MINI REVIEW

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Apyrases in Arabidopsis thaliana

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Abstract

Apyrases belong to the ATPase family of enzymes that hydrolyze phosphoanhydride bonds of nucleoside tri- and diphosphates. These enzymes differ markedly from other phosphohydrolases due to their high specific activity, broad divalent cation requirement, broad nucleotide substrate specificity, and insensitivity to various inhibitors. In the past 30 years, apyrases have been frequently studied in mammals. In comparison, research of apyrases in plants has received little attention, despite the growth of plants being closely related to the apyrases. In this review, we summarize the research of the apyrases in Arabidopsis thaliana and point to the possible future directions of research. Apyrases have seven members found in Arabidopsis thaliana, each with different properties and functions. Currently, the characterization and functions of AtAPY1 and AtAPY2 have been reported, though, to the best of our knowledge, the other apyrase members (AtAPY3 to 7) have not yet been sufficiently described. In this review, we also summarize the progress being made and the difficulties encountered in apyrase research in Arabidopsis thaliana.

Additional key words: ATPase family, AtAPY1 and AtAPY2, enzyme localizations and biochemical properties

Introduction

Apyrases are calcium-activated enzymes that catalyze the conversion of adenosine triphosphate (ATP) to adenosine diphosphate (ADP), adenosine monophosphate (AMP), and Pi (Veloria et al. 2017), and belong to the guanosine diphosphatase 1 - CD39 nucleoside phosphatase superfamily. They contain five apyrase conserved regions (ACRs) (Chiu et al. 2015), which are the main characteristics of the apyrases. Apyrases have been found in all prokaryotes and eukaryotes (Komoszyński and Wojtczak 1996). They are widely distributed in both animal and plant tissues and can be classified as endo-apyrase or ecto-apyrase based on their localization and biochemical properties (Plesner 1995, Komoszyński and Wojtczak 1996). They are widely distributed in both animal and plant tissues and can be classified as endo-apyrase or ecto-apyrase based on their localization and biochemical properties (Plesner 1995, Komoszyński and Wojtczak 1996). According to Knowles (2011), apyrases are multifunctional enzymes involved in pathogen-host interactions, plant growth, lipid and protein glycosylation in cells, and oncogenesis (Clark et al. 2010). Their diverse expressions (Day et al. 2000) and membrane and subcellular localization (Cohn et al. 2001, Govindarajulu et al. 2009) also hint at their roles in various metabolic processes (Sunhee et al. 2009, Kavaiool and Ezhova 2010). In the past 30 years, apyrases have been studied in many eukaryotic systems and examined for their possible use (Moustafa 2014, Veloria et al. 2017).

In plants, despite the lack of understanding about the functions and regulation of the apyrases, some progress is being made in elucidation of the roles of plant apyrases in phosphate transport (Thomas et al. 1999, Clark et al. 2010) cytoskeleton-based cellular metabolism (Shibata et al. 1999, Chen et al. 2013), toxin resistance

Various researchers have highlighted two main categories of apyrases: 1) ecto-apyrases that are present in the extracellular matrix (Plesner 1995), and 2) endo-apyrases that are localized in the cell interior (Komoszyński and Wojtczak 1996). Both ecto-apyrases and endo-apyrases can be soluble or inserted in membranes (Wolf et al. 2007) and have the ability to hydrolyze both the γ- and β-phosphate of ATP and ADP (Plesner 1995). Ecto-apyrases have diverse physiological functions, including the salvaging of extracellular nucleotides (Clark et al. 2014), modulation of ATP-mediated immunoresponses (Di 1998), protein glycosylation (Abeijon et al. 1993), and enhancement of soybean nodulation (Govindarajulu et al. 2009). Legume ecto-apyrases have been shown to be specific in their role in nodulation (McAlvin and Stacey 2005). The biochemical characteristics of plant endo-apyrases have been analyzed in Pisum sativum (Shibata et al. 2002) and Solanum tuberosum (Kettlun et al. 2005), though their physiological functions remain unknown.

Arabidopsis thaliana is a small flowering plant that is widely used as a model organism in plant biology. The advantages of Arabidopsis thaliana include its small genome, extensive genetic and physical mapping of all five of its chromosomes, a rapid life-cycle, which have facilitated its use in studies of the cellular and molecular biology of flowering plants. Because of these advantages, we chose Arabidopsis thaliana as a representative plant species to introduce the research on the apyrases.

Types and functions of apyrases in Arabidopsis thaliana

To date, 18 members of the apyrase family have been discovered in plants. Arabidopsis thaliana contains 7 members (Steinebrunner et al. 2000), Solanum tuberosum contains 1 member (Handa and Guidotti 1996), leguminous plants contain 4 members, and Mimosa contains 6 members (Ishikawa et al. 1984, Ghosh et al. 1998).

