Effects of Organic Acids on Ion Uptake and Retention in Barley Roots

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ABSTRACT

Effects of several organic acids on ion uptake and retention on respiration in barley roots having low and high KCl contents were assayed by measurements of K⁺, Na⁺, Ca²⁺, Cl⁻, and oxygen uptake. Organic acids with high pKₐ values increase the permeability of roots to ions and decrease respiration when present in sufficient concentrations at pH 5 but have no inhibitory effects at pH 7. Absence of respiratory inhibition in short times and at lower organic acid concentrations, under conditions that immediately produce a permeability increase, indicate that the permeability change is not a result of respiratory inhibition. Effects of formate, acetate, propionate, and glutarate are attributed to entry of undissociated acid molecules into the effective membranes. Lack of a permeability increase with succinate, which has lower distribution coefficients to lipid solvents than do the aliphatic acids, can be explained by failure of sufficient amounts of the hydrophilic succinic acid molecules to penetrate the membranes involved. These experiments suggest that undissociated acid in root membranes can increase permeability of the roots.

Entry of weak organic acids into plants has been known for many years (1, 4, 18). They are generally thought to enter by distribution to the lipid-protein of the cellular membranes (see Ref. 1, for review), in part because of enhanced effects with decrease in pH. Most of these acids, however, are toxic at moderate concentrations and low pH at which inorganic salts are tolerated (7, 16). Evidence that organic acids are taken up as anions by plant roots has only recently been advanced (9). Considerations of the entry of organic anions require some clarification of the nature of the interaction of the undissociated acid with roots.

Measurements of salt loss from roots in the presence of several weak organic acids as shown here indicate that the limiting membranes are modified under usual experimental conditions. Changes in metabolic rates are shown to be secondary to these modifications. The conclusion is that the acid anion can enter the root without evident modification of the root membranes. The undissociated acid in sufficient concentration, however, modifies the membrane so as to alter the response toward inorganic salts and possibly toward the acid itself.

MATERIALS AND METHODS

The roots were from 6-day-old seedlings of barley (*Hordeum vulgare*, var. Compana and var. Trebi) which had been dark grown in aerated 2 × 10⁻⁴ m CaSO₄ pH 5.6 at 25 C. High KCl roots were from seedlings grown in 10⁻³ m KCl + 2 × 10⁻⁴ m CaSO₄ at pH 5.6 for 8 hr on the 5th day. Roots were excised and rinsed several times just before use.

Details of procedure and conditions for the experiments were the same as those used previously (8). Briefly, the roots were maintained in aerated organic acid salt solutions at 23 to 25 C, from which successive root samples were withdrawn periodically, rinsed with water four times, and weighed. Sampling times varied from 5 min to 24 hr. Half-gram root samples were dried in the presence of excess Mg(OH)₂ and ashed at 480 C for 1 hr. The ashed residues were diluted to 25 ml with 0.1 N HNO₃ in 10% acetic acid and analyzed for K⁺, Na⁺, Ca²⁺, and Cl⁻. The treatment solutions were also analyzed for assurance that changes in salt concentration during the experiments were not appreciable. Cl⁻ was determined conductimetrically, K⁺ and Na⁺ by flame photometry, and Ca²⁺ by atomic absorption photometry.

Oxygen uptake by the roots was determined by means of a series of Clark oxygen electrodes in a closed system (15). Rates were measured to a 50% decrease in oxygen concentration, generally for 20 to 90 min.

Buffering capacities were determined by grinding duplicate 2-g root samples in water immediately after treatment so that the final dilution was to 25 ml. The resulting homogenates were then rapidly titrated, one to pH 2.5 with 0.05 N HCl, and the other to pH 11.5 with 0.05 N KOH. Water blanks were titrated similarly, and buffering capacity was calculated from the difference between the water and sample titrations. Volume changes during the titration were less than 25%. Equality of back titrations with KOH and with HCl revealed absence of appreciable bicarbonate.

