Reduction of Water Permeability in Potato Tuber Slices by Cyanide, Ammonia, 2,4-Dinitrophenol, and Oligomycin and Its Reversal by Adenosine 5’-Triphosphate and Cytidine 5’-Triphosphate

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ABSTRACT
Five millimolar KCN reduced water permeability in 1-millimeter thick slices of potato tuber (Solanum tuberosum L.). One-tenth millimolar ATP and CTP prevented or reversed the reduced permeability, UTP and GTP were not effective. Five millimolar ammonium carbonate or 0.1 millimolar 2,4-dinitrophenol also reduced water permeability, but ATP and CTP were only partially effective in reversing the reduced permeability. Oligomycin, 5 micrograms per milliliter, reduced water permeability, and the reduction was reversed by ATP and CTP. ATP and CTP appear to be involved in maintaining the structure of water pathways into the cell.

MATERIALS AND METHODS
Uniform disks, 14 mm in diameter and 1 mm thick, were cut from potato tubers (Solanum tuberosum L. var. Russet). The disks were washed in tap water, usually 10 to 15 min, until the starch and cell debris were removed from the cut surface, rinsed twice in distilled water, and stored in aerated distilled water at 5 C until needed an hour or two later.
Six disks (about 1 g fresh weight) were placed in 50 ml of the sodium salt of 0.1 mm ATP, CTP, GTP, or UTP and gently shaken for 45 min. The appropriate amount of ammonia, cyanide, DNP, or oligomycin was then added, and the shaking was continued for an additional 45 min or for only 10 min in the ammonia solution. At the end of this time, the disks were removed from the solution, blotted between Whatman No. 1 filter paper (50 g pressure for 5 sec), and weighed to the nearest milligram. The disks were then placed in a 13 atm (90 g/liter) solution (measured with the thermocouple psychrometer) of mannitol containing the appropriate amount of nucleotide, inhibitor, etc.; shaken for 10 min; removed from the mannitol; blotted as before; and reweighed. The difference between the first and second weighing was taken as the amount of water lost.
Oligomycin (Sigma Chemical Company; 15% oligomycin A, 85% oligomycin B) was dissolved in absolute ethanol to make a concentration of 10 mg/ml. Aliquots of this stock solution were used to make a final concentration of 5 µg/ml. For the oligomycin experiments the same concentration of absolute ethanol was added to the controls and mannitol solution as was added in the test solutions.

In half of the experiments the inhibitor or uncoupler was added first and the ATP, CTP, etc. was added later. When needed, potassium phosphate buffer (0.01 m) was used to control pH. The control experiments were conducted without adding the inhibitors. All experiments were conducted at 23 C.
Each experiment was repeated five times, and the results were analyzed statistically for significance using Duncan's new multiple range test (4). Statistical comparisons between experiments were not made.

1 In cooperation with the Nevada Experiment Station, Reno, Nev. 89507. Nevada Agricultural Experiment Station Journal Paper 295.

2 Abbreviation: DNP: 2,4-dinitrophenol.
3 Trade name and company names are included for the benefit of the reader and do not infer any endorsement of or preferential treatment of the products listed by the United States Department of Agriculture.

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RESULTS

The rate of water loss from potato disks is shown in Figure 1. Water was removed at a constant rate for 15 min. The tissue continued to lose weight, was completely flaccid and appeared to be fully plasmolyzed after 40 min. Water loss from disks preincubated in 0.01 M phosphate buffer was essentially the same as from disks preincubated in water. Water loss from disks treated with ATP, CTP, etc. was the same as from the untreated disks.

Preincubation of the disks in 5 mM KCN, 5 mM (NH₄)₂CO₃, 0.1 mM DNP, 5 μg/ml oligomycin, 0.1 mM ATP, etc. singly, or in combination as previously outlined, resulted in weight gain of 3.0 to 0.5% during the pretreatment period. Disks placed in water gained 2.7% and in 0.01 M phosphate buffer, 2.5%. At the end of the pretreatment period, before the disks were placed in the mannitol solution, all of the disks appeared to be fully turgid. None of the chemicals, at the concentrations or immersion times used, appeared to damage the disks.

Variability was relatively low within each batch of disks. The coefficient of variation ranged from 2 to 15%, depending upon the experiment and treatment. However, the amount of water lost varied from one batch of potatoes to the next, as may be seen by comparing the amounts of water lost from the control treatments in each experiment (Table I).

Preincubation of potato disks in 5 mM KCN gave maximal reduction in water permeability by cyanide without apparent damage to the tissue. The reduction in water permeability was not as great with 1, 2, or 10 mM KCN. Addition of ATP or CTP before adding KCN appeared to prevent reduction in water permeability induced by KCN (Table I). GTP and UTP were not effective in preventing the reduced permeability. When KCN was added first, both ATP and CTP appeared to reverse the KCN-induced decrease in permeability, but GTP and UTP were not effective.

