Plant Physiol. (1978) 61, 474–475

Short Communication

Release of Malate from Epidermal Strips during Stomatal Closure[1]

Received for publication August 25, 1977 and in revised form November 11, 1977

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ABSTRACT

Isolated epidermal strips of *Vicia faba* and *Commelina communis* release malate into their bathing medium when stomata close. This release was largest (about 0.6 of the initial malate content) when epidermal strips of *C. communis* were floated on 10^{-5} M (±)-abscisic acid.

Stomata open by increasing the turgor pressure in guard cells. The increase in pressure is the osmotic effect of an accumulation of inorganic and organic salts of K^+, primarily KCl and K malate (1, 4–6). During stomatal closure, guard cells must dispose of these osmotica. Inorganic ions may be transferred into subsidiary cells, if such cells are present, or other specialized epidermal cells (4, 6). The cell walls of the epidermis may also receive and store inorganic ions, although there is doubt whether their capacity suffices (5). The fate of the organic anions during stomatal closure has been studied after ^{14}C labeling of guard cell contents (2). Epidermal samples were exposed to $^{14}CO_2$ for 2 min and then subjected to closing treatments. The analysis of the epidermes as well as of the liquid on which they floated indicated that guard cells may dispose of malate in three ways: (*a*) catabolism in the tricarboxylic acid cycle; (*b*) decarboxylation followed by gluconeogenesis and starch formation; and (*c*) release from the guard cells. However, it was estimated that only a small fraction of the total malate pool was labeled in these experiments. To determine the extent to which these three methods are employed by guard cells during stomatal closure, the fate of the whole malate pool must be examined. In particular, the importance of a specific release of malate was to be assessed because this mechanism would enable guard cells to reduce their turgor faster than metabolism of malic acid would allow (2). Changes in malate content were followed during stomatal closure in epidermal samples and in the water on which they floated. In most cases, stomatal closing was induced by addition of ABA to the water.

MATERIALS AND METHODS

Plants of *Vicia faba* L. (improved Long Pod variety, Lagomarsino Seeds, Inc., Sacramento, Calif.) and *Commelina communis* L. (seeds from T. A. Mansfield, University of Lancaster, Great Britain) were grown in growth chambers (27/23 C day/night; 85% relative humidity; 16-hr day; 85 w m^{-2} from fluorescent tubes) as described elsewhere (7). The second, third, and fourth fully expanded leaves were removed, rinsed with distilled H_2O, and cut into sections of 1 to 3 × 2 to 4 cm^2. These sections were floated lower side up on distilled H_2O in the light (85 w m^{-2} from mercury vapor lamps, General Electric H400 RDX 33-1) and CO_2-free air for 4 to 5 hr to allow stomata to open. Then the lower epidermis was removed in strips of about 5 × 10 to 15 mm^2. Sixty to 80% of the ordinary epidermal cells ruptured during peeling. The area of each strip was measured with a ruler. In four of five strips/experiment, stomatal apertures were measured under the microscope; then these strips were plunged into boiling 80% ethanol to extract malate. The other strips were transferred to distilled H_2O or solutions containing ABA. After incubation for various times, the strips were put on a microscope slide for the measurement of 20 to 25 stomatal apertures in each strip and then immediately extracted with boiling ethanol. The extracts were evaporated to dryness and analyzed for malate by enzymical oxidation, coupled to the reduction of NAD; the NADH formed was measured fluorimetrically (3; for details see 7). The solutions on which the strips had floated were also evaporated and assayed for malate.

RESULTS AND DISCUSSION

Epidermal strips of *V. faba* with open stomata were exposed in the light to a 0.1 mM solution of (±)-ABA for periods of 15 min to 2 hr (Fig. 1). During the first 15 min of exposure to ABA, stomates did not close much but the malate content of the epidermes decreased by about 25%; some malate appeared in the bathing solution. We observed that 20 to 40% of the ordinary epidermal cells survived peeling but virtually all of them ruptured during incubation. We think it possible that the malate released during the first 15 min originated from bursting epidermal cells. Malate in the epidermis continued to decline. Stomata closed in parallel with this decline, while the concentration of malate in the bathing solution rose. Virtually all malate lost from the epidermis after the first 15 min could be accounted for by malate appearing in the solution.

