Metabolic Activity and Energy Charge of Excised Maize Root Tips under Anoxia

CONTROL BY SOLUBLE SUGARS

PIERRE H. SAGLIO, PHILIPPE RAYMOND, AND ALAIN PRADET
Station de Physiologie Végétale, Institut National de la Recherche Agronomique, Centre de Bordeaux, 33140 Pont-de-la-Maye, France

ABSTRACT

Energy charge and fermentative metabolism under anoxia were monitored in excised maize root tips after various times of aging in air and were related to their soluble sugar content. The energy charge value, which was 0.9 in air irrespective of the time of aging, dropped to a lower value within minutes of transfer to a nitrogen atmosphere. This value was dependent upon sugar content of the tissues which was itself a function of aging. The energy charge value after transfer to nitrogen was 0.6 in freshly excised tissue but only 0.2 in tissue aged for 4 hours. When aged tissues supplied with 0.2 molar glucose were transferred to nitrogen, the energy charge was 0.6, irrespective of the time of aging. When 0.2 molar glucose was added under nitrogen, energy charge rose to 0.6. This rise was faster in root tips aged for 8 hours than those aged for 24 hours.

The rate of ethanol plus lactate production (representing 60 and 10%, respectively, of the total sugar consumption in anoxia) was closely correlated to the level of energy charge. It is concluded that, in anoxia, there is a quantitative relationship between the energy charge value and the level of metabolic activity via fermentative pathways.

It is now widely accepted that, both in animal tissues and microorganisms (4, 20), an active steady-state metabolism is characterized by energy charge values close to 0.9. In spite of the discrepancies in energy charge values reported in the literature, probably due to unsuitable nucleotide extraction techniques (A. Pradet, manuscript in preparation), it appears that higher plants under normoxic conditions (16, 21) do not differ from other organisms in the way their energy charge is regulated in vivo.

In previous work dealing with young excised maize root tips (21), it has been shown that energy charge remained high during aging of the tissues in spite of a dramatic drop of the metabolic activity induced by sugar starvation. On the other hand, when plant tissues are subjected to anoxia, the energy charge drops immediately following the cessation of the oxidative phosphorylation (15, 19) and equilibrates at a new lower level, depending on organs or tissues used in the experiment. By controlling the respiratory rate by O2 partial pressure, it is possible to bring a tissue to a new steady metabolic activity at any level between the highest value reached in normoxia and the lowest one observed in anoxia. Pradet (15) and Raymond and Pradet (18) have shown that, in such conditions, energy charge also stabilized at intermediate values, which correlated well with the level of cellular metabolic activity.

However, the relationship between the level reached by energy charge in anoxia and the residual metabolic activity allowed by fermentative processes is not established. The aim of the study here was to check this relationship. It is a development of a previous study (21) in which it was reported that, in air, the respiratory rate of excised maize root tips was under control of the total soluble sugars contained in the tissues. For the study here, excised maize root tips containing varying amounts of sugar, depending on the time of aging, were put under anoxia (N2). Simultaneous measurements were made of energy charge, soluble sugars, and glycolytic activity (as estimated both by sugar consumption and build-up of terminal products such as ethanol and lactic acid).

MATERIALS AND METHODS

Five-mm primary root tips were cut from maize seedlings (Zea mays L., INRA 402) germinated for 3 days at 25 C in the dark between sheets of filter paper soaked with 2 mM CaCl2.

Anaerobiosis. After various incubation times in normoxic conditions as already described (21), 5 or 10 excised root tips were put into a 50 ml beaker lined with wet filter paper. The beaker was sealed with a three-hole stopper. A glass funnel with Teflon stop cock, through which the fixation fluid was added, was inserted in one hole. Inlet and outlet tubes in the other two holes allowed the beaker to be flushed with a continuous stream of pure gaseous N2. The gas flow was about 150 ml/min and contained less than 100 μl O2/l as checked at the exit of the beakers with a Zirconium O2 analyzer (Sté Mécanalyse-Gaz Purs S.A.R.L., France).

