

SPECIFIC TRANSPORT OF INORGANIC PHOSPHATE, 3-PHOSPHOGLYCERATE AND DIHYDROXYACETONEPHOSPHATE, AND OF DICARBOXYLATES ACROSS THE INNER MEMBRANE OF SPINACH CHLOROPLASTS

Hans Walter HELDT and Lynn RAPLEY

*Institut für Physiologische Chemie und Physikalische Biochemie der Universität München,
8, München 15, Goethestrasse 33, Germany*

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1. Introduction

The intact chloroplast is surrounded by two membranes, the outer and the inner membrane. Apart from these are the thylakoid membranes within the chloroplast. Therefore, three compartments of the chloroplast can be visualized: a) the intermembrane space between the outer and the inner membrane; b) the stroma space between the inner membrane and the thylakoid membranes; and c) the thylakoid space located within the thylakoid membrane. From the membranes mentioned the outer was found to be unspecifically permeable to a large variety of solutes, except macromolecules [1, 2].

Functional studies suggested that 3-phosphoglycerate is able to penetrate the chloroplast [3–6]. Direct measurement of the uptake of 3-phosphoglycerate and of malate into the sucrose-impermeable space of chloroplasts has been reported recently [1]. The ability to take up these anions is lost when the inner membrane is disrupted [1]. Thus the uptake of 3-phosphoglycerate and malate is regarded as a transport across the inner membrane into the stroma space. This transport is different from the anion permeability of the thylakoid membrane studied earlier (for references see [7]).

2. Methods

For the preparation of chloroplasts, see [4]; for

conditions of incubation and silicon layer filtering centrifugation, see [1]. The filtering centrifugation experiments were all carried out with a Coleman centrifuge with 0.5 ml plastic tubes. The tube contained 20 μ l 10% HClO_4 , 70 μ l silicon AR 100 (Wacker Chemie, München) and 200 μ l chloroplast suspension. The incubations were started by addition of substrate and terminated by spinning the chloroplasts through the silicon layer. Unspecific permeation into the intermembrane space was corrected with ^{14}C -sucrose [1]. By a special device [8], 12 samples were incubated and centrifuged at the same time.

3. Results

3.1. Anion uptake by chloroplasts

The uptake of various radioactive labelled anions into the sucrose-impermeable space has been measured, as shown in table 1. The values were all obtained with the same chloroplast preparation. The incubation time was short enough for measurements within the initial phase of uptake (see [1]). Pi^* , 3PGA, aspartate, malate, glutamate and succinate are readily taken up,

* Abbreviations:

chlor.: chlorophyll;
3PGA: 3-phosphoglycerate;
2PGA: 2-phosphoglycerate;
DAP: dihydroxyacetonephosphate;
 Pi : inorganic phosphate.

Table 1
Uptake of radioactive labelled compounds into the sucrose-impermeable space of spinach chloroplasts.

Radioactive compound (1 mM)	Uptake (20 sec, 4°) (nmol/mg chlor.)
Inorganic phosphate	138.7
3-Phosphoglycerate	94.2
Fructose-1,6-diphosphate	0.2
Fructose-6-phosphate	1.4
Glucose-6-phosphate	4.6
6-Phosphogluconate	1.0
Aspartate	127.1
Glutamate	64.1
Malate	58.1
Succinate	53.9
Citrate	6.0
Acetate	3.9

The inorganic phosphate was labelled with ^{32}P , all other compounds with ^{14}C .

but there is little uptake of hexose-phosphates, citrate and acetate.

It may be noted that the absolute rates for the uptake of substrates vary considerably with different chloroplast preparations.

3.2. Competition of anion uptake

The effect of competitive anions on the uptake of radioactive labelled Pi, 3PGA and malate is presented in tables 2 and 3. In the case of radioactive Pi alone (table 2) the uptake is increased when the Pi concentration in the medium is increased from 0.3 mM to 3.3 mM. When 3 mM 3PGA is added to 0.3 mM radioactive Pi, the uptake of Pi is decreased to 1/11 of the Pi uptake in the presence of 3.3 mM Pi alone. Likewise the uptake of 3PGA is lowered by addition of Pi. DAP decreases very strongly the uptake of both 3PGA and Pi, smaller effects being achieved with glyceraldehydephosphate, α -glycerophosphate and, to a minor extent, with phosphoenolpyruvate. It may be noted that the uptake of 3PGA and Pi is only slightly affected by 2PGA or inorganic pyrophosphate. 2,3-Diphosphoglycerate even stimulates the uptake. There is no decrease of Pi or 3PGA uptake observed with dicarboxylates (e.g. malate and succinate) nor

with acetate, sulfate, or nitrate. Arsenate, on the other hand, behaves like Pi. It may be concluded from these results that Pi, 3PGA and DAP compete for a common carrier, which will be called *phosphate translocator*, facilitating the transport of these substances.

The transport of malate shows a different pattern (table 3). 3PGA or Pi have no effect, but there appears to be a competition with succinate, aspartate, glutamate, α -ketoglutarate, oxaloacetate and fumarate. Essentially similar results have been obtained with the uptake of ^{14}C -labelled aspartate, succinate and glutamate. There is almost no decrease of uptake observed with maleinate, malonate, citrate, acetate and pyruvate. These data indicate that chloroplasts contain a specific carrier for the transport of certain dicarboxylates. It will be called *dicarboxylate translocator* in the following.

