Sci. Bull. Fac. Educ., Nagasaki Univ., No. 20, pp.57~66 (1969)

Effect of Monoiodoacetic Acid on Stomatal Movement

Masayoshi FUJINO

Biological Institute, Faculty of Education,
Nagasaki University, Nagasaki

(Received November 30, 1968)

Effect of monoiodoacetic acid on stomatal movement was studied using lower epidermis of *Commelina communis* L.. In the medium containing 10^{-4} M monoiodoacetic acid stomatal opening and potassium absorption into guard cells were remarkably accelerated both in the light and in the dark.

On the other hand, in the medium containing 10^{-4} M monoiodoacetic acid stomatal closing and excretion of potassium from guard cells were remarkably inhibited both in the light and in the dark.

These results support following hypothesis presented by present authors (Fujino 1967): Stomatal movement is caused by active transport of potassium between guard cells and mesophyll. ATP in guard cells is involved in absorption of potassium, while ATPase is responsible in excretion of potassium from guard cells. Most of ATP which is necessary for the stomatal movement is produced by respiration. Light is probably involved in ATPase in-activation.

Introduction

As seen in the literature of Ketellapper (1963), active energy requiring mechanism on stomatal movement have been suggested because of the disappointing state of classical "photosynthesis theory".

Since Williams (1954) suggested that stomatal closing is caused by active pumping out of water, several studies along this line have been reported (Heath & Orchard 1956, Stålfelt 1957 & 1962, Zelitch & Walker 1964, Mansfield 1965). However, there is still disagreement even as to whether closing or opening is the active process.

The present author proposed that stomatal movement is caused by active transport of potassium between guard cells and mesophyll (Fujino 1959 a & 1959 b), ATP in guard cells is involved in absorption of potassium, while ATPase is responsible in excretion of potassium from guard cells (Fujino

1967).

In this experiment, to confirm this view the effects of monoiodoacetic acid which is inhibitor of ATPase on the stomatal movement were studied. During the studies, particular attention has been paid on the role of potassium migration in the stomatal movement.

Material and Methods

Commelina communis L. was used in this experiment as material.

Open sample : Leaves were cut off from the plant and floated on the water for about 2 hours under a light of about 10,000 lux to open stomata.

Closed sample : Branches cut off from the plant were put in the water, then was kept in the dark for 6—12 hours.

Lower epidermis with fully open stomata or completly closed stomata were stripped respectively from the same leaf in about 0.5—0.7cm^2 in size. Two strips were immersed in the medium of tube bottle of 3 cm in diameter, then put in the light or dark. After a fixed time, measurement of stomatal width were carried out. Samples were placed on holded slide glass with a drop of medium and stomatal width was measured at middle of the strips by a micrometer. Although there is some variation in size among many stomata that are present in single strip of epidermis, at least two-third of them have almost the same aperture. The size of this aperture was used to express the extent.

Control medium for opening movement consisted of 18.7 ml of 1/15 M phosphate buffer (PH 5.59) and 1.3ml of 1 M KCl. In some case, distilled water was used. Control medium for closing movement consisted of 20 ml of 1/15M phosphate buffer (pH 5.59) was used. Distilled water was also used in some case. Light and dark treatment, temperature treatment were same as described in previous paper (Fujino 1967).

ATPase activity were detected as follows ; strips were fixed with cold aceton for 20 minutes, washed with water for 20 minutes, then incubated in a medium containing of 5 ml of 0.2 M acetate buffer (pH 5.6), 1 ml of 0.1 M lead nitrate, and 1 ml of 0.05 M ATP (disodium salts) at 37°C. After incubation for 2 hours the strips were washed with water for 20 minutes, then immersed into 5% ammoniumsulfide for about 2 minutes. The distribution of black lead sulfide in epidermis was observed by microscope.

Potassium ions were detected by Macallums' cobalt nitrite method with initial samples from which samples were stripped and with the strips treated with medium for 4 hours.

