Nocturnal Accumulation of Malic Acid Occurs in Mesophyll Tissue without Proton Transport to Epidermal Tissue in the Inducible Crassulacean Acid Metabolism Plant Mesembryanthemum crystallinum

EVIDENCE AGAINST A PREVIOUS HYPOTHESIS

Received for publication December 30, 1980 and in revised form February 24, 1981

KLAUS WINTER2, GERALD E. EDWARDS2,3, AND JOSEPH A. M. HOLTUM4
2Department of Horticulture and 3Department of Chemistry, University of Wisconsin, Madison, Wisconsin 53706

ABSTRACT

The inducible Crassulacean acid metabolism plant, Mesembryanthemum crystallinum, accumulates malic acid, i.e. equivalent amounts of malate anions and protons in the mesophyll cells at night. Levels of malate and titratable acidity are low in the epidermal tissue and do not change significantly during the day/night cycle. This result is in contrast to a recent report (Bloom 1979 Plant Physiol 64: 919-923) that the synthesis of malic acid during dark CO2 fixation is associated with an equivalent exchange of inorganic cations from epidermal tissue with protons in the mesophyll cells.

It has recently been proposed by Bloom (1) that the development of CAM in the halophytic, inducible CAM plant Mesembryanthemum crystallinum (19) requires exposure of plants to high salinity levels and a substantial accumulation of inorganic cations by the shoots. It has also been proposed by the same author (2) that inorganic cations such as Na+ and K+ cycle between mesophyll and epidermal tissue in this species to balance nocturnal malate accumulation and to produce stomatal opening in the dark. Protons, derived from malic acid synthesis at night, could exchange with inorganic cations in the epidermis. If this hypothesis were correct, day/night fluctuations of the malate anion should be restricted to the mesophyll, whereas fluctuations in acidity should be restricted to the epidermis and should not occur in the mesophyll. Paradoxically, Bloom’s hypothesis is based exclusively on the observation of a nocturnal increase of positively charged ions in the mesophyll, i.e. H+ (titratable acidity), Na+, and K+. No measurements were made on the isolated epidermis. To clarify this issue, day/night changes in malate anion and titratable acidity were determined in mesophyll and epidermis of leaves of M. crystallinum exhibiting CAM in response to high NaCl salinity.

1 Supported by the College of Agricultural and Life Sciences, University of Wisconsin, Madison, National Science Foundation Grant PCM 77-09384 (to G. E. E.), and Grant 59-2551-0-1-447-0, Research Grants Office, United States Department of Agriculture (to M. H. O’Leary, Department of Chemistry, whose funds help support J. A. M. H.).
2 To whom requests for reprints should be addressed, at Department of Botany, Washington State University, Pullman, WA 99164.

MATERIALS AND METHODS

Eight- to 10-week-old plants of M. crystallinum L. were used in the experiments. Plants were cultivated in either soil or half-strength modified Johnson nutrient solution (12). Four treatments consisted of different combinations of NaCl in the root medium and of different degrees of water stress, all of which led to the development of CAM in the experimental plants. Treatments are specified in the “Results and Discussion” section (legend to Fig. 1). Plants were kept in a growth cabinet under a 12-h light (25 C)/

RESULTS AND DISCUSSION

Plants from all treatments showed CAM as judged from the increase in malate content and titratable acidity in the dark (Fig. 1). In all experiments, the mesophyll of M. crystallinum accumulated equivalent amounts of malate anions and protons, i.e. malic acid at night (Fig. 1), a result which is similar to whole-leaf extracts of this and other CAM species (8-10). Titratable acidity in the epidermis was low throughout the day/night cycle. The slight increase in malate and titratable acidity in the epidermis at the end of the dark period is likely due to contamination from the mesophyll. These results demonstrate that nocturnal increase in
the concentration of inorganic cations in the mesophyll, especially to balance malate$^-$, as has been suggested by Bloom (2), does not occur. In fact, Bloom’s finding of a nocturnal increase in titratable acidity in the mesophyll of M. crystallinum is in agreement with this view and contradicts his own hypothesis (2).