In Arabidopsis thaliana, seven members of the apyrase family contain representatives from each clade. AtAPY1 and AtAPY2 are clustered into clade I. AtAPY3-6 are clustered into clade II, and AtAPY7 is located alone in clade III.

Among the seven members found in Arabidopsis, AtAPY1 and AtAPY2 have been extensively studied. Two apyrase genes (AtAPY1 and AtAPY2) have been cloned and sequenced. The transcripts of AtAPY1 and AtAPY2 are widely distributed; however, the expression patterns are not identical. In roots, for example, the amount of mRNA of AtAPY1 is greater than that of AtAPY2. Furthermore, AtAPY1 and AtAPY2 are 87 % identical in their amino acid sequences. Both contain four apyrase conserved regions (ACRs), an ATP-binding motif and a hydrophobic segment at the N-terminus. However, only AtAPY1 demonstrates a calmodulin-binding domain (Steinebrunner et al. 2000).

Both AtAPY1 and AtAPY2 have been shown to have numerous physiological functions, which are closely related to pollen development (Steinebrunner et al. 2003), vegetative growth, and stomata movements (Wu et al. 2007, Clark and Roux 2011a). Researchers use RNA interference to inhibit the expression of AtAPY1 or AtAPY2, which results in structural changes of the cell wall (Min et al. 2014). The data provides strong evidence to support the hypothesis that AtAPY1 and AtAPY2 function as plant endo-apyrases and are necessary to add saccharides to proteins or lipids. Nevertheless, their defined functional role as endo-apyrases would not necessarily preclude their roles as regulators of the ecto-ATP/ADP concentration via a secretory mechanism, as argued by Clark et al. (2014), based on immunochemical (Wu et al. 2007) and genetic data (Min et al. 2014) for the suppression of AtAPY1 and AtAPY2 causing an increase in extracellular ATP. Moreover, Yang et al. (2015) showed that the apyrases are associated with extracellular ATP and root skewing in Arabidopsis.

Besides AtAPY1 and AtAPY2, the other five Arabidopsis apyrase genes (AtAPY3-7) have been less studied. AtAPY3-5 occur as recurrent tandem duplications, sharing a 68 % identity (Chiu et al. 2015). All three are expressed during Arabidopsis development with AtAPY3 being predominately found in the roots. Both AtAPY4 and AtAPY5 occur in the rosette leaves (Winter et al. 2007). From this, we may speculate that these enzymes have similar functions at different developmental stages. AtAPY6 was confirmed to have a high expression in mature pollen, but knockout mutants of AtAPY6 displayed a minor change in pollen exine pattern under scanning electron microscopy, which means AtAPY6 plays a minor role in pollen development (Yang et al. 2013). In recent years, the molecular analysis of AtAPY7 has confirmed its ubiquitous expression in a range of Arabidopsis tissues and developmental stages, even though the molecular analysis of AtAPY6 and AtAPY7 mutants has indicated minor aberrations to the pollen exine (Chiu et al. 2015).
knockout mutants (AtAPY6 and AtAPY7) show late-anther dehiscence, exine deformation, and low male fertility (Yang et al. 2013).

For several years, extracellular ATP has been proposed as a potential signalling molecule (Clark and Roux 2011a). Plant cells, for example, can release significant quantities of ATP into the extracellular matrix when they are mechanically stimulated (Jeter et al. 2004), as occurs during wounding (Song and Roux 2006), growth (Kim et al. 2006), or stomatal opening (Clark and Roux 2011b). Meanwhile, Yang et al. (2015) have proved AtAPY1 and AtAPY2 can help regulate the concentration of extracellular ATP, which means the two apyrases can play indirect roles in signal transmission.

Subcellular localization and main specific activities of the apyrases

Previously, only AtAPY1 was believed to be localized in the Golgi instead of the extracellular space (Schiller et al. 2012). Later, both AtAPY1 and AtAPY2 were identified in plant Golgi proteomes (Parsons et al. 2012) and their localizations were confirmed by fluorescent protein tagging (Chiu et al. 2012, Schiller et al. 2012). According to current research, AtAPY1, 2, 4, 5, and 7 are localized in the cis-Golgi, and AtAPY3 is localized in the endosome. Some evidence is for localization of AtAPY6 in the endoplasmic reticulum.

Apyrase activities are strictly dependent upon the presence of divalent cations, with Mg²⁺ and Ca²⁺ being the most effective (Chiu et al. 2015). In addition, these enzymes are generally insensitive to specific inhibitors of P-, F-, and V-type ATPases and display a high specific activity (Komoszyński and Wojtczak 1996, Plesner 1995).