Experimental Results

(a) Effect of monoiodoacetic acid on the stomatel opening.

In the light, after 1 hour stomata opened to 4.3 μ in width in control medium and 6.0 μ after 4 hours as shown in table 1-A. In the medium containing 10^{-5} M monoiodoacetic acid in final concentration stomata opened at almost same rate as control. In the medium containing 10^{-4} M monoiodoacetic acid stomata opened to 4.0 μ in width after 0.5 hour, 8.6 μ after 1 hour and 14.0 μ after 4 hours. Stomatal opening was thus remarkably accelerated with 10^{-4} M monoiodoacetic acid. In the medium containing 10^{-3} M monoiodoacetic acid stomata did not open after 2 hours but opened to only 3.0 μ after 3 hours.

Potassium ions were little seen in guard cells of initial samples, and was seen in guard cells of the strips after 4 hours, especially the quantity of potassium ions became large in the strips treated with 10^{-4} M monoiodoacetic acid as shown in table 1-A. Thus, potassium content in guard cells increased accompanied by stomatal opening.

On the other hand, in the dark, as shown in table 1-B stomata began to open after 1 hour and 4.3 μ in width after 4 hours in control. In the dark opening rate was thus slower than in the light. In the medium containing 10^{-5} M monoiodoacetic acid stomatal opening slightly accelerated. In the medium containing 10^{-4} M monoiodoacetic acid stomata opened to 4.0 μ in width after 0.5 hour and 14.0 μ after 4 hours. Thus, even in the dark stomatal opening was almost same as in the light. In the medium containing 10^{-3} M monoiodoacetic acid stomata did not open at all after 4 hours.

The quantity of potassium ions increased accompanied by stomatal opening. The quantity of potassium ions in guard cells was large which were treated with 10^{-4} M monoiodoacetic acid after 4 hours.

Table 1.* Effects of monoiodoacetic acid on stomatal opening and potassium content in guard cells of *Commelina communis*. In each plot, upper line shows stomata width (μ); lower line, potassium content.

A. Light, 10 K lux, 25°C.

Time(h.)	0	0.5	1	2	3	4
Control	0	0	4.3	4.9	5.9	6.0
	±					++
10^{-5} M monoiodoacetic acid	0	0	4.1	4.5	4.5	6.2
	±					++
10^{-4} M monoiodoacetic acid	0	4.0	8.6	8.6	10.8	14.0
	±					+++
10^{-3} M monoiodoacetic acid	0	0	0		3.0	3.0
	±					+

B. Dark, 25°C.

Time(h.)	0	0.5	1	2	3	4
Control	0 ±	0	2.3	3.0	4.3	4.3 +
10^{-5} M monoiodo-acetic acid	0 ±	0	4.3	5.0	5.0	6.2 ++
10^{-4} M monoiodo-acetic acid	0 ±	4.0	7.0	7.0	10.0	14.0 +++
10^{-3} M monoiodo-acetic acid	0 ±	0	0	0	0	0 ±

Control medium consisted of 18.7ml of 1/15M phosphate buffer (PH 5.59) and 1.3 ml of 1M KCl. Potassium content is expressed as follows in decreasing order: ++++, +++, ++, +, ± (presence of potassium was doubtful).

Distilled water (pH 6.3) was used as control medium instead of phosphate buffer containing KCl. Medium containing monoiodoacetic acid were adjusted with NH_4OH to keep pH 6.3. As shown in table 2, in the light stomata did not open at all after 4 hours in the medium containing 10^{-4} M and 10^{-3} M monoiodoacetic acid as same as control. Potassium ions were little seen in guard cells after 4 hours.

Table 2. Effects of monoiodoacetic acid on stomatal opening and potassium content in guard cells of *Commelina communis*. Light, 10K lux, 23°C.

Time(h.)	0	1.5	1	2	3	4
Control	0 ±	0	0	0	0	0 ±
10^{-4} M monoiodo-acetic acid	0 ±	0	0	0	0	0 ±
10^{-3} M monoiodo-acetic acid	0 ±	0	0	0	0	0 ±

Control medium consisted of 20 ml of distilled water (PH 6.3).

