Identification of ADP-ribosyl cyclase in *Arabidopsis*

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Abstract

In animals, ADP-ribosyl cyclase (ADPR cyclase) is a multifunctional enzyme that catalyses nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP) into two important second messengers, cyclic ADP-ribose (cADPR) and nicotinic acid adenine dinucleotide phosphate (NAADP), respectively. cADPR targets the ryanodine receptor (RyR) present in the sarco/endoplasmic reticulum (SR/ER) and acts as a potent calcium mobiliser in the cell. cADPR elevates cytosolic free Ca^{2+} ($[Ca^{2+}]_{cyt}$) in plants and plays a central role in signal transduction pathways evoked by the drought and stress hormone, abscisic acid (ABA). Despite evidence for the action of cADPR in plants, no predicted proteins with significant similarity to the known ADPR cyclases have been reported in any plant genome database, suggesting that proteins with very low homology or a unique mechanism for cADPR synthesis must exist in plants. Therefore, the main objective of the project was identification of the functional part of the ADPR cyclase enzyme in *Arabidopsis*, responsible for cADPR synthesis. As the activity of this protein is very low in *Arabidopsis*, I used a pharmacological approach with agonists (NaCl, H_2O_2, nitric oxide and cold water) and antagonists (Nicotinamide, 8-Br-cADPR, Mg^{2+}) to identify possible regulators of ADPR cyclase through $[Ca^{2+}]_{cyt}$ measurements and found that nitric oxide (NO) is a potential candidate for increasing the activity of ADPR cyclase. NO can increase the activity of the enzyme 3-fold and nicotinamide inhibits the enzyme activity. cADPR-mediated $[Ca^{2+}]_{cyt}$ oscillations are involved in the circadian clock. CIRCADIAN CLOCK ASSOCIATED1 (CCA1) acts as a repressor of ADPR cyclase activity and in the absence of PSEUDORESPONSE REGULATOR9 (PRR9), *and* PRR579 the activity of CCA1 is increased, thereby reducing the activity of ADPR cyclase in *Arabidopsis*, consistent with the circadian regulation of $[Ca^{2+}]_{cyt}$. I was unable to purify ADPR cyclase protein using traditional biochemical approaches like salt precipitation, different chromatographic techniques, in-gel assay, native and SDS-PAGE due to very low activity being present in *Arabidopsis*.