3.3. Anion transport as a counter exchange

In studies of back exchange (tables 4 and 5), the chloroplasts are incubated for 2 min at 4° with radioactive labelled anions and washed afterwards. To keep the metabolic interconversions of the anions taken up by the chloroplasts as low as possible, the whole procedure is carried out at 4° almost in the dark, the time from starting the preincubation until the start of the back exchange in the washed suspension is only about 8 min.

The back exchange is started by adding unlabelled anions and is terminated 20 sec later by spinning the chloroplasts through a layer of silicon oil. The amount of substances released on addition of anions is calculated from the radioactivity appearing in the supernatant medium and from the specific radioactivity of the substances used in the preincubation.

The chloroplasts preloaded with radioactive Pi or 3PGA (table 4) show a release of the radioactivity when Pi, 3PGA and especially DAP are added. α -Glycerophosphate and phosphoenolpyruvate facilitate a minor release. Inorganic pyrophosphate and 2PGA, hexose and pentose phosphates, 6-phosphogluconate, ATP and carboxylates have no marked effect. When the chloroplasts are preloaded with ^{14}C -labelled dicarboxylates (malate, succinate or aspartate, as shown in table 5), the radioactivity is released after the addition of malate, succinate, aspartate, glutamate, α -ketoglutarate, oxaloacetate

Table 2
Competition of anions for the uptake of inorganic phosphate or 3-phosphoglycerate.

Competitive anion (3 mM)	Concentration of radioactive labelled anion (mM)	Uptake of radioactive labelled anion (20 sec, 4°) (nmoles/mg chlor.)	
		³² P-Pi	¹⁴ C-3PGA
—	3.3	97.0	190.3
—	0.3	37.6	61.0
Inorganic phosphate	0.3		18.0
3-Phosphoglycerate	0.3	8.3	
Dihydroxyacetonephosphate	0.3	2.5	13.0
3-Glyceraldehydephosphate*	0.3	10.0	22.6
α-Glycerophosphate	0.3	16.7	25.3
Phosphoenolpyruvate	0.3	20.6	55.5
2-Phosphoglycerate	0.3	33.0	52.5
2,3-Diphosphoglycerate	0.3	41.2	86.0
6-Phosphogluconate	0.3	37.2	58.8
Inorganic pyrophosphate	0.3	32.6	47.8
Malate	0.3	29.0	56.5
Succinate	0.3	32.2	57.0
Acetate	0.3	32.0	64.0
Sulfate	0.3	37.0	—
Nitrate	0.3	41.0	—
Arsenate	0.3	9.1	20.2

Separate chloroplast preparations have been used with each radioactive substance.

* D-L-3-glyceraldehydephosphate was used, total concn. 6 mM.

and fumarate, but not with maleinate or malonate. There is no release observed after adding Pi, 3PGA, DAP, citrate, acetate or pyruvate. So far the identity of the radioactive substances released has not been proved. From the conditions of preincubation and also from the striking specificity of the back exchange shown in tables 4 and 5, a major interconversion of the radioactive substances within the chloroplasts appears to be improbable.

It is clear from our experiments that the phosphate translocator and also the dicarboxylate translocator catalyze a counter exchange. Furthermore, our data indicate both carriers to have in principle the same specificity for the transport in either direction.

4. Discussion

So far, three specific translocators, facilitating a counter exchange ion transport across the inner membrane have been shown to occur in spinach chloroplasts:

The *ATP translocator* catalyzes the transport of ATP into the chloroplast [9], in this way an ATP level within the chloroplast may be maintained during darkness.

The *dicarboxylate translocator* facilitates the transport of malate, succinate, α-ketoglutarate, fumarate, aspartate, glutamate and oxaloacetate, but it does not transfer malonate, maleinate nor citrate or monocarbonic acids. A permeation of α-ketoglutarate,

Table 3
Competition of anions for the uptake of malate.

Competitive anion (5 mM)	¹⁴ C-Malate (mM)	Uptake of ¹⁴ C-malate (nmoles/ mg chlor.) (20 sec, 4°)
—	5.5	100.7
—	0.5	59.2
Succinate	0.5	8.0
Aspartate	0.5	8.9
Glutamate	0.5	18.3
α-Ketoglutarate	0.5	10.6
Oxaloacetate	0.5	5.7
Fumarate	0.5	13.2
Maleinate	0.5	58.2
Malonate	0.5	52.3
Citrate	0.5	56.7
Acetate	0.5	59.8
Pyruvate	0.5	56.6
3-Phosphoglycerate	0.5	50.0
Inorganic phosphate	0.5	58.5

oxaloacetate, glutamate and aspartate across the chloroplast membrane was suggested earlier from measurements of glutamate oxaloacetate transaminase with intact chloroplast [10]. The metabolic role of the dicarboxylate translocator is not fully understood. By counter exchange of malate with oxaloacetate, reducing equivalents may be transported from the cytosol to the chloroplast or vice versa.