Preincubation of potato disks in 5 mM (NH₄)₂CO₃ gave maximal reduction of water permeability without apparent damage to the tissue. Higher concentrations of (NH₄)₂CO₃ (10 mM) caused the tissue to turn dark and become flaccid. Concentrations of 1 mM (NH₄)₂CO₃ reduced permeability less, and lower concentrations had no effect. The disks were left in the (NH₄)₂CO₃ for only 10 min instead of 45 min because the longer times tended to turn the tissue dark and did not further reduce water permeability.

Preincubation of the potato disks with nucleotides before adding (NH₄)₂CO₃ had no significant effect, although ATP and CTP tended to reduce the effect of (NH₄)₂CO₃. When (NH₄)₂CO₃ was added first, ATP reduced the effect of (NH₄)₂CO₃ about 50% but pre- and post incubation with ATP were not significantly different from each other. Post-treatments with CTP, GTP, and UTP were not effective in reducing the effect of (NH₄)₂CO₃ (Table I).

Preincubation of potato disks in 0.1 mM DNP and 0.01 M phosphate buffer, pH 5.5, gave maximal reduced water permeability without apparent damage to the tissue. The pH 5.5 phosphate buffer held the pH at 5.3 for both the DNP and oligomycin experiments. Concentrations of 0.5 and 1.0 mM DNP caused the tissue to become flaccid, while 0.01 mM DNP had no effect. Pre- and postincubation effects with CTP were significantly different from DNP alone but not from the other nucleotide treatments.

Preincubation of potato disks in oligomycin and phosphate buffer, pH 5.5, gave maximal reduction of water permeability in the range of 2 to 20 μg/ml. Solutions containing more than 20 μg/ml oligomycin became cloudy, and oligomycin would not stay in solution. Preincubation of the disks with 0.1 mM ATP or CTP before adding 5 μg/ml oligomycin reversed the oligomycin effect (Table I). Preincubation of the disks with GTP or UTP had no effect. Adding the nucleotides after preincubation with oligomycin was not statistically effective in reversing the effect of oligomycin.

![Figure 1. Loss of water from 1 mm thick, 14 mm diameter potato tuber disks in a 13 atm (90 g/liter) solution of mannitol.](https://example.com/figure1.png)

Table 1. Effect of ATP, CTP, GTP, and UTP on the Loss of Water from Potato Tuber Disks Treated with Cyanide, Ammonia, Dinitrophenol, and Oligomycin

In half of the treatments disks were treated with 0.1 mM ATP, CTP, etc. for 45 min and then with inhibitor or uncoupler for 45 min. except ammonia treatment was only 10 min, weighed, followed by a 10-min immersion in 13 atm mannitol and weighed again. In the other half the inhibitor or uncoupler was added first and then the ATP, CTP, etc. Each value represents a mean of five replications. Statistical comparisons are for means in each experiment, not between experiments. Olig: oligomycin.

<table>
<thead>
<tr>
<th>Expt.</th>
<th>Water</th>
<th>5mm KCN</th>
<th>ATP-KCN</th>
<th>CTP-KCN</th>
<th>GTP-KCN</th>
<th>UTP-KCN</th>
<th>KCN-ATP</th>
<th>KCN-CTP</th>
<th>KCN-GTP</th>
<th>KCN-UTP</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.212a*</td>
<td>0.133b</td>
<td>0.200a</td>
<td>0.237a</td>
<td>0.120b</td>
<td>0.133b</td>
<td>0.208a</td>
<td>0.210a</td>
<td>0.129b</td>
<td>0.151b</td>
</tr>
<tr>
<td>2</td>
<td>0.109a</td>
<td>0.136cd</td>
<td>0.155bc</td>
<td>0.152cd</td>
<td>0.145cd</td>
<td>0.142cd</td>
<td>0.163b</td>
<td>0.134d</td>
<td>0.145cd</td>
<td>0.113d</td>
</tr>
<tr>
<td>3</td>
<td>0.172a</td>
<td>0.119c</td>
<td>0.133bc</td>
<td>0.144b</td>
<td>0.130bc</td>
<td>0.136bc</td>
<td>0.141b</td>
<td>0.129bc</td>
<td>0.135bc</td>
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<td>4</td>
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<td>0.134cd</td>
<td>0.156ab</td>
<td>0.157ab</td>
<td>0.134cd</td>
<td>0.137cd</td>
<td>0.132cd</td>
<td>0.142bc</td>
<td>0.132cd</td>
<td>0.119d</td>
</tr>
</tbody>
</table>

*Means followed by the same letter are not statistically different at the 1% level as determined by Duncan’s multiple range test (5).
DISCUSSION

The osmotic removal of water from various tissues is both rapid and convenient for assessing permeability to water, though misleading if certain requirements are not met to insure that other parameters remain constant (5). No problems were encountered with the use of mannitol as the osmotic agent because mannitol enters and exits the apparent "free space" of cells rapidly without entering the inside of the cell. After exposure to mannitol for several days a small amount does enter the "inner" or "osmotic" space of the cell (26). For short term experiments mannitol should be satisfactory.

