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SPECIFIC TRANSPORT OF INORGANIC PHOSPHATE, 3-PHOSPHOGLYCERATE AND TRIOSEPHOSPHATES ACROSS THE INNER MEMBRANE OF THE ENVELOPE IN SPINACH CHLOROPLASTS *

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Summary

The uptake of phosphate and phosphorylated compounds into the chloroplast stroma has been studied by silicone layer filtering centrifugation.

1. Inorganic phosphate, 3-phosphoglycerate, dihydroxyacetone phosphate and glyceraldehyde phosphate are transported across the envelope leading to an accumulation in the chloroplast stroma. This uptake proceeds by a counter exchange with phosphate and phosphorylated compounds present there.

2. The transport shows saturation characteristics allowing the determination of K_m and V.

3. The phosphorylated compounds transported act as competitive inhibitors of the transport. From measurements of the K_m and K_i the specificity of the transport is described.

4. The transport of inorganic phosphate and 3-phosphoglycerate is inhibited by *p*-chloromercuriphenyl sulfonate, pyridoxal 5′-phosphate and trinitrobenzene sulfonate.

5. The activation energy of phosphate transport as determined from the temperature dependence is evaluated to be 16 kcal (0—12°C).

6. It is concluded that inorganic phosphate, 3-phosphoglycerate, dihydroxyacetone phosphate and glyceraldehyde phosphate are transported by the same carrier, which has been nominated phosphate translocator.

7. Simultaneous measurements of the proton concentration in the medium and the transport into the chloroplasts show that the transfer of 3-phosphoglycerate involves a transfer of a proton into the same direction.

8. Measurements of the pH dependence of the transport indicate that all

* Most of these results are part of a thesis (Medizinische Fakultät der Universität München) by R. Fliege. Some of the data have been included in a preliminary report [1].

substances including 3-phosphoglycerate are transported by the phosphate translocator as divalent anions.

9. The physiological function of the phosphate translocator is discussed.

Introduction

The chloroplast stroma represents a separate metabolic compartment within the plant cell. The functional barrier between the stroma and the cytosol is the inner envelope membrane [2]. The CO_2 fixation occurring in the chloroplasts involves an uptake of the substrates CO_2 and inorganic phosphate from the cytosol and a release of the products triosephosphate and 3-phosphoglycerate from the stroma. Whereas the inner envelope membrane appears to be freely permeable to CO_2 [3] it is impermeable to anions. This points to the necessity of a specific transport of phosphate and phosphorylated compounds across the inner envelope membrane. There have been many indications that inorganic phosphate, 3-phosphoglycerate and triosephosphate may readily pass the envelope [4]. Direct studies of metabolite uptake using silicone layer filtering centrifugation showed that the substances mentioned above were transported by a specific carrier, which has been named phosphate translocator [5]. In the present report, the properties of this transport mechanism are detailed.

Materials and Methods

(a) Spinach (*Spinacia oleracea*) was obtained at the local market. At the end of these investigations we were able to use spinach grown in water culture according to Lilley and Walker [6]. Chloroplasts with a high portion of intact envelopes were prepared as described previously [2].

(b) The incubation was normally carried out in a medium containing 0.33 M sorbitol, 50 mM *N*-2-hydroxyethylpiperazine-*N*-2-ethane sulfonic acid (HEPES) adjusted to pH 7.6 with NaOH, 1 mM $MgCl_2$, 1 mM $MnCl_2$ and 2 mM EDTA.

(c) The direct measurement of the uptake of metabolites was initiated by adding 10 µl of ^{32}P- or ^{14}C-labelled compounds to 300 µl chloroplast suspension contained in a 400 µl capacity polypropylene microtube (Sarstedt, Nümbrecht, G.F.R.). The uptake was terminated by rapid centrifugation of the chloroplasts through a layer of silicone oil into 20 µl 1 M $HClO_4$. For details of silicone layer filtering centrifugation and evaluation of the uptake into the sorbitol-impermeable ^3H_2O space, which is the space surrounded by the inner envelope membrane [7].

(d) For the indirect measurement of metabolite uptake by "back exchange" the chloroplasts were preincubated with 5 mM ^{32}P-labelled inorganic phosphate (specific activity 2 Ci/mol) and washed afterwards. Transport was initiated by adding 10 µl unlabelled substance to 300 µl chloroplast suspension and terminated by silicone layer filtering centrifugation. The uptake was then evaluated from the release of ^{32}P activity to the supernatant medium.

(e) [^{32}P]Orthophosphate was obtained from Amersham-Buchler (Braunschweig, G.F.R.) and [^{14}C]ribose 5-phosphate from Schwarz/Mann, (Orange-

burg, N.Y., U.S.A.). ^{14}C-labelled 3-phosphoglycerate and hexosephosphates were a generous gift from Boehringer Mannheim GmbH, Tutzing, G.F.R.

Results and Discussion

Time course of phosphate transport

Fig. 1 shows the measurement of the uptake of inorganic phosphate and various phosphorylated compounds into chloroplasts. 3-Phosphoglycerate and inorganic phosphate are very rapidly transported into the sorbitol-impermeable ^3H_2O space (the space surrounded by the inner envelope membrane) and are accumulated there. A similar experiment with broken chloroplasts, consisting of thylakoid membranes only, showed no uptake of these compounds (Chon, C.J., Portis, Jr., A.R. and Heldt, H.W., unpublished). The latter findings indicate that the thylakoid membrane is impermeable to inorganic phosphate and phosphorylated substances. From this it can be concluded that the uptake of inorganic phosphate and 3-phosphoglycerate observed on Fig. 1 reflects the transport into the stroma space only. As the stroma space comprises approx. 7/8 of the sorbitol-impermeable ^3H_2O space [7], the concentration in the stroma can be evaluated. Thus after 240 s the concentration of phosphate and 3-phosphoglycerate in the stroma is found to be about 20 times higher than in the medium. In contrast, ribose 5-phosphate is taken up very slowly, and the entrance of fructose 6-phosphate to the stroma is almost excluded.

