively, the residual sugars, principally glucose, may be in a compartment inaccessible to respiratory processes.

In most experiments, the respiratory rate was maximum at the time of excision. Its rapid fall almost immediately after the onset of the incubation period suggests that the amount of sugars transported from the source (the seed) was permanently adjusted to the needs of the sink (root tips).

We were surprised by the very high concentration of sugar (about 0.2 α glucose) needed to restore the respiratory intensity to its starting level (Figs. 1 and 4). Taking into account the high affinity of glycolytic enzymes for their substrates (8), one might expect to get the same effect with 100-fold less concentrated solutions. The experimental values obtained may be explained by a combination of limiting rate of sugar absorption and sugar compartmentation.

Pradeit (15), Raymond et al. (17), and more recently Raymond and Pradeit (18) have shown that a limitation of the respiratory metabolism by low O₂ partial pressure induces a drop of energy charge which stabilizes at values apparently well correlated with the actual level of cellular metabolic activity. We show that a limitation of O₂ uptake by sugar starvation tended to stabilize the energy charge at a level eventually higher (0.95) than in the presence of glucose (0.90), in spite of a large depression in respiratory rate. These results clearly demonstrate that energy charge is an indicator of the level of metabolic activity only when the rate of oxidative phosphorylation is O₂-limited. When it is sugar-limited, energy charge does not vary and, therefore, cannot be correlated to the rate of respiration.

At high-energy charge, variations of the sum of adenine nucleotides parallel those in ATP content which is the dominant adenine nucleotide of the pool. Fluctuations of ATP content in excised maize root segments during the first 6 h of aging similar to those reported for the sum in our work (Fig. 6) have already been observed (11). Nothing is known about nucleotide catabolism in such tissues and the physiological significance of this drop is not understood. It might reflect a reaction to the stress of excision.

The quick decline of respiratory rate and the need of an exogenous sugar supply to maintain its level at its starting value raises questions about the interpretation of experiments done with young excised root tissues devoid of significant carbohydrate reserves where this point has not been taken into account.

As illustrated by our experiments, the metabolic activity of excised seminal root tips is a very good indicator of the level of tissue sugar supply. Root tips, therefore, should constitute an invaluable tool in studying translocation in the whole plant.

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