Short Communication

Divergent Transport Mechanisms for Pyrimidine Nucleosides in Petunia Pollen

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ABSTRACT

Petunia hybrida pollen exhibits divergent transport mechanisms for pyrimidine nucleosides. Uridine and cytidine show all the properties of being actively transported, a nucleoside transport mechanism not hitherto reported in plant cells. Contrasting with this, thymidine transport has the properties of a nonactive, carrier-mediated system. Reasons for these different mechanisms are considered to lie in the high demand for uridine and cytidine, obtained perhaps from stylar tissue, for the biosynthetic reactions of the pollen tube, while thymidine demand is lower due to the absence of DNA replication in germinating Petunia pollen.

Gene expression and RNA synthesis in pollen can be followed by incorporation of labeled uridine into RNA (13, 19), while pollen DNA repair is monitored by labeled thymidine incorporation into DNA (8-10). In addition it has been reported that the pool of these and other nucleic acid precursors increase in the style of Petunia hybrida after pollination (20), and since pollen and pollen tube interact closely with stylar tissue in Petunia (17), then the style could be a good source of nucleic acid and nucleotide precursors for the biosynthetic reactions of the elongating pollen tube. There is a need therefore to understand the uptake of these precursors by pollen since the incorporation of nucleoside precursors in other biological systems is limited by the rate of entry into the cell (1, 3, 16, 19). Pollen cultures also provide us with a convenient system for studying the mechanism of pyrimidine nucleoside transport in plant cells, for comparison with other cells. The mechanism of pyrimidine nucleoside transport varies according to the organism, having been shown to be active in certain bacterial cells (4, 14, 15) and nonactive, carrier-mediated in animal cells (11, 17, 18, 22). However, in these cases it has either been shown or inferred that all nucleosides are transported by a similar mechanism in any particular cell. Little is known about nucleoside transport in plant cells; a simple diffusion mechanism has been proposed for all pyrimidine nucleosides in Euglena (21) while Suss and Tupy (19) have shown that the uridine uptake system is saturable in tobacco pollen. We find here that uridine and cytidine are transported by an active process in Petunia pollen, and thymidine by a nonactive carrier-mediated system.

1 Abbreviations: DCCD, N,N'-dicyclohexylcarbodiimide; NBD-CI, 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole; DNP, 2,4-dinitrophenol; CCCP, carbonylcyanide-m-chlorophenylhydrazone.
Table I. Kinetic Constants for Transport of Pyrimidine Nucleosides by Petunia Pollen

<table>
<thead>
<tr>
<th>Nucleoside</th>
<th>$K_m$ ($\mu$M)</th>
<th>$V_{max}$ (pmol mg$^{-1}$ fresh wt h$^{-1}$)</th>
<th>$E_a$ (kJ mol$^{-1}$ K$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uridine</td>
<td>11.8 ± 1.1</td>
<td>1586.2 ± 42.8</td>
<td>75.4</td>
</tr>
<tr>
<td>Cytidine</td>
<td>16.3 ± 1.9</td>
<td>827.4 ± 47.1</td>
<td>67.3</td>
</tr>
<tr>
<td>Thymidine</td>
<td>18.1 ± 0.9</td>
<td>109.9 ± 2.0</td>
<td>42.0</td>
</tr>
</tbody>
</table>

* Energy of activation.

Table II. Effect of Metabolic Inhibitors and Antagonists on Pyrimidine Nucleoside Transport into Petunia Pollen

<table>
<thead>
<tr>
<th>Inhibitor or Antagonist</th>
<th>Concentration (mM)</th>
<th>Inhibition of Transport</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Uridine</td>
<td>Cytidine</td>
</tr>
<tr>
<td>DCCD</td>
<td>50</td>
<td>35</td>
</tr>
<tr>
<td>NBD-CI</td>
<td>50</td>
<td>61</td>
</tr>
<tr>
<td>CCCP</td>
<td>10</td>
<td>83</td>
</tr>
<tr>
<td>DNP</td>
<td>50</td>
<td>65</td>
</tr>
<tr>
<td>Ethanol (0.5%)</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Uridine</td>
<td>50</td>
<td>52</td>
</tr>
<tr>
<td>Cytidine</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>Thymidine</td>
<td>50</td>
<td>10</td>
</tr>
<tr>
<td>Adenosine</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>Guanosine</td>
<td>50</td>
<td>8</td>
</tr>
</tbody>
</table>

System (22), showed very little inhibition in pollen.

When we tested the ATPase inhibitors DCCD$^1$ and NBD-CI on the pollen transport system, further differences between thymidine and the other two pyrimidine nucleosides became apparent. Both these reagents inhibited uridine and cytidine transport, and were without effect on thymidine transport (Table II). Preincubation of pollen for 1 h with DCCD or NBD-CI did not affect thymidine transport, but increased inhibition of uridine transport by a further 20%. We concluded that uridine and cytidine are taken up in pollen by an active transport system similar to that already described for some bacteria (4, 14, 15), while thymidine transport is a nonactive process. This was further supported by the observation that the proton translocators (uncouplers) CCCP and DNP were also effective inhibitors of uridine and cytidine transport in Petunia pollen (Table II), and had a minimal effect on thymidine uptake.

These metabolic inhibitors did not increase efflux of label, as compared to controls, after 30 min resuspension in the presence of inhibitors. We conclude therefore that these compounds inhibit transport of uridine and cytidine by affecting energy metabolism rather than by altering membrane integrity under the conditions of our experiment. An investigation of the effect of temperature showed that thymidine transport in Petunia pollen has an activation energy of 42.0 kJ mol$^{-1}$ K$^{-1}$ (Table I), a value which is significantly higher than would be obtained if thymidine was transported by a simple diffusion mechanism (5) as in Euglena (21). We infer then, that thymidine transport proceeds by a nonactive, saturable, carrier-mediated process.

DISCUSSION

If the result of this difference in the mechanism of transport between the pyrimidine nucleosides is the more rapid transport of uridine and cytidine compared with thymidine as observed in Petunia pollen, then it is not difficult to seek an explanation for this expenditure of energy on active uptake of the two nucleosides

concerned. Pollen is a highly differentiated tissue, its primary function being to deliver the gametes to the ovule through a rapidly synthesized pollen tube which has to interact with and make its way through stigma and style (12). Uridine and cytidine nucleosides are in immediate and urgent demand in the early stages of pollen germination for RNA synthesis and for pollen tube polysaccharide and membrane lipid biosyntheses, and, as this study suggests, they could be salvaged from stylar tissue. On the other hand, DNA replication does not take place during germination of Petunia pollen (8), so that demand for thymidine should not be high. DNA repair, which can occur in germinating Petunia pollen (8-10), requires only smaller quantities of thymidine.

In pollen from F. hybrida, and perhaps from other species, there may well have been significant pressure for the evolution of a more rapid transport system for uridine and cytidine, resulting in the divergent transport mechanisms for pyrimidine nucleosides described here. Although thymidine is taken up readily by some plant cells (6), in others there is some difficulty (7). It is possible that the divergence in transport mechanisms among the pyrimidine nucleosides is widespread among plant cells. Our studies do provide the necessary background data for ensuring a precursory uptake for nucleic acid synthesis investigations in Petunia pollen.

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LITERATURE CITED