(*b*) *Effect of monoiodoacetic acid on stomatal closing.*

In the light, stomata closed to 14.0 μ in width after 1 hour and 8.6 μ after 4 hours as shown in table 3-A in control. In the medium containing 10^{-5} M monoiodoacetic acid stomatal width decreased almost as same as control. In the medium containing 10^{-4} M monoiodoacetic acid stomatal width decreased to 17.2 μ after 0.5 hour, then did not decrease after 3 hours and 14.0 μ after 4 hours. Thus, stomatal closing was remarkably inhibited with 10^{-4} M

monoiodoacetic acid. In the medium containing 10^{-3} M monoiodoacetic acid stomata closed to 19.3 μ after 0.5 hour and 17.0 μ after 2 hours. Thus, stomatal closing was remarkably inhibited, but stomata closed to 6.0 μ after 3 hours and 4.3 μ after 4 hours, stomata width thus decresed rapidly after 2 hours.

Large quantity of potassium ions were seen in guard cells of initial samples and decreased accompanied by the decrese of stomatal width. Large quantity of potassium ions were seen in guard cells of the strips treated with 10^{-4} M monoiodoacetic acid after 4 hours.

On the other hand, in the dark stomata closed to 10.0 μ after 0.5 hour and 5.0 μ after 4 hours in control as shown in table 3—B. Thus, in the dark the rate of stomatal closing was rapid compared with the rate in the light. In the medium containing 10^{-5} M monoiodoacetic acid stomatal closing was almost same as control. In the medium containing 10^{-4} M monoiodoacetic acid stomata closed to 15.1 μ after 0.5 hour and 14.0 μ after 4 hours. Stomatal

Table 3. Effects of monoiodoacetic acid on stomatal closing and potassium content in guard cells of *Commelina communis*.

A. Light, 10K lux, 25°C.

Time(h.)	0	0.5	1	2	3	4
Control	23.0 ++++	17.0	14.0	9.0	8.6	8.6 ++
10^{-5} M monoiodoacetic acid	23.0 ++++	17.2	15.0	10.8	8.6	8.6 ++
10^{-4} M monoiodoacetic acid	23.0 ++++	17.2	17.2	17.2	17.2	14.0 +++
10^{-3} M monoiodoacetic acid	23.0 ++++	19.3	19.0	17.0	6.0	4.3 +

B. Dark, 25°C.

Time(h.)	0	0.5	1	2	3	4
Control	23.0 ++++	10.0	8.6	8.0	5.0	5.0 +
10^{-5} M monoiodoacetic acid	23.0 ++++	10.0	8.6	8.0	6.0	6.0 ++
10^{-4} M monoiodoacetic acid	23.0 ++++	15.1	15.1	15.1	14.4	14.0 +++
10^{-3} M monoiodoacetic acid	23.0 ++++	19.0	17.2	17.2	10.8	8.6 ++

Control medium consisted of 20ml of 1/15M phosphate buffer (PH 5.59).

closing thus remarkably inhibited with 10^{-4} M monoiodoacetic acid. In the medium containing 10^{-3} M monoiodoacetic acid stomata closed to 19.0 μ after 0.5 hour, 17.2 μ after 2 hours and 8.6 μ after 4 hours.

Large quantity of potassium ions were seen in guard cells of initial samples, but after 4 hours there were a little potassium ions except the strips treated with 10^{-4} M monoiodoacetic acid.

Effect of monoiodoacetic acid on stomatal closing in the dark was observed using distilled water as control medium instead of phosphate buffer. As shown in table 4, stomata closed to 2.2 μ after 0.5 hour and 0 μ after 1 hour. In the medium containing 10^{-4} M monoiodoacetic acid stomata closed to 4.3 μ after 0.5 hour and closed completly after 1 hour. In the medium containing 10^{-3} M monoiodoacetic acid stomata closed to 8.6 μ after 1 hour and 5.6 μ after 4 hours. Stomatal closing thus inhibited.