As the rates of phosphate and 3-phosphoglycerate uptake are very high, it is advantageous for resolving the kinetics when the measurements are carried out at low temperatures. For this reason, the transport rates, dealt with in the following, have been mainly obtained at 4°C from 10-s measurements.

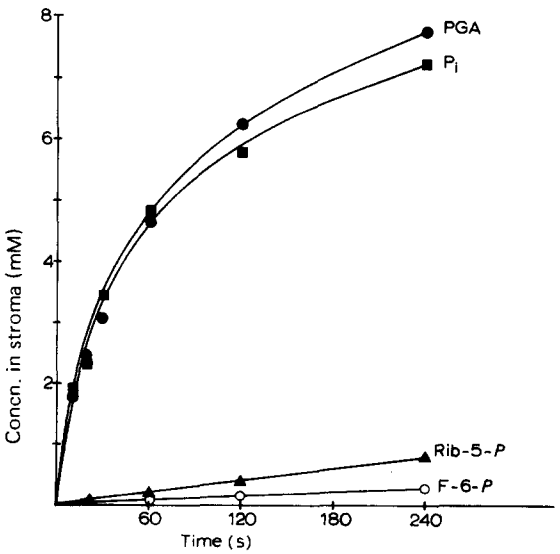

Fig. 1. Uptake of phosphorylated substances (0.32 mM external concentration) into the stroma space. Temperature 0°C, darkness. PGA = 3-phosphoglycerate; Rib-5-*P* = ribose 5-phosphate; F-6-*P* = fructose 6-phosphate.

Kinetic parameters

In the experiment shown in Fig. 2 the concentration dependence of phosphate transport was studied. The double reciprocal plot yields a linear function indicating substrate saturation of the transport. Such a saturation is also observed with 3-phosphoglycerate uptake, as has been shown earlier [8]. It is also shown in Fig. 2 that the uptake of inorganic phosphate is competitively inhibited by 3-phosphoglycerate and dihydroxyacetone phosphate. The data allow the evalution of the K_m (substrate concentration causing half-maximal rate of transport) and V (maximal velocity of transport) for inorganic phosphate and the inhibition constant K_i for 3-phosphoglycerate and dihydroxyacetone phosphate, as listed in the legend. Likewise, also the transport of 3-phosphoglycerate is competitively inhibited by inorganic phosphate and dihydroxyacetone phosphate [8].

As has been shown earlier, the K_m values obtained for the transport of inorganic phosphate and 3-phosphoglycerate agree well with the inhibition constants K_i of these compounds [8]. Furthermore, about the same K_i values of dihydroxyacetone phosphate were found for phosphate and 3-phosphoglycerate transport [8]. These results indicate that all three compounds are bound to the same carrier. It may be noted that the absolute K_m, K_i and V

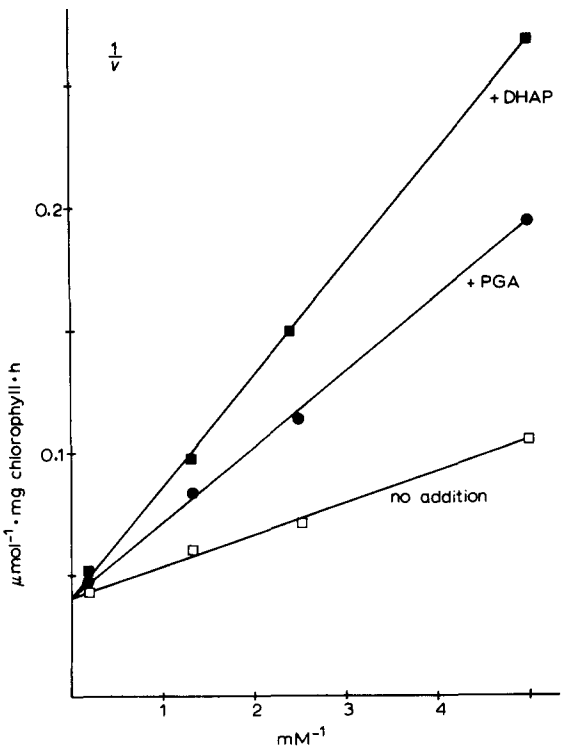

Fig. 2. Concentration dependence of phosphate transport into the chloroplast stroma. Inhibition by 3-phosphoglycerate or by dihydroxyacetone phosphate (0.33 mM external concentration). Temperature 0°C, darkness. The evaluation of the curve yields for inorganic phosphate, K_m 0.33 mM, for 3-phosphoglycerate K_i 0.24 mM and for dihydroxyacetone phosphate K_i 0.12 mM. DHAP = dihydroxyacetone phosphate; GAP = D-glyceraldehyde phosphate.

values show considerable differences in different leaf material from spinach. The results of Figs. 2 and 4 and Tables I—III have been obtained with market spinach. Similar experiments with spinach grown in water culture (Table V, Figs. 9—12) yielded K_m and V values which were sometimes twice those obtained with market spinach. The relative differences between the K_m and K_i values for the different substances were not markedly changed. These observations may suggest that the absolute kinetic constants of the transport are influenced by growth condition. It is feasable that the K_m values of the carrier might be affected by a change of the lipid composition in the inner envelope membrane.