In the experiment of Figure 1B (growth conditions similar to those in Bloom’s study [2]), possible fluctuations of organic acids other than malate in mesophyll and epidermis which could require charge balance by inorganic cations were examined. HPLC revealed substantial amounts of oxalate and citrate plus isocitrate and small amounts of aconitate in the leaf mesophyll, levels of which did not fluctuate between end of the light and end of the dark periods (Table 1). The levels of these organic acids were low in the epidermal tissue (Table 1). Since titratable acidity in leaves of M. crystallinum is only sufficient to balance malate$^-$ levels, the negative charges of oxalate, citrate plus isocitrate, and aconitate are probably balanced by inorganic cations.

The possibility of transport of certain cations (e.g. Na$^+$ and K$^+$) between epidermis and mesophyll tissue during CAM has not been excluded in the present study. However, the above data indicate that an exchange of protons and inorganic cations between tissues would be insignificant and secondary relative to the total level of acidification.

A recent study on the CAM plant Kalanchoë daigremontiana revealed a close relationship between organic anions and inorganic cation levels in deacidified leaves at the end of the light period (11). The results obtained under these conditions, however, must be clearly separated from the day/night fluctuations in organic

---

**Table 1. Organic Anion Content in Mesophyll and Epidermis (Upper Plus Lower)**

<table>
<thead>
<tr>
<th></th>
<th>Oxalate</th>
<th>Citrate Plus</th>
<th>cis-Aconitate</th>
<th>Malate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mesophyll, L$^*$</td>
<td>19.7</td>
<td>20.4</td>
<td>0.7</td>
<td>ND$^b$</td>
</tr>
<tr>
<td>Mesophyll, D</td>
<td>18.6</td>
<td>21.2</td>
<td>Trace</td>
<td>16.5</td>
</tr>
<tr>
<td>Epidermis, L</td>
<td>2.1</td>
<td>0.6</td>
<td>0.3</td>
<td>ND</td>
</tr>
<tr>
<td>Epidermis, D</td>
<td>2.3</td>
<td>0.8</td>
<td>0.3</td>
<td>ND</td>
</tr>
</tbody>
</table>

$^a$ L, 12-h Light period; D, 12-h dark period.

$^b$ ND, Not detected.
acids associated with CAM.

Not only the data of Figure 1, but also anatomical considerations, fail to support Bloom's hypothesis (2). Examination of freshly prepared leaf cross-sections showed that the ratio volume mesophyll to volume epidermis (excluding the bladder cells which do not participate in CAM) is at least 10:1. Nocturnal malic acid synthesis on a total leaf water basis may be 100 μeq or more. If the protons from malic acid concentrate in the epidermis and are balanced by Cl⁻, as proposed by Bloom (2), a highly improbable epidermal concentration of at least 1 N HCl would result. In contrast to Bloom's anatomical description of _M. crystallinum_ (2), the leaf epidermis of this species consists not of a double, but of a single, cell layer (5, 7). Furthermore, the epidermal bladder cells are not positioned on top of the epidermis, as indicated by Bloom, but represent enlarged epidermal cells (5, 7).

Statements that _M. crystallinum_ exhibits CAM only in response to high salinity (1) ignore earlier investigations (13–15, 17), which show the induction of CAM in plants grown in cooled or nonaerated nutrient solutions without added NaCl. Thus, reduced water availability, not an ion-specific effect, is probably the trigger for the induction of CAM in _M. crystallinum_. Lower net dark CO₂ fixation rates under stress conditions without special NaCl treatment in comparison to growth at high salinity levels may reflect the halophilic properties of this species. _M. crystallinum_ shows optimum growth only when cultivated in nutrient solutions containing 5 to 100 mM NaCl (15, 17; Winter, unpublished data). Contrary to Bloom's conclusions (1), plants with CAM generally do not occupy saline habitats or accumulate high levels of inorganic salts typical of halophytes (16, 18). It should also be noted that sodium-deficient, nonhalophilic CAM plants, such as _Bryophyllum tubiflorum_, may show reduced growth (4) but still exhibit all features of CAM (3). Therefore, implications that Na⁺ is a prerequisite for the onset of CAM (1) are misleading.

LITERATURE CITED