On the other hand, large amount of potassium ions were seen in guard cells of initial samples, but after 4 hours potassium ions were little seen except the strips treated with 10^{-3} M monoiodoacetic acid in which a little potassium ions were seen.

Table 4. Effects of monoiodoacetic acid on stomatal closing and potassium content in guard cells of *Commelina communis*. Dark, 23°C.

Time	0	0.5	1	2	3	4
Control	24.2 ++++	2.2	0	0	0	0 ±
10^{-4} M monoiodoacetic acid	24.2 ++++	4.3	0	0	0	0 ±
10^{-3} M monoiodoacetic acid	24.2 ++++	8.6	6.0	6.0	6.0	5.6 ++

Control medium consisted of 20ml of distilled water (PH 6.3).

(c) *Effect of monoiodoacetic acid on ATPase activity.*

Effect of 10^{-3} M and 10^{-4} M monoiodoacetic acid on ATPase activity in guard cells of closed samples were observed after 2 hours incubation.

Fig. 1 showed these results. In control, strong ATPase activity was seen in nucleus, protoplasm menbran, and cytoplasm. In the medium containing 10^{-4}M monoiodoacetic acid, weak ATPase activity was seen in these parts of guard cells, and in the medium containing 10^{-3} M monoiodoacetic acid ATPase activity was very weak. Thus, ATPase activity was inhibited with monoiodoacetic acid.

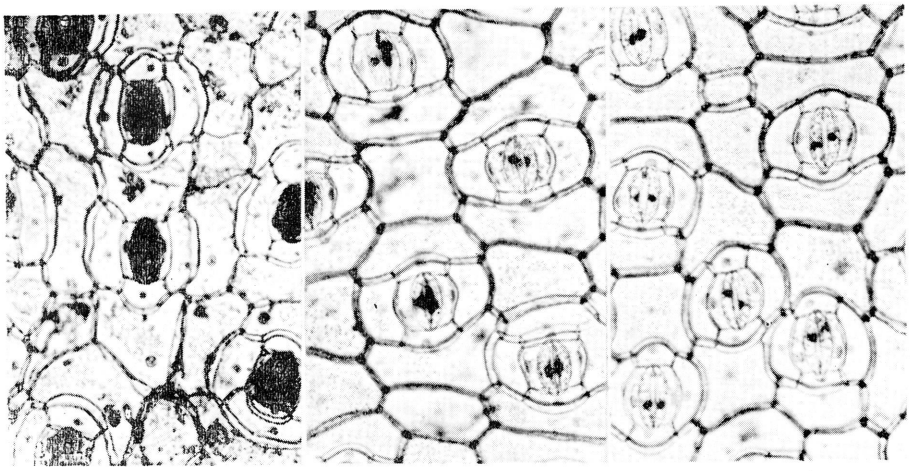

Fig. 1. Effect of monoiodoacetic acid on ATPase activity in guard cells of *Commelina communis* L. with closed stomata in the dark : left, in control medium ; middle, in medium containing 10^{-4}M monoiodoacetic acid ; and right, in medium containing 10^{-3}M monoiodoacetic acid. × 100

Discussion

Since Williams (1954) first assumed that active, energy-consuming process is involved in stomatal movement, there has been increasing support of this idea. However, there has been a confusion as to whether active process is involved in stomatal opening and or stomatal closing.

Recently, Zelitch (1961, 1965) reported that ATP derived from non-cyclic photophosphorylation in guard cells is probably the driving force responsible for the increased turgor in guard cells and any method of interfering with the synthesis of ATP near the guard cells or of stimulating its breakdown might then cause stomatal closure. Metabolism of glycolic acid is probably as essential for the synthesis of ATP, especially at low concentration of carbon dioxide (Zelitch and Walker 1964).