Despite the variability of the absolute K_m and K_i values, the specificity of the transport can be described from inhibition studies. In the experiment of Table I the K_i for various substances was measured in chloroplasts from market spinach. Of those compounds which are only very slowly transported into the stroma, the rates of uptake were also measured at 20° C (Table II). In order to test whether the slow uptake of the investigated metabolites was mediated by the phosphate translocator or the glucose carrier [9], inorganic phosphate and D-glucose were added in concentrations similar to the corresponding K_m values of the transport. As the uptake of pyrophosphate, ribose 5-phosphate, fructose 6-phosphate and fructose 1,6-biphosphate is inhibited by phosphate, these compounds seem to be transported by the phosphate translocator. The inhibition of glucose 6-phosphate uptake by D-glucose indicates that this hexose phosphate is mainly transported by the glucose carrier.

Counter exchange

As shown in Fig. 1, the concentration of phosphate taken up into the stroma

TABLE I

COMPETITIVE INHIBITION OF THE TRANSPORT OF INORGANIC PHOSPHATE (K_m 0.20 mM) INTO THE CHLOROPLAST STROMA

Temperature 4°C, darkness.

Inhibition (mM)	K_i (mM)
Dihydroxyacetone phosphate	0.13
3-Phosphoglycerate	0.15
2-Phosphoglycerate	6.5
2,3-Diphosphoglycerate	9.1
Glycerol 1-phosphate	1.3
Glycerol 2-phosphate	7.7
Phosphoenolpyruvate	4.7
Erythrose 4-phosphate	2.3
Ribose 5-phosphate	10
Glucose 6-phosphate	40
Fructose 6-phosphate	13
Fructose 1,6-bisphosphate	8.5
6-Phosphogluconate	20
Inorganic pyrophosphate	1.8
Arsenate	0.35
Citrate	1.5
L-Malate	7.6
Malonate	30

TABLE II

UPTAKE OF PHOSPHORYLATED METABOLITES INTO THE STROMA SPACE OF SPINACH CHLOROPLASTS

Temperature 20°C, darkness.

Metabolite (2 mM)	Rate of uptake (μmol/mg chlorophyll per h)		
	—	+ Inorganic phosphate (0.2 mM)	+ D-Glucose (20 mM)
Inorganic pyrophosphate	1.3	0.4	n.d.
Ribose 5-phosphate	1.7	1.1	1.9
Glucose 1-phosphate	0.07	0.04	0.08
Glucose 6-phosphate	0.43	0.44	0.24
Fructose 6-phosphate	0.32	0.20	0.34
Fructose 1,6-bisphosphate	0.85	0.53	0.86
Gluconate 6-phosphate	0.23	0.22	0.28

n.d., not determined.

can be much higher than in the medium. This is especially the case if the concentration in the medium is kept low. The apparent accumulation in the stroma is caused by a counter exchange of anions, as shown in Fig. 3. In this experiment the chloroplasts were preincubated with ^{32}P-labelled inorganic phosphate and washed afterwards. ^{14}C-labelled 3-phosphoglycerate was added and the uptake and the release of these compounds was measured simultaneously. The amount of phosphate released is equivalent to the amount of 3-phosphoglycerate taken up, indicating strict coupling of inward and outward transport. This concurs with the previously reported finding that leakage of phosphate from intact chloroplasts is about three orders of magnitude slower than the rate of counter exchange [26]. The measurement of the counter exchange is a valu-

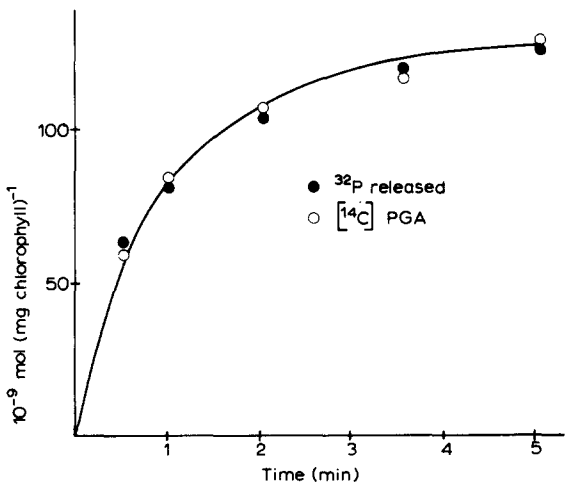

Fig. 3. Simultaneous measurement of the uptake of ^{14}C-labelled 3-phosphoglycerate (0.8 mM) and the release of [^{32}P]phosphate. The chloroplasts were preincubated with inorganic [^{32}P]phosphate in the dark. Temperature 4°C, darkness.

able means for studying the kinetic properties of the phosphate translocator. Instead of measuring the uptake of radioactively labelled phosphates directly one can obtain the same information indirectly by following the release of ^{32}P-labelled phosphate from the chloroplasts to the supernatant after the addition of unlabelled compounds. By such "back exchange" the K_m and V can be also determined for those compounds which are not available radioactively labelled. Fig. 4 shows double reciprocal plots for the concentration dependence of the transport of various metabolites obtained in this way. Table III lists the kinetic constants for a number of phosphorylated compounds as measured by back exchange.