None of the chemicals used appeared to harm the tissue at the concentrations employed. Higher concentrations of DNP and (NH₄)₂CO₃ caused the tissue to become flaccid. Stuart and Haddock (25) encountered a similar problem with ammonia which, at high concentrations, caused sugar beet roots to turn dark and greatly increased root permeability to water. In all of these experiments attempts were made to meet the conditions for using osmotic methods put forth by Glinka and Reinhold (5).

The data from these experiments (Table 1) suggest that the nucleotides ATP and CTP are involved in maintaining the permeability of potato storage tissue to water. The inhibition of cytochrome oxidase by cyanide (6) should cause a cessation or reduction of ATP production, reducing over-all ATP to a level where the water permeability of the cell could be changed. However, reducing the level of ATP could also lower the concentration of CTP (2) to an inhibitory level. If this happens, then the addition of ATP or CTP, or both, should restore the water permeability of potato disks. In these experiments, where cyanide was used to reduce water permeability, this restoration did occur.

Ammonia (NH₃) and undissociated NH₄OH act as respiration inhibitors by interfering with the oxidation of NADH (30, 31). This could lower the production of ATP or CTP to levels low enough to reduce water permeability of a cell. Five millimolar (NH₄)₂CO₃ solution has a pH of 8.3; at this pH inhibitory concentrations of NH₃ and undissociated NH₄OH could occur (30). Stuart and Haddock (25) found that preincubating sugar beet roots with ATP prevented the reduction of water permeability by about 50% when ammonia was used as the inhibitor. In the present experiment, ATP prevented the reduction of water permeability in potato slices by (NH₄)₂CO₃ to about the same degree.

ATP was not effective in reversing the reduced water permeability induced by DNP, because DNP may promote the hydrolysis of ATP besides uncoupling the formation of ATP from respiration (23). ATPases also can hydrolyze CTP in certain rabbit tissues, but the ATPases are not as efficient with CTP as with ATP (17). CTP partially reduced the effect of DNP on water permeability.

Oligomycin inhibits the formation of ATP in various tissues (22) and inhibits ion transport (1, 8). I also found that oligomycin reduces water permeability in potato disks. ATP and CTP prevented the reduced permeability if added before oligomycin. Both ATP and CTP appear to be involved in maintaining water permeability since the reduced permeability with ammonia is partially reversed with ATP but not CTP, and reduced permeability with oligomycin or cyanide is reversed with ATP and CTP. It may be that ATP and CTP work at different sites, both of which are responsible for water permeability; but this has not been established in this study.

I have assumed that the inhibitors are working as specific metabolic inhibitors, but I have no assurance that this assumption is valid. Five millimolar cyanide is a rather high concentration, and 0.1 mM DNP is borderline. The inhibitors may directly affect the membrane as there is little phospholipid metabolism taking place until after several hours in sliced potato storage tissue (10). However, as sliced potato tissues age, respiration becomes less sensitive to cyanide (6). The endoplasmic reticulum and mitochondria in potato storage tissue cells change shortly after slicing and are not restored until many hours later (9). What effect this has on water permeability of the slices is not known.

If nucleotides or inhibitors do not enter the cell, then they may affect the membrane directly from the outside. Hodges (8) found that oligomycin functioned to inhibit K uptake in oat roots by blocking ATP utilization in a process occurring primarily at the cell surface. Ling (14) suggested that ATP or some other high energy compound plays a major role in cell maintenance, not by the continued breakdown and delivery of energy but by its absorption on proteins as ATP per se. There is other evidence that ATP may combine directly with the membrane. Karjolainen (11) suggested that adenine nucleotides may form an integral part of the membrane surrounding calf thymus nuclei. Robinson (19) demonstrated that adding ATP or ADP to microsomal suspensions altered their light-scattering properties and very low concentrations were sufficient to trigger the response. With the exception of CTP, other related nucleotides had no effect. He suggested that the effect of ATP, ADP, and CTP is to alter the conformation of the microsomal membranes. ATP or CTP may or may not have to enter the cells of freshly cut potato slices but may combine with the membranes or membrane proteins to maintain or restore the cell's permeability to water.

Whether ATP or CTP combines with the membrane or whether they change the configuration of the membranes in potato is not known. However, they could be involved in phosphorylating some unknown compound and/or supplying energy to a contractile protein in the membrane that is responsible for keeping the water pathways into the cell open.

LITERATURE CITED


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