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Changes in Membrane Lipids of Roots Associated with Changes in Permeability

I. EFFECTS OF UNDISSOCIATED ORGANIC ACIDS

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ABSTRACT

Previous work has shown that undissociated forms of organic acids, such as formic, acetic, and propionic acids, increase the permeability of barley roots to ions. The work here was undertaken to test whether these undissociated acids affect the lipids from the root membranes in such a way as to account for the permeability increase. Relative amounts of the principal fatty acids from barley root membranes were measured as a function of organic acid concentration, pH, and time of treatment of barley roots under conditions similar to those of the previous studies.

Undissociated formic, acetic, and propionic acids all rapidly increase the proportions of palmitic, stearic, and oleic acids and decrease proportions of linoleic and linolenic acids. Only the undissociated species are effective. The effects on the fatty acids from membrane lipids parallel effects on ion permeability. It is concluded that the increase in permeability produced by undissociated organic acid is due to changes in the lipids of barley root membranes.

Previous work has shown that undissociated forms of organic acids, such as formic, acetic, propionic, butyric, and glutaric acids, increase the permeability of barley roots to ions (8). Relative effects of the acids increased according to their lipid solubility. This suggests a link between lipids in root membranes and areas where hydrophilic ions permeate. The work presented here was designed to test this hypothesis.

The approach was to determine relative amounts of the principal fatty acids, *i.e.* palmitic (C 16:0), stearic (C 18:0), oleic (C 18:1), linoleic (C 18:2), and linolenic (C 18:3), from the polar lipids of membranes (4) in the roots. The fatty acids were measured as a function of organic acid concentration, pH, and time of treatment under conditions similar to those of the previous studies of the organic acid effects on permeability of roots to ions (8). The work presented here is among the first to demonstrate that a chemical can cause an immediate change in membrane composition and, thereby, immediately affect functionality.

MATERIALS AND METHODS

The roots were from 6-day-old seedlings of barley (*Hordeum vulgare* var. Trebi) which had been dark-grown in aerated 0.2 mM $CaSO_4$ (pH 5.6) at 25 C. Roots were excised and rinsed several times with demineralized H_2O just before the experiment.

Details of procedures and conditions for the experiments have been described (5). Briefly, about 10 g roots were maintained in 4 liters aerated organic acid salt solution at 23 to 25 C for 15 min to 6 h. Then the roots were removed and freeze-dried. The pH was maintained during treatment by periodic titration with an appropriate base or acid.

Lipids were extracted and recovered from freeze-dried root tissue according to the procedures of Folch *et al.* (3). Polar lipids were separated from nonpolar lipids by rubber membrane dialyses according to the method of Bottcher *et al.* (1). Polar lipids were saponified with alcoholic KOH as outlined by Burchfield and Storrs (2). Methyl esters of the resulting fatty acids were prepared for gas chromatography (11) with boron trifluoride in methyl alcohol by the method of Metcalfe and Schmitz (10). Heptadecanoic acid (C 17:0) was included in all samples as an internal standard.

The results are expressed generally as the per cent by weight of the total fatty acids (C 16:0, C 18:0, C 18:1, C 18:2, C 18:3) and as ratios of saturated acids to unsaturated acids. This was done partly because the gas chromatographic data are presented as relative values. The combination of weighing the samples and quantitative introduction of an internal standard so that quantities can be calculated is less precise. However, some results are given also as μmol acid/g freeze-dried roots. The dry weights are about 7% of the fresh weights.

RESULTS

Formic, acetic, and propionic acids at pH 5 all increase the proportion of palmitic acid (C 16:0) and decrease the proportions of linolenic acid (C 18:3) and, to a lesser extent, linoleic acid (C 18:2) from the polar lipids of roots in 10 mM salts of these acids for 6 h (Table I). Stearic (C 18:0) and oleic (C 18:1) acids increase proportionately also, but the major and most consistent effects are on the three fatty acids shown which comprise 91 to 96% of the total fatty acids from the membrane lipids of the roots. Changes in the fatty acids are reflected by increase in the degree of saturation and increase in the ratio of linoleic to linolenic acid (Table I). All of these effects of the organic acids at pH 5 are significant at the 1% level of confidence. Effects of 10 mM solutions of these acids at pH 5 are consistent with the concentrations of undissociated acid, *i.e.* propionic (4.3 mM) > acetic (3.6 mM) > formic (0.53 mM). The dissociation constants for the acids are: formic, 0.177 mM; acetic, 17.6 μM; propionic, 13.4 μM; succinic

Table I. *Effects of Various Organic Acids as a Function of pH on Fatty Acids from Polar Lipids of Barley Roots*

Percentages are expressed as per cent by weight of the total fatty acid content. S/U is the ratio of the sum of the saturated acids (C 16:0 + C 18:0) to the sum of the unsaturated acids (C 18:1 + C 18:2 + C 18:3). The roots were in 10 mM K^+ salt solutions for 6 h. The 0 treatment in II refers to roots that were untreated.