Specificity of the phosphate translocator

From the data shown in the preceeding, the specificity of the phosphate translocator can be deduced. The carrier accepts either inorganic phosphate (also arsenate) or a phosphate molecule attached to the end of a 3-carbon compound, like 3-phosphoglycerate, glyceraldehyde 3-phosphate or dihydroxyacetone phosphate. Thus 1-phosphoglycerate is also transported to some extent. 4-Carbon compounds like erythrose 4-phosphate are only slowly transported, and ribose 5-phosphate even less. The affinity of the carrier seems to be very low for hexose phosphates, for which the inner envelope membrane is practically impermeable. 3-Carbon compounds in which the phosphate is attached to carbon atom 2, like 2-phosphoglycerate, phospho*enol*pyruvate and glycerol 2-phosphate show little binding to the carrier. Interestingly, the V of 2-phosphoglycerate does not largely differ from the corresponding value of 3-phosphoglycerate. This clearly shows that 2-phosphoglycerate is about equally well transported by the carrier as 3-phosphoglycerate, once it is bound to the carrier. This is different with pyrophosphate. The marked inhibition of phosphate transport clearly shows that pyrophosphate is bound to the carrier, with a K_i about 10 times higher than the K_m of inorganic phosphate. How-

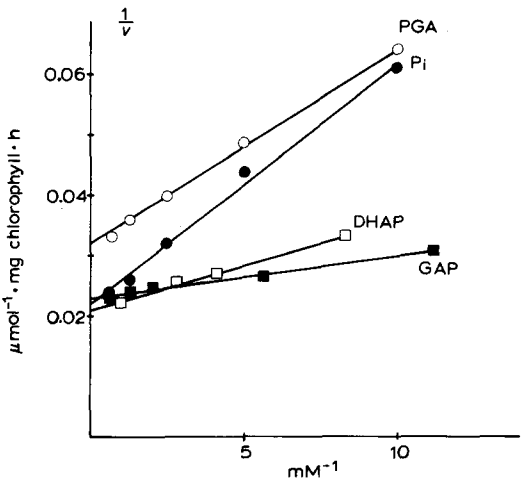

Fig. 4. Concentration dependence of the uptake of inorganic phosphate and phosphorylated compounds into the chloroplast stroma. Measurements by back exchange. Temperature 4°C, darkness.

TABLE III

KINETIC CONSTANTS OF PHOSPHATE TRANSPORT INTO THE CHLOROPLAST STROMA

Values obtained by back exchange. For details see methods and text. Temperature 4°C, darkness.

	K_m (mM)	V (μmol/mg chlorophyll per h)	n
Inorganic phosphate	0.30 (0.15—0.50)	57 (40—91)	16
Dihydroxyacetone phosphate	0.13 (0.06—0.22)	51 (36—74)	8
D-Glyceraldehyde phosphate	0.08 (0.03—0.17)	41 (37—44)	7
3-Phosphoglycerate	0.14 (0.09—0.19)	36 (29—45)	10
2-Phosphoglycerate	2.8 (1.8 —3.7)	24 (20—26)	4
Glycerol 1-phosphate	1.1 (0.7 —1.4)	59 (38—71)	8

ever, the rate of transport is extremely low for this substance. In the experiment of Table II the rate of uptake obtained at 20°C with 2 mM pyrophosphate was only 1.3 μmol/mg chlorophyll per h. Since the concentration used in this experiment was about equivalent to the corresponding K_i value, the V of pyrophosphate is estimated to be about 2.5 μmol/mg chlorophyll per h. This value is by a factor of 100 lower than the V of orthophosphate at this temperature. Apparently, the carrier loaded with pyrophosphate is almost unable to cross the membrane. It may be noted that citrate (K_i = 1.5 mM) seems to be bound to the carrier in a manner similar to that of pyrophosphate (K_i = 1.8 mM). Citrate is also transported very slowly (unpublished).

Inhibition of the phosphate translocator

From the data shown above, citrate and pyrophosphate are suited as inhibitors of the phosphate translocator. Furthermore, a number of reagents, known to react with certain amino acids, had inhibitory effect on the transport (refs. 8, 10, 11 and Flügge, U.-I. and Heldt, H.W., unpublished). As shown in Table IV, the transport is inhibited by *p*-chloromercuriphenyl sulfonic acid, known to react with sulfhydryl groups, by pyridoxal 5′-phosphate forming a

TABLE IV

INHIBITION OF THE TRANSPORT INTO THE CHLOROPLASTS

The control rates of transport were: inorganic phosphate (external concentration 0.40 mM): 32.1 μmol/mg chlorophyll per h; 3-phosphoglycerate (external concentration 0.32 mM): 34.8 μmol/mg chlorophyll per h and L-malate (external concentration 0.17 mM): 5.8 μmol/mg chlorophyll per h. Chloroplast concentration 90 μg chlorophyll/ml. Temperature 4°C. Darkness.

Inhibitor	Time of preincubation (min)	Concentration (μM)	Inhibition of transport (%)		
			Inorganic phosphate	3-Phosphoglycerate	L-Malate
Trinitrobenzene sulfonate	7	100	50	46	0
	7	200	70	72	0
p-Chloromercuriphenyl sulfonate	10	100	78	84	36
	10	300	91	97	50
Pyridoxal 5′-phosphate	45	280	72	81	0
	45	750	86	88	6

Schiff base with lysine [12,13] and by trinitrobenzene sulfonic acid, presumably reacting with an amino group [14]. Due to their anionic charge, all these substances do not permeate readily the inner envelope membrane and are therefore suited to react preferentially with the carrier proteins exposed to the outer surface of the inner envelope membrane. As shown in Table IV, all substances inhibited the transport of phosphate and 3-phosphoglycerate to about the same extent, whereas the transport of malate, as catalyzed by the dicarboxylate translocator, is differently affected. These results may be regarded as further evidence for our earlier conclusion, that phosphate and 3-phosphoglycerate are transported by the same carrier.

