Short Communication

Relation of Light-dependent Potassium Uptake by Pea Leaf Fragments to the pK of the Accompanying Organic Acid

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Sliced or chopped leaf tissue has recently been employed to study ion transport into leaf cells (1-5). Leaf fragmentation eliminates transpirational effects on ion movements and also permits an external solution to be put into contact with the leaf cells. With this technique, Rains (3, 4) has demonstrated a light-enhanced potassium uptake into sliced corn leaves. Nobel (1) has reported a light-dependent K+ uptake into chopped pea leaves of 7.5 μmoles/g fresh weight-hr when the external bathing solution contains 5 mm KCl, the rate of uptake increasing to 56 μmoles/g fresh weight-hr when bicarbonate replaces chloride. The present series of experiments indicates that such stimulation of K+ uptake by HCO3− represents a nonspecific anion effect that apparently depends on the pK of the acid.

MATERIALS AND METHODS

Pisum sativum L., cultivar Blue Bantam (W. Atlee Burpee Co., Riverside, Calif.) was grown in moist vermiculite at 20 C. An illumination of 2000 lux was provided for 12 hr each day by fluorescent tubes. Just before the light period on the 14th day, fully expanded leaves were removed and chopped so that the largest dimension was approximately 1 mm (1). The chopped leaves were rinsed for a total of 1 hr with six 10-min changes of 0.26 m sucrose, 5 mm potassium acetate, pH 6.3 (or other solution as indicated, solutions of the same composition being used for both rinsing and incubating). Incubation of 0.4 g of chopped and rinsed leaves in 10 ml of solution was at 20 C in the dark or with 4000 lux provided by fluorescent lamps (1).

At various times during incubation, 0.100-ml aliquots of the external solution were withdrawn for ion determinations. Potassium was determined with an Evans Electroseleum Mark II flame photometer. 14C-labeled organic acids were measured by scintillation techniques. Bicarbonate-14C, formate-14C, acetate-14C, acetate-214C, propionate-14C, and butyrate-14C were purchased from New England Nuclear Corporation (Boston, Mass.), and glycolate-14C and pyruvate-14C were obtained from Amersham/Searle (Arlington Heights, Ill.). Hydrogen ion activity was determined with a glass electrode placed in the incubation medium. Osmotic pressure was calculated from freezing-point depression data obtained with an Advanced Instruments Model 31LAS Osmometer. DCMU3 was purchased from K & K Laboratories, Inc. (Hollywood, Calif.), and tri-FI-CCP was generously provided by Dr. P. G. Heytler (E. I. du Pont de Nemours & Co., Wilmington, Del.).

RESULTS

Experiments with KCl and KHCO3 have shown that the light-dependent uptake of potassium into chopped pea leaves depends on the anion (1). To study this dependency, potassium salts (5 mm) of various organic acids were placed in the external solution. Figure 1 indicates that the net uptake of potassium in the light increases in going from pyruvate to formate to acetate, whereas the K+ uptake in the dark was relatively small in all three cases. The rates of the light-dependent (light minus dark) uptake calculated from the initial slopes of the uptake curves (Fig. 1) were 21 μmoles of K+/g fresh weight-hr for 5 mm potassium pyruvate, 34 for formate, and 48 for acetate. The results on the light-dependent uptake of potassium in the presence of other anions are summarized in Table I. In addition, the initial rate of influx of 14C-labeled organic acids was measured (right-hand column of Table I). When Na+ replaced K+ in the external solution, the light-dependent uptake of acetate decreased only 5%.

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1 This investigation was supported by Public Health Service Research Grant GM 15183 from the National Institute of General Medical Sciences, and by the Research Committee of the Los Angeles Division, University of California Academic Senate.
2 Abbreviations: DCMU: 3-(3,4-dichlorophenyl)-1,1-dimethylurea; tri-FI-CCP: p-trifluoromethoxy carbonyl cyanide phenylhydrazone.
3 DCMU was purchased from K & K Laboratories, Inc.
Table I. Summary of the Light-dependent Uptake by Chopped Pea Leaves of the Potassium Salts of Various Organic Acids

Leaf fragments were incubated at 4000 lux or in the dark in the presence of 0.26 M sucrose, 5 mM of a particular potassium salt, and at a pH of 6.3. Three experiments or more were averaged under each condition.

<table>
<thead>
<tr>
<th>Organic Acid</th>
<th>pH</th>
<th>Light-dependent Uptake</th>
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<tr>
<td></td>
<td></td>
<td>K⁺ Organic acid</td>
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<td></td>
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<td>µmoles/g fresh wt-hr</td>
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<tr>
<td>Pyruvate</td>
<td>2.5</td>
<td>21 18</td>
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<tr>
<td>Acetoacetate</td>
<td>2.5</td>
<td>22 18</td>
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<tr>
<td>Glycollate</td>
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<td>29 23</td>
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<tr>
<td>Formate</td>
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<td>34 30</td>
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<tr>
<td>Acetate</td>
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<td>49 43</td>
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<tr>
<td>Butyrate</td>
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<td>49 42</td>
</tr>
<tr>
<td>Propionate</td>
<td>6.3</td>
<td>56 53</td>
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</table>

Fig. 2. Effect of the photosynthetic inhibitor DCMU and the uncoupler tri-Fl-CCP on potassium uptake in the light and the dark with 5 mM CH₃COOK in the solution bathing the chopped pea leaves.

