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Effect of nitric oxide on energy metabolism in postharvest banana fruit in response to chilling stress

Yansheng Wang, Zisheng Luo *, Zia Ullah Khan, Linchun Mao, Tiejin Ying

Zhejiang University, College of Biosystems Engineering and Food Science, Zhejiang Key Laboratory for Agro-Food Processing, Hangzhou 310058, People's Republic of China

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ABSTRACT

Effects of postharvest nitric oxide (NO) treatment on energy metabolism and chilling injury in cold-stored banana fruit were investigated. Banana fruit were treated with 0.05 mM NO donor sodium nitroprusside, and then stored at 7 °C for up to twenty days. NO treatment apparently inhibited the development of chilling injury. The contents of adenosine triphosphate (ATP) and energy charge in the NO-treated fruit were significantly higher than control fruit. Meanwhile, the activities of enzymes involved in energy metabolism, including H^+-ATPase, Ca^{2+}-ATPase, succinic dehydrogenase and cytochrome C oxidase were markedly enhanced by NO treatment. In addition, notably elevated activities of fructokinase, glucokinase, glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase were observed in NO-treated banana fruit. These results indicated that NO could enhance chilling tolerance of banana fruit through maintaining high levels of energy status and inducing enzyme activities involved in energy metabolism during cold storage.

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1. Introduction

Cold storage is used to control decay and retard ripening of fruit and vegetables. However, some tropical and subtropical fruit, like banana fruit, are highly susceptible to chilling injury (CI). CI symptoms in banana fruit include rapid peel browning, pitting, and failure of fruit softening, which considerably reduce commercial quality and consumer acceptance (Jiang et al., 2004). With the extensive application of cold storage, the study of more effective techniques for banana fruit storage under low temperature is in urgent need.

Accumulating evidence demonstrates that the reduction of cellular energy may account for the chilling injuries and physiological disorders in those cold-sensitive fruits in response to chilling stress (Liu et al., 2011; Zhou et al., 2014). It has been suggested that the enhanced energy status could contribute to chilling tolerance in various kinds of fruits such as cucumber, mango and peach (Chen and Yang, 2013; Jin et al., 2013, 2014; Li et al., 2014; Yang et al., 2011). Cellular energy may mediate the chilling resistance of cold-stored fruit through directly affecting the biosynthesis of membrane lipids and cell membrane restoration (Jin et al., 2014). Chen and Yang (2013) reported that a higher level of ATP content and energy charge may be involved in inducing activities of antioxidant enzymes, thus protecting postharvest cucumber fruit against oxidative damage at low temperature. In addition, glycolysis, the oxidative pentose phosphate (OPP) pathway, the mitochondrial tricarboxylic acid (TCA) cycle, and the electron transport system are metabolic pathways associated with energy metabolism, which provides the energy for plant biochemical processes (Vanlerberghe, 2013). It is assumed that energy metabolism may play important roles in the development of chilling resistance.

Nitric oxide (NO) is a key signaling molecule mediating multiple plant responses to biotic and abiotic stresses (Arasimowicz and Floryszak-Wieczorek, 2007). Some researchers suggested that NO may suppress ATP synthesis in plant mitochondria, which can be attributed to its inhibition effect on the cytochrome pathway (Yamasaki et al., 2001). However, Gupta et al. (2011, 2012),) found that mitochondrial NO could contribute to improving the energy status in root nodules, similarly as under hypoxic/anoxic conditions. The involvement of endogenous NO in cold response has been investigated in loquat fruit, in which chilling resistance was remarkably reduced by an NO scavenger (Xu et al., 2012). Exogenous NO treatment can alleviate chilling injuries in cold-stored banana fruit, which may be attributed to the enhancement of the antioxidant defense system and secondary metabolites accumulation (Wang et al., 2013b). However, it appears that there is little information on the effect of NO treatment on energy metabolism in cold-stored

* Corresponding author. Tel.: +86 571 88982175.
E-mail address: luozisheng@zju.edu.cn (Z. Luo).

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banana fruit has been available. The objective of this study was to investigate the effect of NO treatment on energy metabolism in postharvest banana fruit in response to cold stress.

2. Material and methods

2.1. Fruit material and treatments

Banana fruit (*Musa* spp., AAA group cv. 'Brazil') were transported from Guangxi Province to the laboratory at Zhejiang University after being harvested at a commercially mature stage (70–80%). Upon arrival, fruit were separated into fingers, and selected for uniformity of size, color and absence of damage. The selected fingers were randomly divided into two groups of 240, comprising three replicates of 80. Fingers (80) in the first group were immersed in 0.05 mM sodium nitroprusside (SNP) solution in a 30 L sealed vacuum container, and vacuum infiltrated at low pressure (10 kPa) for 5 min (NO). As an NO donor that can alleviate the chilling injuries of cold stored banana fruit, SNP at 0.05 mM concentration was selected based on our preliminary research (data not shown). Fingers in the second group were soaked in sterile deionized water under the same conditions (Control). The banana fruit of the two groups were placed into unsealed polyethylene bags (0.04 mm) and stored at 7 °C for 20 day. A sample of nine fruit was randomly collected at a period of 5 day. The peels of the selected fruit were cut into pieces, frozen in liquid nitrogen and stored at −80 °C for the subsequent analysis of energy status and enzyme activities. The peel material was thoroughly homogenized to ensure that samples taken for analysis were representative. Three independent replicates were conducted.

