Effects of Uncouplers of Phosphorylation on Stomatal Opening and Closing of *Commelina communis*

Masayoshi Fujino and Nobutaka Jinno

Biological Laboratory, Faculty of Education
Nagasaki University, Nagasaki

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Abstract

Effects of uncouplers of phosphorylation, CCCP, dicumarol and sodium salicylate, on stomatal opening and closing were investigated with epidermal strips of *Commelina communis*.

These uncouplers strongly inhibited both stomatal opening and closing in the light and dark. The results strongly suggest that both stomatal opening and closing are active, energy-requiring processes. And it is also suggested that the energy for uptake of K⁺ is derived from both photo- and oxidative phosphorylation and the energy for excretion of K⁺ from oxidative phosphorylation.

Introduction

It has been established that stomatal opening is caused by active uptake of K⁺ into the guard cells (Fujino, 1959, 1967; Fisher, 1968; Fisher and Hsiao, 1968; Thomas, 1970a, b; Willmer and Mansfield, 1970). Moreover, Fujino (1959, 1967) proposed that stomatal closing were active, energy-requiring process. Recently, Fujino's hypothesis was supported by Penny and Bowling (1974) and Raghavendra, Rao and Das (1976).

If, as Fujino (1967) proposed, both stomatal opening and closing are active processes, then both movements should be sensitive to an uncoupler of phosphorylation. There have been several reports about the effect of uncoupler of phosphorylation on stomatal closing (Zelitch, 1961), on both stomatal opening and closing (Fujino, 1959, Pemadasa and Koralege, 1977), and on stomatal opening (Humble and Hsiao, 1970; Willmer and Mansfield, 1970; Mouravieff, 1971). Thus, recent studies on stomatal movement are mainly considered from the point of stomatal opening process. In this work, further observations about effects of several uncouplers on both stomatal opening and closing will be described.

Abbreviation: CCCP, carbonylcyanide-m-chlorophenylhydrazone.
Materials and Methods

Commelina communis cultivated in a greenhouse was used in this experiment.

Open samples: At about twelve on the day of experiment, entire leaves were excised and transferred to the experimental room, and was preexamined by a microscope to ensure that stomata were widely open. Strips with fully opened stomata (approximately 25μm) were used as open samples.

Closed samples: On the evening of the previous day, entire leaves were excised and transferred to the experimental room, and kept floating on the water in an incubator in order to close stomata at 30°C for one night. Virtually most stomata closed completely. Abaxial epidermis were stripped from the same leaf about 5×5 mm in size. Five strips were immersed in 5ml of the bathing medium, and kept in the light of 8000 lux for four hours, maintaining at 30°C. At least 20 stomata per each strip were measured at the middle of strips by a microscope every one hour. Each value is an average of at least 100 individual stomatal apertures.

Control medium for stomatal opening was 60 mM phosphate buffer of pH 6.0 containing 75 mM KCl, while control medium for stomatal closing was 60 mM phosphate buffer of pH 6.0 without KCl.

CCCP (0.1, 0.01 and 0.001 mM), dicumarol (3, 3′ methylen–bis–(4-hydroxycumarin)) (0.1, 0.01 and 0.001 mM) and sodium salicylate (10, 5 and 1 mM) were made up in both the bathing medium for opening and closing. CCCP contained up to 0.2% ethanol, which was necessary to dissolve it. Stomatal movement is not affected by 0.2% ethanol.

Results

Stomatal opening and closing in the control medium.

As shown in Fig. 1, when epidermal strips with completely closed stomata were incubated in the control medium containing 75 mM KCl, stomatal opening in the light was progressively stimulated as the time proceeds. After four hours, stomatal apertures were 20μm in the light. While, stomatal closing in the control medium without KCl was very rapid in the dark, especially within first one hour (Fig. 2). After one hour, apertures decreased from 25μm to 9μm. Thereafter, stomatal closing gradually occurred, and the aperture was 3.2μm after four hours.

Effect of CCCP.

As shown in Fig. 1 and 2, in the presence of CCCP, stomatal opening in the light and closing in the dark were strongly inhibited. When 0.1mM CCCP was applied, the inhibition of opening and closing was greatest, remaining almost closed and opened stomata in the light and dark respectively. At 0.01mM, only a slight opening and closing occurred. Even at 0.001mM, stomatal opening and closing were considerably inhibited, and stomata opened to 9.3μm after four hours in the light. While, apertures of opened stomata decreased from 25μm to 24μm after four hours in the dark.

Effect of dicumarol.

As shown in Fig. 3 and 4, the addition of dicumarol to the bathing medium gave
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Fig. 1. Effect of CCCP on stomatal opening in the light. Closed samples were incubated in the control medium for opening (○—○), 10^{-3} M (□ — □), 10^{-4} M (△—△) and 10^{-5} M CCCP (▼—▼).

Fig. 2. Effect of CCCP on stomatal closing in the dark. Open samples were incubated in the control medium for closing (■—■), 10^{-3} M (■ — ■), 10^{-4} M (▲—▲) and 10^{-5} M CCCP (▼—▼).

a strong inhibition of stomatal opening and closing. At a concentration of 0.1 mM, stomata maintained almost initial state, that is, stomatal opening in the light and closing in the dark scarcely occurred. At 0.01 mM, only a slight opening and closing occurred. Even at 0.001 mM, stomatal opening and closing were considerably inhibited. Stomata opened to 14.7 μm after four hours in the light. While, apertures of opened stomata decreased from 25 μm to 10.7 μm after four hours in the dark.

