Ion Transport Characteristics of Grape Root Lipids in Relation to Chloride Transport

Pieter J. C. Kuiper

United States Salinity Laboratory, Soil and Water Conservation Research Division, ARS, United States Department of Agriculture, Riverside, California

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Abstract. Ion transport properties of grape root lipids were measured as liquid-membrane permeability. Phosphatidylethanolamine exchanged chloride very slowly against carbonate but more rapidly against nitrate, phosphate, and sulfate. Exchange of chloride against nitrate was rather low for the phosphatidylethanolamine lipid fractions; monogalactose diglyceride was by far the most effective chloride transporter studied. Comparison between the lipid composition of the roots of the 5 grape rootstocks and the chloride transport capacity of the specific membranes strongly suggest that, indeed, the chloride transport capacity of the lipids present in the membranes of the root cells accounts for the observed differences in chloride transport to the leaves. Whereas monogalactose diglyceride had a high chloride transport capacity, compared with phosphatidylethanolamine, the reverse was true for exchange of sodium against potassium. Thus, phosphatidylethanolamine has more the properties of a cation exchanger, and monogalactose diglyceride those of an anion transporter.

Bernstein, Clark, and Ehlig (personal communication) observed that grape rootstocks differ markedly with respect to the amount of chloride translocated to the leaves; leaves on Cardinal rootstock contained 15 times as much chloride as those on Salt Creek rootstock. Differences between scions were small, compared with the effect of the rootstock.

Comparison of the lipids of the roots of the 5 grape rootstocks showed that chloride accumulation in the leaves correlated directly with the level of monogalactose diglyceride and inversely with the levels of phosphatidylethanolamine and phosphatidylethanolamine (2).

Chloride- and sodium-transport characteristics of these lipids were studied as liquid-membrane permeability, using Schumman's model (1,3). In this model, chloride or sodium is transported by the lipid from one aqueous compartment to another through a nonaqueous phase under a concentration gradient. Small amounts of lipids from oriented molecular layers at the interfaces.

Materials and Methods

Extraction and separation of the root lipids are described in a companion paper (2).

The model for measurement of the liquid-membrane permeability as adapted from Schumman consists of 2 compartments, A and B, separated by a wall (fig 1). The solution level on either side was 4 to 5 mm below the top of the divider. Compartment A contained 225 ml of 2 M NaCl (pH 6.5), compartment B, 75 ml of a 2 M solution of a salt of the counter-ion. Both compartments were covered with a lid 2 mm above the separating wall. A tube of 2.5 cm I. D. in the center of the lid provided room

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1 Contribution from the United States Salinity Laboratory, Soil and Water Conservation Research Division, ARS, USDA, Riverside, California, in cooperation with the 17 Western States and Hawaii.

2 Plant Physiologist. Present address: Laboratory for Plant Physiological Research, Agricultural University, Wageningen, Holland.

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Fig. 1. Apparatus for measuring liquid-membrane permeability of lipids. Apparatus was constructed of lucite. Compartment B was partially filled with paraffin.
for stirring of the nonaqueous phase. Stirring of the aqueous phases did not prove to be necessary. The nonaqueous phase consisted of 100 ml of water-saturated n-pentanol which was slowly pipetted on to the 2 aqueous phases to prevent spilling of salt solution over the rim. Generally, the pentanol level in the tube was 5.5 cm above the lid. A stirring blade of 2 cm² area was lowered just below the surface, and stirring was controlled at 150 rpm. Only minor ripples were visible at both interfaces. Before the experiment was begun, possible air bubbles under the cover were carefully removed. The area of each interface was 40 cm².

Chloride transport was determined by withdrawing 25-ml samples from compartment B and replacing them with an equal volume of the appropriate salt solution. Chloride was determined potentiometrically by titration with AgNO₃, using a calomel electrode and an Ag/AgCl electrode. For determination of sodium, 1-ml samples were taken from compartment B and analyzed with a flame photometer.

Results and Discussion

No detectable amount of chloride was transported in 24 hours when pure water-saturated pentanol was used as the nonaqueous phase. The effect of different anions on chloride exchange of phosphatidylcholine was measured first. In table I, data are given for the chloride transport when 25 mg phosphatidylcholine was dissolved in the pentanol. When compartment B was filled with distilled water, only a trace of chloride could be detected after 16 hours. Even this small amount may have been caused by manipulation of the salt solution. Because of osmotic water movement, the aqueous solution levels in both compartments had to be adjusted frequently. Bicarbonate and carbonate were very ineffective in inducing chloride exchange. Other anions, such as nitrate and sulfate, were far more effective. Increase

Table I. Rate of Chloride Transport from 1 M NaCl to 1 M Solution of the Sodium Salt of the Indicated Anion in Schulman’s Model

