Review

Diversity of Specificity and Function of Phosphate Translocators in Various Plastids

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ABSTRACT

This report gives a comparison of the specificity of phosphate translocators in various plastids. Whereas the phosphate translocator of the C₄ plant spinach mediates a counter exchange between inorganic phosphate, dihydroxyacetone phosphate, and 3-phosphoglycerate, the phosphate translocators in chloroplasts from C₃ and CAM plants transport phosphoenolpyruvate in addition to the above mentioned metabolites. In plastids from pea roots the phosphate translocator also transports glucose 6-phosphate. This diversity of phosphate translocators is discussed in view of the special functions of the various plastids.

TRANSPORT IN CHLOROPLASTS FROM C₃ PLANTS

The Pi/triose-P/3-PGA translocator, for brevity named phosphate translocator, can be regarded as the main transport function of the chloroplast inner envelope membrane (10). The protein mediating this transport is the largest protein fraction of the chloroplast envelope, amounting in spinach to about 15% of the total envelope protein. Transport by the phosphate translocator proceeds by strict counter exchange; for each molecule transported inward another molecule is transported in the opposite direction. The substances transported by the phosphate translocator compete with each other (7). For this reason the inhibition constants can be taken as a useful measure for the specificity of the transport. As shown in Table 1 the translocator from the C₃ plant spinach accepts at its binding site either Pi or a phosphate molecule attached to the end of a three carbon chain, such as 3-PGA¹ or DHAP. Three carbon compounds in which the phosphate is attached to the carbon atom C₂, such as 2-PGA (not shown here) or PEP show little interaction with the translocator, and hexose phosphates, e.g. glucose 6-P, are not transported at all (7).

The phosphate translocator facilitates the export of photosynthesis products from the chloroplasts. In this it appears to have various functions. In catalyzing a counter exchange with Pi it enables the export of DHAP as precursor for sucrose synthesis and of 3-PGA for the provision of carbon skeletons for nitrate assimilation. It also enables a DHAP/3-PGA shuttle. Connected with the activity of cytosolic NADH-glyceraldehyde phosphate dehydrogenase and phosphoglycerate kinase, an indirect transport of ATP and of reducing equivalents in form of NADH from the stroma to the cytosol is possible (10). It is not certain, however, to what extent this shuttle plays a physiological role, since redox equivalents in form of NADH, as required for nitrate reduction in the cytosol, can be also exported from the chloroplasts by a malate-oxaloacetate shuttle, and mitochondrial ATP synthesis may be a better source for supplying the cytosolic demand of ATP than photophosphorylation (10). Alternatively, in connection with the nonphosphorylating NADP-glyceraldehydephosphate dehydrogenase, located in the cytosol (15), photosynthetic electron transport seems to be able to maintain the cytosolic NADP-system in a reduced state, as required for biosynthetic processes. Cytosolic NADPH is also a good substrate for mitochondrial oxidative phosphorylation. Isolated plant mitochondria show very high respiration rates with external NADH and NADPH (17). In vivo, however, the mitochondrial oxidation of external NADPH may be higher than that of external NADH, since in a leaf the cytosolic NADPH level is very high (9), whereas the cytosolic NADH concentration is much below the Kᵅ for mitochondrial oxidation (11). For this reason, a DHAP/3-PGA shuttle connected with nonphosphorylating glyceraldehyde phosphate dehydrogenase could contribute to a redox transfer from the chloroplasts to the mitochondria.

TRANSPORT IN CHLOROPLASTS FROM C₄ MESOPHYLL CELLS

The functioning of the C₄ cycle implies the export of PEP from, and the import of Pi into the mesophyll chloroplasts. Because bundle sheath cells from maize are deficient in PSII activity, redox equivalents required for CO₂ fixation have to be delivered from the mesophyll chloroplasts by a 3-PGA/DHAP shuttle.

Table 1 illustrates the specificity of the phosphate translocator in maize mesophyll chloroplasts. In contrast to spinach chloroplasts, C₄ mesophyll chloroplasts also transport PEP. Measurements of the effect of inhibitors on the transport of the various substrates, of the competition between substrates and of back exchange showed that in C₄ mesophyll chloro-

¹ Abbreviations: 3-PGA, 3-phosphoglycerate; DHAP, dihydroxyacetone phosphate; PEP, phosphoenolpyruvate; glucose 6-P, glucose 6-phosphate; triose-P, triose phosphate.
Table I. Specificity of Phosphate Translocators in Different Plastids. Determined from the Concentration Dependence of $^{32}$Pi Uptake without and with Various Competing Substances.

<table>
<thead>
<tr>
<th></th>
<th>Spinach Leaf Chloroplasts$^a$</th>
<th>Maize Mesophyll Chloroplasts$^b$</th>
<th>Pea Root Plastids$^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mM</td>
<td>mM</td>
<td>mM</td>
</tr>
<tr>
<td>Pi</td>
<td>$K_m$</td>
<td>0.20</td>
<td>0.045</td>
</tr>
<tr>
<td>DHAP</td>
<td>$K_i$</td>
<td>0.13</td>
<td>0.084</td>
</tr>
<tr>
<td>3-PGA</td>
<td>$K_i$</td>
<td>0.15</td>
<td>0.053</td>
</tr>
<tr>
<td>PEP</td>
<td>$K_i$</td>
<td>4.7</td>
<td>0.086</td>
</tr>
<tr>
<td>Glucose 6-P</td>
<td>$K_i$</td>
<td>40</td>
<td></td>
</tr>
</tbody>
</table>

$^a$ Fliege et al. (7).  $^b$ Gross et al. (8).  $^c$ Borchert et al. (2).

