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Metabolic heat rate and respiratory substrate changes in aging potato slices

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Abstract

Potato tubers (*Solanum tuberosum* L. cv. Russet Burbank) were cut into slices 1 mm thick. Tissue was placed in sealed ampules in a calorimeter operated isothermally at 25°C. The metabolic heat rate began to increase within 2 h after slicing, reaching a maximum after 30 h. The peak heat rate was six times that of the initial rate. Heat production was cyanide-sensitive at first while that which developed with time was largely cyanide-insensitive. The initial heat rate per oxygen consumed increased to a peak value, indicating greatly reduced energy conservation. The ratio of CO_2 produced to O_2 consumed, together with the CO_2 rate/heat rate indicated a change from more reduced to more oxidized substrates, i.e. from lipid to starch. Calorimetric results were in good agreement with published results obtained with other methods. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Metabolism; Potato; *Solanum tuberosum*; Temperature; Tissue slices

1. Introduction

Respiration of potato tuber tissue is strongly dependent on oxygen diffusion into the tissue [1]. Oxygen uptake progressively increased as the tuber was cut, peeled, cubed or sliced (Fig. 1). Since 20–30 h is required to achieve the maximum rate of respiration [2], obviously much more than simple oxygen diffusion is occurring. Comparison of the metabolism of freshly cut slices with those aged 24–30 h, shows both qualitative and quantitative differences. As the slices age there is a four- to six-fold increase in the rate of oxygen uptake [2]. Some of this increase may be due to the development of the alternative pathway of respiration, since fresh slice respiration is very sensitive to cyanide while aged slice metabolism is largely cyanide-insensitive [3]. The purpose of cyanide-insensitive respiration in some tissues is heat production [4]. Ordentlich et al. [5] studied potato slices with calorimetry from that viewpoint. Fresh slice metabolism is greatly stimulated by uncouplers such as 2,4-dinitrophenol [6], although uncouplers have little effect on aged slices. This result is contradicted by the large increase in phosphorylation noted in aged

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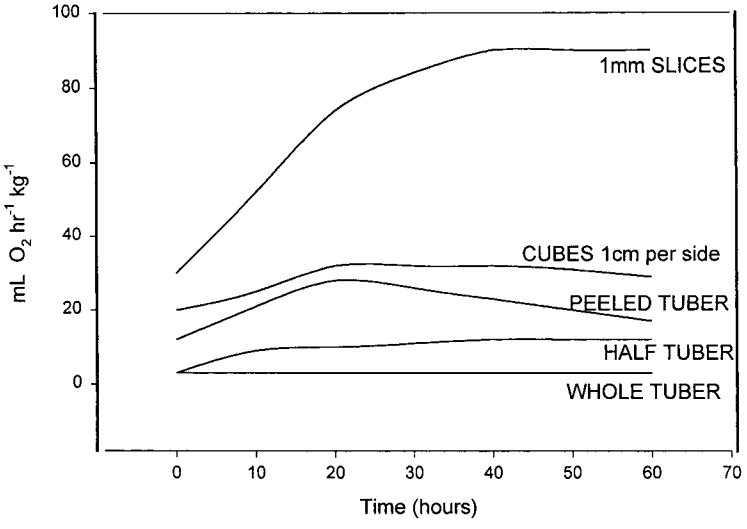

Fig. 1. Oxygen uptake by potato tuber tissue over time in response to degree of cutting.

slices [7,8]. As slices age there is also an apparent shift from alpha-oxidation of fatty acids to starch as the substrate for respiration [9,10].

Clearly much is known about the metabolism of potato tuber slices. They would seem to be an ideal system to test the limits and potential of calorespirometry [11].

2. Materials and methods

Potato tubers (*Solanum tuberosum* L. cv. Russet Burbank) were purchased at a local market, peeled and cut on a sliding microtome into slices 1 mm thick. For some experiments, cylinders 10 mm in diameter were cut with a cork borer before slicing. For the larger calorimeter ampule, slices about 20 mm×20 mm were used. The slices were rinsed in distilled water and either used directly or aged in an Ehrlenmeyer flask containing 0.1 mM $CaSO_4$ with mild agitation on a shaker. Aged slices were withdrawn at intervals and used for metabolic measurements. Tissue was weighed at the conclusion of the measurement (fresh weight) and after drying overnight at 60°C (dry weight).

Potassium cyanide, an inhibitor of the cytochrome pathway of electron transport, and salicylhydroxamic acid (SHAM), an inhibitor of the alternative pathway of electron transport in mitochondria, were obtained from Sigma. Inhibitors were added to potato tuber slices after a 15 min incubation in 30 mM HEPES buffer (pH 7.0), 10 mM SHAM, 1 mM KCN, or water (controls).

