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Effects of Sodium Azide and Potassium Cyanide on the Stomatal Opening and Closing of *Commelina communis* L.

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Abstract

The effects of sodium azide and potassium cyanide on the stomatal opening and closing of epidermal strips of *Commelina communis* L. was observed both in the light and dark. It was found that these inhibitors inhibited both stomatal opening and closing in the light and dark respectively, suggesting that the stomatal movement is considered to function by affecting the metabolic reactions.

Introduction

The mechanism of the stomatal movement has been investigated by many workers, however, it seemed not to be still clear.

Fujino (1967) reported that the active transport of K⁺ into and out of guard cells is responsible for the stomatal movement.

Fisher (1968), and Fisher and Hisao (1968) also found that stomata in epidermal strips of *Vicia fava* opened in response to light when the strips floated on solutions containing K⁺, and estimated K⁺ uptake quantitatively with ⁸⁶Rb and found that the uptake of K⁺ closely paralleled the concomitant increase in stomatal aperture.

Moreover, quantitatively estimations with electron microprobe was done in both tobacco (Sawhney and Zelitch, 1969) and *Vicia fava* (Humble and Raschke, 1971), and confirmed that K⁺ concentration in guard cells is correlated with stomatal aperture. Thus, the stomatal movement can be considered to function by affecting the metabolic reactions.

There are several reports on the effects of chemical inhibitors, azide and cyanide, on the stomatal movement (Mouravieff, 1959; Stålfelt, 1956; Zelitch, 1961; Walker and Zelitch, 1963; Zelitch, 1965). These reports, however, do not reach to the unified view.
In this paper, effects of sodium azide and potassium cyanide on the stomatal opening and closing of Commelina communis L. will be described.

Materials and Methods

_Commelina communis_ L. cultivated in a green house was used in this experiment as materials.

_Open samples_: At about twelve on the day of experiment, entire leaves were cut off and transferred to the experimental room, and was preexamined by a microscope to ensure that the stomata were wide open. Leaves with open stomata (approximately 25μm) were used as open samples. When the stomata did not open fully, the leaves were floated on the water for about 2 hours under the light of about 8000 lux at 30°C. Virtually most stomata opened to about 25μm in width.

_CLOSED samples_: On the evening of the previous day, entire leaves were cut off and transferred to the experimental room, and kept floating on the water in an incubator for 1 night at 30°C. By this pretreatment, when the stomata did not completely close, epidermal strips were immersed in distilled water for 30 min. Virtually most stomata closed completely.

Abaxial epidermis with fully open stomata or completely closed stomata were taken respectively from the same leaf about 5×5 mm in size. Five strips were immersed in 5 ml of the bathing medium, and kept in the light of 8000 lux and dark for 4 hours, maintaining a temperature of 30°C. At least 20 stomata per each strip were measured at the middle of the strips by a microscope every 1 hour. Each value is an average of at least 100 individual stomatal apertures.

Control medium for the stomatal opening was 60 mM phosphate buffer of pH 6.0 containing 75 mM KCl, while control medium for the stomatal closing was 60 mM phosphate buffer of pH 6.0 containing no KCl.

The final concentrations of sodium azide and potassium cyanide added to both control medium were 1 mM, 0.1 mM, 0.01 mM, and 10 mM, 1 mM, 0.1 mM, respectively.

Results

I. The effect of sodium azide

   a) The inhibition of opening in the light.

When epidermal strips with closed stomata were incubated in the control medium containing KCl, the stomatal opening was normally occurred in the light. The stomatal apertures were 8.0 μm after 1 hour and 18.3 μm after 4 hours.

The addition of sodium azide affected the stomatal opening in the light. The opening was partly inhibited by 0.01 mM, and was fully inhibited by 1 mM and 0.1 mM sodium azide.

