Plant Physiology and Biochemistry 49 (2011) 449–457

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy

Research article

Mitochondrial energy metabolism in young bamboo rhizomes from *Bambusa oldhamii* and *Phyllostachys edulis* during shooting stage

Lee-Feng Chien*, Yuan-Chin Wu, Hui-Ping Chen

Department of Life Sciences, College of Life Sciences, National Chung Hsing University, Taichung 40227, Taiwan

ARTICLE INFO

Article history:
Received 14 September 2010
Accepted 24 January 2011
Available online 1 February 2011

Keywords:
Bamboo mitochondria
Complex
Supercomplex
AOX
PUCP
$PmitoK_{ATP}$

ABSTRACT

The energy metabolism of mitochondria in young rhizomes of the bamboo species *Bambusa oldhamii*, which favors shooting during the summer, and *Phyllostachys edulis*, which favors shooting during the winter, was characterized. The mitochondrial energy-converting system was clarified in terms of respiratory activity and structural organization. The respiration rates were measured at 15, 28, and 42 °C by NADH, succinate, and malate oxidation. NADH was shown to act as an efficient substrate regardless of the temperature. The structural organization of functional mitochondrial respiratory supercomplexes was studied using blue native PAGE and in-gel activity staining. In both species, almost 90% of the total complex I was assembled into supercomplexes, and *P. edulis* contained a greater amount of complex-I-comprising supercomplexes than *B. oldhamii*. Approximately 50% of complex III and 75% of complex V were included in supercomplexes, whereas *P. edulis* mitochondria possessed a greater amount of complex-V-comprising supercomplexes. The alternative oxidase (AOX), plant mitochondrial uncoupling protein (PUCP), plant mitochondrial potassium channel ($PmitoK_{ATP}$), rotenone-insensitive external/internal NADH:ubiquinone oxidoreductase [NDH(e/i)], and superoxide dismutase (SOD) activities of the energy-dissipating systems were investigated. *P. edulis* mitochondria had higher levels of the PUCP1 and AOX1 proteins than *B. oldhamii* mitochondria. The activity of $PmitoK_{ATP}$ in *P. edulis* was higher than that in *B. oldhamii*. However, *P. edulis* mitochondria possessed lower NDH(e/i) and SOD activities than *B. oldhamii* mitochondria. The results suggest that the adaptation of *P. edulis* to a cooler environment may correlate with its greater abundance of functional mitochondrial supercomplexes and the higher energy-dissipating capacity of its AOX, PUCP and $PmitoK_{ATP}$ relative to *B. oldhamii*.

© 2011 Elsevier Masson SAS. All rights reserved.

1. Introduction

Bamboo, an important Asian economic crop, serving as both as a vegetable and in manufacturing, is one of the fastest-growing plants on the planet. The edible part of the bamboo shoot is the immature expanding culm that emerges from the rhizome. It consists of meristematic tissue that carries on rapid cell division and requires a huge amount of energy. However, the mechanisms underlying this burst of rapid growth and energy metabolism are unclear. Since mitochondria are the powerhouses that supply energy for growth, we aimed to investigate the energy-converting systems through the electron transport chain and ATP synthase, and the energy-dissipating systems of thermogenesis, transport, and reactive oxygen species (ROS) production. To understand whether the energy metabolism in young bamboo shoots varies with species and environmental factors, the mitochondria from a native Taiwanese species of green bamboo, *Bambusa oldhamii* (whose new shoots develop in the summer, from April to October), and an imported species of moso bamboo, *Phyllostachys edulis* (whose new shoots develop in the winter, from December to March), were isolated and characterized [17,36].

The standard plant mitochondrial electron transfer chain consists of four multi-protein complexes, commonly referred to as complexes I, II, III, and IV. When the substrates NAD(P)H and succinate are oxidized, electrons are transferred from complexes I and II to complex III *via* UQ, from complex III to complex IV *via* cytochrome *c*, and finally, to O_2. Subsequently, a proton

Abbreviations: BN- or CN-PAGE, blue native or clear native polyacrylamide gel electrophoresis; bis-Tris, 2-[bis(2-hydroxyethyl)amino]-2-(hydroxymethyl) propane-1,3-diol; DM, *n*-dodecyl-β-D-maltoside; complex I, NADH:UQ oxidoreductase; complex II, succinate:UQ oxidoreductase; complex III, UQ:cytochrome *c* oxidoreductase; complex IV, cytochrome *c* oxidase; complex V, F_oF_1 ATP synthase; AOX, alternative oxidase; PUCP, plant mitochondrial uncoupling protein; $PmitoK_{ATP}$, plant mitochondrial potassium channel; NDH(e/i), rotenone-insensitive external/internal NADH:ubiquinone oxidoreductase; SOD, superoxide dismutase.