Tissue Fixation and Sampling. After various incubation times in anoxia, the root tips were rapidly frozen by pouring cold diethyl ether (−100 C) into the beaker through the funnel. The excised tissues were then stored at −25 C prior to analysis. Adenine nucleotides and soluble sugars (glucose, fructose, and sucrose) were extracted and assayed as already described (21).

Determination of Lactic Acid and Ethanol Produced under Anoxia. Twenty to sixty root tips were placed in 50-ml vials containing 1 ml H2O or 0.2 mM glucose. The vials were closed by a stopper fitted with inlet and outlet tubes for N2 flow. To avoid losses of ethanol produced at the beginning of anoxia, the flowing time was limited to 1 min with a gas flow of 1 liter/min. The vials then were sealed and incubated at 25 C for various times. At the end of the experiment, they were rapidly frozen as above and kept at −80 C until assayed.

Extraction of lactic acid and ethanol as well as lactic acid determination were performed according to Gutmann and Wahllefeld (9). Ethanol was assayed according to Bernt and Gutmann (2). Samples were read on a double-beam spectrophotometer against a reference containing the extract without enzymes to balance the color shift of the extract which was not stable with time. A blank containing the extraction medium was used to
correct the results for ethanol contained in extraction and reaction media.

RESULTS

Control of Energy Charge Level by Soluble Sugars after 30 min Anoxia. The transition of excised maize root tips from air to N_2 induced a rapid drop of energy charge from a high value close to 0.9 in air to lower values ranging from 0.6 to 0.15, depending on the aging time of the tissues before submitting them to anoxia (Fig. 1). These changes in energy charge values reflected the variations of individual nucleotides ATP, ADP, and AMP (Table I). The concentration of soluble sugars (glucose + fructose + sucrose) decreased during aging in accordance with the decrease in energy charge values after 30 min of anoxia (Fig. 1).

Further evidence supporting this relationship was obtained by incubation of root tips since their excision in 0.2 M glucose and transfer at various times to anoxia for 30 min. In this case, energy charge stabilized at 0.6 irrespective to the time of aging (Fig. 1). When 0.2 M glucose was added to 8-h aged tissues, which were subsequently submitted to anoxia, the energy charge dropped to 0.6 instead of 0.2 in sugar-depleted root tips. In 24-h aged tips, a 4- or 5-h preincubation period in air with glucose was needed before energy charge could reach the value of 0.6 under anoxia. A similar result was obtained when glucose was added to 8-h aged tissues already submitted to anoxia (Fig. 2).

The transfer from air to N_2 also induced a drop of the sum of adenine nucleotides (Fig. 3C). However, in contrast to energy charge, the level reached after 30 min anoxia, which corresponded to about 60 to 80% of that observed in air, was quite independent of aging time and sugar concentration of the tissues.

Change in Content of Soluble Sugars and Adenine Nucleotides

![Graph](image)

Fig. 1. Energy charge (EC) after 30 min of anoxia as a function of aging time in air. (○), energy charge in air; (□), energy charge after 30 min anoxia in absence of glucose; (●), energy charge after 30 min anoxia in presence of 0.2 M glucose; (O), total soluble sugar content of the tissues. Anoxia (N_2) started at the points shown by the arrows. Each point represents the mean of three replicates. Antibiotics were added only in medium supplemented with glucose.

Table 1. Adenine Nucleotide Content of Excised Maize Root Tips after 30 min Anoxia as a Function of Aging Time in Air prior to N_2 Treatment

Five excised root tips were incubated in air at 25°C for various times, transferred to N_2 for 30 min, and fixed. The data reported in this table correspond to the experiment described in Fig. 1. Each value is the mean of three determinations. The coefficient of variation was less than ±10%.