The final aperture after 4 hours at 1 mM, 0.1 mM and 0.01 mM sodium azide were 4.1 μm, 5.3 μm and 10.5 μm respectively (Fig. 1-A).
b) **The inhibition of closing in the dark.**

When strips with open stomata were incubated in the control medium containing no KCl, the stomatal closing was considerably rapid, especially within the first hour. Thereafter, gradual stomatal closing was followed. The aperture decreased from 25.0 μm to 8.0 μm after 1 hour and to 2.5 μm after 4 hours.

When sodium azide was supplied to epidermal strips, the stomatal closing was affected. The closing was fully inhibited by 1 mM sodium azide remaining widely open as compared with the control, while partial inhibition was found at 0.1 mM and 0.01 mM. At a concentration of 1 mM, 0.1 mM and 0.01 mM sodium azide, stomatal apertures after 4 hours decreased from 25.0 μm to 16.0 μm, 9.3 μm and 5.3 μm respectively (Fig. 1-B).

![Fig. 1 The effect of sodium azide on the stomatal opening in the light (A) and closing in the dark (B).](image)

II. **The effect of potassium cyanide**

a) **The inhibition of opening in the light**

By the addition of potassium cyanide to the bathing medium, stomatal opening was affected, causing reductions in aperture.

At a concentration of 10 mM, the stomatal opening was strongly inhibited, and stomata opened to 5.5 μm at most after 4 hours. While the stomatal opening was partly inhibited by 1 mM and 0.1 mM potassium cyanide, and stomata opened to 9.3 μm and
11.4 μm after 4 hours respectively (Fig. 2-A). Thus, the effect of potassium cyanide on the stomatal opening in the light was similar to that of sodium azide.

b) The inhibition of closing in dark

When potassium cyanide was supplied to epidermal strips with open stomata, stomatal closing in the dark was also affected. At a concentration of 10 mM, the stomatal closing was strongly inhibited, resulting in wide aperture of 15.3 μm after 4 hours. While the stomatal closing was partly inhibited by 1 mM and 0.1 mM potassium cyanide, and stomata closed to 6.0 μm and 4.5 μm after 4 hours (Fig. 2-B). Thus, the effect of potassium cyanide on the stomatal closing in the dark was also similar to that of sodium azide.

Fig. 2 The effect of potassium cyanide on the stomatal opening in the light (A) and closing in the dark (B).

Discussion

Sodium azide and potassium cyanide inhibited both stomatal opening in the light and closing in the dark.

These results suggest that metabolic reactions responsible for the opening and closing are involved in the stomatal movement.

Sodium azide and potassium cyanide are known to be inhibitors in both photo- and oxidative phosphorlation (Losada and Arnon, 1963), and may result in less ATP becoming available to the mechanism bringing about the transport of K⁺ into and out of the guard cells.
Zelitch (1965) reported that sodium azide affected both metabolic reactions and membrane permeability. Moreover, he reported that glycolate metabolism was essential for the stomatal opening suggesting that ATP was derived from the glycolate metabolism.

If the functioning of guard cell membranes is impaired by sodium azide, the permeability should be increased, and result in more rapid stomatal closure. From the present results it seemed that the inhibition of stomatal closing by sodium azide is not caused by the impairment of the cell membrane. It is indicated that permeability changes do not contribute the normal stomatal movement.

Zelitch (1961) reported that sodium cyanide did not influence the stomatal closing in tobacco leaf disks, while sodium azide was highly effective, preventing the stomatal opening. The latter is similar to the result reported here.

Walker and Zelitch (1963) showed that sodium azide of appropriate concentration not only inhibited opening in the light and induced closing in the light but also inhibited the stomatal closing in the dark, and suggested that the biochemical reactions responsible for the opening and closing are presumably different.

It is indicated that stomatal opening and closing processes is not caused by a simple reversal of the same series of reactions (Zelitch, 1965). As reported by Fujino (1967), ATP and ATPase in the guard cells may be involved in the stomatal opening and closing.

Further observation of the stomatal movement should be done metabolically.

**Literature cited**


