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*Note: The table contains information about a document related to the effects of calcium-complexing agents, sodium oxalate, and sodium citrate on the stomatal opening and closing of Commelina communis L.*
Effects of Calcium-complexing Agents, Sodium Oxalate and Sodium Citrate on Stomatal Opening and Closing of *Commelina communis* L.

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Abstract

Effects of calcium-complexing agents, sodium oxalate and sodium citrate on stomatal opening and closing of *Commelina communis* L. were investigated.

Addition of these to the bathing medium accelerated stomatal opening in the light, while depressed stomatal closing in the dark.

These results suggest that these agents can combine with Ca\(^{2+}\) in the guard cells, and formation of calcium-complex may activate ATPase responsible for active transport of K\(^{+}\).

Introduction

It has been confirmed that addition of Ca\(^{2+}\) depressed stomatal opening (Fisher, 1972; Fujino, 1967; Pallaghy, 1970; Willmer and Mansfield, 1970, 1971; Thomas, 1970, 1971), while accelerated stomatal closing (Fujino, 1967). However, it has not been well understood that how Ca\(^{2+}\) could regulate stomatal movement.

Fujino (1967) found that Ca\(^{2+}\) chelator EDTA accelerated stomatal opening even in the dark, and suggested that Ca\(^{2+}\)-dependent ATPase might be mainly involved on stomatal movement.

In this study, we discuss the effects of calcium-complexing or extracting agents, sodium oxalate and sodium citrate on stomatal movement.

Material and Methods

*Commelina communis* L. was grown in a greenhouse, and fully expanded leaves were pretreated in the dark and light to obtain closed and open stomata for about ten hours, respectively, floating lower epidermis down on water.

Lower epidermal strips were taken from the same leaf about 5mm X 5mm in
size at the end of both the dark and light treatment. Five strips with closed and open stomata were immersed in 50 mM Tris-maleate buffer (pH 6.5) with and without 100 mM K⁺, respectively.

Calcium-complexing agents, sodium oxalate and tribasic sodium citrate were used at final concentration of 10 mM and 6.7 mM, respectively. Supposing these agents can ionize by 100%, molarity of Na⁺ is 20 mM. Then the effect of addition of 20 mM NaCl was also investigated.

Strips were observed with microscope at × 200 magnification, and standard aperture of each strip was measured by a micrometer. Each value is an average of these standard apertures.

Results and Discussion

Stomatal Opening

Figure 1 shows that stomatal opening in the light was accelerated by addition of 10 mM sodium oxalate and 6.7 mM sodium citrate.

These agents had a greater effect in increasing stomatal aperture, and after four hours the aperture was 20 μm and 19 μm in sodium citrate and sodium oxalate, respectively. Stomatal opening in the presence of 20 mM NaCl was also occurred, resulting in a slight acceleration as compared to the control. However, the aperture was about one-half of that by addition of calcium-complexing agent.

From these results, it is suggested that these agents can combine with Ca²⁺ in the guard cells, and that ATPase responsible for active transport of K⁺ may be activated by the elimination of Ca²⁺ as calcium-complex, resulting in more accelerated stomatal opening. It was also found that ATPase activity by colorimetric assay was stimulated by addition of 1 mM sodium oxalate (Jinno, unpublished data).

These results are similar to the observation of Fujino (1967). In the study of the effect of EDTA on stomata, he found that addition of it accelerated stomatal opening both in the light and dark, and he
suggested that Ca²⁺-dependent ATPase might be mainly involved in stomatal closing.

As addition of 20 mM NaCl had no a strong effect in increasing stomatal aperture, stomatal opening may be mainly caused by the effect of calcium-complexing agents. Higher concentration of Na⁺ may required for accelerating of stomatal opening (Raghavendra et al., 1976).

Stomatal Closing

Figure 2 shows that addition of sodium oxalate and sodium citrate depressed stomatal closing in the dark, following by a slow decrease in aperture. Stomatal closing in the presence of 20 mM NaCl was not effectively depressed, remaining a slight wider aperture than in the control. Closing in 20 mM NaCl was faster than that in calcium-complexing agent.

These results suggest that ATPase may be activated by the formation of calcium-complex as described above, and the activation of ATPase may stimulate uptake of K⁺ into the guard cells, causing the small reduction in aperture.

Fujino (1967) reported that application of 1 mM EDTA inhibited the stomatal closing remaining open stomata, and he suggested there were mechanisms for active uptake and loss of K⁺ by the guard cells, and Ca²⁺ could accelerate the latter, as described above. However, chelation of Ca²⁺ by EDTA may stimulate ATPase activity, remaining open stomata (Jinno, unpublished data).

From present results, Ca²⁺ may play an important role on the stomatal movement. As Ca²⁺ is known to be a very unmobile element, it seems not to move between the guard cells and subsidiary cells. Then, investigation of Ca²⁺ behavior in the guard cells should be done in future.
Literature Cited