The use of the above-mentioned inhibitors and of further substances served as a valuable tool to label the protein involved in phosphate transport (refs. 10 and 11, and Flügge, U.-I. and Heldt, H.W., unpublished). The application of group-specific inhibitors made it also possible to describe the active centre of the transport protein, as will be reported in a later publication.

Temperature dependency

In the experiment of Fig. 5 the temperature dependence of phosphate transport was investigated. Because of the high activity of the transport, rate measurements could only be carried out below 12°C. The Arrhenius plot of the data yields a linear function. The activation energy as derived from the slope is about 16 kcal. From this one can extrapolate that the rate of phosphate transport at 20°C is about five times higher than the rates normally measured at 4°C. As shown in Table V, changing of the temperature mainly effects the V and has only little influence of the corresponding K_m values.

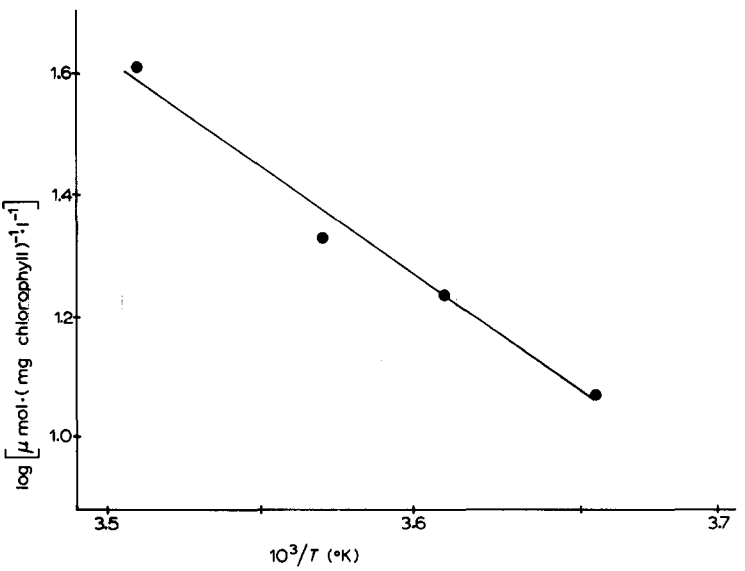

Fig. 5. Temperature dependence of the rate of phosphate uptake into the chloroplast stroma as presented in an Arrhenius plot. The concentration of inorganic phosphate was 50 μM. The activation energy derived from the graph is 16 kcal/mol (0—12°C).

TABLE V

TEMPERATURE DEPENDENCE OF THE KINETIC CONSTANTS OF PHOSPHATE AND PHOSPHOGLYCERATE TRANSPORT INTO THE CHLOROPLAST STROMA

Darkness.

	Temperature (°C)	K_m (mM)	V (μmol/mg chlorophyll per h)
3-Phosphoglycerate	0	0.40	35
	10	0.40	57
Inorganic phosphate	0	0.69	55
	10	0.69	100

On the mode of 3-phosphoglycerate transport

At a neutral pH range 3-phosphoglycerate has one more negative charge than inorganic phosphate or dihydroxyacetone phosphate. The question arises how the balance of charges is achieved during a counter exchange of 3-phosphoglycerate with one of the two other metabolites. The problem will be discussed here with 3-phosphoglycerate reduction as an example. This reaction occurs in intact chloroplasts at very high rates (up to 400 μmol/mg chlorophyll per h, 20°C [15] and requires a counter exchange of 3-phosphoglycerate with dihydroxyacetone phosphate. In principle, two different mechanisms are feasable, as schematized in Fig. 6. Fig. 6A depicts an electrogenic exchange. For the sake of charge balance, this mechanism would require a compensating ion flux. The charge balance might be either achieved by a co-flux of cations, like H^+, K^+, or Mg^{2+} or a counterflux of anions, e.g. chloride. Whereas previous

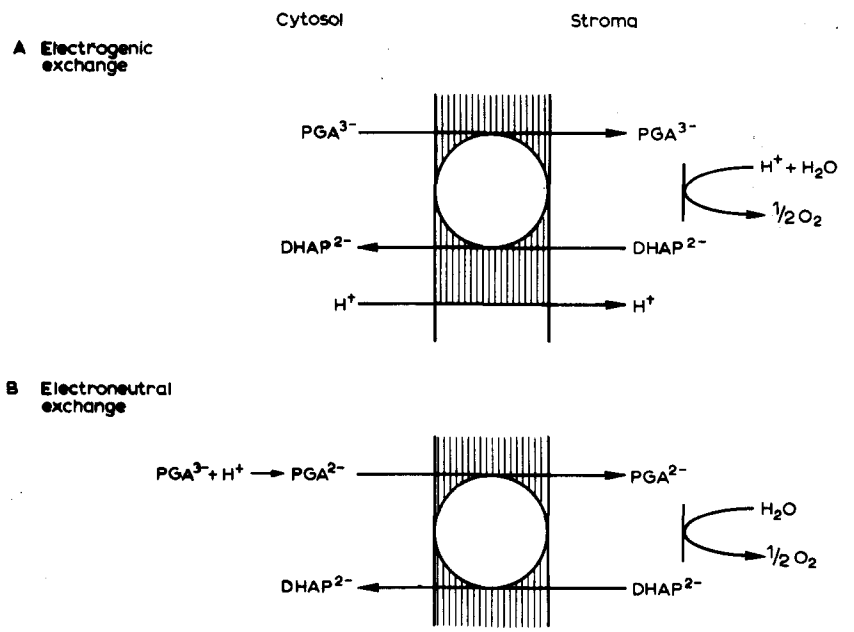

Fig. 6. Schematic diagram of the proton balance during 3-phosphoglycerate reduction.

