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Stomatal Behaviour after Sonication in *Commelina communis*, *Tradescantia reflexa* and *Vicia faba*

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Abstract

The stomatal opening of epidermal strips of *Commelina communis* L., *Tradescantia reflexa* Raf. and *Vicia faba* cv. Ryosai Issun was observed after ultrasonic treatment. Ultrasonic treatment preferentially disrupted the subsidiary and epidermal cells but did not impair guard cell movement.

After sonication the initially closed stomata pretreated in darkness opened significantly.

The osmotic pressure obtained from plasmolytic method was higher in the guard cells than in the surrounding cells.

No accumulation of potassium salts occurred even in the guard cells of the open stomata after sonication.

After sonication the opening of initially closed stomata may be brought about by the removal of pressure owing to the destruction of surrounding cells.

Complete stomatal closure at higher osmotic pressure of the guard cells may be brought about by the mechanical advantage of the surrounding cells.

Key words : *Commelina communis* — Sonication — Stomatal behaviour — *Tradescantia reflexa* — *Vicia faba*.

Introduction

Stomatal opening and closing are associated with movements of potassium salts into and out of the guard cells, respectively, and it is generally accepted that such accumulations and losses are responsible for the turgor changes leading to changes in aperture.

Squire and Mansfield (1972) described first that in *Commelina communis* the

destruction of the subsidiary and epidermal cells by low pH treatment enhanced the stomatal opening. Recently MacRobbie (1980) and MacRobbie and Lettau (1980) also reported the enhancement of the stomatal opening by the destruction of surrounding cells. Moreover, Itai and Meidner (1978) and Weyers and Travis (1981) showed that the surrounding cells were damaged by peeling and the stomata were relatively widely open compared with those surrounding living epidermal cells.

A Simple method for obtaining functional isolated guard cells in epidermal strips of *Vicia faba* was developed by Ogawa et al., (1978) using a ultrasonic disrupter. This method is very useful for studies on the stomatal movement.

The present paper is concerned with the role of the subsidiary and epidermal cells on the stomatal movement, especially on the stomatal closing in *Commelina communis*, *Tradescantia reflexa* and *Vicia faba*.

Materials and Methods

Plants

Commelina communis L., *Tradescantia reflexa* Raf. and *Vicia faba* cv. Issun Ryosai were grown in a green house at a minimum temperature of 10℃ and under natural illumination. Young fully expanded leaves were used. Immediately after excising leaves, they were kept for about 15 hr in darkness at about 25℃ in order to obtain initially closed stomata, soaking a petiole or the base of leaf blade in distilled water under normal air.

Sonication of epidermis

Lower epidermal strips of dark pretreated leaves were cut to about 5×5 mm and transferred to a glass container of 5 (ID) ×15 (H) mm with distilled water. Using a ultrasonic disrupter (Model UR—2—P, Tomy Seiko Co., Tokyo Iapan), the strips were subjected to 30—60 sec burst at maximum range 10, and transferred to the appropriate bathing medium as described below. After sonication the viability of the guard cells and surrounding cells were juged by their appearances, ability to take up neutral red (0.01% distilled water solution) and to plasmolyze in 0.3—0.5 M mannitol. By these treatments, the guard cell were found to be functional but the surrounding cells to be destroyded and dead.

Stomatal opening

After the stomata of dark pretreated epidermis were ensure to be completely closed, the epidermal strips were immersed in distilled water for 1 hr in darkness.

Immediately after sonication, the aperture were measured as described previously (Jinno and Kuraishi 1982) and transferred to distilled water. For each treatment at least 10 stomatal aperture in each of at least 3 epidermal pieces were measured.

Determination of potassium content

After potassium salt detection using cobaltinitrite test, the potassium content dermination was carried out as described previously (Jinno and Kuraishi 1982).

Determination of osmotic pressure

The osmotic pressure of the guard, subsidiary and epidermal cells was determined by the plasmolytic method using a graded series of mannitol solutions. The concentration required for 50% plasmolysis was estimated for the guard and subsidiary cells.

Results

Fig. 1 shows the increase in the stomatal aperture of *C. communis* after sonication. The initially closed stomata of dark pretreated leaves remained almost closed state at no sonication during the incubation of 60 mim in distilled water. On the other hand, immediately after sonication the initially closed stomata opened to about 7.5 μm, and the stomata remained almost same aperture during the experiment.

In the case of *T. reflexa*, data alone at the start of the expriment was shown (Table 1). On the non-sonicated epidermis, no increase in the stomatal aperture occurred. By sonication of epidermis, the appearance of the guard cells became more

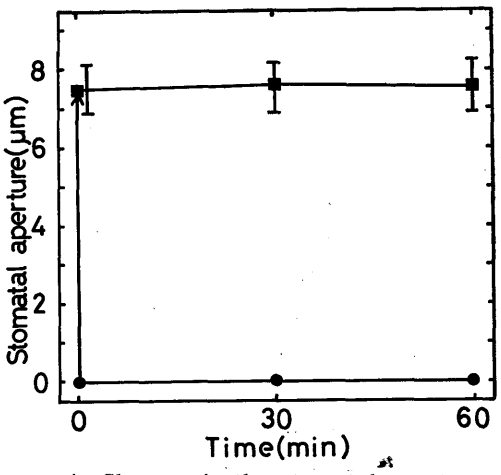

Fig. 1 Changes in the stomatal aperture of dark pretreated leaves of *Commelina communis*. Upward arrow indicates increase in the aperture immediately after sonication. Vertical bars indicate S. E. in three different experiments. -■-, sonicated strips; -●-, non-sonicated strips.

