Gradients of adenylate nucleotides and energy charge within ‘Rocha’ pear fruit

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Abstract

Cellular energy status is central for metabolic regulation. Pear fruit is stored for several months and its fruit anatomy, surface-to-volume ratio, and gas exchange properties may result in uncharacterized gradients in adenylate nucleotides. The aim of this work was to characterize the axial and radial gradients of adenylate nucleotides and energy charge in pear fruit. ‘Rocha’ pear (*Pyrus communis* L.) fruits with 55-60 mm were sampled after 3 months under controlled atmosphere storage, in a mature but unripe stage. Fruits were sectioned transversely in skin (1.5 mm thick), outer flesh (10 mm thick under skin), and inner flesh (10 mm thick under outer flesh) and longitudinally in three-thirds, proximal, medial, and distal. The sections were freeze-dried and adenosine 5'-triphosphate (ATP), adenosine 5'-diphosphate (ADP) and adenosine 5'-monophosphate (AMP) measured by bioluminescence. Adenylate energy charge (AEC) was calculated as: AEC = [[ATP] + 0.5[ADP]] / [[ATP] + [ADP] + [AMP]]. A radial AEC gradient was measured in the fruit but no significant gradient was observed from the proximal to the distal fruit sections. Total pool of nucleotides, on a fresh mass basis, was 865.7 nmol g^{-1} in the skin, 438.1 nmol g^{-1} in the outer flesh, and 585.6 nmol g^{-1} in the inner flesh. AEC was 0.80, 0.72, and 0.69 in the skin, outer, and inner flesh, respectively. AMP levels were less than 11% of the total nucleotide pool. ATP accounted for 70, 53, and 46% of the total adenylate nucleotides, in the skin, outer, and inner flesh, respectively. In conclusion, there was variation in energy charge in the radial direction, with lower values at the fruit center. Whether this transversal gradient is related to susceptibility of internal browning disorders remains to be clarified.

Key words: ATP, ADP, AMP, fruit quality, *Pyrus communis* L.

INTRODUCTION

Cell metabolism comprise changes in chemical energy among metabolites. Energy released by catabolic reactions is stored in the phosphate bonds of adenylate nucleotides, which can then supply energy to enable thermodynamically unfavorable reactions (Geigenberger et al., 2009). Three adenylate nucleotides are interconverted to store and release chemical energy: ATP, ADP and AMP. The nucleotides are composed of an adenine base attached to a ribose sugar. These adenylate nucleotides are linked to 3, 2 or 1 phosphate groups in ATP, ADP, and AMP, respectively. High-energy phosphoanhydride bonds in the adenylate nucleotides pool store and release energy and AMP, ADP, and ATP are interconverted in biochemical reactions (Haferkamp et al., 2011).

Cells require energy to maintain homeostasis and to drive anabolic processes involved in growth, development, and defense from biotic and abiotic stresses (Geigenberger et al., 2009). In harvested fruits, ATP is generated in mitochondria via oxidative phosphorylation, in the tricarboxylic acid cycle, and by the glycolytic pathway in cytoplasm (Fernie et al., 2004; Sweetlove et al., 2010). The energy status of a cell can be quantified by its adenylate energy charge (AEC). The AEC is defined as ([ATP] + 0.5 [ADP]) / ([ATP] + [ADP] + [AMP]) and its value range from 0 to 1 (Atkinson and Walton, 1967). These authors argued that all three nucleotides should be considered to account for the energy status in cell metabolism, rather than ATP and ADP alone. Several enzymes involved in energy metabolism are regulated in opposite ways by ATP and ADP or by ATP and AMP, and ratios between nucleotides are important for metabolic regulation.

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The AEC of most cells under normal conditions ranges from 0.8 to 0.95 (Atkinson and Walton, 1967). However, AEC can decrease to lower values under increased demand for ATP or under conditions that impair ATP-generating processes like starvation or low oxygen partial pressures (Saquet et al., 2003; Geigenberger et al., 2009). Since ATP-generating pathways are inhibited by a high AEC (Atkinson, 1968), anabolic processes are favored in cells with a high energy charge.

