

**Short Communication**

# Energy Supply for Stomatal Opening in Epidermal Strips of *Commelina benghalensis*<sup>1</sup>

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## ABSTRACT

The influence of light or darkness on stomatal opening in epidermal strips of *Commelina benghalensis* was evaluated in the presence or absence of O<sub>2</sub> and/or metabolic inhibitors. Opening was restricted in nitrogen and was promoted by NADH and acids of the tricarboxylic acid cycle (succinate and  $\alpha$ -ketoglutarate) in CO<sub>2</sub>-free air in light as well as in darkness. The enhancement by light of stomatal opening was prevalent under nitrogen or in the presence of the respiratory inhibitors (sodium azide and oligomycin). Respiratory inhibitors decreased the opening in light or darkness under CO<sub>2</sub>-free air but exhibited no effect under nitrogen, whereas phosphorylation uncouplers were inhibitory in light or darkness under both CO<sub>2</sub>-free air and nitrogen. The results suggest that oxidative phosphorylation is a basic source of energy for stomatal opening, although photophosphorylation could be an energy source.

In spite of considerable effort in studying the physiology of stomata, the mechanism of stomatal opening and closing is not understood clearly (1, 7, 11, 12, 18). It is established that stomatal opening involves an increase in the turgor of guard cells which requires energy (11, 13, 18), but information on the source of energy is rather limited.

The acceleration of stomatal opening by light is a common observation and several investigators proposed that light might supply energy for the opening process, probably through cyclic electron flow (5, 8, 9, 16, 20). But stomata can open to a remarkable extent even in dark and it has therefore been claimed that light does not supply energy to the stomatal mechanism (18). There is morphological evidence that the energy required for stomatal opening could come from oxidative phosphorylation (6, 12, 14, 18), mainly because of the occurrence of numerous mitochondria in guard cells. ADP or ATP has been found to enhance stomatal opening in isolated epidermal strips (4, 6, 16, 17, 19). The experiments reported herein were directed toward delineating more clearly the possible role of oxidative phosphorylation.

## MATERIALS AND METHODS

**Plant Material.** *Commelina benghalensis* L. plants were grown in seed pans outdoors on soil supplemented with manure (soil-farmyard manure, 3:1). The average temperature was 30 C during

day and 20 C night with about a 12-h photoperiod. The plants were watered daily.

**Preparation of Epidermal Strips.** Second to fourth fully expanded leaves from 6- to 8-week-old plants were picked in the morning (usually between 9:00 and 10:00 AM) and used within 15 min. The lower epidermis was peeled off carefully. The adhering mesophyll cells were removed by rubbing the underside of the strip with a zero-size painting brush. The epidermal strips then were cut into pieces of approximately 5 × 10 mm and were left in distilled H<sub>2</sub>O for 2 h in dark at room temperature (28–30 C).

**Incubation.** The strips were transferred to 2- × 15-cm side-arm test tubes containing 10 ml 25 mM Mes buffer (pH 6.0), 100 mM KCl, 100  $\mu$ M CaSO<sub>4</sub>, 10 mM ADP (unless otherwise specified), and the test chemicals at the given final concentrations. The tubes for similar gaseous treatment were interconnected and the gas (air, CO<sub>2</sub>-free air, or N<sub>2</sub>) was bubbled at a rate of 300 to 400 ml min<sup>-1</sup>. The tubes were placed in a water bath maintained at 30 C and were either kept in darkness or illuminated at an irradiance of 100 w m<sup>-2</sup> with a bank of incandescent bulbs.

**Measurement.** The content of each tube was emptied quickly at given times into a Petri dish. Stomatal apertures were measured with a precalibrated ocular micrometer. Five strips were used for each treatment and 10 stomata, chosen at random, were measured in each strip. The experiments were repeated three times.

**Status of Epidermal Strips.** The guard cells were living during and after the 5-h experimental period as checked by the uptake of neutral red and protoplasmic streaming.