<table>
<thead>
<tr>
<th>Anion</th>
<th>1 hr</th>
<th>16 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>(water only)</td>
<td>...</td>
<td>0.10</td>
</tr>
<tr>
<td>HCO₃⁻</td>
<td>...</td>
<td>0.40</td>
</tr>
<tr>
<td>CO₃²⁻</td>
<td>...</td>
<td>0.75</td>
</tr>
<tr>
<td>H₂PO₄⁻</td>
<td>1.44</td>
<td></td>
</tr>
<tr>
<td>HPO₄²⁻</td>
<td>1.56</td>
<td></td>
</tr>
<tr>
<td>SO₄²⁻</td>
<td>2.73</td>
<td></td>
</tr>
<tr>
<td>NO₃⁻</td>
<td>4.17</td>
<td></td>
</tr>
<tr>
<td>PO₄³⁻</td>
<td>5.40</td>
<td></td>
</tr>
<tr>
<td>OH⁻</td>
<td>6.15</td>
<td></td>
</tr>
</tbody>
</table>

Table I. Rate of Chloride Transport from 1 M NaCl to 1 M Solution of the Sodium Salt of the Indicated Anion in Schulman’s Model

Pentanol phase contained 25 mg phosphatidylcholine in all cases.

in negative charge of the phosphate ion induced a higher rate of chloride transport.

Other lipids which do not have ionic groups may transport chloride via another mechanism. Monogalactose diglyceride, e.g., may transport the chloride ion within the water mantle bound to the galactose part of the molecule. This has not been studied yet and for this reason the more general term chloride transport instead of chloride exchange is used for lipids other than phosphatidylcholine.

The nitrate ion was chosen for the following chloride transport studies. Neutral lipids were very poor chloride-transporters. No detectable amounts of chloride were measured 3 hours after the beginning of the experiment when 10 mg of the sterol fraction was dissolved in the nonaqueous phase. Very small amounts of transported chloride were measured when 10 mg of the glyceride fractions were dissolved in pentanol. The chloride-transport capacity increased in the order tri-, di-, and monoglycerides (fig 2). The phosphatidylcholine and the phosphatidylethanolamine fractions of the varieties Cardinal and Salt Creek induced an increase in chloride transport with time until a constant level was reached after 2 or 2 and one-half hours. This level was higher than that of the monoglycerides. Small differences between the 2 phospholipid fractions were not significant. This applied also for differences between fractions of both rootstocks.

On the other hand, the chloride transport of the 2 monogalactose diglyceride fractions increased...
strongly with time, and no saturation level was reached after 3 hours. This lipid was by far the most effective chloride-transporter studied (fig 2). Differences between the fractions of both rootstocks were again not significant. Observations on the chloride-transport capacity of the charged lipid fractions of Dog Ridge and Thompson Seedless were in agreement with the curves of figure 2. So far it is not understood why the rate of transport of Cl through the monogalactose diglyceride membrane continues to increase rapidly, even after 3 hours.

Comparison between the lipid composition of the roots of the 5 grape rootstocks and the chloride transport capacity of the specific lipid membrane strongly suggests that, indeed, the chloride transport capacity of the lipids present in the membranes of the root cells may contribute directly to the observed differences in chloride transport to the leaves, when the low-affinity transport system is operating. Varieties with a large quantity of monogalactose diglyceride in the root lipids (Cardinal, Thompson Seedless) demonstrated a high accumulation rate of chloride in the leaves. In rootstocks of varieties that demonstrated limited chloride accumulation, phospholipids replaced the monogalactose diglyceride. In this connection, it is also interesting that the sterol fraction, which did not transport chloride at all in the model, was unusually small in the most susceptible variety, Cardinal. It is not clear from these experiments if the transport capacity of the lipids alone determines the rate of chloride transport or that a lipid-protein enzyme complex is involved in chloride transport to the leaves.

A few experiments on sodium transport of the phosphatidylycholine and the monogalactose diglyceride fractions of Cardinal were carried out (fig 3). Compartment A contained 2 mM NaCl solution, and compartment B 2 mM KCl or 2 mM CaCl₂ solutions. Using phosphatidylycholine, potassium induces a higher rate of sodium transport than does calcium. The data in figures 2 and 3 show that phosphatidylycholine more effectively exchanges sodium (against potassium) than chloride (against nitrate). The monogalactose diglyceride fraction transports sodium at a lower rate than the phosphatidylycholine fraction does, thus reversing the observed differences between these 2 fractions as regards chloride transport. The experiments show that phosphatidylycholine has more the characteristics of a cation exchanger, while monogalactose diglyceride is more an anion transporter.

**Fig. 3.** Rate of sodium transport from compartment A to compartment B when 10 mg of lipid is dissolved in pentanol: (●) phosphatidylycholine, 2 mM NaCl – pentanol – 2 mM KCl; (○) phosphatidylycholine, 2 mM NaCl – pentanol – 2 mM CaCl₂; (+) monogalactose diglyceride, 2 mM NaCl – pentanol – 2 mM KCl.

**Literature Cited**