experiments showed that the permeability of the envelope for K^+ and Mg^{2+} was very low [16], Cl^- was found to be a permeable anion [27]. It has to be taken into account, however, that the conversion of 3-phosphoglycerate to dihydroxyacetone phosphate requires the consumption of protons. According to the hypothetic mechanism A, these protons would have to be consumed in the stroma. The implications of this may be illustrated by a simple calculation: With a 3-phosphoglycerate reduction rate of 120 μmol/mg chlorophyll per h the amount of protons required in 10 s only would be equal to the total amount of protons (0.3 μmol/mg chlorophyll [17]) which are transported from the stroma into the thylakoid space during a dark-light transient. It is inescapable that this large proton requirement would have to be compensated by proton movement across the envelope. Otherwise 3-phosphoglycerate reduction would lead to an excessive alkalinization in the stroma, which is not observed. For this reason, in the hypothetical mechanism A the compensating ion would have to be a proton. Such a separate proton movement across the envelope, however, seems to be rather unlikely, since direct measurements of the proton permeability yielded H^+ transfer rates across the envelope (5 μmol/mg chlorophyll per h [16]) which were more than one order of magnitude lower than would be required for co-transport during 3-phosphoglycerate reduction. These considerations make mechanism A not a very likely case. With the electroneutral exchange schematized in Fig. 6B, there is nosuch requirement for any separate transport, since according to this mechanism the protons are consumed in the external medium for the formation of the lesser charged form of 3-phosphoglycerate.

Proton balance of 3-phosphoglycerate transport

Both mechanisms of 3-phosphoglycerate transport schematized in Fig. 6 imply a concomitant transfer of a proton together with the 3-phosphoglycerate, either directly or indirectly. The experiment of Fig. 7 records the change of the proton concentrations in the slightly buffered suspension medium of a chloroplast preparation following the addition of 3-phosphoglycerate or inorganic phosphate.

When inorganic phosphate was added, there is no change of the H^+ concentration in the medium observed, except a small jump caused by slight differences between the medium and the addition. A very similar result was also obtained with dihydroxyacetone phosphate (unpublished). The addition of 3-phosphoglycerate, however, resulted in a decrease of the H^+ concentration in the medium with a pronounced time dependence. In a similar experiment the pH in the stroma was assayed. The addition of 3-phosphoglycerate resulted in an acidification of the stroma pH by 0.1 unit [25]. Such a pH change was not observed with 2-phosphoglycerate, which is not transported, and also not with inorganic phosphate.

In the experiment of Fig. 8 the proton balance of the transport was determined by measuring the uptake of ^{14}C-labelled-3-phosphoglycerate into the stroma and the change of the proton concentration in the medium. The data clearly show that the transport of 3-phosphoglycerate into the stroma is indeed accompanied by a proton transfer.

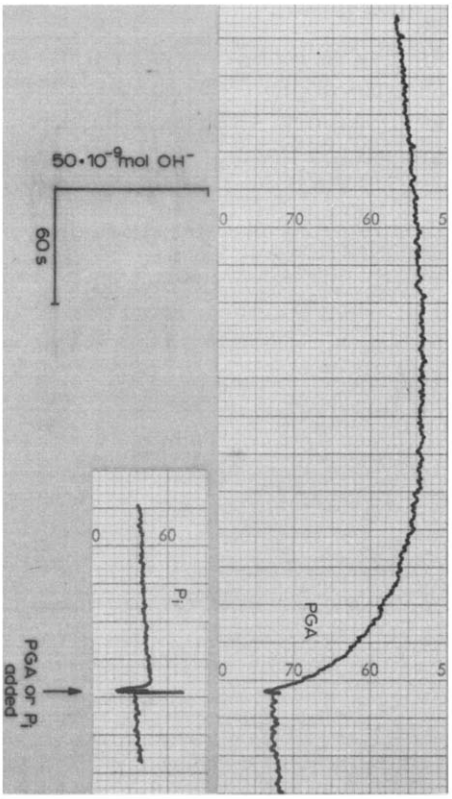

Fig. 7. Proton consumption in the medium during the transport of 3-phosphoglycerate and inorganic phosphate (0.47 mM external concentration) into the chloroplast stroma as measured with a glass electrode. The medium contained 0.33 M sorbitol, 1 mM $MnCl_2$, 1 mM $MgCl_2$, 2 mM EDTA, 0.2 mM HEPES, pH 6.9, and chloroplasts equivalent to 0.36 mg chlorophyll/ml. Total volume 2.0 ml. Temperature 0°C, darkness.

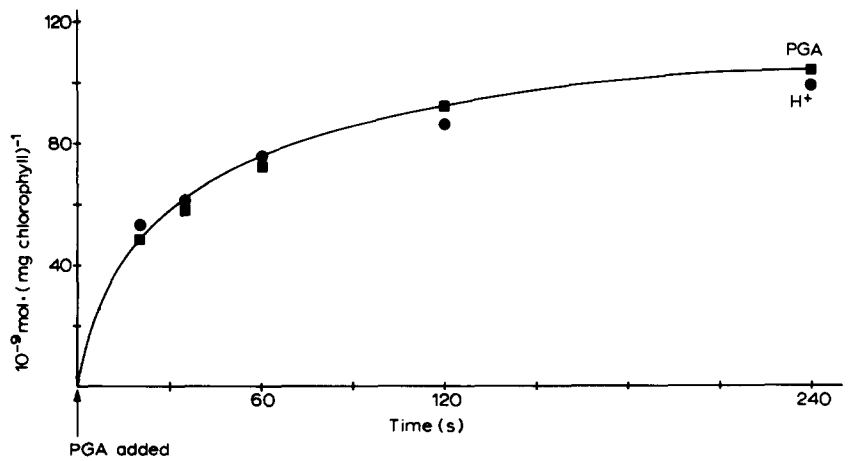

Fig. 8. Simultaneous measurement of the uptake of 3-phosphoglycerate into the stroma and the consumption of protons in the external medium. See legend Fig. 7. Temperature 0°C, darkness.

