FEBS Letters 580 (2006) 6783–6788

The polyamine spermine protects against high salt stress in *Arabidopsis thaliana*

Koji Yamaguchi[a], Yoshihiro Takahashi[a], Thomas Berberich[b], Akihiko Imai[c], Atsushi Miyazaki[a], Taku Takahashi[c], Anthony Michael[d], Tomonobu Kusano[a,*]

[a] *Graduate School of Life Sciences, Tohoku University, Sendai, Miyagi 980-8577, Japan*
[b] *Iwate Biotechnology Research Center, Kitakami, Iwate 024-0003, Japan*
[c] *Graduate School of Natural Science and Technology, Okayama University, Okayama 700-8530, Japan*
[d] *Institute of Food Research, Norwich Research Park, Colney, Norwich NR4 7UA, United Kingdom*

Received 18 August 2006; revised 28 September 2006; accepted 24 October 2006

Available online 27 November 2006

Edited by Julian Schroeder

Abstract **It is well known that changes in abiotic conditions such as the concentration of ions, temperature and humidity lead to modulation of polyamine contents in plants. However, little is known about the relevant parts these polyamines play in abiotic stress responses. Here we addressed a specific role of spermine during high salt stress using an *Arabidopsis* double knockout-mutant plant (*acl5*/*spms*) which cannot produce spermine. The mutant showed higher sensitivity to high salt than wild type plants. This phenotype was cured by exogenous spermine but not by the other polyamines putrescine and spermidine, suggesting a strong link between spermine-deficiency and NaCl-hypersensitivity. The mutant was also hypersensitive to high levels of KCl but not to $MgCl_2$ or to high osmoticum. NaCl-hypersensitivity of the mutant was compromised by treatment with Ca^{2+} channel blockers. Moreover, the mutant showed poor growth on Ca^{2+}-depleted Murashige–Skoog agar media. The data suggest that the absence of spermine causes an imbalance in Ca^{2+} homeostasis in the mutant plant. Based on the data obtained, we propose a model for a role of spermine in high salt stress responses.**

Keywords: Calcium homeostasis; Polyamine; Salt stress; Spermine; *Arabidopsis thaliana*

1. Introduction

Polyamines (PAs) are organic polycations found in all living organisms. In higher plants putrescine (Put), spermidine (Spd), and spermine (Spm) are the most abundant PAs [1] and are implicated in various developmental processes [2,3]. Because the levels of PAs increase during the adaptation to stresses in a variety of plants, it is thought that they are also involved in these processes [4]. However, the physiological role of stress-induced PA accumulation remains unknown.

In general, Put is produced by two alternative routes in plant cells: one is the direct conversion of ornithine to put by ornithine decarboxylase, and the other is an indirect route initiated from arginine (Arg) by Arg decarboxylase (ADC) via agmatine. The model plant *Arabidopsis thaliana* lacks ornithine decarboxylase activity which restricts the biosynthesis of PAs to the second pathway [5] with ADC as a key enzyme. Put is subsequently converted to Spd, and then to Spm by the symmetrical addition of an aminopropyl moiety from decarboxylated *S*-adenosylmethionine (SAM), which is produced from SAM by SAM decarboxylase. These steps are mediated through the action of two closely related but distinct enzymes, Spd synthase (SPDS) and Spm synthase (SPMS). In *Arabidopsis*, there are two genes coding for ADC (*ADC1* and *ADC2*), two expressed genes for SAM decarboxylase, two genes (*SPDS1* and *SPDS2*) for SPDS, and two genes (*ACL5* and *SPMS*) for SPMS [6–8].

Recent studies showed that *acl5*/*spms* double knockout mutants lack detectable Spm but show no morphological phenotype except for reduced stem elongation associated with the *acl5* mutation [9], and that both *adc1*/*adc2* and *spds1*/*spds2* double knockout mutants were embryo-lethal [10,11]. Thus it was concluded that Put and Spd are essential for normal growth of *Arabidopsis*, while Spm is not.