RESULTS

Changes in Ion Contents. Roots having either low or high KCl content when placed in 10⁻² eq/liter solutions of formate (Figs. 1, 2), acetate (Fig. 2), propionate (Fig. 2), or glutarate (Fig. 3) at pH 5 lose endogenous and previously accumulated ions. Potassium release from roots begins immediately even in K⁺ salts of these acids (Fig. 1D). Sodium salts were used in most experiments so that cation influx (Na⁺) and efflux (K⁺) could be measured simultaneously. In Na⁺ salts of the organic acids (Figs. 1A, 2, 3), an increased Na⁺ influx accompanies the increased K⁺ efflux over the first 15 to 30 min, and initial rates of Na⁺ uptake are 20 to 105% faster than in Cl⁻ solutions (Fig. 2). The initial Na⁺ uptake (5–30 μeq/g) is in great excess of the amount in the free space (2 μeq/g) which is reduced to a negligible amount by rinsing in all experiments. Subsequently, the Na⁺ content decreases with continued loss of K⁺ and Ca²⁺. Chloride efflux likewise increases in 10⁻² eq/liter formate, acetate, or propionate at pH 5 (Fig. 2). No Cl⁻ accumulates when KCl is in solution with these acids at pH 5 (Fig. 1D).

Results are similar in the presence of Ca²⁺ at pH 5. For ex-
EFFECTS OF ORGANIC ACIDS

Fig. 1. Ion contents of low KCl roots in 10⁻⁴ eq/liter salts at pH 5. A: Formate, Na⁺ salt; B: formate, Na⁺ + 2 × 10⁻⁴ eq/liter Ca²⁺ salt; C: sulfate, K⁺ salt + 10⁻⁴ eq/liter KCl; D: formate, K⁺ salt + 10⁻³ eq/liter KCl. The undissociated acid concentration of the organic acid solutions is given under the acid name.

ample, presence of 2 × 10⁻³ eq/liter Ca²⁺ in the external solution does not prevent loss of K⁺ and subsequent loss of Na⁺ from roots in 0.9 × 10⁻³ eq/liter formate at pH 5 (Fig. 1B). This is in spite of a Ca²⁺ content that is unchanged from the initial content and is greater than the Ca²⁺ content of roots in Cl⁻ (Fig. 2) or SO₄²⁻ solutions (Fig. 1C). Roots in formate without Ca²⁺ (Fig. 1A) lose 50% of their Ca²⁺ within 3 hr. Although the time of maximal Na⁺ retention and 50% K⁺ loss is delayed somewhat in the Ca²⁺ solution (Fig. 1B), the eventual K⁺ loss is the same with or without Ca²⁺. Calcium also does not prevent the loss of Cl⁻.

For example, in the same experiment as that in Figure 1B, the Cl⁻ content decreased from 1.6 to 0.5 μeq/g.

Roots become leaky in 1.5 × 10⁻³ eq/liter acetate and 1.3 × 10⁻³ eq/liter propionate at pH 5 (Fig. 4). Potassium and Ca²⁺ losses exceed Na⁺ accumulation and the roots lose Cl⁻. Potassium influx in 10⁻⁵ eq/liter glutarate at pH 5 (Fig. 3) is large compared to the amount of Na⁺ initially accumulated and Na⁺ efflux eventually exceeds influx, demonstrating that roots in this acid also become more permeable. Although K⁺ influx and Na⁺ influx are increased in 10⁻⁵ eq/liter glycolate at pH 5 (Fig. 4), cation influx continues to exceed efflux and the roots eventually accumulate 25% more cations than roots in Cl⁻ solutions (e.g., Fig. 2). In contrast to the effects of acetate, etc., cation uptake capacities of roots in 10⁻⁴ eq/liter glycinate or succinate at pH 5 (Fig. 3) are generally 20 to 50% greater than when roots are in Cl⁻ solutions (Fig. 2). Even high KCl roots lose little or no Cl⁻ (<25%). Cation effluxes and initial uptake rates are about the same as in Cl⁻.

Despite the organic acid effects at pH 5, no increase in permeability occurs in roots in 10⁻³ eq/liter organic acid salts at pH 7 in which the undissociated acid concentration does not exceed 7 × 10⁻³ M (Fig. 5). Initial rates of cation accumulation at pH 7 are the same in formate and propionate as in sulfate. Cations continue to be accumulated rapidly in the organic salt solutions at 24 hr, as if the cation uptake capacities were increased. The roots accumulate chloride in the presence of the organic acid salts at pH 7, but the rate is somewhat suppressed, possibly because of competition of organic anions with chloride.