## RESULTS AND DISCUSSION

ADP is included in the incubation medium of these studies presented here because it is one of the substrates for phosphorylation and has amplified the stomatal responses to the gaseous phase in light or darkness. Figure 1 presents the responses of stomata to O<sub>2</sub> and CO<sub>2</sub> in the presence or absence of ADP. With illumination, stomatal opening was maximum in CO<sub>2</sub>-free air and minimal under N<sub>2</sub> (Fig. 1A). Stomata did not open in darkness under N<sub>2</sub> (Fig. 1B). In presence of ADP, illumination enhanced stomatal opening in air, CO<sub>2</sub>-free air, N<sub>2</sub> (Fig. 1C) compared to that in darkness (Fig. 1D). However, the enhancement by light of opening was maximum in N<sub>2</sub>. The stomatal opening was significant even in darkness in presence of ADP under air or CO<sub>2</sub>-free air (Fig. 1D).

Apparently ADP can enter guard cells as exhibited by the responses to ADP in the present observations (Fig. 1) as well as by earlier workers (4, 6, 16, 17, 19). Studies with isolated leaf cells also indicate that externally supplied nucleotides and sugar phosphates can modify their metabolism (3, 10).

Succinate and  $\alpha$ -ketoglutarate (intermediates of the tricarboxylic acid cycle) and NADH promoted stomatal opening in light and darkness, but only under CO<sub>2</sub>-free air (Table I). They had no

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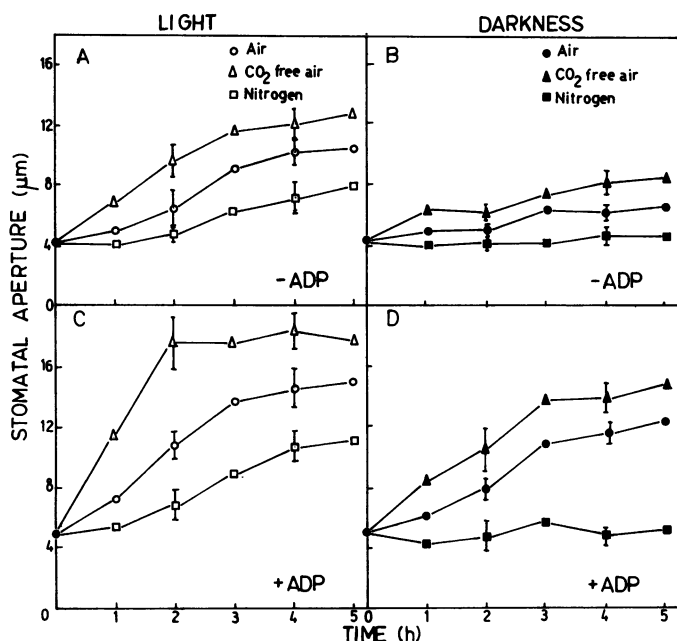


FIG. 1. The effect of gaseous phase on stomatal opening in epidermal strips of *C. benghalensis* either in light or darkness in the presence or absence of 10 mM ADP. The values are means of three experiments and the vertical bars represent the SE.

Table I. Influence of NADH, ATP, Organic Acids, and Metabolic Inhibitors on Stomatal Opening in Epidermal Strips of *C. benghalensis*

The values represent the stomatal aperture after a 5-h incubation period. The data are averages of three experiments  $\pm$  SE.