pH dependence

The pH dependence of transport was studied in order to obtain information on the question as to which ion species are transported by the carrier. Figs. 9 and 10 show the pH dependence of phosphate transport. A pH change between 6.6 and 8.2 had almost no effect on the V, but altered the K_m. At the pH range of the experiment the inorganic phosphate ($pK_2 = 7.2$) co-exists as monovalent and divalent anion. Since it is likely that the carrier will only accept one of these ion species, the K_m values have been recalculated for each of these. The K_m values calculated for HPO_4^{2-} show very little pH dependence. The entire difference between the K_m obtained for pH 7.1—8.2 is only a factor of 1.4. In contrast, the K_m values calculated for $H_2PO_4^-$ exhibit a strong pH dependence. The corresponding value at pH 7.1 is 12 times higher than at pH 8.2. These findings suggest that HPO_4^{2-} is the ion species transported by the carrier. Very similar results are also obtained with dihydroxyacetone phosphate, as shown in Figs. 10 and 11. Also the V of 3-phosphoglycerate transport is only little altered by pH changes (Fig. 10). The pH dependence of the K_m values (Fig. 12), however, is rather different from the pH dependence of the K_m of phosphate and dihydroxyacetone phosphate transport. The measured values for total 3-phosphoglycerate ($pK_3 = 7.1$) are very low between pH 6.4 and 7.1, and show a strong increase at higher pH. The K_m values calculated for the trivalent anion behave very similarly, whereas K_m values for the divalent anion are almost independent of the pH. These data suggest that 3-phosphoglycerate is transported by the phosphate carrier as divalent anion. A similar conclusion has been reached earlier by Heber [15] from measurement of the K_m of 3-phosphoglycerate for reduction by intact chloroplasts assuming that transport into the stroma was the limiting step.

Though being no strict evidence, these findings support the view that a counter exchange of 3-phosphoglycerate with either dihydroxyacetone phosphate or inorganic phosphate proceeds by electroneutral exchange as depicted in Fig. 6B.

On the physiological function of the phosphate translocator

Of all the transport systems found so far in spinach chloroplasts (refs. 9 and

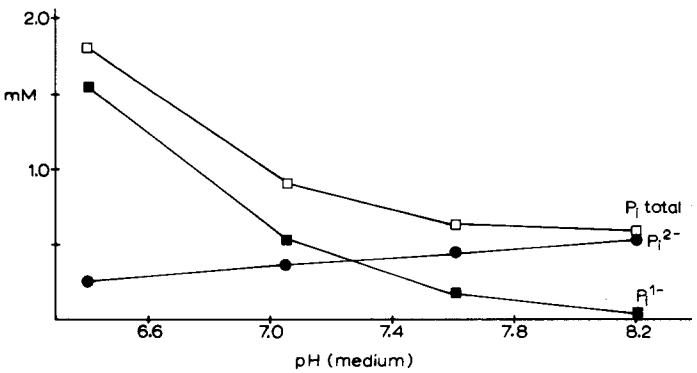

Fig. 9. pH dependence of the K_m for the transport of inorganic phosphate into the chloroplast stroma. Temperature 0°C.

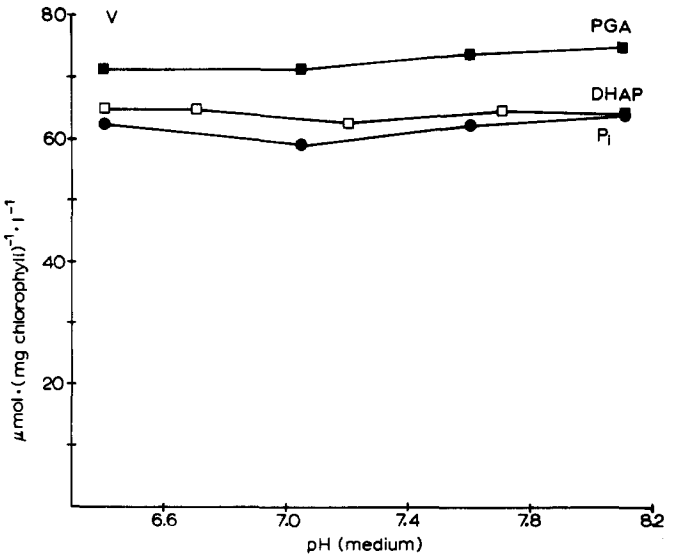

Fig. 10. pH dependence of the *V* for the transport of inorganic phosphate, 3-phosphoglycerate and dihydroxyacetone phosphate into the chloroplast stroma. Temperature 0°C, darkness. Experiments of Figs. 9, 11 and 12.

18, and 26), the phosphate translocator has the highest activity. There are indications that this carrier represents a major protein constituent of the inner envelope membrane [10,11].