<table>
<thead>
<tr>
<th>Aging Time in Air prior to N_2 (30 min)</th>
<th>Nucleotide Content</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ATP (nmol/10 tips)</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>h</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>3.54</td>
</tr>
<tr>
<td>1.5</td>
<td>1.42</td>
</tr>
<tr>
<td>2.5</td>
<td>1.32</td>
</tr>
<tr>
<td>4</td>
<td>1.02</td>
</tr>
<tr>
<td>6</td>
<td>0.74</td>
</tr>
<tr>
<td>9</td>
<td>0.78</td>
</tr>
<tr>
<td>24</td>
<td>1.76</td>
</tr>
</tbody>
</table>

Freshly excised tips in air 17.79 2.46 Traces 20.25

![Graph](image)

Fig. 2. Effect of an exogenous glucose supply under anoxia on energy charge level of excised maize root tips previously aged 8 h in air at 25°C. Anoxia was induced and glucose was added as shown by the arrows.

![Graph](image)

Fig. 3. Change in soluble sugars (glucose + fructose + sucrose), energy charge, and sum of nucleotides during incubation of excised maize root tips in anoxia. The tips had been submitted to various times of aging at 25°C in air prior to N_2 (○), freshly excised; (□), 4 h aging; (●), 8 h aging; (△), 24 h aging. Each point is the mean of two replicates. N_2 was added as shown by the arrow.
Table II. Adenine Nucleotide Content of Excised Maize Root Tips after Various Incubation Time in Anoxia

<table>
<thead>
<tr>
<th>Time in N₂</th>
<th>0 h</th>
<th>4 h</th>
<th>8 h</th>
<th>24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ATP</td>
<td>ADP</td>
<td>AMP</td>
<td>ATP</td>
</tr>
<tr>
<td>h</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>18.8</td>
<td>5.2</td>
<td>0.4</td>
<td>18.7</td>
</tr>
<tr>
<td>0.5</td>
<td>5.5</td>
<td>5.6</td>
<td>4.1</td>
<td>2.2</td>
</tr>
<tr>
<td>1</td>
<td>4.5</td>
<td>5.1</td>
<td>4.2</td>
<td>0.9</td>
</tr>
<tr>
<td>2</td>
<td>2.2</td>
<td>3.4</td>
<td>7.0</td>
<td>0.6</td>
</tr>
<tr>
<td>4</td>
<td>1.1</td>
<td>2.7</td>
<td>9.2</td>
<td>1.1</td>
</tr>
<tr>
<td>6</td>
<td>0.6</td>
<td>1.9</td>
<td>9.6</td>
<td>0.8</td>
</tr>
<tr>
<td>24</td>
<td>0.1</td>
<td>0.4</td>
<td>1.3</td>
<td>0.1</td>
</tr>
</tbody>
</table>

* — only trace amounts were present in the samples.

Fig. 4. Rate of glycolysis in excised maize root tips as a function of aging. After various times of incubation in air, the tips were transferred to N₂ for 1 h. Lactate and ethanol, accumulated during that time in presence (○) or in absence (●) of 0.2 m glucose, were determined in triplicate on 30 tips. Glucose was added in air as shown by the arrows. No antibiotics were added.

Fig. 5. Relationship between energy charge in anoxia and the rate of glycolysis in excised maize root tips. Energy charge was determined after 30 min anoxia. The rate of glycolysis was estimated by measuring the accumulation of lactate plus ethanol after 1 h anoxia.

**in Long-term Experiments under Anoxia.** The rate of total soluble sugar consumption during the first 2 h after excision was the same in air and in N₂ (Figs. 1 and 3A). For longer incubation times, in contrast to normoxia where sugar concentration decreased slowly, no marked decrease was observed in anoxia. Similar observations were made on root tips aged for 4, 8, or 24 h prior to anoxic incubation. The pool of sugars was proportionately smaller and stabilized more rapidly in aged than in freshly excised material (Fig. 3A).