* Corresponding author. Fax: +886 4 22874640.
E-mail address: lfchien@dragon.nchu.edu.tw (L.-F. Chien).

0981-9428/$ – see front matter © 2011 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.plaphy.2011.01.024

electrochemical potential is generated and coupled to the conversion of ADP and P_i to ATP, which is catalyzed by complex V [34]. The mitochondrial protein complexes, complexes I–IV and complex V, have been found to be finely arranged in the inner membranes as so-called "respirasomes" or "supercomplexes" [21,33]. Such multiprotein complexes may be advantageous for substrate channeling, catalytic enhancement, the sequestration of reactive intermediates, and servicing of the rapid intramolecular functional group transfer reactions [30]. Blue native (BN)- and clear native (CN)- polyacrylamide gel electrophoresis (PAGE) techniques have been used to reveal the varying structural organization of the respiratory supercomplexes in different organisms, whereas the formation of supercomplexes has been shown to be closely related to cellular function and regulation [39]. For instance, three supercomplexes, $III_2 + IV$, $III_2 + IV_2$, and III_2, have been found in yeast mitochondria, while five much larger supercomplexes, $I + III_2$, $I + III_2 + IV$, $I + III_2 + IV_2$, $I + III_2 + IV_4$, and V_2, were observed in bovine heart mitochondria [33]. In higher plants, distinct mitochondrial supercomplexes have been found in different tissues and developmental stages [12]. Moreover, the abundance and assembly of mitochondrial supercomplexes changes under various conditions [22,37]. Therefore, knowing the structural assembly and the ratio of the variant mitochondrial respiratory complexes would help to clarify the relationship between mitochondrial energy metabolism and cellular function.

In addition to the standard pathway, plant mitochondria contain an alternative oxidase (AOX) that transfers electrons from UQ to O_2 and operates a non-phosphorylating bypass mechanism for dissipating free energy as heat, called the alternative (Alt) pathway [24]. AOX activity is generally low in unstressed plants, but it increases when plants are subjected to harsh environments [13,14]. There is evidence that the overexpression of AOX decreases the formation of ROS [35]. Plant mitochondria possess both the Alt pathway as well as other components that can dissipate $\Delta\mu_{H}^{+}$ and cause a reduction in ATP synthesis, including plant mitochondrial uncoupling protein (PUCP), plant mitochondrial potassium channel ($PmitoK_{ATP}$), and alternative rotenone-insensitive external/internal NADH:ubiquinone oxidoreductase [NDH(e/i)]. PUCP was first discovered in potato tuber mitochondria and was later found in other higher plants [38]. These PUCPs seem to function similarly to mammalian uncoupling proteins (UCPs), providing an energy-dissipating system that can be activated by fatty acids and that leads to protons moving from the intermembrane space to the matrix, thus bypassing ATP synthase [16]. PUCPs have been highly strong correlated to thermogenesis, as a defense against ROS production, and in metabolic energy balance [4,35,37]. $PmitoK_{ATP}$ was identified only a few years ago, first in durum wheat and subsequently in other plants [26]. $PmitoK_{ATP}$ catalyzes the electrophoretic uniport of K^+ across the inner mitochondrial membrane and is also associated with K^+ cycling through the K^+/H^+ antiporter, thereby dissipating $\Delta\psi$ [31]. Alternative rotenone-insensitive NDH(e/i)s may also function as an energy-dissipating system that can decrease respiratory ATP output by up to 30% [28]. All of these energy-dissipating systems are related to ROS production.

Thus, mitochondria were isolated from young bamboo rhizomes of *B. oldhamii* and *P. edulis*, and their energy-converting and energy-dissipating systems were characterized. The respiratory activity and structural organization of the energy-converting systems were clarified. The mitochondrial respiration rates of NADH, succinate, and malate oxidation were measured at different temperatures. The structural organization and assembly of functional mitochondrial respiratory supercomplexes were studied using BN-PAGE and in-gel activity staining with the addition of n-dodecyl-β-D-maltoside and digitonin. The energy-dissipating systems were characterized in terms of their AOX, PUCP, $PmitoK_{ATP}$, rotenone-insensitive NDH (e/i), and SOD activities. Our results suggested that the adaptation of *P. edulis* to a cooler environment may be associated with its greater abundance of functional mitochondrial supercomplexes and higher energy-dissipating capacity relative to *B. oldhamii*.