Treatment	16:0	18:2	18:3	18:2/18:3	S/U	Total
	% total			ratio		μmol/g dry wt
I H_2O						
pH 7	25.4	50.8	18/6	2.73	0.370	37.4
pH 5	26.7	49.8	18.1	2.75	0.390	38.8
Formate						
pH 7	26.7	48.2	18.0	2.68	0.390	42.5
pH 5 (0.53 mM formic acid)	31.7	47.4	14.7	3.22	0.503	43.7
Acetate						
pH 7	25.1	50.6	18.6	2.72	0.370	
pH 5 (3.6 mM acetic acid)	34.3	46.3	10.5	4.40	0.593	47.6
Propionate						
pH 7	26.2	50.2	19.1	2.63	0.381	43.6
pH 5 (4.3 mM propionic acid)	36.8	45.2	10.0	4.52	0.641	40.5
II O	26.6	50.8	17.9	2.84	0.383	37.0
Succinate						
pH 5 (0.52 mM succinic acid)	24.8	51.6	15.9	3.25	0.376	41.2

Table II. *Fatty Acids from Polar Lipids: Relative Contents of Roots in Various Salt Solutions at pH 5*

The roots were in 10 mM salt solutions for 6 h. S/U is the ratio of the sum of the saturated acids (C 16:0 + C 18:0) to the sum of the unsaturated acids (C 18:1 + C 18:2 + C 18:3).

Treatment	16:0	18:2	18:3	18:2/18:3	S/U	Total
	% total			ratio		μmol/g dry wt
O[a]	25.9	50.2	18.4	2.75	0.379	48.9
KCl	25.0	51.8	17.8	2.90	0.365	35.5
+Ca	26.7	49.9	15.9	3.14	0.417	43.9
NaCl	25.3	52.5	17.1	3.08	0.368	44.6
+Ca	24.6	50.0	17.8	2.81	0.376	44.3
$CaSO_4$	25.6	51.9	17.9	2.89	0.369	40.4

[a] Roots untreated.

(K_1), 68.9 μM.

Despite the highly significant effects of the organic acids at pH 5, no changes are evident in the fatty acid distribution when the roots are in 10 mM organic acid salt solutions at pH 7 where the undissociated acid concentration is less than 0.1 mM (Table I). The change in pH *per se* has no effect, as shown by results from the roots in H_2O in Table I.

In contrast to the relatively lipophilic aliphatic acids, hydrophilic succinic acid at pH 5 has no significant effect on the fatty acid distribution (Table I). The concentration of undissociated succinic acid in 10 mM succinate at pH 5 is 0.52 mM, which is about the same as the concentration of undissociated formic acid under similar conditions. Various salts of strong acids at pH 5 likewise have no significant effects on the fatty acids, whatever the cation (Table II).

Studies of various times of treatment of the roots in 10 mM acetate at pH 5 reveal that the effects are produced as soon as the roots are introduced to the solution and progress at constant rates during the 6 h of treatment (Fig. 1). The linear curves all pass through zero effect at zero time with correlation coefficients between 0.94 and 0.99. The only delayed effect is the relative decrease in C 18:2. Fatty acids from the roots in acetate at pH 7 do not change at all during the 6 h. Individual results at pH 5 differ significantly from the corresponding results at pH 7.

The dependence of the effects on only the concentration of undissociated acid was tested by comparison of the effects of undissociated acetic acid over a range of concentrations which were obtained in two ways. For one, the concentration of acetate was varied at constant pH and for the other, the pH was varied at constant acetate concentration (Fig. 2). Over the low range of concentrations (Fig. 2A), the effects increase in direct proportion to the increase in undissociated acid concentration. The acetate concentration varied from 0.1 to 10 mM. However, the intensity of the effects reaches a maximum at about 11 mM undissociated acetic acid, as shown in Figure 2B where the highest acetate concentration was 50 mM. These results show that undissociated acetic acid concentrations obtained by varying the pH produce the same curves as concentrations obtained by varying the acetate concentration. The effects are independent of the changes in pH or in the total acetate concentration *per se*.