Pear fruit is a pome composed of a core, flesh, and skin. Different fruit structures are likely to have metabolic differences that affect the adenylate nucleotide pool. Moreover, resistance to oxygen diffusion in the fruit may limit respiratory activity (Ho et al., 2006; 2009; 2010) and, therefore, the energy charge of tissues. In support of this assumption, ATP and ADP concentrations are higher in the skin than in the flesh of 'Golden Delicious' apple (Tan, 1999). Energy charge is likely to affect internal disorders of pear (Saquet et al., 2000; Saquet et al., 2003; Veltman et al., 2003) and the fruit's ability to maintain homeostasis during long-term storage. Therefore, adequate sampling for studies of the adenylate nucleotide pool require an understanding of their gradients within the fruit. However, the distribution of adenylate nucleotides and AEC within a pear fruit is unknown.

The aim of this study was to characterize the axial and radial gradients of adenylate nucleotides and AEC within a pear fruit to support sampling decisions for further studies on the energy status of pear during ripening or storage.

MATERIAL AND METHODS

Plant material and sampling

'Rocha' pear (*Pyrus communis* L.) fruits were harvested at commercial maturity from an orchard in Bombarral, Portugal, in August 2014. Fruit with 55-60 mm diameter were stored for 3 months at -0.5 °C and 1 kPa O_2 + 0.7 kPa CO_2 and subsequently sampled for analyses. Pear fruits were sectioned transversely in skin with 1.5 mm thick, outer flesh with 10 mm thick under the skin, and inner flesh with 10 mm thick under outer flesh. Fruit were also sectioned longitudinally in three-thirds: proximal, medial, and distal. The fruit sections were immediately frozen in liquid nitrogen, freeze-dried, and stored at -30 °C until nucleotides analyzes.

Extraction and measurement of adenylate nucleotides

Extraction and assessment of ATP, ADP and AMP were performed as described by (Saquet et al., 2003). Lyophilized and powdered tissue samples (1 g) were placed in 10 mL of a trichloroacetic acid (TCA, 5%) and ethylene diamine tetra acetic acid (EDTA, 2 mM) solution and extracted during 30 min on ice. The samples were then centrifuged under refrigeration (4 °C) at 21,000 *g* for 30 min. A 0.1-mL aliquot of supernatant was diluted 30 times with Tris(hydroxymethyl aminomethane)-EDTA buffer at pH 7.75.

The reaction mixture to determine ATP was composed of 10 µL extract, 40 µL ATP monitoring reagent (AMR) and 150 µL of Tris-EDTA buffer (2 mM, pH 7.75). Luminescence was measured with a multi-mode reader (model Synergy II, BioTek, Winooski, USA). An internal standard (10 µL of ATP at 2 µM) was fed and the luminescence recorded again in each sample. ADP was converted to ATP by incubating the sample with pyruvate kinase (PK). Samples were incubated with PK at 120 units mL^{-1} in phosphoenolpyruvate (PEP) buffer for 30 min at 25 °C. Total ATP concentration was determined and ADP concentration calculated by difference. AMP was converted to ADP by incubation of the samples with a mixture of myokinase (MK, 180 units mL^{-1} in PEP buffer), which in turn was converted to ATP by PK. Nucleotide concentrations are expressed on a fresh mass basis.

AEC was calculated from the concentration of ATP, ADP, and AMP as AEC = ([ATP] + 0.5[ADP]) / ([ATP] + [ADP] + [AMP]) (Atkinson, 1968).

RESULTS AND DISCUSSION

Adenylate nucleotide gradients within a pear fruit

Gradients in adenylate nucleotides were observed in the radial direction (Table 1), but not in the longitudinal direction (Table 2).

Table 1. Adenylate nucleotides and AEC in the radial section of the fruit. Values are means (SD), *n*=4.

Nucleotide or AEC	Adenylate nucleotides (nmol g^{-1} FM)		
	Skin	Outer flesh	Inner flesh
ATP	604.4 (61.5)	231.4 (71.6)	267.1 (38.7)
ADP	167.8 (37.4)	169.6 (45.6)	272.1 (42.3
AMP	93.5 (11.6)	37.0 (6.1)	46.4 (10.3)
Total pool	865.7	438.1	585.6
AEC	0.80 (0.01)	0.72 (0.05)	0.69 (0.05)

The total pool of nucleotides was 865.7 nmol g^{-1} in the skin, 438.1 nmol g^{-1} in the outer flesh, and 585.6 nmol g^{-1} in the inner flesh (Table 1). ATP accounted for 70.53, and 46.0 % of the total adenylate nucleotides, in the skin, outer, and inner flesh, respectively. AMP concentration was less than 11% of the total adenylates pool. AEC decreased along the transversal section of the fruit, from 0.80 in the skin to 0.69 in the inner flesh.