The total K⁺ uptake from 5 mM CH₃COOK was 82 µmoles/g fresh weight at 4 hr. This uptake represents an actual accumulation of osmotically active material in the cells. Specifically, the cell sap expressed from chopped leaves after 4 hr with 5 mM CH₃COOK was 368 milliosmolal for those incubated in the light, and 280 milliosmolal for leaves kept in the dark.

The light-dependent ion uptake into chopped pea leaves was also investigated by using inhibitors which were present in both the rinse and the incubation media (together with 0.26 M sucrose, 5 mM CH₃COOK, pH 6.3). As indicated in Figure 2, the photosynthetic inhibitor DCMU decreased the K⁺ uptake in the light and had little effect in the dark. The light-dependent potassium uptake was more than 50% inhibited at 1 µM DCMU. The uncoupler tri-Fl-CCP also inhibited the light-dependent K⁺ uptake more than 50% at 1 µM. However, tri-Fl-CCP had an appreciable effect in the dark, e.g., 10 µM tri-Fl-CCP led to a net K⁺ efflux of 7 µmoles/g fresh weight·hr (Fig. 2). Both DCMU and tri-Fl-CCP decreased the light-dependent uptake of ¹⁴C-acetate to an extent consistent with their effects on the light-dependent potassium uptake.

DISCUSSION

The data summarized in Table I show that the initial rate of light-dependent K⁺ uptake into chopped pea leaves, when expressed in micromoles per gram fresh weight per hour, has a value which fortuitously is approximately 10 times the pK of the organic acid. The K⁺ uptake is about 5 µmoles/g fresh weight·hr greater than that of the accompanying carboxylic acid, the difference being accounted for by a light-dependent efflux of H⁺, Na⁺, Mg²⁺, and Ca²⁺ from the leaf cells (1). The light-dependent uptake of potassium and the organic acid does not depend on the chain length (and by implication, also not on the lipid solubility) of the anion, e.g., the uptake rates with acetate, propionate, and butyrate are all essentially the same (Table I).

To interpret the correlation between the light-dependent uptake of the potassium salts of organic acids and the pK values, attention should be directed to the anion. For instance, the light-dependent uptake of acetate was approximately the same when Na⁺ replaced K⁺. Also, the light-dependent K⁺ uptake varied linearly with HCO₃⁻ when the latter replaced Cl⁻ at a constant K⁺ concentration (1). The positive correlation between light-dependent uptake and pK (pK = -log K, where K is the dissociation constant of the acid, viz. [H⁺][A⁻]/[HA]) suggests that the uptake depends on some associated form of the organic acid. In other words, the higher the pK, the smaller is the fraction dissociated under the experimental conditions employed and, consequently, the greater is the tendency for association.

Three possibilities for the uptake of an associated species are: (a) the anion enters as the associated form of the potassium salt, e.g., CH₃COOK; (b) the anion moves in as the undisassociated acid, e.g., CH₃COOH, and then there is an exchange of H⁺ inside for K⁺ outside; and (c) the correlation with pK reflects a relative tendency of binding of the anion onto a positively charged site on a “carrier” molecule, the subsequent inward transfer of the organic acid being followed by K⁺ uptake to maintain internal electroneutrality. At the concentration used (5 mM), very little of the potassium salts of organic acids would be in the associated forms, and so the first mechanism is unlikely. Similarly, the concentrations of the undisassociated forms (RCOOH) of most of the organic acids employed are quite low at the pH values in these experiments (near 6). More importantly, both the first and the second mechanisms predict a nearly linear correlation between the rate of ion uptake and the concentration of the associated form of the organic acid, not the logarithmic correlation which a parallel with pK indicates; this latter direct proportionality between pK and the uptake of the potassium salts of organic acids is the key feature of the present studies. After a comment on free energy, the third mechanism will be briefly considered.

The change in standard Gibbs free energy for the dissociation of protons from an organic acid is linearly proportional to the pK (ΔG° = -RT ln K = -2.303 RT log K = 2.303 RT pK). Similarly, the association energy, which is just the negative of the dissociation energy, depends linearly on the pK. Thus, the higher the pK, the more negative is the association energy, which means the more thermodynamically favorable the association reaction becomes. One could speculate that this tendency for hydrogen ions to associate with the carboxyl groups might parallel the tendency for such groups to bind onto carrier molecules, i.e., the relative values of the association energies between H⁺’s and COO⁻’s of a series of organic acids may well be similar to the relative values for the association energies between the negative carboxyl groups of the same organic acids and positively charged sites on carrier molecules. If the probability for transfer of the
organic acids across some membrane is proportional to the association energy between the dissociated carboxyl group of the organic acid and a positively charged site on the carrier, then the rate of uptake could vary linearly with the pK, as was observed in the present experiments. Although this proposal is admittedly speculative, it accounts for the logarithmic correlation between dissociation constants and the light-dependent uptake of potassium salts of organic acids, and also it provides a new way of considering binding onto carriers in terms of association energies.

Finally, the energetics of the uptake of potassium and organic acids into chopped pea leaves will be considered. The inhibition of the light-dependent K⁺ uptake by DCMU (Fig. 2) indicates that photosynthesis, or some partial reaction thereof, can supply the needed energy. The decrease in light-dependent potassium uptake caused by the uncoupler tri-Fl-CCP suggests that photosynthetic electron flow alone is not responsible, but that ATP or a high energy intermediate is required. The efflux of potassium in the dark in the presence of tri-Fl-CCP (Fig. 2) may be due to an uncoupling of oxidative phosphorylation and consequent cessation of ATP formation by mitochondria.

LITERATURE CITED