Apyrase family members have different substrate specificity (Chiu et al. 2015), and the clade I members, AtAPY1 and AtAPY2, show a clear preference towards UDP and UDP/GDP. This may explain why knocking out either AtAPY1 or AtAPY2 affects UDPase/GDPase activity in microsomal preparations from Arabidopsis. Knocking out AtAPY1 or AtAPY2 resulted in a minor change in the galactose content of cell walls (Chiu et al. 2012). To be more specific, AtAPY1 is an integral membrane protein that shows a clear preference towards UDP. The overexpression of AtAPY2 can lower the sensitivity of Arabidopsis leaves to applied ATP, and AtAPY1 and AtAPY2 are essential for normal plant development. AtAPY1 and AtAPY2 play a key role in glycosylation, and are associated with extracellular ATP (Yang et al. 2015). The clade II member, AtAPY3 has a strong preference of nucleoside triophosphates (NTPs) but demonstrates significant activities toward ADP and GDP. Other members of the clade II apyrase family displayed an array of substrate preferences. AtAPY4 shows only a slight affinity to cytosine triphosphate (CTP), AtAPY5 has the highest catalytic activity for nucleoside diphosphate (NDP), which can turn NDP into NMP, and AtAPY6 show a broad range of substrate activities toward all NTP and NDP substrates, which means it can catalyze both NTP and NDP. The clade III representative, AtAPY7 shows no detectable NTase or NDPase activity. In brief, all AtAPY1-6 enzymes exhibit classic apyrase-like NTase and/or NDPases activities, with no nucleoside monophosphate (NMP) activity, and AtAPY7 does not show NTase or NDPase activity.

What needs to be pointed out is that apyrases are divided into two main types according to their localization. AtAPY1-7, what we have introduced before, belong to the endo-apyrases. The ecto-apyrases belong to those apyrases which are localized in the extracellular matrix. For Arabidopsis thaliana, several functions are related to the ecto-apyrases, including quenching of an ATP signal (Jeter et al. 2004, Tang et al. 2003), playing a role in toxin resistance (Thomas et al. 2000), and being involved in phosphate nutrition (Thomas et al. 1999, Song and Roux 2006). Most of these enzymes are ecto-phosphatases with their N-terminal (and sometimes their C-terminal) domains being anchored in the plasma membrane, with the rest of the protein positioned in the extracellular matrix (Komoszyński and Wojtczak 1996).

Table 1. Location and enzyme activities of apyrases in Arabidopsis thaliana.

<table>
<thead>
<tr>
<th>Member</th>
<th>Location</th>
<th>NTPase or NDPase activities</th>
<th>Preference of substrates</th>
</tr>
</thead>
<tbody>
<tr>
<td>AtAPY1</td>
<td>cis-Golgi</td>
<td>yes</td>
<td>UDP/GDP</td>
</tr>
<tr>
<td>AtAPY2</td>
<td>cis-Golgi</td>
<td>yes</td>
<td>UDP/GDP</td>
</tr>
<tr>
<td>AtAPY3</td>
<td>endosome</td>
<td>yes</td>
<td>NTP, ADP, GDP</td>
</tr>
<tr>
<td>AtAPY4</td>
<td>cis-Golgi</td>
<td>yes</td>
<td>slight affinity for CTP</td>
</tr>
<tr>
<td>AtAPY5</td>
<td>cis-Golgi</td>
<td>yes</td>
<td>NDP</td>
</tr>
<tr>
<td>AtAPY6</td>
<td>endoplasmic reticulum</td>
<td>yes</td>
<td>all NTP and NDP substrates</td>
</tr>
<tr>
<td>AtAPY7</td>
<td>cis-Golgi</td>
<td>none</td>
<td>none</td>
</tr>
</tbody>
</table>
Progress and difficulties in apyrase research in *Arabidopsis thaliana*

Summary of progress:
- a) Seven members of the apyrases are discovered and classified;
- b) Each apyrase is subcellularly localized;
- c) The substrate preferences are determined;
- d) A preliminary understanding of the roles and functions of the apyrases are achieved, with a more complete understanding of the biochemical and subcellular characterization of AtAPY1 and AtAPY2.

The main difficulties in research of the apyrases are:
- a) Only small amounts of apyrases are available; in particular, AtAPY3-7;
- b) The new technologies are developed slowly, and researchers often need to develop specialized and innovative equipment and methods;
- c) Only a small number of researchers and laboratories are engaged in this field of research.

Conclusions

Apyrases play multiple regulatory roles in the cellular activities of *Arabidopsis thaliana*. A better understanding of their different regulatory functions in different tissues may enable to use them in regulating plant growth and enhancing crop production. Although some technological difficulties are present in research of the various members of the apyrase enzyme family, some progress in this area is evident. New technologies are developed to further characterize the apyrases, and the research involving *Arabidopsis thaliana* may prove to be especially useful.

References


Chen, Y., Yordanov, Y.S., Ma, C., Strauss, S., Busov, V.B.: DR5 may prove to be especially useful.