Effect of sodium salicylate.

As shown in Fig. 5 and 6, by the addition of sodium salicylate, both stomatal opening and closing were strongly inhibited. However, higher concentration of it required to bring about the same inhibition of stomatal movement as CCCP and dicumarol.

At 10 mM, stomata remained almost closed and opened in the light and dark respectively. At 5 mM, considerable inhibition was found, and slight opening and closing was occurred. The addition of 1 mM had no effect on stomatal movement, and stomata opened to 18.6 μm after four hours in the light. While, stomatal apertures of opened stomata decreased from 25 μm to 5.3 μm in the dark.
Fig. 3. Effect of dicumarol on stomatal opening in the light. Closed samples were incubated in the control medium for opening (○—○), 10^{-3} M (□—□), 10^{-4} M (△—△) and 10^{-5} M dicumarol (▽—▽).

Fig. 4. Effect of dicumarol on stomatal closing in the dark. Open samples were incubated in the control medium for closing (●—●), 10^{-3} M (■—■), 10^{-4} M (▲—▲) and 10^{-5} M dicumarol (▼—▼).

Fig. 5. Effect of sodium salicylate on stomatal opening. Closed samples were incubated in the control medium for opening (○—○), 10^{-2} M (□—□), 1/2 x 10^{-2} M (△—△) and 10^{-3} M sodium salicylate (▽—▽).

Fig. 6. Effect of sodium salicylate on stomatal closing. Open samples were incubated in the control medium for closing (●—●), 10^{-2} M (■—■), 10^{-3} M (▲—▲) and 10^{-4} M sodium salicylate (▼—▼).

Discussion

The use of uncouplers of phosphorylation in *Commelina communis* provided useful informations to investigate the mechanism of stomatal movement.

As described in the introduction, recent works reached the conclusion that stomatal opening was associated with the active uptake of K⁺ into the guard cells. However, it is not clear regarding to the synthesis of energy for the uptake of K⁺.

Some experiments were described, indicating that ATP derived from cyclic photophosphorylation was driving force for stomatal opening in the light (Humble and Hsiao, 1969, 1970; Willmer and Mansfield, 1970), and that ATP derived from non-cyclic photophosphorylation was involved in the uptake of K⁺ in the light (Zelitch, 1965, 1969; Sawhney and Zelitch, 1969). On the other hand, Allaway and Mansfield (1967) reported that photophosphorylation was not essential for stomatal opening in response to changes in CO₂ concentration, suggesting that ATP may be produced by another mechanism.

Willmer and Mansfield (1970) found that PNP, an effective uncoupler of oxidative phosphorylation, had no effect on stomatal opening in the dark in *Commelina communis* and suggested that oxidative phosphorylation was not involved in stomatal opening in the dark, and that the energy for the uptake of K⁺ came from the electron-transfer reactions.

Mouravieff (1971) also found that DNP had no effect on stomatal opening in the light in *Veronica beccabunga* L. and *Lythrum salicaria* L., and proposed that glycolytic and photosynthetic glyceraldehyde phosphate dehydrogenase may play an important role on stomatal opening.

On the other hand, Turner (1972, 1973) found that DNP inhibited stomatal opening both in the light and dark in epidermal strips of *Phaseolus vulgaris* L. var. Pinto, suggesting that oxidative phosphorylation may play essential role, while photophosphorylation provides an additional energy source.

There has been a few works as to stomatal closing process. Williams (1954) and Stålfelt (1957) proposed that stomatal closing was active, energy-requiring transport of water of the guard cells. However, Heath and Orchard (1956) disproved the William’s hypothesis, indicating that only stomatal opening was an active process. Zelitch (1965) also proposed that only stomatal opening was an active process, since the temperature has...
no effect on stomatal closing in the dark. While, Pemadasa and Koraleg (1977) found that DNP inhibited both stomatal opening and closing, and suggested that both stomatal opening and closing were mainly dependent on ATP derived from oxidative phosphorylation.

It has been demonstrated that sodium salicylate could uncouple oxidative phosphorylation in animal connective tissues (Whitehouse, 1964), and that dicumarol depressed the rate of aerobic phosphorylation in rat liver mitochondria (Martius and Nitz-Litzow, 1953). In this experiment, the application of these compounds had strong effects on the stomatal opening and closing. The effects are similar to that of CCCP. These may act as an uncoupler of phosphorylation in plant cell.

Present results strongly suggest that both stomatal opening and closing are also active energy-requiring process. The energy source for the active uptake of K+ in the light may be provided by both photosynthetic and oxidative phosphorylation. While, the energy source for the active excretion in the dark may be provided by oxidative phosphorylation. The inhibition of phosphorylation with uncouplers may cause less ATP becoming available to bring about the uptake and excretion of K+. Still, it seems that diffusional transport of K+ out of the guard cells may also partly involved in stomatal closing.

It was noticeable that ATPase activity of lower epidermal strips of Commelina benghalensis possessed two optimal pH (Raghavendra, Rao and Das, 1976). One of the two was 5.5 (ATPase 5.5), and the other was 7.5 (ATPase 7.5). They proposed that ATPase 7.5 associated with stomatal opening and ATPase 5.5 with stomatal closing.

Using potassium sensitive microelectrodes, it was found that potassium transport both into and out of the guard cells was an active process (Penny and Bowling, 1974).

Literature Cited


Humble, G. D. and T. C. Hsiao. 1969. Specific requirement of potassium for light-activated open-
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