Addition	Stomatal Aperture			
	Light		Darkness	
	CO <sub>2</sub> -free air	N <sub>2</sub>	CO <sub>2</sub> -free air	N <sub>2</sub>
	$\mu\text{m}$			
<b>Experiment 1</b>				
Control (3.8) <sup>a</sup>	14.2 $\pm$ 1.2	10.4 $\pm$ 0.9	12.8 $\pm$ 1.5	3.6 $\pm$ 0.5
+ 1 mM NADH	16.5 $\pm$ 2.4	10.2 $\pm$ 1.2	15.3 $\pm$ 1.2	3.6 $\pm$ 0.4
+ 10 mM ATP	16.9 $\pm$ 2.0	12.6 $\pm$ 1.0	15.5 $\pm$ 2.2	6.2 $\pm$ 0.9
+ 10 mM Succinate	17.0 $\pm$ 1.3	10.4 $\pm$ 1.4	16.1 $\pm$ 2.8	3.8 $\pm$ 0.4
+ 10 mM $\alpha$ -Keto-glutarate	18.3 $\pm$ 3.1	9.8 $\pm$ 1.9	15.6 $\pm$ 1.2	3.8 $\pm$ 0.2
<b>Experiment 2</b>				
Control (6.0) <sup>a</sup>	18.9 $\pm$ 2.5	11.8 $\pm$ 1.6	15.5 $\pm$ 1.8	6.2 $\pm$ 0.8
+ 10 $\mu\text{M}$ Antimycin A	8.3 $\pm$ 1.3	7.8 $\pm$ 0.7	7.8 $\pm$ 0.9	6.0 $\pm$ 0.8
+ 1 $\mu\text{M}$ CCCP	6.9 $\pm$ 0.5	7.2 $\pm$ 0.9	7.4 $\pm$ 0.5	6.4 $\pm$ 0.6
+ 0.1 mM sodium azide	13.5 $\pm$ 1.8	10.9 $\pm$ 1.5	8.6 $\pm$ 0.6	5.8 $\pm$ 1.0
+ 0.1 $\mu\text{g/ml}$ oligomycin	14.6 $\pm$ 1.8	11.2 $\pm$ 2.0	8.2 $\pm$ 1.1	6.0 $\pm$ 0.9

<sup>a</sup> Opening at the start of the incubation.

effect on stomatal opening in N<sub>2</sub>. ATP, however, enhanced opening in N<sub>2</sub> as well as in CO<sub>2</sub>-free air both in light and darkness. Antimycin A, which is an inhibitor of photosynthetic (cyclic) and respiratory electron flow, and CCCP,<sup>3</sup> which is an uncoupler of

<sup>3</sup> Abbreviation: CCCP, carbonyl cyanide *m*-chlorophenylhydrazine.

oxidative and photophosphorylation, suppressed stomatal opening under all conditions. Oligomycin and sodium azide, which are inhibitors of respiration, reduced significantly the opening in light or darkness under CO<sub>2</sub>-free air.

Three points become evident from the experiments on stomatal opening in various gas phases (Fig. 1): (a) illumination is not necessary to drive stomatal movement since opening was observed even in darkness; (b) the involvement of oxidative metabolism is indicated by the restriction of opening in the absence of O<sub>2</sub>; and (c) light can play a role as suggested by the enhancement of opening in air or N<sub>2</sub> upon illumination.

The restriction of stomatal opening by uncouplers of phosphorylation (Table I) indicates that the process of opening is the result of active ion transport, presumably potassium (1, 7, 18). The extensive work of Pallaghy and Fischer (13) already has provided evidence that the uptake of both <sup>42</sup>K and <sup>35</sup>Cl requires energy and is sensitive to metabolic inhibitors. At least part of the energy for stomatal opening can come from oxidative phosphorylation as supported by three observations in Table I: (a) the ability of externally added ATP to increase slightly the opening under N<sub>2</sub>; (b) the enhancement of opening by NADH and organic acids of the tricarboxylic acid cycle only in CO<sub>2</sub>-free air; and (c) the suppression of opening by inhibitors of respiratory electron transport chain and oxidative phosphorylation.

Illumination nearly doubled stomatal aperture in air or CO<sub>2</sub>-free air (Fig. 1); the enhancement was prominent in N<sub>2</sub> or in the presence of respiratory inhibitors, like sodium azide or oligomycin. This indicates that photophosphorylation was an energy source. The data indicate that stomatal opening is driven by a common pool of ATP contributed from oxidative phosphorylation and photophosphorylation. Guard cells are characterized by the presence of numerous mitochondria as well as chloroplasts (2, 14, 15) and possess the enzymes of the tricarboxylic acid cycle (18).

