Plant Physiol. (1972) 49, 649–650

Short Communication

Photophosphorylation Can Provide Sufficient Adenosine 5'-Triphosphate to Drive K^+ Movements during Stomatal Opening[1]

Received for publication September 8, 1971

J. E. PALLAS, JR.
Soil and Water Conservation Research Division, Agricultural Research Service, United States Department of Agriculture, Watkinsville, Georgia 30677

R. A. DILLEY
Department of Biological Science, Purdue University, Lafayette, Indiana 47907

A stomatal opening movement in plants is achieved when the turgor of guard cells sufficiently exceeds that of neighboring cells. Osmotically active molecules and ions produce the turgor difference. They must be either transported from neighboring cells or free space into the guard cell, or produced or released within the guard cell from a source already there. Shaw and Maclachlan (9) discounted the possibility that carbohydrates manufactured from carbon dioxide within the guard cell play a major part in opening in light. How much turgor changes depend on degradation of guard cell starch is still a moot question.

It has long been known that K^+ accumulates in stomata (7) and that ions of group I metals stimulate opening of stomata (5). Recent research suggests that light-activated stomatal movement is brought about by a transport of K^+ (3, 4, 10, 11) or Na^+ (14) into and out of guard cells with energy derived by photophosphorylation driving an active transport mechanism.

The intent of these studies was to calculate the theoretical ATP generation through photophosphorylation in guard cells and compare it with the rate and extent of K^+ influx reported (2) for guard cells during opening of stomata.

MATERIALS AND METHODS

The plant material used was *Vicia faba* L., var. Long Pod. Chloroplasts in the epidermis of this species are possessed almost exclusively by guard cells. The lower epidermis of expanded leaves was peeled and put into a 0.4 M sucrose, 10 mM tris solution, pH 7.0. Epidermal strips, upon which major veinal areas were identified, were discarded or such areas trimmed off the strip. To remove adhering fragments of mesophyll cells containing chloroplasts, the strips were sonicated in 10-sec bursts for 30 to 45 sec, washed twice with tris buffer, and finally with distilled water. Guard cell chloroplasts were not affected by this short sonication. The strips were then lyophilized, weighed, pulverized by mortar and pestle in acid-washed quartz sand, and extracted with 80% acetone for 24 hr at room temperature. Chlorophyll *a*, *b*, and total chlorophyll were estimated by the method of Arnon (1). Chloroplast frequency within guard cells and stomatal frequency were determined by light microscopic observation on fresh epidermal strips mounted in water.

RESULTS

The concentrations of chlorophyll per gram of dry and fresh weight are given in Table I. The ratio of chlorophyll *a*-chlorophyll *b* for the epidermis of 6-week-old plants was 4.5, while that for the whole leaf was 3.0. The chlorophyll content in the guard cells of the *Vicia faba* leaves was 52 pmole chlorophyll/cm^2 of leaf tissue. A stomatal density of 6100 ± 200/cm^2 was measured, similar to that reported by Fisher (2). Twenty-five visual observations gave an average of 13 ± 2 chloroplasts per guard cell. Thus, we calculate that a single guard cell chloroplast contains 0.33 pg chlorophyll. This value is in good agreement with the data of Thomas *et al.* (12) who determined the chlorophyll content of chloroplasts from various plants.

In calculating the expected ATP generation rate and comparing it with the rate and extent of K^+ influx into guard cells during opening of stomata, two assumptions were made: (*a*) that a reasonable rate of ATP synthesis on a chlorophyll basis was 250 μmoles ATP/hr·mg chlorophyll (13), and (*b*) that the K^+ flux data of Fisher (2) represents a 95% contribution

Table I. *Chlorophyll Content in Guard Cell Chloroplasts, Epidermis, and Leaves of 6-week-old Vicia faba*

	Guard Cells	Guard Cells	Leaf Cells	Guard Cell
	μg/g dry wt	μg/g fresh wt	μg/g dry wt	pg/chloroplast
Chlorophyll *a*	73.0	3.8	1.2×10^4	
Chlorophyll *b*	16.0	0.8	4.0×10^3	
Chlorophyll *a* + *b*	89.0	4.6	1.6×10^4	0.33

[1] Calculated from a stomatal frequency of 6100 ± 200/cm^2 of epidermis and 13 ± 2 chloroplasts/guard cell.

[1] Contribution from the Southern Branch, Soil and Water Conservation Research Division, Agricultural Research Service, United States Department of Agriculture, in cooperation with the University of Georgia Agricultural Experiment Stations.

due to guard cells and 5% due to other epidermal cells attached to the epidermal strip.