After 4, 8, and 24 h aging in air, the rate reached by energy charge after 30 min of anoxia was very low and remained close to 0.2 over the 24 h incubation under N₂ (Fig. 3B). Soon after excision, it was 0.55 and then decreased as illustrated (Fig. 3B). The response of total adenine nucleotides to anoxic conditions was very similar in root tips freshly excised or previously aged 4 h in air (Fig. 3C). A slight decrease during the first 2 h, it stabilized at least until the 6th h incubation and was close to zero after 24 h anoxia. On the other hand, the decline in the sum of nucleotides in root tips aged in air for 8 or 24 h prior to anoxia was continuous and more rapid in the latter case, reaching approximately zero within 24 h (Fig. 3C). Variations of individual nucleotides ATP, ADP, and AMP are reported in Table II.

**Activity of Fermentative Pathways under Anoxia as a Function of Aging Time in Air.** Just after excision, the soluble sugar content determined on five batches of 20 root tips was 245 nmol/tip (sd = ±21). After 1 h anoxia, it was 137 nmol/tip (sd = ±23). The amount of lactate plus ethanol and alanine produced during that time was 165 nmol/tip (sd = ±16). It represented about 75% of the sugars consumed with 60% for ethanol alone and 10 and 5% for lactate and alanine, respectively. There was no accumulation of malate, which eventually decreased slightly.

The rate of glycolysis was estimated after 0, 3, 5, and 20 h aging in air by measuring the ethanol and lactate produced after 1 h anoxia (Fig. 4). The amount accumulated decreased with aging time. It represented only 50, 20, and 10% of the maximum activity after 3, 5, and 20 h, respectively. The maximum activity was determined on recently excised root tips incubated with 0.2 m glucose. When 0.2 m glucose was added in air to 5-h aged root tips, the glycolytic activity resumed almost immediately to its maximum level. With 20-h aged root tips, it took 4 to 5 h for 0.2 m glucose to restore the maximum activity. The correlation between energy charge after 30 min anoxia and the rate of lactate plus ethanol production is shown in Figure 5.

**DISCUSSION**

According to Krebs (12), metabolic equilibrium, including the ATP/ADP × Pi ratio, may play an important part in the control of the rate of processes such as glycolysis. Unfortunately, the large pool of vacuolar Pi makes difficult the measurement of this ratio in plant tissues. Adenine nucleotides have not been reported in the vacuole (14) and the mitochondrial pool seems to be relatively small in comparison to cytosolic pool (27). Variation in their
concentrations, then, should be essentially related to the cytosol. A direct role of adenine nucleotides or of their ratios as regulators of glycolysis is still unclear, especially in plant tissues where phosphofructokinase, a control point of glycolysis, appears to be regulated by Pi and phosphoenolpyruvate instead of by adenine nucleotides, as it is in animals (7, 26). However, the values of ATP/ADP ratios and of energy charge (ATP + 0.5 ADP/ATP + ADP + AMP) which integrate ATP/ADP and ATP/AMP ratios are obviously affected in some way by changes in concentrations of Pi or phosphoenolpyruvate through equilibrium reactions such as ATP ⇌ ADP + Pi or ADP + phosphoenolpyruvate ⇌ ATP + pyruvate. As emphasized by Krebs (12), the cellular metabolism is a network of near-equilibrium reactions in which adenine nucleotides play a central part. Then whether or not energy charge as such has a direct control upon a metabolic process, it should be correlated to the redox state of the tissue and reflect any modification of the equilibria corresponding to new steady states.

Here, it is shown that, in excised maize root tips under anoxia, there is a quantitative relationship between the energy charge and the rate of accumulation of lactate and ethanol (Fig. 5). It is known that ethanol, lactate, and CO₂ are the main products of the anoxic metabolism in plants (10, 22). Some plants, however, accumulate other compounds: γ-amino butyrate and alanine (23–25), malate (5), or glycerol (6). In the material here, 70% of the sugars consumed under anoxia gave rise to ethanol and lactate. Alanine accounted for 5% and the remaining 20% might have been converted to other terminal products, accumulated as glycolytic intermediates, and/or used in biosynthetic reactions. The measurement of the rate of ethanol plus lactate increase gave a good estimation of the rate of energy-rich bond production by fermentative metabolism. These measurements were done over a period of 1 h. They represented an average rate which took no account of a possible nonlinear kinetic of the lactate plus ethanol production. However, the decrease of sugar content of the tissues (Fig. 3A) indicated that the glycolytic rate was rapid during at least the 1st h of anoxia in freshly excised root tips. The average value then should represent a reasonable approximation of the rate at 30 min.

