Effect of Anoxia on ATP Levels and Ion Transport Rates in Red Beet[1]

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ABSTRACT

The ATP content of disks of storage tissue of red beet is reduced under N_2 atmosphere to between 10 and 25% of its value in air. Plasmalemma fluxes of K^+ and Cl^- are inhibited within 1 minute or less following removal of O_2, and the extent of this inhibition is entirely attributable to the depletion of ATP. The ATP content remains approximately constant or decreases slightly for up to 1 hour under N_2. On return to air, the ATP content recovers, reaching 75% of the aerobic level within 45 seconds.

In the preceding paper (2), we have presented evidence that the plasmalemma influxes of K^+ and Cl^- in storage tissue of red beet are both closely dependent on the ATP level in the tissue. This conclusion is contrary to that of Polya and Atkinson (3). Among the lines of evidence adduced by the latter investigators in favor of another energy source is that a N_2 atmosphere did not appear to alter ATP levels, although it strongly inhibited ion fluxes. Here, we repeat the measurements of ATP content under N_2 atmosphere, with opposite results to those of Polya and Atkinson (3). We also show that plasmalemma fluxes are inhibited within 1 min or less following removal of O_2. This response is too rapid to be attributable to ATP-dependent changes in tonoplast transport, and thus indicates a more direct dependence of plasmalemma influxes on ATP.

MATERIALS AND METHODS

Disks of storage tissue of red beet (*Beta vulgaris* L.) were prepared as in the preceding paper (2). The procedures for measurement of plasmalemma influx and ATP content were also the same as in reference 3, with the following exceptions. For all experiments, the tissue was placed in unbuffered 0.5 mM KCl or 0.5 mM NaCl as indicated, at 30 C. No Ca^{2+} was included in the experimental solutions inasmuch as we were duplicating experimental solutions described by Polya and Atkinson (3). Although Ca^{2+} often increases influx rates in red beet, it does not seem to be required to maintain membrane integrity, perhaps because it is already present in the cell wall (4). In all cases, there was a 30-min period of pretreatment in the unlabeled experimental solution in air, before any experimental treatments were applied. Influx was estimated by a 5-min exposure to ^{42}K, ^{36}Cl, or ^{24}Na, followed by a 5-min wash in unlabeled solution.

In all experiments, 1-g samples of aged beet disks were placed

in closed 25-ml flasks containing 5–7 ml experimental solution at 30 C. Peristaltic pumps were used to empty and refill the flasks at intervals without exposure to air. N_2 treatments were applied by passing O_2-free N_2 gas into the flasks and at the same time replacing the solutions with solutions equilibrated with N_2. For measurements of ATP content under N_2, liquid N_2 was poured into the flasks to freeze the tissue before exposing it to air. The frozen tissue was then extracted as described previously (2).

RESULTS AND DISCUSSION

Figure 1 shows the time course of changes in tissue ATP concentration and of K^+ and Cl^- influxes following removal of O_2. Influx and ATP levels are shown as a percentage of the control level in air. The control levels were: K^+: 3.25 $\mu eq/g \cdot h$; Cl^-: 1.01 $\mu eq/g \cdot h$; ATP: 26.5 nmol/g. Each point for K^+ or Cl^- influx represents the influx over the preceding 5-min period (e.g. the points at 5 min represent the influxes from 0 to 5 min). Each point for ATP represents the ATP content at the time indicated, i.e. the moment at which the tissue was frozen. The ATP level under N_2
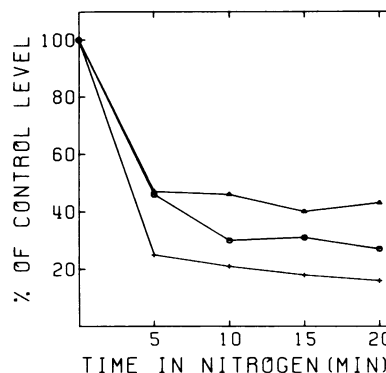

FIG. 1. Changes in tissue ATP concentration and in plasmalemma influxes in K^+ and Cl^- with time after removal of O_2. (+———+): ATP concentration in tissue; (O———O): K^+ influx; (▲———▲): Cl^- influx. Each point for influx indicates ^{42}K or ^{36}Cl uptake over the preceding 5-min period.