Relative K⁺, Ca²⁺, and Cl⁻ losses from roots in 10⁻³ eq/liter acetate at pH 5 follow the same curve when the ordinates are expressed as percentage of initial values (Fig. 6). A 50% loss takes about 3 hr, although the absolute amounts of the various elements differ greatly. The ion losses parallel losses of titratable anions and acid. Titratable anions (A⁻) decreased from 50 to 15 μeq/g and HA⁺ from 112 to 20 μeq/g, reaching a 50% loss at

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1 Abbreviations: A: titratable anions; HA: titratable acid.
3.5 to 4.5 hr. The titratable anion losses are sufficient to balance net cation losses in excess of chloride.

**Respiratory Changes.** Oxygen uptake rates of roots in 10^{-3} eq/liter salt or propionate at pH 5 are uninhibited over a 24-hr period (Table I), although the roots lose Cl^{-} and endogenous cation losses exceed accumulation of externally supplied cations after the 1st hr (e.g., Fig. 4). No respiratory inhibition occurs in 10^{-3} eq/liter gluatrate or glycerate at pH 5 over 24 hr. In 10^{-3} eq/liter formate, acetate, or propionate at pH 5, respiration generally becomes stimulated during the first 5 to 10 min of treatment, but then, during the next 5 or 10 min, it decreases to control rates and continues to decrease rapidly thereafter (Table I). In spite of the delayed onset, respiratory inhibition proceeds faster and becomes greater than relative ion and buffering losses after the first half-hour. Respiratory inhibition is partially reversible upon transfer of the roots from the organic acid solution to water, although the inhibition may eventually approach 100%.

Roots pretreated with 3, 6, and 24 hr in 10^{-3} eq/liter acetate at pH 5 were rinsed once with water and then transferred as quickly as possible to water for measurements of oxygen uptake. The transfer time was about 3 min. Roots which had lost 75% of their salt, buffering capacity, and respiration in the acetate at pH 5 for 6 hr, recover about 50% of their respiration (Fig. 7). Most of the recovery seems to be immediate. By contrast, roots transferred to 10^{-3} eq/liter KCl or K_{2}SO_{4}, with or without 10^{-3} eq/liter Ca^{2+}, after only 3 hr in 10^{-3} eq/liter acetate at pH 5, fail to recover any capacity to accumulate ions over a 24-hr period. The slight gains of externally supplied cations (+1.8 μeq/g K^{+} and/or <4.3 μeq/g Ca^{2+}) are balanced by endogenous cation losses (−4 to −5 μeq/g Na^{+}). Yet respiration recovers to 89% of the rate of control roots. Respiration of roots in 10^{-3} eq/liter acetate at pH 7 increases slightly relative to respiration in water (Table I, Fig. 7), as does respiration in sulfate. Sulfate stimulates respiration substantially at pH 5 and, to a lesser degree, at pH 7 (Table I). The stimulatory effect also is reversible upon transfer of the roots to water.

Other acids also were assayed for respiratory and permeability effects. Results of some of these experiments are not reported in detail since roots in 10^{-3} eq/liter concentrations of these acids at pH 5 respond essentially the same as roots in gluatrate (Fig. 3). They were: alanine (2.2 × 10^{-3} M H_{3}A^{+}), aspartate (4.2 × 10^{-7} M H_{3}A^{+}), citrate (1 × 10^{-8} M H_{3}A), glycine (2.3 × 10^{-7} M H_{3}A^{2-}), lactate (1.2 × 10^{-4} M H_{3}A), malate (7 × 10^{-5} M H_{2}A), and pyruvate (3.2 × 10^{-5} M HA). Effects of other acids with high pK_{a} values are similar to those of formate or acetate.
DISCUSSION