Table 1 Changes in the stomatal aperture and K^+content of dark pretreated leaves of *Tradescantia reflexa* after sonication

Treatment	Width of a pair of guard cells (μm)	Stomatal aperture (μm)	K^+content (μmm^2)
No sonication	30.9±2.0	0	N. M.
Sonication	47.8±2.5	5.9±1.5	N. M.

Data are mean±SE of measurements on at least 30 stomata taken from the same leaf. N. M., not measurable.

inflated and width of a pair of guard cell was about 1.5 times that of intact closed stomata of dark pretreated leaves. The stomata of sonicated epidermis opened to about 6 μm, and the open stomata did not close during the experiment, remaining alomst same aperture (data not shown).

Fig. 2 shows the stomatal behaviour of *V. faba* after appropriate treatments. The stomata of *V. faba* has no morphologically differentiated subsidiary cells. In most epidermal strips, some epidermal cells were ruptured by peeling, and the stomata opened to about 4 μm. The stomatal opening of intact guard cells surrounded by living epidermal cells was not induced in distilled water.

Immediately after sonication initially closed stomata also opened to about 4.5 μm, and thereafter the stomata hardly change in aperture during the experiment.

As shown in Table 2 and 3, histochemical test showed that there appeared to be little changes in potassium salts in the guard cells, although there were significant changes in the stomatal aperture after sonication both in *C. communis* and *V. faba*.

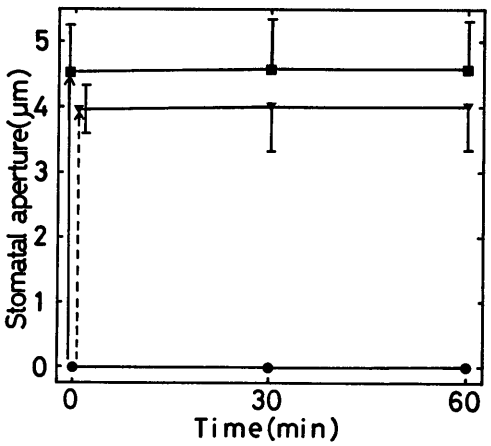

Fig. 2 Changes in the stomatal aperture of dark pretreated of *Vicia faba*. Upward solid and broken arrow indicate increase in the aperture immediately after sonication and peeling, respectively. Vertical bars indicate S. E. in three different experiments. -■-, sonicated epidermal strips; -▼-, epidermal strips with damaged epidermal cells by peeling; -▲-, non-sonicated epidermal strips with intact epidermal cells.

Table 2 Osmotic pressure among the guard cells, subsidiary cells and epidermal cells of closed stomata in *Commelina communis*, *Tradescantia reflexa* and *Vicia faba*

Plants	Guard cells (bars)	Subsidiary cells (bars)	Epidermal cells (bars)
C. communis	3.7	3.2	3.2
T. reflexa	4.2	3.7	3.7
V. faba	4.5	—	3.5

Data are averages of two experiments. —, Not tested because of having no subsidiary cells.

Table 3 Relation between the stomatal aperture and K^+content of open stomata after sonication in *Commelina communis* and *Vicia faba*

Plants	Stomatal aperture (μm)	K^+content (μmm^2)
C. communis	7.5±1.5	N. M.
V. faba	4.5±0.8	N. M.

N. M., not measruable.

The osmotic pressure of the guard, subsidiary and epidermal cells were determined as described above. As shown in Table 2, the osmotic pressure of the guard cells was higher than that of surrounding cells on each species. In *C. commuis* and *T. reflexa* the guard cells are surrounded by six and four subsidiary cells, respectively. and the there was no significant change in the osmotic pressure among the subsidiary cells.

Discussion

The results presented here indicate that the stomatal opening may be brought about by the removal of pressure due to the destruction of surrounding cells during sonication but not by the accumulation of potassium salts into the guard cells.

The present results are essentially similar to those obtained by Squire and Mansfield (1972), MacRobbie (1980) and MasRobbie and Lettau (1980). Those reports showed that in *C. communis* the preferential destruction of surrounding cells by low pH treatment induced the stomatal opening, releasing a pressure against the guard cells. Moreover, Itai and Meidner (1978) and Weyers and Travis (1981) reported that the stomatal opening was induced by the destruction of surrounding cells during peeling.

Results from plasmolytic method indicate that the complete stomatal closure may be induced by the mechanical advantage of the surrounding cells (DeMichele and Sharpe 1973). Since the surrounding cells such a epidermal and subsidiary cells have a larger area of attack than the guard cells, the pressure of surrunding cells can balance or overcome the higher pressure in the guard cells.

Meidner and Bannister (1979) suggested that there were two closing process : one is the partial closure owing to increasing full turgor in the surrounding cells, the other is complete closure due to mild plasmolysis of the guard cells.

However, since it seems unlikely that the plasmolysis occur in the natural condition, the complete closure may be induced by the mechanical advantage of the surrounding cells described above.

In conclusion, the stomatal movements are strictly controlled by the subsidiary and epidermal cells, especially at the complete stomatal closure.

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