The *phosphate translocator* is specific for DAP, 3PGA and Pi, it does not transport 2PGA nor inorganic pyrophosphate.

The main function of the chloroplast is to provide the plant with substrates generated by photosynthesis which are utilized for energy production and biosynthesis of the cell. With isolated spinach chloroplasts, 3PGA and DAP were shown to be the main products of photosynthesis released from the chloroplast [6]. Thus the phosphate translocator appears to be a member of the reaction chain of photosynthesis. The coupling of the transport of 3PGA and of DAP with a counterflux of Pi may be important to maintain a constant level of phosphate in the chloroplast. In analogy to the role of mitochondrial adenine

Table 4
Release of radioactive labelled inorganic phosphate or 3-phosphoglycerate from the chloroplasts on addition of various anions.

Anion added (0.5 mM)	Radioactive substance released (nmoles/mg chlor., 20 sec, 4°)	
	³² P-phosphate	¹⁴ C-3PGA
Inorganic phosphate	47.0	11.1
3-Phosphoglycerate	47.2	12.9
Dihydroxyacetonephosphate	57.3	13.1
Glyceraldehydephosphate*	48.1	8.9
α-Glycerophosphate	19.1	4.6
Phosphoenolpyruvate	11.2	0.4
Inorganic pyrophosphate	6.3	0.2
2-Phosphoglycerate	5.1	1.9
2,3-Diphosphoglycerate	4.2	0.2
Fructosediphosphate	1.7	1.3
Glucose-6-phosphate	1.2	0.1
Fructose-6-phosphate	1.1	0.6
Ribose-5-phosphate	3.0	1.0
6-Phosphogluconate	0.9	0.2
ATP	3.0	0.8
Malate	0.2	0.6
Succinate	0.8	0.9
Acetate	1.0	0.6
Arsenate	36.3	8.6

All values have been corrected for unspecific leakage of substances. Separate chloroplast preparations have been used for each radioactive substance.

* D-L-3-Glyceraldehydephosphate was used, total concn. 1.0 mM.

nucleotide translocation for the regulation of ATP synthesis in the mitochondria [11], the phosphate translocator of the chloroplast is likely to play a role in the regulation of photosynthesis by the plant cell. This regulation may be achieved by transport of either 3PGA, DAP or Pi from the cytosol into the chloroplast.

With intact isolated chloroplast, CO₂ fixation is inhibited on the addition of higher concentrations of inorganic phosphate. The inhibition is released after the addition of either 3PGA or DAP [4]. This effect

Table 5
Release of radioactive labelled malate, succinate or aspartate from the chloroplasts on addition of various anions.

Anion added (1 mM)	Radioactive labelled substance released (nmoles/mg chlor., 20 sec, 4°)		
	¹⁴ C-Malate	¹⁴ C-Succinate	¹⁴ C-Aspartate
Malate	40.3	25.6	25.4
Succinate	36.8	27.5	19.7
Aspartate	40.8	25.9	31.3
Glutamate	23.7	12.2	16.0
α-Ketoglutarate	30.7	26.0	16.8
Oxaloacetate	26.7	9.8	17.2
Fumarate	31.7	24.5	14.8
Maleinate	0.6	0.3	0.4
Malonate	0.5	0.1	0.4
Citrate	1.5	0.5	2.0
Acetate	0.5	0.3	0.5
Pyruvate	0.6	0.3	0.4
3-Phosphoglycerate	0.2	0.2	0.5
Inorganic phosphate	0.6	0.2	0.1
Dihydroxyacetonephosphate	1.1	1.1	0.8

All values have been corrected for unspecific leakage of substances. Separate chloroplast preparations have been used with each radioactive substance.

is now well explained by the properties of the phosphate translocator. The addition of Pi results in a release of 3PGA and DAP from the chloroplasts, leading to a depletion of the pool of ribulose phosphates, the CO₂ fixation thus being inhibited by the lack of an acceptor. It should be kept in mind that this is a purely artificial metabolic situation, since 3PGA is always found in the cytosol of the plant cell [3]. It may be taken as a model, however, for possible function of the phosphate translocator in the regulation of photosynthesis by levels of metabolites in the cytosol.

Besides this, the phosphate translocator appears to be involved in an indirect transport of ATP from the chloroplast to the cytosol. From studies of the adenine nucleotide translocation in spinach chloroplasts, any considerable direct transport of ATP from the chloroplast to the cytosol appeared to be improbable [9]. This conclusion was substantiated by studying the photophosphorylation of intact chloroplasts [12]. From *in vitro* experiments a shuttle was suggested,

involving a transfer of DAP from the chloroplast into the cytosol, followed by oxidation to 3PGA accompanied by the formation of ATP and NADH, the 3PGA thus formed being transferred into the chloroplast again, where it is reconverted to DAP, at the expense of ATP and NADPH [12]. Similar to the mitochondria [13], the phosphorylation potential of the ATP in chloroplasts seems to be lower than in the cytosol [12]. The question arises if the phosphate translocator may be involved in maintaining the differences between the phosphorylation potentials of the ATP in the two compartments.

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