Total amounts of the fatty acids from polar lipids decrease appreciably when the roots are in 50 mM solutions of the organic acids at pH 5. Thus, there is a net loss of C 18:2 and C 18:3 and no net gain at C 16:0. Consequently, effects of acetic acid on fatty acids from nonpolar lipids and free fatty acids were determined to assess whether the fatty acids lost from the polar lipids appeared in the free fatty acids and whether the effects are principally on the polar lipids (membrane lipids) (Table III). Even at 50 mM, acetate at pH 5 has little or no effect on fatty acids from the nonpolar lipid fraction. Only a decrease in 18:3 is evident, which results in a higher ratio of 18:2 to 18:3, whereas percentages of all of the fatty acids from the polar lipid fraction are affected. Total amounts of the fatty acids from the nonpolar lipid fraction are not affected appreciably at either pH. Both distribution and the total amount of the free fatty acid fraction are affected by 50 mM acetate at pH 5 but are not at all affected at pH 7. At pH 5, there

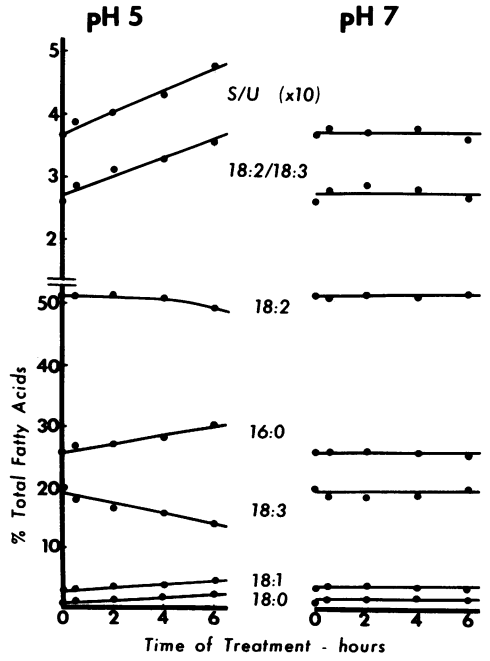

FIG. 1. Effects of 10 mM acetate on proportions of fatty acids from root membranes as a function of time and pH.

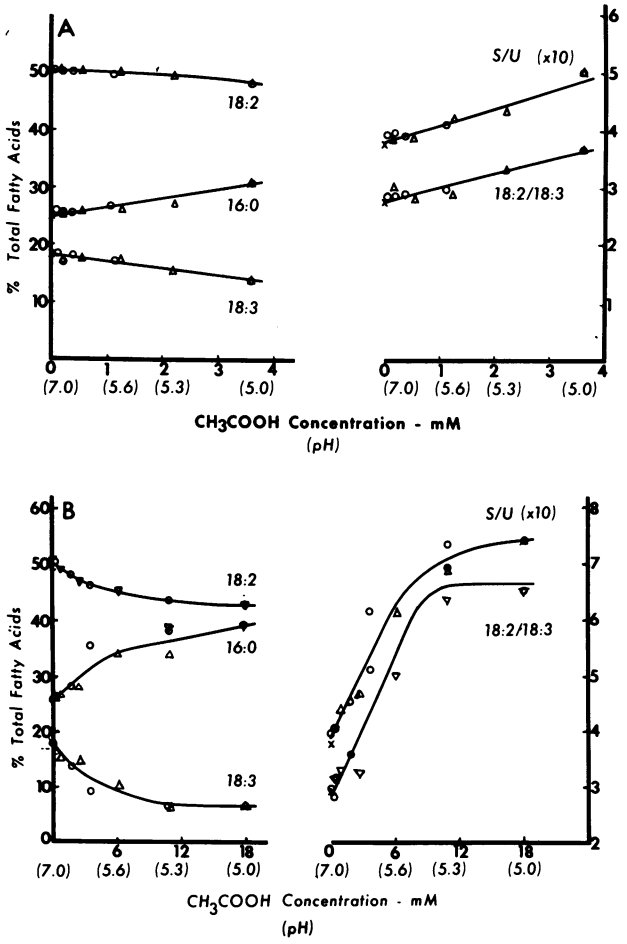

FIG. 2. Effects of undissociated acetic acid concentrations on proportions of fatty acids from root membranes. (○), treatment with undissociated acetic acid concentrations obtained by varying the acetate concentration from 0.1 to 10 mM (A) or 0.5 mM to 50 mM (B) at a constant pH of 5.0. (△), treatment with undissociated acetic acid concentrations obtained by varying the pH from 7 to 5 at a constant acetate concentration of 10 mM (A) or 50 mM (B).

is almost a 400% net gain in the amount of C 16:0 which lowers percentages of the other fatty acids in this fraction but does not change in the total amounts of these acids. The gain in the total amount of the free fatty acid fraction at pH 5 is not sufficient to balance the loss of fatty acids from the polar lipid fraction.