2. Materials and methods

2.1. Isolation of mitochondria from young bamboo rhizomes

Fresh edible rhizome shoots of *B. oldhamii* and *P. edulis* were obtained from the local market (Fig. S1). The leaf sheaths covering the shoots were removed, and only the top 10 cm of the shoot was used. Using a juice extractor, about 400 ml of bamboo shoot juice was squeezed from 1 kg bamboo shoots and mixed with 400 ml of extraction buffer containing 0.4 M mannitol, 50 mM MOPS (pH 7.2), 2 mM ethylene glycol tetraacetic acid (EGTA), 4 mM L-cysteine, 0.6% (w/v) polyvinylpyrrolidone (PVP), 20 mM β-mercaptoethanol, and 0.5% (w/v) defatted bovine serum albumin (BSA) at 4 °C. The mixtures were centrifuged for 5 min at 400×*g*, and then the supernatant was transferred into new bottles for further centrifugation for 15 min at 2000×*g*. After centrifugation, the supernatant was transferred into new bottles and centrifuged for another 30 min at 10,000×*g*. The pellet was gently homogenized in a wash buffer containing 0.3 M mannitol, 20 mM MOPS (pH 7.2), 1 mM EGTA, and 0.1% (w/v) defatted BSA and centrifuged for 10 min at 2000×*g*. The resulting supernatant was centrifuged for 15 min at 15,000×*g*. The crude mitochondrial pellet was gently homogenized in a small amount of wash buffer. The mitochondrial protein concentration was determined by the method of Biuret using BSA as a standard [27]. Isolated mitochondria were either directly analyzed or stored at −80 °C.

2.2. Measurement of the respiration rate of isolated mitochondria

The respiration rate (i.e., the rate of oxygen consumption) was measured in states 2 (substrate added), 3 (ADP added), and 4 (ADP exhausted, without the addition of oligomycin) using a Clark-type electrode (Rank Brother, Cambridge, UK) at various temperatures [6]. The respiration rate was measured in 2 ml of reaction buffer containing 0.3 M mannitol, 10 mM MOPS (pH 7.2), 10 mM NaH_2PO_4, 2 mM $MgCl_2$ with addition of mitochondrial proteins from *B. oldhamii* and *P. edulis*. The respiratory substrates, NADH (1 mM), succinate (5 mM), or malate (5 mM malate plus 5 mM glutamate and 5 mM NAD^+), as well as ADP (200 μM), were added when required.

2.3. One-dimensional (1-D) BN- or CN-PAGE

Mitochondria were centrifuged for 25 min at 10,000×*g*, and the sedimented pellets were resuspended in either *n*-dodecyl-β-D-maltoside (DM) solubilization buffer containing 50 mM bis-Tris–HCl (pH 7.0), 750 mM aminocaproic acid, 0.5 mM ethylene diamine tetraacetic acid (EDTA), and 1.0–4.0 g g^{-1} of DM/protein or digitonin solubilization buffer containing 30 mM HEPES (pH 7.4), 150 mM potassium acetate, 10% (v/v) glycerol, 2 mM phenylmethylsulfonyl fluoride, and 1.0–10 g g^{-1} of digitonin/proteins [12]. After being dissolved with detergents on ice (an additional 20-min incubation in the case of digitonin solubilization), the samples were centrifuged at 14,000×*g* for 30 min to remove insoluble materials and subsequently supplemented with 5 μl of coomassie blue solution containing 5% (w/v) Coomassie Blue R350 (Serva) (no dye for CN-PAGE) in 750 mM aminocaproic acid. Samples were directly loaded onto BN or CN gels.

Previously described methods for 1-D BN- or CN-PAGE were used [39]. The separating gel consisted of a gradient of 5–13% (w/v) acrylamide, and the stacking gel consisted of 4% (w/v) acrylamide

Download English Version:

https://daneshyari.com/en/article/2016438

Download Persian Version:

https://daneshyari.com/article/2016438

Daneshyari.com