Table I. *Plasmalemma Influxes of Na^+ and Cl^-, and ATP Content of Beet Disks after 1 Hour in Solutions Equilibrated with Air or N_2*

Isotope	Treatment	Influx	ATP Content
		$\mu eq/g \cdot h$	$nmol/g$
^{24}Na	Air	4.89	22.5
^{24}Na	N_2	1.96	3.6
^{24}Na	N_2	1.64	2.7
^{36}Cl	Air	0.92	24.8
^{36}Cl	N_2	0.11	2.8
^{36}Cl	N_2	0.08	2.3

is about 20% of that in air, and shows relatively little change from 5 to 20 min after the start of the N₂ treatment. Comparison with the preceding paper (2) shows that reduction of the ATP content to 20% of the control level is sufficient to cause inhibition of K^+ and Cl^- influxes to about 30 and 20% of the controls, respectively. Cl^- influx in the experiment of Figure 1 is higher than this, perhaps because of a seasonal effect. In any event, it appears that the effect of anoxia on ion fluxes is entirely attributable to the change in ATP level. The inhibition of K^+ influx during the first 5 min reached approximately 80% of the degree of inhibition seen in the subsequent period. This means that K^+ influx could have continued at the control rate for not more than 1 min after removal of O_2. In the case of Cl^-, influx for the first 5-min period is not significantly different from that in the following period, indicating that inhibition occurred in less than 1 min. This rapid inhibition
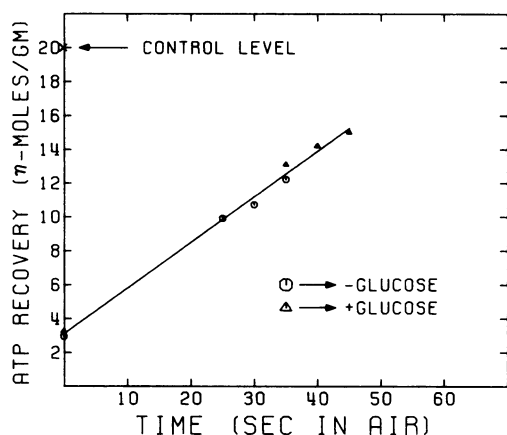

FIG. 2. Recovery of tissue ATP level after anoxia. Beet disks were kept under N₂ atmosphere for 1 h in 0.5 mM KCl ± 10 mM glucose. The tissue was then removed from solution and left exposed to air for 0–45 s before freezing in liquid N₂.

provides evidence that the plasmalemma fluxes themselves respond to ATP rather than to ATP-dependent changes in tonoplast transport.

After 1 h in N₂ atmosphere (Table I) ATP levels remain low, and Cl^- influx is correspondingly inhibited. In this experiment, influx of Na^+, rather than K^+, was measured. The inhibition of Na^+ influx was less pronounced than that of Cl^- influx. We have not investigated further whether Na^+ influx is in fact dependent on ATP, or for example on the membrane potential or proton gradient. If air is readmitted to the tissue after 1 h in N₂, there is a rapid recovery in the ATP content (Fig. 2), which reached 75% of the control (aerobic) level in 45 s. Addition of glucose during the incubation under N₂ does not affect the ATP content either during inhibition or during recovery. The rate of respiration of storage tissue (1) is such that the turnover time for cellular ATP should be 2 or 3 s. Thus the time required for recovery of ATP in Figure 2 may indicate the time taken for O_2 to diffuse into the tissue. It is evident that the ATP level recovers readily from anoxia. The results also illustrate the importance of rapid freezing of the tissue under N₂ to obtain true values for cellular ATP.

We do not know whether our precautions to avoid exposure of the tissue to air after N₂ treatment might account for the difference between the present results and those of Polya and Atkinson (3). In any case, we feel that the reliability of the present data is indicated by the small error variation, the good recovery of added ATP (2), and the correlation between influxes and ATP content in all experiments (present results and ref. 2).

LITERATURE CITED

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