Here we asked what functions Spm might play in higher plants. We found that the lack of Spm in the *Arabidopsis acl5*/*spms* mutant causes hypersensitivity to NaCl, possibly due to impaired Ca^{2+}-homeostasis, and discuss how Spm helps the plants to cope with high salt stress.

2. Materials and methods

2.1. Plant material and growth condition

Arabidopsis thaliana ecotype Columbia (Col-0) and its mutant derivative, *acl5*/*spms*, were used in all experiments. Sterilized seeds were placed on 1/2 MS agar (0.8%) plates (pH 5.7) containing 1% sucrose and B5 vitamin. Plates were kept inclined with an angle of ca. 85° and incubated at 22 °C in a growth chamber with a photocycle of 16 h light/8 h dark.

2.2. Various treatments

For salt treatment 10-day-old seedlings were carefully detached from agar plates and transferred to 1/2 MS agar plates containing

*Corresponding author. Fax: +81 22 217 5709.
E-mail address: kusano@ige.tohoku.ac.jp (T. Kusano).

Abbreviations: ADC, Arg decarboxylase; Arg, arginine; CAX, calcium exchanger; CDPK, calcium dependent protein kinase; CLC, chloride channel; GluR, glutamate receptor; MPK, mitogen-activated protein kinase; PA, polyamine; PAO, polyamine oxidase; PCR, polymerase chain reaction; Put, putrescine; RT, reverse transcription; SAM, *S*-adenosylmethionine; SOS, salt overly sensitive; Spd, spermidine; SPDS, Spd synthase; Spm, spermine; SPMS, Spm synthase

0014-5793/$32.00
doi:10.1016/j.febslet.2006.10.078

150–225 mM NaCl. For PA treatment, PAs (1 mM each) were added to MS medium with or without 225 mM NaCl. In case of inhibitor treatments, seedlings were incubated on wet filter paper containing 0.5 mM La^{3+} and 50 μM verapamil, respectively, for 12 h before being subjected to NaCl treatment.

2.3. Chlorophyll quantification

The chlorophyll content was determined according to the method of Lichtenthaler [12].

2.4. Total RNA preparation and reverse transcription (RT)-polymerase chain reaction (PCR) analysis

10–14-day-old seedlings were harvested, frozen in liquid nitrogen and stored at −80 °C until use. Total RNA was prepared by a procedure described by Shirzadegan et al. [13]. RT-PCR was performed as described [14]. *Arabidopsis tubulin* gene was amplified using a pair of specific primers (see supporting appendix) to confirm equal loading. The products were fractionated by electrophoresis on a 1% agarose gel, stained with ethidium bromide, and scanned using a fluorescent image analyzer.

2.5. Real-time quantitative RT-PCR analysis

CAX1, *−2*, *−3* mRNA levels were quantitated by real-time RT-PCR with QuantiTect SYBR Green RT-PCR Kit (Qiagen) and a DNA EngineOpticon System (MJ Research). A standard curve was constructed from *CAX1*, *−2*, and *−3* RNAs and the values were normalized to *tubulin* levels. Primers used for real-time PCR are described in the supporting appendix.

2.6. Statistical treatment of the data

The statistical significance of the experiments was analyzed by Student's *t*-test. Statistical analyses were performed using Microsoft Excel software and Statcel2.

3. Results

3.1. A Spm-deficient mutant is hypersensitive to high salt stress and exogenous Spm cures its phenotype in a specific manner

In *Arabidopsis*, two loci, *acl5* and *spms*, are responsible for Spm synthase activity [8]. An *acl5*/*spms* double knockout mutant is unable to synthesize Spm, but is still able to finish its whole life cycle [9]. This mutant (referred to as Spm-deficient or *acl5*/*spms* mutant in this work) contained similar levels of Put and Spd compared to wild type (WT) plants, and it manifested a dwarf phenotype at a later growth stage, while no apparent difference in growth was observed at earlier stages (up to 3 weeks after germination). Therefore, we used 10–14-day-old seedlings in the following experiments. We initially addressed whether the Spm-deficient mutant is more sensitive/tolerant to increased concentration of NaCl than WT. As seen in Fig. 1A, the *acl5*/*spms* mutant was hypersensitive to 225 mM NaCl accompanied by a 40% lower chlorophyll content than that of WT (Fig. 1B). A similar response was observed at 150 and 200 mM NaCl concentrations (data not shown).