Reversibility of the undissociated acid effects was tested by treating roots for 3 h in 10 mM acetate at pH 5 and then transferring them to 10 mM KCl for 3 h (Table IV). The roots did not recover at all. Roots which were in acetate and then KCl solutions have about the same proportions of fatty acids as the roots in acetate only.

DISCUSSION

The work presented here provides compelling evidence that undissociated acid is the effective species of formic, acetic, or propionic acid in producing changes in the lipid composition of barley root membranes. Neither the presence of the organic acid anions nor the pH change *per se* have any such effects (Table I). The changes are large when roots are in acetate solutions in which undissociated acetic acid concentrations exceed 1 mM (Fig. 2). No effects are evident where the undissociated acid concentration is less than 0.1 mM, as in 10 mM formate, acetate, or propionate at pH 7 (Table I). This is in marked contrast to the uptake of the organic acids which chiefly involves organic acid anions (6).

Table III. *Effects of Acetate as a Function of pH on Fatty Acids from Lipid Fractions of Barley Roots*

Treated roots were in 50 mM acetate solutions for 6 h. S/U is the ratio of the sum of the saturated acids to the sum of the unsaturated acids.

Treatment	16:0	18:0	18:1	18:2	18:3	18:2/18:3	S/U	Total
	% total					ratio		μmol/g dry wt
Polar Lipid Fraction								
O[a]	24.9	0.6	3.3	49.5	21.7	2.30	0.343	43.3
Acetate								
pH 7	26.8	1.4	3.1	48.7	20.1	2.43	0.392	37.0
pH 5	41.4	2.5	6.6	43.1	8.4	4.92	0.774	23.0
Nonpolar Lipid Fraction								
O[a]	21.5	15.0	13.3	36.8	13.3	2.78	0.575	8.5
Acetate								
pH 7	20.8	15.7	10.2	40.0	13.4	2.89	0.565	8.6
pH 5	24.9	11.4	12.6	43.2	8.0	5.41	0.570	9.2
Free Fatty Acids								
O[a]	13.9	11.2	18.5	51.8	4.6	11.2	0.336	11.0
Acetate								
pH 7	14.2	14.4	17.4	49.4	4.6	10.7	0.398	11.4
pH 5	35.6	10.7	14.6	36.6	2.6	14.2	0.861	16.4

[a] Roots untreated.

Table IV. *Nonreversibility of Effects of Acetate at pH 5 on Fatty Acids from Root Membranes*

Barley roots were in 10 mM acetate at pH 5 for 3 h, after which they were immediately transferred to 10 mM KCl at pH 5 for 3 h. S/U is the ratio of the sum of the saturated acids to the sum of the unsaturated acids.

Treatment	16:0	18:0	18:1	18:2	18:3	18:2/18:3	S/U	Total
	% total					ratio		μmol/g
O[a]	25.9	1.9	3.6	50.3	18.3	2.74	0.385	57.4
Acetate (pH 5)	26.3	3.0	5.2	49.7	15.8	3.14	0.413	46.9
Transferred to KCl	28.7	3.1	5.0	48.7	14.5	3.37	0.467	51.0

[a] Roots untreated.

Formate, acetate, and propionate are readily taken up at pH 7 as well as at pH 5. Furthermore, the endogenous acetate concentration in barley roots is about 10^{-2} mol/l cell water (7), which is as high as the solutions used in the present experiments. Participation of only the undissociated acid in the effects on membrane lipids is shown in another way. The undissociated acid concentration can be varied either by changing the pH at a constant concentration of organic acid salt or by varying the organic acid salt concentration at constant pH. When this is done, the effects reflect only the concentration of undissociated acid, whatever the pH or organic acid salt concentration (Fig. 2).

The undissociated acids principally affect only the fatty acids from the membrane lipids (Table III). Fatty acids from nonpolar lipids, which comprise lipids from sources other than membranes, are largely unaffected. The effect of undissociated acid, then, is not a general effect on lipids, although this might be a consequence of cell morphology and the relatively short duration of the experiments, *i.e.* of access to the plasma membranes preceding access to the interior of the cells. Another indication that only membrane lipids are involved initially is that the organic acids have an immediate effect (Fig. 1). The time curves show no initial lag.