A second type of experiment was performed on epidermal samples of *C. communis*. Epidermal strips with open stomata were floated on either distilled H_2O or solutions of 0.1 μM to 10 μM (±)-ABA in light and CO_2-free air. After 30 or 40 min, stomatal apertures were measured and the malate content of tissues and solutions determined. The results are shown in Table I. With increasing concentrations of ABA, the malate remaining in the epidermis declined, and increasing amounts of malate were released to the bathing medium. In *C. communis*, the epidermal cells remained intact after stripping; thus, malate released represents a net loss from the intact epidermis. Strips floating on water also closed somewhat during the experiment. In this case, too, the

[1] Research supported by the United States Energy Research and Development Administration under Contract EY-76-C-02-1338. Part of a M.S. thesis of C. A. V. K.

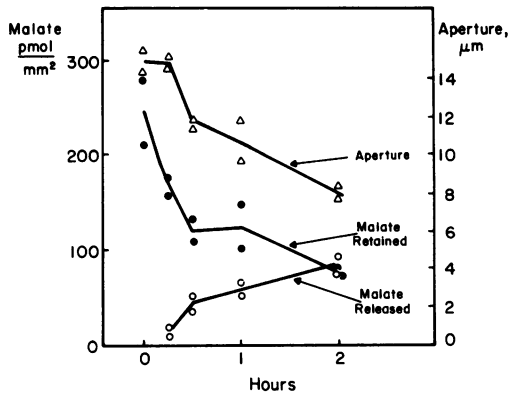

FIG. 1. Epidermal malate content, and malate released into the bathing medium during a stomatal closing movement induced by 10^{-4} M (±)-ABA. Epidermal strips were made from leaf sections of *V. faba* having open stomata. ▲: stomatal aperture; ●: epidermal malate, ○: malate released. The symbols represent the results of two separate experiments conducted at an interval of 1 week. Each malate value is the result of two separate determinations on four or five strips each. Stomatal apertures are means from measurements on 20 to 25 stomata in each strip.

malate content of the epidermis decreased from the initial level and malate appeared in the bathing solution.

The observation that epidermis of *C. communis* with closed stomata had a high malate content when analyzed immediately after stripping needs corroboration and explanation.

Our experiments demonstrate that in *V. faba* as well as *C. communis* malate release by guard cells constitutes a significant process occurring during stomatal closure; the earlier report of a participation of malate release in the reduction of the solute content of guard cells (2) received strong support through our determination of the total amounts of malate involved. The fact that stomatal closure in absence of ABA (Table I) was also accompanied by malate release indicates that ABA does not trigger a closing process entirely different from closing due to other "natural" causes.

Table I. Epidermal malate content and malate released into the bathing medium during stomatal closing movements in absence or presence of ABA. Epidermal strips were made from leaf sections of Commelina communis having open stomata and were floated on water or solutions of 10^{-7} to 10^{-5} M (±)-ABA for the times indicated. CO_2-free air.

Trial	Time	Treatment	Range of apertures[1]	Malate, pmol mm^{-2}		
	min	M (±)-ABA	μm	Tissue	Solution	Sum
I	0	Dark[2]	0	474		
	0	Light[2]	15-16	551		
	40	Water	0-11	165	165	330
	40	10^{-7}	0-5	146	203	349
	40	10^{-6}	0	162	198	360
	40	10^{-5}	0	77	357	434
II	0	Dark[2]	1-5	243		
	0	Light[2]	14-16	381		
	30	Water	11-13	216	141	357
	30	10^{-7}	0-3	149	-	-
	30	10^{-6}	0-2	117	165	282
	30	10^{-5}	0	83	211	294

[1] Accurate determination of stomatal apertures was not possible during the time span of the experiment.

[2] Leaf sections floated on water in darkness or light for 4 or 5 hr.

The fate of the malate released by guard cells of an attached epidermis remains to be explored. However, since in our material guard cells constituted about 4% of the total epidermal volume of *V. faba* and 3% of that of *C. communis* this may not be a significant question.

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