However, Mansfield (1965), Heath, Mansfield and Meidner (1965) reported that appreciable formation of glycollate is not essential for light induced opening. Meidner and Mansfield (1966) showed that glycollate had no influence on the stomata by the experiment treated with α-hydroxysulphonate and glycollate, and they reported that stomatal opening and closing should be considered as involving components both depent upon, and indepent of CO_2 concentration (Mansfield & Meidner 1966). Meidner (1968) concluded that blue light may promote stomatal opening by its effect on enzymes controlling the starch and polysaccharide content of guard cells. Kuiper (1964) reported

that photosynthesis of the guard cell chloroplasts is responsible for the maintance of stomatal movement. Probably ATP produced by photophosphorylation may be involved in stomatal movement.

On the other hand, it was observed that potassium content in guard cells is intimately correlated with stomatal movement (Imamura 1943, Yamashita 1952). The present author proposed that stomatal movement is caused by a active transport of potassium between guard cells and mesophyll. (Fujino 1959 a & b). MacRobbie (1965) suggested that in *Nitella* potassium uptake is supported by light through the utilization of ATP produced in photophosphorylation. Sigenthaler and Packer (1965) proposed the mechanism of stomatal control based upon the regulation of chloroplast volume by light-and energy-dependent processes. Raschke (1966) suggested that osmoregulation in guard cells is achieved by energy-dependent ion translocations in mitocondria and chloroplast ; turgor adjustments are made through changes in the permeabilities for water and solutes, brought about by CO_2 and light. Rains (1967) reported that the rate of absorption of potassium by slices of corn leaf in the light was about twice the rate in the dark : In the light, energy was closely linked to photosynthetic reaction : in the dark, it was linked to respiratory process. Fischer believe active K^+ uptake is the cause of stomatal opening : and this was confirmed by $^{86}Rb^+$ tracer work (personal communication).

As mentioned above, most of these investigators suggested that stomatal opening is active process and that uptake of potassium is supported by light through the utilization of ATP produced in photophosphorylation.

When leaf with open stomata is put in the dark, stomata close rapidly accompanied by the excretion of potassium from guard cells. In these investigations, however, there are little on the mechanism of rapid closing and excretion of potassium from guard cells in the dark.

The present author proposed that the driving mechanism of potassium transport exists in guard cells. ATP in guard cells is involved in absorption of potassium, while ATPase in guard cells is resposible in excretion of potassium from guard cells. Most of ATP which is necessary for the stomatal movement is produced by respiration. Stomatal movement depends on the balance of action of ATP and ATPase. Light is probably involved in ATPase in-activation (Fujino 1967).

In this experiment, in control medium opening is rapid in the light than in the dark. However, in the medium containing 10^{-4} M monoiodoacetic acid, stomatal opening and absorption of potassium in the dark were remarkably accelerated as same as in the light. In the medium containing 10^{-3} M monoiodoacetic acid stomatal opening was remarkably inhibited probably because of inhibition of other metabolic process by high concentration of inhibitor.

When distilled water was used as control medium, stomata did not open both in control and in medium containing monoiodoacetic acid because of absence of potassium ions.

On the other hand, in control, stomatal closing is rapid in the dark than in the light. However, in the medium containing 10^{-4} M monoiodoacetic acid stomatal closing and excretion of potassium from guard cells were remarkably inhibited both in the dark and in the light.

ATPase activity in guard cells was remarkably inhibited with 10^{-4} M monoiodoacetic acid.

The present author reported that stomatal opening and potassium absorption were accelerated by addition of ATP to the medium in the light and that para-mercuric benzoate and NaF which were inhibitors of ATPase accelerated stomatal opening and potassium absorption both in the light and dark (Fujino 1967).

As mentioned above, similar results were obtained by present experiment treated with monoiodoacetic acid. The present experimant support the authors' hypothesis concerning the role of ATP and ATPase on stomatal movement.

Literature Cited

1) Fujino, M. 1959. a. Correlation among the stomatal movement, pH, salts concentration, starch content and osmotic value in *Commelina* (in Japanese). *Kagaku*, **29**, 147—148.