They are linear and extrapolation shows zero effect at zero time. This indicates that the effects are primary events and not a consequence of a disrupted metabolism and decreased respiration, although this occurs eventually (8). Respiration is uninhibited during the first 0.5 h of treatment. Almost exclusive involvement of the membranes is shown also by lack of any effect of K^+ and Ca^{2+} salts of strong acids (Table II), although distribution of internal organic acids is affected over the duration of experiments such as these, owing to imbalance in the cation and anion uptakes (7).

The previous studies of ion permeability of barley roots (8) may have assessed plasma membranes of only a portion of the cells, whereas the measurements of changes in polar lipids involve all membranes of all of the cells. Therefore, effects on fatty acids from polar lipids may be attenuated because all membranes may not be affected or accessible to the undissociated organic acid. Results in these experiments are expected to represent minimal changes in the lipids which could be greater in more accessible localized areas.

The similarity between the effects of the undissociated acids on membrane lipids of barley roots and on the permeability of the roots to ions is striking. All of the effects on the fatty acids from the polar lipids already discussed parallel effects on ion permeability (8). The concentration of undissociated acid that is effective and the rapidity of the responses are the same. In addition, both membrane lipid changes and permeability increases reflect the lipophilic character of the organic acids. The more lipophilic they are, the greater are the effects, as shown by comparison of the relative effects of the lipophilic aliphatic acids and hydrophilic succinic acid. Both changes in permeability and in the membrane lipids are unaffected by succinic acid (at 0.52 mM undissociated succinic acid) and more affected by formic acid than by acetic or propionic acids. This is shown in the present study by an effect of formic acid (at 0.53 mM undissociated formic acid) in Table I nearly as great as that of acetic and propionic acids (at 3.6 and 4.3 mM undissociated acid, respectively), although the concentration of undissociated formic acid is only 12% of the concentration of undissociated propionic acid. Furthermore, effects of the undissociated acids on permeability of the roots to ions are not reversible (8). The roots failed to take up any K^+ or Cl^+ when they were transferred from 10 mM acetate at pH 5 to 10 mM KCl. Likewise, the effects of undissociated acid on the membrane lipids are not reversible (Table IV). These results imply that the increase in permeability produced by undissociated organic acid is due to chemical changes in the plant membranes.

It might be held that the fatty acid changes produced by undissociated organic acids are the result of respiratory inhibition (8). However, additional differences between the responses of respiration and the fatty acid distribution to treatment with the organic acids make this unlikely. The concentration-pH curves in Figure 2A show that effects on C 16:0, C 18:3, and the ratios increase in direct proportion to the increase in acetic acid concentration in the range of 0.1 to 3.6 mM. Respiration is uninhibited at acetic acid concentrations below 1 mM, whatever the pH or total acetate concentration. The inhibition of respiration is largely reversible upon transfer of the roots to an inorganic salt solution (8), whereas effects on the fatty acids are not reversible (Table IV). Respiration recovered to 89% of control under treatment of the roots similar to that of Table IV. Thus, the effects on the fatty acids differ from the effects on respiration with respect to time of treatment, organic acid concentration, and reversibility. They parallel the effects on permeability, not respiration.

Changes in the membrane lipids evidently involve solubilization of undissociated acid within lipid areas of the membrane. This is suggested by effectiveness of only the undissociated species and greater effectiveness of the shorter chain formic acid than the longer chain acids, acetic and propionic. The nature of the changes, that is, the increase in saturation of the fatty acids from the polar lipids in particular, the decrease in linolenic acid, and the rapidity with which these effects are produced is suggestive of desaturase inhibition. Fatty acid desaturases are considered to be associated with membranes (9). Inhibition of the desaturases for both linolenic and linoleic acids seems likely in as much as proportions of oleic, stearic, and palmitic acids increase by about the same factor (Table III). Levels of linoleic acid would be expected to increase, on the one hand, upon inhibition of its desaturase but to decrease, on the other hand, upon inhibition of the oleic acid desaturase, resulting in little net change as observed. Thus, the lipid changes associated with permeability increase in barley roots appear to be localized effects of the acid moiety within the membrane. It remains to be seen whether increase in the saturation of fatty acids from membrane lipids is associated with permeability increases in other organs.

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