To test further a link between Spm deficiency and increased sensitivity to high salt stress in the *acl5*/*spms* double mutant, PAs were exogenously supplied at 1 mM in an assay system. Under non-stressed conditions, PAs including Spm did not have any effects on the growth of Spm-deficient mutant plants (Fig. 1C). The NaCl-hypersensitivity of the mutant, however, could be rescued specifically by exogenous Spm (Fig. 1C) while Put and Spd at 1 mM each did not have such effects (Fig. 1C).

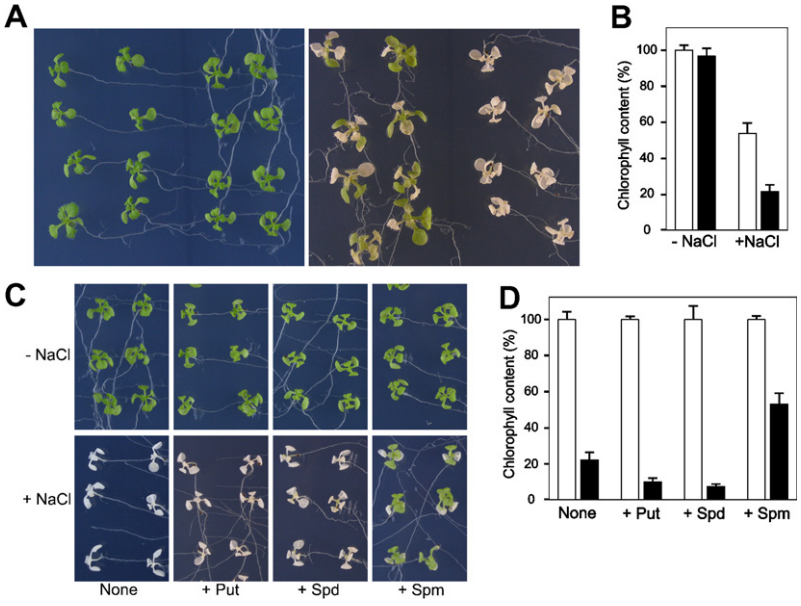

Fig. 1. Spm-deficient *Arabidopsis* plants are hypersensitive to high salt stress and exogenous Spm specifically rescues *acl5*/*spms* seedlings from NaCl-hypersensitivity. (A) Phenotypes of WT and *acl5*/*spms* double mutant plants, grown for 4 days on MS agar media (left panel) or MS agar media containing 225 mM NaCl (right panel) after transfer of 10 days-old seedlings. In each panel, the left side and right side correspond to WT and Spm-deficient mutant plants, respectively. Experiments were repeated more than three times with similar results. (B) Relative chlorophyll content in WT and *acl5*/*spms* seedlings treated with 225 mM NaCl for 4 days as in (A). Means ± S.E. of chlorophyll content in 30 seedlings were calculated for each treatment and the mean value of WT seedlings grown on MS agar media for 4 days after transfer is set as 100%. Open and filled bars indicate WT and *acl5*/*spms* mutant, respectively. (C) PA Spm rescues *acl5*/*spms* seedlings from NaCl hypersensitivity. Phenotypes of Spm-deficient mutant seedlings grown for 4 days on MS agar media (upper panels) and MS agar media containing 225 mM NaCl (lower panels) with or without PAs. From left to right: no addition, Put, Spd, and Spm. Experiments were repeated more than three times with similar results. (D) Relative chlorophyll content in *acl5*/*spms* seedlings grown for 4 days on 1 mM PA-containing MS media in the absence (upper panel) or presence (lower panel) of 225 mM NaCl as in (A). No treatment with NaCl (open bar) and 225 mM NaCl treatment (filled bar).