Table 2. Adenylate nucleotides and AEC in the longitudinal section of the fruit. Values are means (SD), *n*=4.

Nucleotide and AEC	Adenylate nucleotides (nmol g^{-1} FM)		
	Proximal	Medial	Distal
ATP	410.2 (67.1)	368.2 (64.2)	498.5 (69.3)
ADP	463.7 (72.3)	253.6 (60.9)	151.4 (59.8)
AMP	29.9 (6.8)	80.5 (18.3)	84.4 (8.3)
Total pool	903.9	702.4	734.4
AEC	0.71(0.07)	0.71 (0.06)	0.78 (0.04)

The sections along the proximal to distal axis of the fruit combined skin and flesh. Assuming that the skin has higher ATP concentration and AEC than the flesh (Table 1), the results obtained in composite sample of skin and flesh are likely to underestimate the actual gradient along the longitudinal axis. However, given the relatively small proportion of skin to flesh (ca. 10%) the comparative results are likely to reflect the actual concentrations in the flesh.

Adenylate nucleotide gradients in harvested fruits

There is little information on the distribution of adenylate nucleotides and AEC within fruits. 'Golden Delicious' apple has higher concentration of ATP and higher ratios of ATP:ADP in the skin than in the flesh (Tan, 1999). Although the author did not map the gradients in adenylate nucleotides in the fruit, the report is consistent with the results presented herein for 'Rocha' pear. In melon under hypoxia AEC was 0.97 in the outer mesocarp and 0.83 in inner tissues of the mesocarp (Biais et al., 2010). In melon, the total pool of adenylate nucleotides was higher in the pericarp, lower in the outer flesh and increased in the inner of fruit flesh. Similarly, in potato tubers, a bulky plant organ, ATP concentration and AEC was lower at the center than in the outer cortex. The center of the tuber had AEC between 0.45 and 0.60 with oxygen partial pressure *ca*. 5 kPa whereas AEC in aerobic tissues at tuber periphery ranged from 0.75 to 0.85 (Geigenberger et al., 2000). Lower ATP concentration and AEC have been reported in the inner flesh of 'Hass' avocado (Lange and Kader, 1997), ‘Jonagold’ apple and ‘Conference’ pear (Saquet et al., 2000), ‘Golden Delicious’ apple (Tan and Bangerth, 2001) and ‘Regina’ sweet cherry (Harb et al., 2006). Taken together, these results suggest that AEC decreases from the outer to the inner cell layers in fleshy fruit and other bulky organs, such as potato tuber. Radial oxygen partial pressure gradient in these organs are associated with the AEC gradient.

Resistance to gas diffusion and respiratory consumption of oxygen create steep oxygen gradients in fruits and in potato tuber (Dadzie et al., 1996; Geigenberger et al., 2000). These gradients in oxygen partial pressures from the skin to the center of fruits have been shown in apple cultivars (Rajapakse et al., 1990; Drazeta et al., 2004), in Asian pears (Rajapakse et al., 1990) and in European pears (Ho et al., 2006; 2010). The low oxygen partial pressure at the core of pear fruit (Ho et al., 2006; 2010) partially explains the lower AEC in the inner flesh measured in ‘Rocha’ pear (Table 1).

Implications of energy gradients to storage disorders

Internal physiological disorders, such as core and flesh browning, occur in pear during long-term storage under controlled atmosphere (Streif et al., 2003). Internal browning disorders in ‘Rocha’ pear under controlled atmosphere storage start at the fruit core or inner flesh at the widest fruit section, the region with lower AEC (Table 1). The lower levels of ATP and lower energy charge at the fruit core (Table 1) and the decrease in energy charge during storage (Saquet et al., 2000; 2001; 2003; Veltman et al., 2003) are consistent with the location of the disorders in the fruit and their occurrence during the storage period.

Although the mechanisms of internal browning disorders are not fully understood, the AEC of the fruit tissues is likely to play a role in their development. Low energy charge shifts metabolic regulation toward catabolism and limited availability of chemical energy can hinder membrane repair leading the irreversible development of browning symptoms. Interestingly, low ATP concentrations are associated with increased membrane permeability in plant cells (Trippi et al., 1996) and a minimum ATP level is required to maintain membrane integrity (Rawyler et al., 1999).

CONCLUSION

A radial gradient in ATP concentration and AEC exists in the pear fruit, decreasing from the skin toward the inner flesh but no significant gradient exists along the longitudinal axis of the fruit. Tissue sampling should take into account the gradient in adenylate nucleotides and AEC.

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