Stimulation of H\(^+\)-transport activity of vacuolar H\(^+\)-ATPase by activation of H\(^+\)-PPase in *Kalanchoë blossfeldiana*

E. FISCHER-SCHLIEBS, J.-R. MARIAU* and U. LÜTTGE

Institut für Botanik, Technische Hochschule Darmstadt,
Schmittstrasse 3-5, D-64287 Darmstadt, Germany
Max Planck Institut für Züchtungsforschung, Carl-von-Linné-Weg 10, D-50829 Köln, Germany*

Abstract

In *Kalanchoë blossfeldiana* cv. Tom Thumb the initial rate of ATP-dependent H\(^+\)-transport into tonoplast vesicles was stimulated up to three times if the H\(^+\)-ATPase (EC 3.6.1.3) was energized a few minutes after pre-energization of the H\(^+\)-PPase (EC 3.6.1.1). H\(^+\)-PPase-activated ATP-dependent H\(^+\)-transport was observed in plants of *K. blossfeldiana* cultivated in short day (SD) or long day (LD) conditions expressing different degrees of crassulacean acid metabolism (CAM). However, based on the higher activity and protein amount of H\(^+\)-PPase and H\(^+\)-ATPase present in the vacuolar membrane of SD plants the maximum H\(^+\)-transport activity in the stimulated mode of the H\(^+\)-ATPase was significantly higher in tonoplast vesicles of SD plants than of LD plants. Hence, a co-ordinated action of the H\(^+\)-PPase and H\(^+\)-ATPase at the tonoplast of *Kalanchoë* could allow a higher transport capacity at the vacuolar membrane when plants perform high CAM. Immunoprecipitation experiments with an antiserum raised against the A-subunit of the vacuolar H\(^+\)-ATPase of *Mesembryanthemum crystallinum* L. showed that in SD and LD plants of *K. blossfeldiana* the H\(^+\)-PPase was co-precipitated with the vacuolar H\(^+\)-ATPase holoenzyme. The co-precipitation of the two transport proteins indicates a close structural localization of the H\(^+\)-PPase and the A-subunit of the vacuolar H\(^+\)-ATPase.

Additional key words: Crassulacean acid metabolism, photoperiodism, proton transport, tonoplast.

Received 21 June 1996, accepted 5 September 1996.

Abbreviations: CAM - Crassulacean acid metabolism; LD - long day; PP, - inorganic pyrophosphatase; SD - short day; SDS-PAGE - sodium dodecyl sulphate gel electrophoresis.

Acknowledgements: We thank R. Ratjezuk for stimulating discussion. This work was supported by the Deutsche Forschungsgemeinschaft (Bonn, Germany) in the frame of the Sonderforschungsbereich 199 (project B2).

Fax: (+49 6151) 16 4630, e-mail: Fischer-Schliebs@Biol.Bio TH-Darmstadt.De.

169
Introduction

The vacuolar H⁺-PPase (EC 3.6.1.1) is an important component of the tonoplast of plant cells (for review see Leigh et al. 1994). It has been reported to generate an inside positive electrochemical proton gradient of similar magnitude as is established by the vacuolar H⁺-ATPase (EC 3.6.1.3) on the same membrane (Johannes and Reise 1990). Thus, beside the H⁺-ATPase the H⁺-PPase may contribute to the energization of a broad range of H⁺-coupled and electrically coupled transport processes at the vacuolar membrane of higher plant cells. However, up to now it is not clearly understood, why two independent H⁺-translocating enzymes, hydrolyzing ATP or PP, are needed at the tonoplast. According to one idea the H⁺-PPase may indeed conserve the free energy of PP, generated in synthetic reactions in the form of an electrochemical proton gradient at the tonoplast, and hence, may play an important role in regulating the cytoplasmic PP level (Wang et al. 1986, Hoffmann and Bentrup 1989, Rea and Poole 1993).

The idea that the H⁺-PPase alternatively to the H⁺-ATPase might energize the tonoplast could be of importance in plants performing Crassulacean acid metabolism (CAM) where a high capacity for the nocturnal secondary active transport of organic acids, mainly malic acid, into the vacuole is required and the availability of ATP is restricted (Lüttge et al. 1981, Lüttge 1987). However, for thermodynamic reasons due to the steep H⁺-gradients established at the tonoplast during the night and the lower free energy of PP, hydrolysis with respect to ATP it seems unlikely that the H⁺-PPase contributes much to acid accumulation in CAM plants. Nevertheless, it was shown for the CAM plant Kalanchöe daigremontiana that the activation of the H⁺-PPase by its substrate magnesium pyrophosphate kinetically enhanced H⁺-transport across the membrane of isolated tonoplast vesicles by the H⁺-ATPase if the latter was activated by its substrate MgATP a few minutes later (Marquardt-Jarczyk and Lüttge 1990). The stimulation of the H⁺-ATPase by the H⁺-PPase indicated a co-ordinated action of the two H⁺-translocating tonoplast proteins which may be relevant in Kalanchöe species for the functioning of CAM.

In leaves of the CAM plant K. blossfeldiana cv. Tom Thumb the degree of CAM expression is promoted by exposing plants to short day conditions (SD) with a photoperiod of less than 10 h (Bruféret et al. 1982). Thus, K. blossfeldiana can serve as a model system to investigate the impact of CAM on the interaction of the H⁺-PPase and H⁺-ATPase in the vacuolar membrane. The aim of our study was to gain more information on a possible co-operative relationship of the two membrane proteins in another Kalanchöe species and the influence of the degree of CAM expression on this relationship.

Materials and methods

Plants of Kalanchöe blossfeldiana cv. Tom Thumb were grown from seeds in the glasshouse in soil (day/night temperature: 25 - 30/13 - 20 °C, 16-h photoperiod). When plants were 2 month old, they were transferred to a phytotron and exposed for