The measurement of energy charge gives an instantaneous value. However, its slow decrease between 30 and 60 min anoxia (0.05 unit) justifies the use of the value at 30 min as representative of the 1-h period. Values of energy charge and glycolytic rate thus could be compared.

The drop of the sum of nucleotides in root tips transferred from air to N₂ seems to be a common feature in plant tissues (3, 8). Its physiological significance is not understood. It can only be speculated that AMP-deaminase is involved in this phenomenon. As postulated (4), this enzyme, by decreasing the total adenylate pool size, may help to stabilize the energy charge under conditions of environmental stress.

The stabilization of sugar content after 2 h or less under anoxia is difficult to interpret. It might be due to glucoseogenesis. It is unlikely to be due to starch breakdown because of the low concentration found in maize root tips (21). The concomitant cessation in ethanol and lactate production suggest that the pool of soluble sugars was not being utilized by the tissues, perhaps due to its compartmentalization. However, the increase in energy charge when glucose was added under anoxia (Fig. 2) indicates that exogenous sugars penetrated into the tissues even in the absence of O₂.

It took longer for root tips to recover their maximum glycolytic activity in the presence of glucose after 20 h than after 4 or 8 h aging. A similar observation could be made for energy charge under anoxia as well as for respiratory rate (21). This suggests that cellular machinery remained intact during the first 8 h aging but that degradation occurred later, which took some time to be repaired before complete recovery. The nature of these possible degradations is unknown. They might affect metabolic enzymes and/or molecules involved in glucose absorption. These observations can be paralleled with the behavior of the sum of nucleotides in anoxia (Fig. 3C) which remained stable during at least 6 h only in tissues aged less than 8 h in air prior to N₂. After 24 h anoxia, however, the very low value of the sum in all treatments indicates the decay of the tissues.

These results extend those obtained by Raymond and Pradet with lettuce seeds (18). By limiting the rate of oxidative phosphorylation by low O₂ partial pressure, these authors have shown that energy charge stabilized at values representative of the rate of cellular metabolic activity. Likewise, it is shown here that a limitation by sugar starvation of the rate of glycolysis (the main ATP-regenerating pathway in anoxia) induced a decrease of energy charge to values correlated with the level of metabolic activity allowed by fermentative processes in excised maize root tips (Fig. 5). This conclusion is in good agreement with other data reported in the literature. Low value of energy charge (0.3 or less) corresponds to low metabolic activities (15). Organisms able to grow or maintain a high activity under anoxia or without respiration have a high energy charge (17, 20; for review, see ref. 11).

The data presented here do not claim that there is any direct regulatory role of energy charge, as such, in control of glycolysis. They only demonstrate, contrary to a previous prediction (4), that energy charge or ATP/ADP ratio are indicators of metabolic activity and can be quantitatively correlated to metabolic fluxes when ATP-regenerating pathways are the limiting factors.

After this paper was submitted to publication and during its revision, the authors became aware of a very similar study done on human blood platelets (1) which entirely support the conclusions reached here. Using CN⁻ instead of anoxia to inhibit the respiration, these authors demonstrate the existence of a good correlation between energy charge and glycolytic fluxes controlled by glucose availability. As reported by Raymond and Pradet (18) with lettuce seed, they show that energy charge of human blood platelet can also be stabilized at any level.

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LITERATURE CITED

11. KNOWLES CJ 1977 Microbial metabolic regulation by adenine nucleotide pools. In BA Haddock, WA Hamilton, eds, Microbial Energetics. Cambridge Uni-
ANOXIC METABOLISM OF MAIZE ROOT TIPS