Organic acids, such as formate or acetate, cause the root membranes to become more permeable to ions inasmuch as ion influx is increased as well as efflux. The permeability increases are large and proceed rapidly in $10^{-2}$ eq/liter acetate or propionate at pH 5 in which undissociated acid concentrations exceed $3 \times 10^{-3}$ M (Fig. 2). When undissociated acid concentrations are $5.4 \times 10^{-4}$ M, as in $10^{-2}$ eq/liter formate (Fig. 2), $1.5 \times 10^{-3}$ eq/liter acetate or $1.3 \times 10^{-3}$ eq/liter propionate at pH 5 (Fig. 4), the effects are somewhat attenuated and progress less rapidly. No increase in permeability is evident at pH 7 in $10^{-2}$ eq/liter solutions of these acids ($<7 \times 10^{-5}$ M HA, Fig. 5). The effects are also absent at pH 5 when roots are in $10^{-2}$ eq/liter solutions of acids with low pKₐ values, such as citrate ($1 \times 10^{-3}$ M H₃A), yet citrate uptake ultimately exceeds acetate or propionate uptake at pH 5 (9). In fact, the effects are in marked contrast to the uptake of all of the acids by the barley roots, which chiefly involves the organic anions (9). Formate, acetate, and propionate are readily taken up at pH 7 as well as at pH 5. Furthermore, roots have rather high endogenous levels of acetate and propionate. The acetate content of barley roots is of the order of $10^{-3}$ mol/liter of cell water (11), as high as or higher than the concentrations used in the experiments reported here. Consequently, the permeability increases involve only the undissociated acid species and not the presence or entry of organic anions. Respiratory inhibition likewise reflects only the undissociated acid species.

The organic acid effects on ion fluxes and retention might be expected to result from respiratory inhibition. However, respiration and the permeability increases respond differently with respect to undissociated acid concentrations, treatment times and reversibility of the effects. This may be only a consequence of root morphology, of access to the plasmalemma preceding access to respiratory sites; nevertheless, the permeability increase appears to be the primary event. It seems likely that respiration would be inhibited as a result of continued loss of ions and buffering capacity, but dependence of the degree of respiratory inhibition on the continued presence of the acid in the external solution implies involvement of other factors. In fact, rapid partial recovery from respiratory inhibition upon removal of the roots from the organic acid solution without recovery of any capacity to accumulate ions also indicates some separation of the two effects.

Effects of the aliphatic acids on permeability and respiration are greater and appear sooner than do the effects of the dicarboxylic acids or hydroxycylic acids. Ion losses progress more rapidly and are greater at pH 5 in $10^{-2}$ eq/liter formate ($5.3 \times 10^{-4}$ M HA) than in $10^{-2}$ eq/liter glutarate ($6.8 \times 10^{-4}$ M HA). Sodium and K⁺ fluxes of roots in $10^{-2}$ eq/liter glutarate at pH 5 ($6.3 \times 10^{-4}$ M HA) increase, but the roots do not lose their capacity to accumulate and retain ions. Roots lose ions in formate solutions with an undissociated formic acid concentration as low as $1.5 \times 10^{-4}$ M, ($2.3 \times 10^{-4}$ eq/liter formate at pH 5), yet succinate solutions with $5 \times 10^{-4}$ M undissociated succinic acid ($10^{-2}$ eq/liter salt at pH 5) do not increase permeability. These differences among the acids suggest that the permeability changes require entry of the undissociated acid into the root membranes. Inasmuch as plant membranes are generally thought to contain lipid and protein layers, entry of acid molecules might be expected to reflect differences in the partition of the acids between

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**Table 1. Effects of Organic Acids on Oxygen Uptake Rates of Barley Roots**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Initial 5-15 min</th>
<th>3 hr</th>
<th>6 hr</th>
<th>24 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>eq/liter</strong></td>
<td><strong>μeq/min•g</strong></td>
<td><strong>% rate in H₂O</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H₂O</td>
<td>809</td>
<td>828</td>
<td>826</td>
<td>702</td>
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<td>Sulfate</td>
<td>10⁻², pH 5</td>
<td>104</td>
<td>102</td>
<td>99</td>
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<tr>
<td></td>
<td>10⁻³, pH 7</td>
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<td>111</td>
<td>112</td>
</tr>
<tr>
<td>Formate</td>
<td>10⁻², pH 5</td>
<td>99</td>
<td>60</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>10⁻³, pH 7</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Acetate</td>
<td>10⁻², pH 5</td>
<td>108</td>
<td>17</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>10⁻³, pH 7</td>
<td>101</td>
<td>101</td>
<td>135</td>
</tr>
<tr>
<td></td>
<td>10⁻⁴, pH 5</td>
<td>96</td>
<td>99</td>
<td>108</td>
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<td>Propionate</td>
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<td></td>
<td>10⁻³, pH 5</td>
<td>101</td>
<td>97</td>
<td>82</td>
</tr>
<tr>
<td>Glutarate</td>
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<td>Glycolate</td>
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<td>96</td>
<td>111</td>
<td>118</td>
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<tr>
<td>Succinate</td>
<td>10⁻², pH 5</td>
<td>111</td>
<td>138</td>
<td>144</td>
</tr>
<tr>
<td></td>
<td>10⁻³, pH 7</td>
<td>...</td>
<td>131</td>
<td>...</td>
</tr>
</tbody>
</table>
organic liquids and water (5, 17). Studies of Overton and Collander have shown that permeation of nonelectrolytes into many plant tissues is correlated with lipid solubility (1).