The Hart Scientific model 7707 differential scanning microcalorimeter takes four ampules, each with a total volume of 1 ml. One is the thermal blank and the other three hold samples. A filter paper disk moistened with 100 μl of buffer/inhibitor solution was placed in the bottom of each experimental ampule with one to five potato tuber disks (1 mm thick×10 mm diameter). The calorimeter was run in the isothermal mode at 25°C. After a thermal equilibration time of 30 min, data were collected every 30 s on a personal computer. The ampules were opened at this point to prevent oxygen depletion and to insert small vials. Small vials (50 μl) were placed in each of the experimental chambers and filled with either water (30 μl) or 0.4 M sodium hydroxide (30 μl). Lids equipped with pressure transducers were put on each ampule. In this way, metabolic heat rate, CO_2 production and O_2 consumption were simultaneously measured by microcalorimetry [12].

Larger ampules (total volume=70 ml) were used with another calorimeter (not available commercially)

Table 1
Inhibitor studies on the metabolic heat rate of 1 mm thick×10 mm diameter potato tuber slices in the calorimeter set at 25°C

Hours after slicing	Control $\mu W\ g^{-1}$ DW	+SHAM % of control	+KCN % of control
0	126	100	0
2	195	95	18
3.5	228	78	27
5	255	87	32
6.5	341	92	28
7.5	363	78	22
12	539	69	27
13	587	76	31
25	700	80	42
27.5	722	57	45
29.5	757	53	65

operated in the isothermal mode at 25°C. The larger volume made possible longer runs before oxygen became limiting. Only two ampules were accommodated in this instrument.

The experimental ampule had a pressure transducer and a well for either water or NaOH. For some experiments, the sealed ampule was connected by glass capillary tubing to the injection valve of a dual column gas chromatograph. Samples of gas (30 μl each) were periodically injected into the gas chromatograph to follow rates of O_2 use and CO_2 production. After a thermal equilibration for 90 min, heat rates and pressure measurements were collected until O_2 in the ampule was depleted.

3. Results

A series of experiments on aging potato tuber slices were made in the DSC with 1 ml ampules. Each time listed in Table 1 was a separate run on the same batch of tissue. While respiration via the cytochrome path nearly doubled, the great bulk of the increase in respiration was through development of the alternative path. Our results are in good agreement with those of Ordentlich et al. [5].

In the larger calorimeter (70 ml ampule), the heat rate of potato tuber slices was followed continuously for 20 h without opening the ampule. Fig. 2 shows a cubic spline curve fit to the respiratory quotient (CO_2 produced/O_2 consumed) calculated from the gas exchange rates. The change in respiratory quotient was consistent with a shift from lipid to starch as the substrate. Heat rate per CO_2 and per O_2 indicate that energy was conserved for a time after slicing. As the

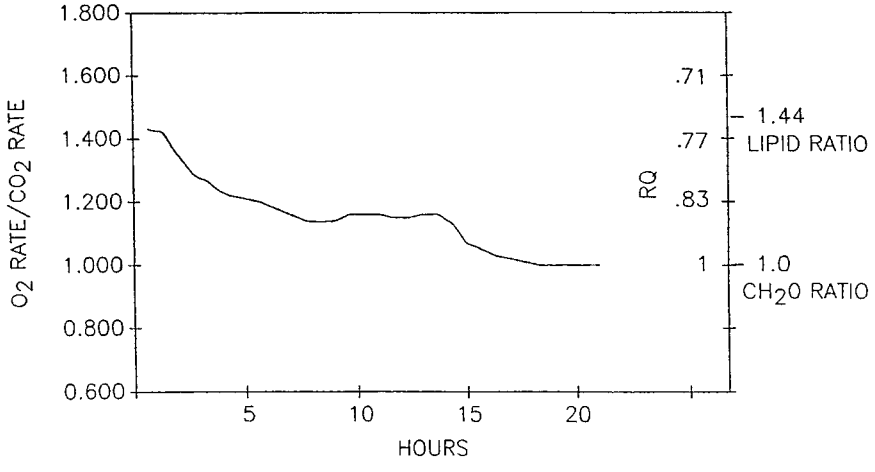

Fig. 2. The respiratory quotient (RQ), measured as O_2 uptake/CO_2 evolution is an index of respiratory substrate change from lipid to carbohydrate in aging potato slices. Lids on the sample ampules were equipped with pressure transducers and small vials (50 μl) containing either NaOH or H_2O were placed in the ampules. Thus, each ampule provided data on heat production rate together with either oxygen uptake (NaOH in the vials), or CO_2/O_2 ratios (water in vials).

alternative path became more prominent, the proportion of energy conserved decreased.

4. Discussion

While respiration rate via the cytochrome path doubled during aging, more of the six-fold increase in total respiration rate was due to development of the alternative path. About 10% of the total metabolic heat could not be accounted for by the two paths and is probably associated with direct oxidation reactions associated with wounding. Steward [13] suggested that difficulty of oxygen penetration into the tuber limited aerobic respiration of tissue much below the surface. However, Burton [1] demonstrated that the partial pressure of oxygen in the center of a potato tuber was in excess of 0.15 atm. That fresh potato tuber slices do alpha-oxidation of fatty acids was shown by use of radioactive isotopes [10] and release of endogenous stable isotopes [9] and is supported by our data.

As demonstrated in this paper, microcalorimetry has great potential to give fresh insights into old problems.

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