One function of this carrier appears to be the indirect transfer of ATP and of reducing equivalents from the chloroplast to the cytosol by a triosephosphate-phosphoglycerate shuttle [19]. A direct transfer of reduced pyridine nucleotides seems to be impossible, since the inner envelope membrane was found to be impermeable to pyridine nucleotides [15]. Furthermore, studies of specific ATP transport [18] and of the photophosphorylation of externally

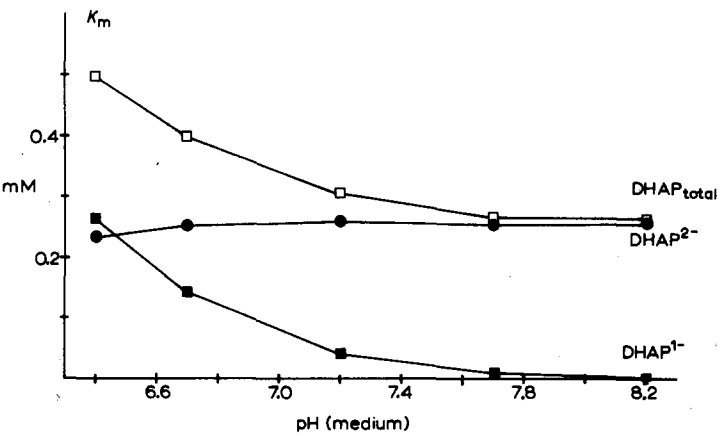

Fig. 11. pH dependence of the K_m for the transport of dihydroxyacetone phosphate into the chloroplast stroma, as obtained by back exchange with [^{32}P]phosphate. Temperature 0°C, darkness.

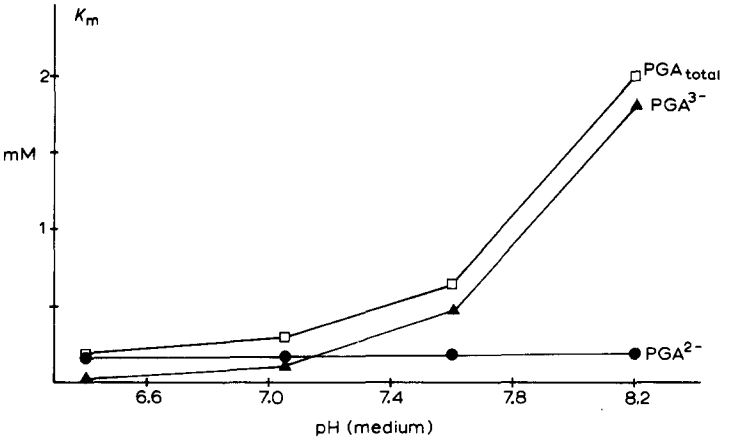

Fig. 12. pH dependence of the K_m for the transport of 3-phosphoglycerate into the chloroplast stroma. Temperature 0°C, darkness.

added ADP [20,21] indicated that a direct transfer of ATP from the stroma to the cytosol does not exist in spinach chloroplasts to any considerable extent. In the proposed shuttle, 3-phosphoglycerate is reduced in the stroma to triosephosphate at the expense of ATP and NADPH, both being generated from the photosynthetic electron transport. After being transported across the envelope, a reoxidation of the triosephosphate to 3-phosphoglycerate by enzymes of the glycolytic pathway results in the formation of ATP and NADH in the cytosol. Alternatively the triosephosphate can be converted in the cytosol by the non-reversible and non-phosphorylating glyceraldehyde phosphate dehydrogenase, yielding the reduction of NADP [22]. Both variants of the cycle have been shown to operate in suspensions of isolated chloroplasts [19,21,22], but it has been found that the non-phosphorylating NADP-dependent glyceraldehyde phosphate dehydrogenase (EC 1.2.1.9) has a lower K_m for glyceraldehyde phosphate than the phosphorylating NAD-dependent glyceraldehyde phosphate dehydrogenase (EC 1.2.1.12). It seems that the export of reducing equivalents from the stroma to the cytosol in the form of NADPH, as required for biosynthetic processes, has priority over the supply of ATP together with NADH to the cytosol.

Apparently it is the main function of the phosphate translocator to enable the export of fixed carbon from the stroma to the cytosol. When isolated chloroplasts are allowed to fix CO_2, only triosephosphate, 3-phosphoglycerate and a trace of pentose monophosphate are released to the external medium [23,24], whereas hexose phosphates and ribulose bisphosphate are retained in the chloroplasts. There is a striking similarity between the products of CO_2 fixation released from the chloroplasts and the specificity of the phosphate translocator, demonstrating the role of this carrier in the release. The rates of translocation, as measured in the present publication, are comparable to the corresponding rates required for CO_2 fixation [24]. There is an exception, however, in the case of 3-phosphoglycerate. As has been shown in Table III, the K_m and V values for the transport of this substance into the chloroplasts,

as measured in the dark, are similar to the corresponding kinetic constants for the transport of dihydroxyacetone phosphate. Although the concentration of 3-phosphoglycerate found in the stroma in the steady state of CO_2 fixation is about 10 times higher than that of triosephosphate [24], the efflux of 3-phosphoglycerate from the chloroplasts during CO_2 fixation is much lower than the efflux of triosephosphate [24]. This apparent discrimination of 3-phosphoglycerate efflux is observed in illuminated chloroplasts only [25] and appears to be due to a light-dependent proton gradient between the external space and the stroma. As shown in Figs. 7 and 8 the transfer of 3-phosphoglycerate involves a transfer of a proton. In illuminated chloroplasts such a proton transfer would be directed against a proton gradient of about $\Delta pH = 1$ [25]. It is therefore feasible that in illuminated chloroplasts the efflux of 3-phosphoglycerate is inhibited by this gradient. Experiments on the effect of light-dependent cation gradients on the transport catalyzed by the phosphate translocator are continuing.

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