2.2. CI index assessment

CI index was assessed using a five-stage scale based on the extent of chilling symptoms on the surface of twenty individual banana fruit (Nguyen et al., 2003); 0: no chilling injury; 1: mild injury; 2: moderate injury; 3: severe injury; 4: very severe injury. The CI index was calculated using the following formula:

$$\text{CI index} = \sum(\text{CI scale}) \times \frac{(\text{number of fruit at that scale})}{(\text{total number of fruit in the group})}$$

2.3. Measurement of electrolyte leakage

The rate of electrolyte leakage was determined according to the method described by Chen et al. (2008). Twenty discs of banana peel were excised with a 1 cm diameter stainless steel borer from six banana fruit. The discs were added into 20 mL of distilled water and maintained in a water bath shaker at 25 °C for 30 min. The electrical conductivity (L_0) was measured using a conductivity meter (DDS-11A, Shanghai Leici Instrument Inc., Shanghai, China). Then the mixture was heated to 100 °C for 20 min and quickly cooled down to room temperature. Another reading of the electrical conductivity was taken and recorded as L_1. The rate of electrolyte leakage was expressed using the following equation:

$$\text{Electrolyte leakage } (\%) = \left(\frac{L_0}{L_1}\right) \times 100\%$$

2.4. Extraction of mitochondria

Crude mitochondria were extracted from banana peel by the method of Zhou et al. (2014) with a slight modification. A frozen sample (20.0 g) was homogenized in 30 mL of 50 mM Tris–HCl buffer (pH 7.8), containing 0.25 M sucrose, 0.3 M mannitol, 1 mM EDTA, 0.1% (w/v) bovine serum albumin, 0.1% (w/v) cysteine and 5 g/L polyvinyl pyrrolidone at 4 °C. The homogenate was centrifuged at 4000 × *g* for 10 min at 4 °C, using a refrigerated centrifuge (Universal 320R, Hettich, Tuttlingen, Germany). The supernatant was collected and centrifuged at 12,000 × *g* for 10 min at 4 °C for sedimentation of mitochondria. The mitochondria pellet was then re-suspended in washing buffer (10 mM Tris–HCl buffer, containing 0.25 M sucrose, 0.3 M mannitol and 1 mM EDTA) and again centrifuged 12,000 × *g* for 10 min at 4 °C. The final sediment was dissolved with 4 mL washing buffer as crude mitochondria extract for the enzyme assays.

2.5. Measurement of mitochondrial ATPase

H^+-ATPase and Ca^{2+}-ATPase activities were determined by measuring the inorganic phosphorus liberated after catalytic hydrolysis of ATP to ADP (Jin et al., 2013).

For H^+-ATPase activity assay, 3 mL of total reaction mixture containing 0.5 mL of crude mitochondria extract, 30 mM Tris–HCl buffer (pH 8.0), 3 mM Mg_2SO_4, 0.1 mM Na_3VO_4, 50 mM $NaNO_3$, 50 mM KCl and 0.1 mM $(NH_4)_2MoO_4$.

For Ca^{2+}-ATPase activity assay, 3 mL of total reaction mixture containing 0.5 mL of crude mitochondria extract, 30 mM Tris–HCl buffer (pH 8.0), 0.1 mM Na_3VO_4, 50 mM $NaNO_3$, 50 mM KCl, 3 mM $Ca(NO_3)_2$ and 0.1 mM $(NH_4)_2MoO_4$.

The reaction was initiated by the addition of 100 μL of 30 mM ATP–Tris (pH 8.0). After incubating at 37 °C for 20 min, 30 mM trichloroacetic acid was added into the mixture to terminate the reaction. The absorbance at 660 nm was measured using a spectrophotometer (UV-1750, Shimadzu Corporation, Tokyo, Japan). One unit of H^+-ATPase and Ca^{2+}-ATPase activities were expressed as the release of 1 μmol of phosphorus per second.

2.6. Measurement of cytochrome C oxidase (CCO) activity

CCO activity was assayed by the method of Jin et al. (2013). The assay mixture contained 50 mM phosphate buffer (pH 7.5), 20 mM dimethyl phenylene diamine and 0.3 mM reduced cytochrome C. The reaction was initiated by adding 0.5 mL of crude mitochondria extract and absorbance change was recorded at 510 nm. One unit of CCO activity was defined as an increase of 0.01 in absorbance per second under the assay conditions.

2.7. Measurement of succinate dehydrogenase (SDH) activity

SDH activity was measured according to the method of Acevedo et al. (2013). The substrate solution contained 50 mM potassium phosphate buffer (pH 7.8), 0.08 mM DCPIP, 1 mM phenazine methosulphate (PMS), 4 mM sodium azide and 100 mM sodium succinate, incubating at 30 °C for 10 min. Blanks were performed by replacing the succinate with 100 mM sodium malonate. The activity was determined by adding 0.5 mL of crude mitochondria extract. The absorbance was measured at 600 nm. One unit of SDH activity was defined as an increase of 0.01 in absorbance per second.

Protein content in the crude mitochondria extract was determined according to the method of Bradford (1976), using bovine serum albumin as a standard. Specific activity of the enzymes was expressed as units per kilogram protein.

2.8. Measurement of ATP, ADP and AMP contents and energy charge

ATP, ADP and AMP were extracted and assayed according to Zhou et al. (2014) with a minor modification. Frozen sample (2.0 g) was ground in liquid nitrogen and extracted with 5 mL of 0.6 M

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