Plastics PEP, DHAP, 3-PGA, and Pi are transported by a single translocator (3, 8, 12, 20). A phosphate translocator with almost identical properties was also found in chloroplasts from Mesembryanthemum crystallinum exhibiting crassulacean acid metabolism (18). Since in the latter plant crassulacean acid metabolism can be induced by water stress, it is at the present unknown whether chloroplasts from M. crystallinum possess a second phosphate translocator with properties similar to that of chloroplasts from other C₃ plants. Obviously, the specificity of the phosphate translocator in C₄ mesophyll and in CAM chloroplasts differs markedly from that of C₃ chloroplasts.

Redox transfer by 3-PGA/DHAP shuttle between C₄ mesophyll and bundle sheath chloroplasts implies that in the two types of chloroplasts the 3-PGA/DHAP exchange proceeds in opposite directions. Transport measurements with isolated mesophyll and bundle sheath chloroplasts from the C₄ plant Panicum miliaceum (millet) revealed that in the two types of chloroplasts the kinetic properties of the phosphate translocators were different (19). These results show that in different cells of a leaf of a single species the chloroplast phosphate translocators can be different.

TRANSPORT IN PLASTIDS FROM NONGREEN PLANT TISSUES

The nongreen plastids differ from the chloroplasts in being dependent on the import of carbon compounds. Because of difficulties to prepare nongreen plastids suitable for transport measurements, it could not be decided until recently whether a phosphate translocator is operating in these plastids. Emes and England (5) succeeded in establishing a method to prepare plastids from the roots of germinated peas that were suitable for transport measurements. Emes and Traska (6) found an uptake of $[^{32}P]$orthophosphate into the plastids from pea roots. This uptake was reduced in the presence of triose-P or 3-PGA, suggesting that a phosphate translocator, similar to that in chloroplasts, is also operating in the root plastids. Work from our own laboratory established the characteristics of the transport of Pi and phosphorylated compounds into pea root plastids by measurement of the concentration dependence of the uptake of radioactively labeled compounds, its competitive inhibition by other compounds, and of back exchange (2) (Table 1). These studies provided evidence that the plastids from pea roots contain a phosphate translocator which is similar to the chloroplast translocators in transporting Pi, DHAP, and 3-PGA in a counter exchange mode, but which differs in also transporting glucose 6-P, but not glucose 1-P.

The difference in specificity between the phosphate translocator in the root plastids and that of chloroplasts reflects differences in its function. A major function of the root plastids is the reduction of nitrite for which redox equivalents are supplied by oxidative pentose phosphate pathway. With intact root plastids, nitrite was found to be reduced very effectively in the presence of glucose 6-P (1). Pea root plastids (2), like plastids from wheat endosperm (22), do not have fructose 1,6-bisphosphatase activity; therefore, the operation of the oxidative pentose phosphate pathway in these plastids requires uptake of glucose 6-P and release of DHAP (Fig. 1). Synthesis of starch involves the uptake of glucose 6-P and the release of Pi (Fig. 1). The specificity of the pea root phosphate translocator described above suits both functions.

Little is known about the transport properties of other nonphotosynthetic plastids. Since results of transport meas-

![Figure 1. Schematic representation of the two functions of the phosphate translocator in pea root plastids.](https://www.plantphysiol.org)
urements are not available, our knowledge about permeability properties of these plastids relies on studies of metabolic capacities and of enzyme latency only. Studies with amyloplasts from suspension cultures of soybean led to the assumption that in these plastids triose-P is taken up as a precursor for starch synthesis (16). Work with cauliflower bud plastids revealed the possibility that besides triose-P also PEP and 2-PGA readily cross the plastid envelope (13). Investigations with maize kernel amyloplasts indicated that although DHAP was the preferred substrate for uptake into amyloplasts and incorporation into starch, glucose 6-P and fructose 1,6-bis-P may also be taken up (4). An uptake of hexose phosphates into nongreen plastids was also suggested from measurements of the distribution of the label after incorporation of 14C glucose into starch of cultured cells (21). With isolated amyloplasts from wheat endosperm it was found that only glucose 1-P gave considerable labeling of starch that was dependent upon the integrity of the amyloplasts (14, 22). It is most likely that all these nongreen plastids possess a phosphate translocator. From the various results of indirect studies discussed above, the question arises to what extent the phosphate translocators in the various plastids differ. Are there plastid phosphate translocators which are specific for glucose 1-P? To answer this question, transport measurements with highly intact plastids are required.

**CONCLUDING REMARKS**

As shown in this review, in different plastids the phosphate translocators of the inner envelope membrane can be rather different with respect to their transport specificities. It is to be expected that in a single plant there are different genes encoding different phosphate translocators, e.g. for chloroplasts (in C3 plants differently for mesophyll and bundle sheath chloroplasts), root plastids, and possibly also for other plastids. One working hypothesis is that the different translocators are derived from a common ancestor and are subsequently evolved by diversification. Recently the cDNA sequence of the C3 phosphate translocator from both spinach and pea chloroplasts has been determined (23). This sequence does not share any homology with known mitochondrial or bacteriolar transport proteins. Both translocator proteins contain several α-helical transmembrane stretches. Some of these α-helices have an amphiphilic character and can presumably form a hydrophilic pore through which the substrates are translocated across the membrane. A comparison of the cDNA sequences of the other phosphate translocators discussed in this review may provide information on the structural features determining the specificity of the transport of phosphorylated intermediates in plastids.

**LITERATURE CITED**