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#### LITERATURE CITED

- ALLAWAY WG, FL MILTHORPE 1976 Structure and functioning of stomata. In TT Kozłowski, ed, Water Deficits and Plant Growth, Vol IV. Academic Press, New York, pp 57-102
- ALLAWAY WG, G SETTERFELD 1972 Ultrastructural observations on guard cells of *Vicia faba* and *Allium porum*. Can J Bot 50: 1405-1413
- CHOLLET R 1976 C, control of photorespiration: studies with isolated mesophyll cells and bundle sheath strands. In RH Burris, CC Black, eds, CO<sub>2</sub> Metabolism and Plant Productivity. University Park Press, Baltimore, pp 327-341
- DAS VSR, AS RAGHAVENDRA 1974 Control of stomatal opening by pyruvate metabolism in light. Indian J Exp Biol 12: 425-428
- DAS VSR, AS RAGHAVENDRA 1974 Role of cyclic photophosphorylation in the control of stomatal opening. In RL Bielecki, AR Ferguson, MM Cresswell, eds, Mechanisms of Regulation of Plant Growth, Bulletin No. 12. Royal Society of New Zealand, Wellington, pp 455-460
- FUJINO M 1967 Role of adenosine triphosphate and adenosine triphosphatase in stomatal movement. Sci Bull Fac Educ Nagasaki Univ 18: 1-47
- HSIAO TC 1976 Stomatal ion transport. In U Lüttge, MG Pitman, eds, Transport in Plants. II. Part B. Tissues and Organs. Encyclopedia of Plant Physiology, New Series, Vol 2 Chap 4.3. Springer-Verlag, Berlin, pp 195-221
- HSIAO TC, WG ALLAWAY, LT EVANS 1973 Action spectra for guard cell Rb<sup>2+</sup> uptake and stomatal opening in *Vicia faba*. Plant Physiol 51: 82-88
- HUMBLE GD, TC HIAO 1970 Light-dependent influx and efflux of potassium of guard cells during stomatal opening and closing. Plant Physiol 46: 483-487
- JENSEN RG, RBI FRANCKI, M ZAITLIN 1971 Metabolism of separated leaf cells. I. Preparation of photosynthetically active cells from tobacco. Plant Physiol. 48: 9-13
- MANSFIELD TA 1976 Mechanisms involved in turgor changes of guard cells. In N Sunderland, ed, Perspectives in Experimental Biology, Vol 2. Botany Pergamon Press, Oxford, pp 453-462
- MEIDNER H, TA MANSFIELD 1968 Physiology of Stomata, McGraw Hill, London
- PALLAGHY CK, RA FISCHER 1974 Metabolic aspects of stomatal opening and ion accumulation by guard cells of *Vicia faba*. Z Pflanzenphysiol 71: 332-344
- PALLAS JE JR, HH MOLLENHAUER 1972 Physiological implication of *Vicia faba* and *Nicotiana tabacum* guard-cell ultrastructure. Am J Bot 59: 504-514
- PEARSON CJ, FL MILTHORPE 1974 Structure, carbon dioxide fixation, and

- metabolism of stomata. *Aust J Plant Physiol* 1: 221-236
16. RAGHAVENDRA AS, VSR DAS 1972 Control of stomatal opening by cyclic photophosphorylation in light. *Curr Sci* 41: 150-151
  17. RAGHAVENDRA AS, IM RAO, VSR DAS 1976 Characterisation of abscisic acid inhibition of stomatal opening in isolated epidermal strips. *Plant Sci Lett* 6: 111-115
  18. RASCHKE K 1975 Stomatal action. *Annu Rev Plant Physiol* 26: 309-340
  19. THOMAS DA 1971 The regulation of stomatal aperture in tobacco leaf epidermal strips. III. The effect of ATP. *Aust J Biol Sci* 24: 689-707
  20. WILLMER CM, TA MANSFIELD 1970 Effects of some metabolic inhibitors and temperature on ion-stimulated stomatal opening in detached epidermis. *New Phytol* 69: 983-992