The monocarboxylic acids are relatively lipophilic. In fact, they permeate red cells much more rapidly than the more hydrophilic dicarboxylic acids (3, 6), differences which were attributed to solubility of the lipophilic acids in the membranes (3). Acids such as succinic and citric are predominantly hydrophilic and, as such, probably fail to penetrate biological membranes (6). Consequently, damaging effects of undissociated acid molecules on barley roots, as contrasted with the uptake of the organic anions (9), may involve presence of undissociated acid molecules within lipid phases of the membranes.

It might be held that lack of a permeability increase or respiratory inhibition in organic acid solutions at pH 7 or with acids such as succinate, etc., at pH 5 is due to failure of roots to take up any organic acids under these conditions. Measurements reported elsewhere, however, show that all of the compounds are taken up both at pH 5 and 7 (9). Uptake of undissociated acid molecules can be only a small portion of the total organic acid uptake which principally involves the organic anion. As a corollary, permeability increase and respiratory inhibition would seem to be expressions of undissociated acid entry in the present experiments. Absence of such effects accompanied by organic acid uptake further supports the concept that barley roots take up organic anions under these conditions.

Effects of weak acids on biological systems have long been known to be influenced by the pH of the medium. The effects are generally greater under acidic conditions. Simon and Bevers (16), measuring yeast respiration and fermentation, *Avena* coleoptile extension and fungus growth, and reviewing work of others, observed that similar relationships hold for a wide variety of weak acids. Lactic acid, but not lactate salt, was implicated in the production of irreversibility in oligohemiang shock in mammals (14). Decreasing pH enhances the hemolytic effects of long chain fatty acids on erythrocytes (6). Permeability of mammalian gastric mucosa to H+ increases greatly when the tissue is exposed to acetate, propionate, or butyrate in acidic, but not in neutral solution (2). Van Overbeek and Blondeau found that acetic and propionic acids increased permeability of beet root disks (19). Only the undissociated molecules were toxic, and toxicity was greatest when molecular weight was lowest, as was found also in the present experiments.

The similarity of the organic acid and pH effects on such diverse tissues is striking. Furthermore, the nature of the organic molecules in producing the permeability changes in barley roots is so diverse that it seems unlikely that their carbon entities are causing the changes. The most common feature is that they carry an acid entity. This seems to be the common denominator with many other tissues as well. However, differences between organic acid effects on permeability and on respiration suggest that the permeability increases in barley roots do not primarily result from the production of a high internal H+ concentration uniformly pervading the entire cytoplasm, although such a condition may result ultimately as a consequence of the increased permeability. Permeability increases are as great and as rapidly produced in high salt roots as in low salt roots, in spite of protection of roots from effects of low pH in the external solution by presence of salt (10, 12, 13). Nor can the permeability increase be attributed to the amounts of Ca+2 in the roots. Presence of Ca+2 in the external solution does not prevent large losses of ions, although much Ca+2 is retained by the roots. Rather, the permeability increases in the present work appear to be localized effects of the organic acids. Van Overbeek and Blondeau interpreted the toxic effects of organic acids on plants due to solubilization of the acid moiety within the membranes, resulting in modification of the plasma membrane and other lipophases as well, which they describe in detail (19). This interpretation would seem to apply also to the organic acid effects on barley root permeability.

**LITERATURE CITED**