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SHORT REPORTS

PYRUVATE DEHYDROGENASE ACTIVITY DURING RIPENING OF HAMLIN ORANGES

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Key Word Index—Pyruvate dehydrogenase; ripening; Hamlin oranges; aerobic respiration; anaerobic metabolism; respiratory cofactors; pyruvate metabolism.

Abstract—Pyruvate dehydrogenase (PD) was examined as a possible regulatory enzyme in the decline in aerobic respiration and increase in anaerobic metabolism in the ripening Hamlin orange. Oranges were harvested weekly over the growing season from October to February. Juice vesicles were excised and analysed for PD and the cofactors, NADH, NAD, ATP and ADP as well as for reduced and oxidized ubiquinone. PD levels increased slightly from October through February. The cofactor ratio of ATP/ADP increased slightly during that period, NADH/NAD increased more than 2-fold and ubiquinone became more reduced. Data suggest that PD could function as a regulatory enzyme in pyruvate metabolism and that either substrate levels of NAD dehydrogenases increased beyond the capacity of the respiratory pathway to adjust to a higher flux, or the oxidative function of the pathway declined over the season.

INTRODUCTION

Ripening of citrus fruit is characterized by a decline in aerobic respiration [1, 2] and an increase in anaerobic metabolism [3]. Increase in anaerobic metabolism in the Hamlin orange resulted in accumulation of ethanol in the juice vesicles and increase in activities of pyruvic decarboxylase (PDC) and alcohol dehydrogenase (ADH) [4]. Pyruvate dehydrogenase (PD) is probably the regulatory enzyme of the aerobic–anaerobic transition in juice vesicles. PD functions as a metabolic regulator in mammalian, avian and microbial tissues [5] and in various plant tissues [6–10]. Plant PD activity is inhibited by acetyl CoA, NADH and ATP [7–9] and is sensitive to regulation by each. If NADH and ATP levels increase in juice vesicles without increase in PD during ripening of Hamlin oranges, conditions would be conducive for modification of aerobic oxidation of pyruvate. The ubiquinone (UQ) redox state is a measure of the electron flux through the aerobic oxidase pathway [11]. The redox state in ripening Hamlin oranges was examined to determine the capacity of the terminal electron transport system to oxidize NADH.

We report and discuss changes in PD activity and levels of NADH, NAD, ATP, ADP, UQH and UQ in juice vesicles during ripening of Hamlin orange.

RESULTS AND DISCUSSION

PD activity increased slightly in juice vesicles during ripening from October to November and then remained constant until the final samples were taken in February (Table 1). PD activity was considerably lower than other enzymes of malate and pyruvate metabolism reported by Roe *et al.* [4] and the increase during ripening was smaller. Crompton and Laties [6] observed that PD activity was much lower than other mitochondrial enzymes in potato slices.

The NADH level increased from 0.5 μM in October to 2.4 μM in December. The NAD level increased less so that the ratio of NADH to NAD increased about three-fold over that period (Table 2). The ethanol content increased four-fold over this period [4]. The NADH and NAD levels and ratios are comparable to values reported for immature and ripe Valencia oranges [12].

Rubin and Randall [7] showed that PD from broccoli

Table 1. Pyruvate dehydrogenase activity in juice vesicles of Hamlin oranges during ripening

	Units/mg protein
Oct.	2.2 ± 0.3
Nov.	2.9 ± 0.3
Dec.	3.2 ± 0.4
Jan.	3.0 ± 0.3
Feb.	2.9 ± 0.2

Each value represents the mean ± s.e. of two samples.

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Table 2. Respiratory cofactors in juice vesicles of Hamlin oranges during ripening

	NADH (μM)	NADH / NAD	NADPH (μM)	NADPH / NADP	ATP (μM)	ATP / ADP	UQH (μM)	UQH / UQ
Oct.	0.5±0.1	0.09	4.1±0.5	1.0	151±6	0.7	4.1±0.6	0.4
Nov.	1.8±0.2	0.18	5.0±0.9	1.1	120±5	0.8	3.2±0.4	0.4
Dec.	2.4±0.2	0.27	5.6±0.6	1.0	91±5	1.0	4.8±0.5	0.5
Jan.	2.0±0.3	0.17	6.3±0.3	1.0	82±1	1.0	5.7±0.6	0.7
Feb.	2.1±0.1	0.22	6.3±0.3	1.0	68±6	1.0	5.2±0.4	0.6
Mar.	4.3±0.4	0.24	7.4±0.3	1.2	66±9	1.0	5.8±0.7	0.7

Mean ± s.e. of four analyses.

(*Brassica oleracea* var. *italica*) was very sensitive to increases in the mole fraction of NADH. A change of 10–15% in the mole fraction of NADH in whole tissue was considered sufficient to alter PD activity 15–25%. The mole fraction of NADH increased 10% and pyruvate content increased 25-fold after aging potato slices anaerobically for 24 hr [6]. These authors considered that the increase in NADH affected pyruvate accumulation by decreasing metabolism of pyruvate through PD.

The ATP and ADP levels decreased similarly over the season so that the ATP/ADP ratio was stable after a slight increase initially (Table 2). Randall *et al.* [10] showed that ATP inactivated PD from pea leaf mitochondria and that the inactivation resulted from a phosphokinase reaction with ATP. The ATP-inactivated PD was reversed by a Mg^{2+} dependent phosphatase as reported for mammalian PD [5]. Both the ATP-kinase and the phosphatase were inhibited by ADP which suggests that the ATP/ADP ratio is a potential regulator of PD activity.

Ubiquinol (UQH) content of juice vesicles increased slightly as the fruit ripened over the season (Table 2). The ubiquinol to ubiquinone ratio (UQH/UQ) increased from 0.4 in October to 0.7 in March. UQH/UQ is an indicator of the redox state of the aerobic respiratory pathway, so increase in the ratio indicates a change in the equilibrium between substrate availability and oxidative function.

Either substrate levels of NAD dehydrogenases increased beyond the capacity of the pathway to adjust to the higher flux, or the oxidative function of the pathway declined over the season. Increase in the NADH/NAD ratio is further evidence that the oxidative capacity of the pathway is inadequate to maintain the redox equilibrium and the availability of NAD for dehydrogenase reactions. An increase in the NADH/NAD ratio throughout the sampling period from October to February, which is accompanied by both a sharp increase in ethanol concentration [4] and a slight rise in PD activity is taken as evidence that PD could function as a regulatory enzyme in pyruvate metabolism in Hamlin oranges.

EXPERIMENTAL

Source of oranges, sampling design and preparation of juice vesicles were described [4]. All enzymes, coenzymes and substrates were obtained from Sigma and all other chemicals were obtained from Fisher. Mitochondrial fractions were prepared [13] from juice vesicles of oranges harvested weekly. PD was extracted and assayed by the procedure of Rubin and Randall [7]. NAD, NADH, NADP and NADPH were extracted from frozen juice vesicles and assayed as described [12]. ADP and ATP were extracted from juice vesicles and assayed as in ref. [14]. UQ and UQH were extracted from mitochondrial fractions and assayed as in ref. [11].

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