The expected ATP yield in the guard cells would be: (250 μmole ATP/μmole chl·hr) $\times$ (52 pmole chl/cm^2) = 0.013 μmole ATP/cm^2·hr. Using $^{86}Rb^+$ (as K^+) uptake data from Fisher (2), an increase in stomatal aperture of 7 μ was associated with an uptake of 20 nmole/cm^2·3 hr.

$$\frac{0.007\ \mu\text{mole } K^+/\text{cm}^2\cdot\text{hr}}{0.013\ \mu\text{mole ATP/cm}^2\cdot\text{hr}} = 0.5\ K^+/\text{ATP}$$

DISCUSSION AND CONCLUSION

Humble and Hsiao (4) report that under anaerobic conditions guard cell opening in *Vicia faba* was not appreciably affected by DCMU but was completely inhibited by carbonyl cyanide *m*-chlorophenylhydrazone (Cl-CCP) plus DCMU. Since DCMU inhibits net electron flow in photosynthesis, they deduced that stomata can at least partially open in light without noncyclic electron flow, but when Cl-CCP uncoupled photophosphorylation there was very little K^+ uptake or stomatal opening. Thus, it was concluded that the energy for K^+ uptake by guard cells in light, as in leaf tissue (8), may come partly from cyclic photophosphorylation. Kuiper (6) has indicated that the action spectrum of stomatal movements and photophosphorylation in spinach chloroplasts are similar. Based on our analysis of chlorophyll concentration in guard cells, along with the other stated assumptions, it is indicated that only 0.5 K^+ need be coupled to an ATP molecule in uptake. Even if the ATP yield were only 100 μmole/hr·μmole chl the K^+/ATP ratio would be 1 K^+/ATP. Thomas (10, 11) has presented evidence that K influx into guard cells is associated with a membrane-bound ATPase. It appears then that there is sufficient photosynthetic ATP-generating capacity in *Vicia faba* guard cell chloroplasts to accommodate the observed K^+ fluxes (2) correlated with stomatal movement.

LITERATURE CITED

1. Arnon, Daniel I. 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. Plant Physiol. 24: 1–15.
2. Fisher, R. A. 1968. Stomatal opening: role of potassium uptake by guard cells. Science 160: 784–785.
3. Fujino, M. 1967. Role of adenosine triphosphate and adenosinetriphosphatase in stomatal movement. Sci. Bull. Fac. Educ. Nagasaki Univ. 18: 1–47.
4. Humble, G. D. and T. C. Hsiao. 1970. Light-dependent influx of potassium of guard cells during stomatal opening and closing. Plant Physiol. 46: 483–487.
5. Iljin, W. S. 1922. Werkung der Kationen von Salzen auf den Zerfall und die Bildung von Stärke in der Pflanze. Biochem. Z. 132: 494–510.
6. Kuiper, P. J. C. 1964. Dependence upon wavelength of stomatal movement in epidermal tissue of *Senecio odoris*. Plant Physiol. 39: 952–955.
7. Macallum, A. B. 1905. On the distribution of potassium in animal and vegetable cells. J. Physiol. 32: 95.
8. Rains, D. W. 1968. Kinetics and energetics of light-enhanced potassium absorption by corn leaf tissue. Plant Physiol. 43: 394–400.
9. Shaw, M. and G. A. Maclachlan. 1954. The physiology of stomata. I. Carbon dioxide fixation in guard cells. Can. J. Bot. 32: 784–794.
10. Thomas, D. A. 1970. The regulation of stomatal aperture in tobacco leaf epidermal strips. I. The effect of ions. Aust. J. Biol. Sci. 23: 961–979.
11. Thomas, D. A. 1970. The regulation of stomatal aperture in tobacco leaf epidermal strips. II. The effect of ouabain. Aust. J. Biol. Sci. 23: 981–989.
12. Thomas, J. B., K. Minnaert, and P. F. Elbers. 1956. Chlorophyll concentration in plastids of different groups of plants. Acta Bot. Neerlan. 5: 315–321.
13. Vernon, L. P. and M. Avron. 1965. Photosynthesis. Annu. Rev. Biochem. 34: 269–296.
14. Willmer, C. M. and T. A. Mansfield. 1969. Active cation transport and stomatal opening: a possible physiological role of sodium ions. Z. Pflanzenphysiol. 61: 398–400.