2) _____. 1959. b. Stomatal movement and active migration of potassium (in Japanese). *ibid*, **29**, 660—661.

3) _____. 1967. Role of adenosinetriphosphate and adenosinetriphosphatase in stomatal movement. *Science Bull. Facu. Edu. Nagasaki Univ.*, No. **18** 1—47.

4) Heath, O. V. S. & B. Orchard. 1956. Studies in stomatal behavior. Vll. Effects of anairobic conditions upon stomatal movement-A test of williams' hypothesis of stomatal mechanism. *Jour. Exp. Bot.*, **7**, 313—325.

5) Heath, O. V. S., T. A. Mansfield & H. Meidner. 1965. Light-induced stomatal opening and the postulated roles of glycolic acid. *Nature*. **207**, 960—962.

6) Imamura, S. 1943. Untersuchgen über den Mechanismus der Turgorschwankung der Spaltöffnung Schliesszellen. *Jap. Jour. Bot.*, **12**, 251—346.

7) Ketellapper, H. J. 1963. Stomatal physiology. *Ann. Rev. Plant Physiol.*, 14, 249—270.

8) Kuiper, P. J. C. 1964. Dependence upon wavelength of stomatal movement in epidermal tissue of *Senecio odoris. Plant Physiol.*, **39**, 952—955.

9) MacRobbie, E. A. C. 1965. The nature of the coupling between light energy and active ion transport in *Nitella traslucens. Biochem. Biophys.* Acta. **94**, 64—73.

10) Mansfield,, T. A. 1965. Glycolic acid metabolism and the movement of stomata. *Nature*. **205**, 617—618.

11) Mansfield, T. A. & H. Meidner. 1966. Stomatal opening in light of different wavelengths: Effects of blue light independent of carbon dioxide concentration. *Jour. Exp. Bot.*, **17**, 510—521.

12) Meidner, H. & T. A. Mansfield. 1966. Rate of photosynthesis and respiration in relation to stomatal movements in leaves treated with α-hydroxysulphonate and glycollate. *Jour, Exp, Bot,*, **17**, 502—509.

13) Meidner, H. 1968. The comparative effects of blue and red light on the stomata of *Allium cepa* L. and *Xanthicum pennsylvanicum*. *Jour. Exp. Bot.*, **19**, 146—151.

14) Rains, D. W. 1967. Light-enhanced potassium absorption by corn leaf tissue. *Science*, **156**, 1382—1383.

15) Raschke, K. 1966. Die Reaktionen des CO_2-Regelsystems in den Schliesszellen von *Zea Mays* auf Weissen Licht. *Planta*, **68**, 111—140.

16) Sigenthaler, P. A. & L. Packer. 1965. Light-dependent volume changes and reactions in chloroplasts 1. Action of alkenylsuccinic acids and phenylmercuric acetate and possible relation to mechanisms of stomatal control. *Planl Physiol.*, **40**, 785—791.

17) Stålfelt, M. G. 1957. The water output of the guard cells of the stomata. *Physiol. Plant*, **10**, 752—773.

18) _____. 1962. The effect of temperature on opening of the stomatal cells. *ibid.*, **15**, 772—779.

19) Williams, W. T. 1954. A new theory of the mechanism of stomatal movement. *Jour. Exp. Bot.*, **5**, 343—352.

20) Yamashita, T. 1952. Influence of potassium supply upon various properties and movement of the guard cell. *Sieboldia*, **1**, 51—70.

21) Zelitch, I. 1961. Biochemical control of stomatal opening of leaves. *Biochemistry*, **47**, 1423—1433.

22) Zelitch, I. & D. A. Walker. 1964. The role of glycolic acid metabolism in opening of leaf stomata. *Plant Physiol.*, **39**, 856—862.

23) Zelitch, I. 1965. Environmental and biochemical control of stomatal movement in leaves. *Biol. Rev.*, **40**, 463—482.