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# REGULATION OF THE RECONSTITUTED CHLOROPLAST PHOSPHATE TRANSLOCATOR BY AN $H^+$ GRADIENT

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**This report describes the influence of $\Delta$pH on the transport of phosphate, triose phosphate and 3-phosphoglycerate catalyzed by the phosphate translocator in a reconstituted system. The $H^+$ gradient across the liposome membrane is adjusted by the addition of external buffer solution and maintained for several minutes. The following results are obtained: (1) An inward directed $H^+$ gradient leads to an increase of 3-phosphoglycerate transport and to a decrease of phosphate and triose phosphate transport. (2) An $H^+$ gradient in the opposite direction results in a restriction of 3-phosphoglycerate influx whereas the influx of phosphate and triose phosphate is enhanced. (3) The magnitude of the pH effect depends on the internal substrate. Compared to the homoexchange mode, the effect of applied $\Delta$pH is more pronounced in the heteroexchange mode. (4) Transport of phosphate and 3-phosphoglycerate is influenced by $\Delta$pH in a different manner. In the case of phosphate and triose phosphate transport the observed effects are associated with changes in the apparent $K_m$ values whereas in the case of 3-phosphoglycerate transport the application of a pH gradient is linked to a change of $V_{max}$. (5) In competition experiments with both substrates in the external medium, $\Delta$pH influences the effect of phosphate as a competitive inhibitor of 3-phosphoglycerate transport whereas the effect of 3-phosphoglycerate on phosphate transport is not affected by a pH gradient. (6) The measured apparent $K_m$ and $V_{max}$ values under the influence of $\Delta$pH can be used for the calculation of substrate fluxes across the envelope during illumination. It can be demonstrated that the increase of stromal pH in the light gives rise to a considerable change in the ratio of the substrates transported. Under conditions without pH gradient, the species transported out is mainly 3-phosphoglycerate and the species transported in is mainly triose phosphate. These fluxes are reversed when a pH gradient is applied (light conditions).**

## Introduction

The phosphate translocator of the chloroplasts, located in the inner membrane of the chloroplast envelope, facilitates a counterexchange of phosphate, triose phosphate (dihydroxyacetone phosphate and glyceraldehyde phosphate) and 3-phosphoglycerate [1]. In the illuminated state, it enables the outward transport of triose phosphate from the chloroplasts for two different purposes. Firstly, by counterexchange with phosphate, fixed carbon is exported as triose phosphate from the chloroplasts into the cytosol, where it serves as the substrate for sucrose biosynthesis. Secondly, by an export of triose phosphate in exchange with 3-phosphoglycerate, a shuttle is introduced by which the chloroplasts provide the cytosol with ATP and reducing equivalents [2–4]. The functioning of these two shuttles implies a preferential export of triose phosphate into the cytosol and a preferential

Abbreviations: Tricine, *N*-tris(hydroxymethyl)methylglycine; Pipes, 1.4-piperazinediethanesulfonic acid.

import of 3-phosphoglycerate into the chloroplast stroma, even though the level of 3-phosphoglycerate in the chloroplast stroma during $CO_2$ fixation is much higher than that of triose phosphate.

Indeed, it had been actually observed that illumination results in a restriction of 3-phosphoglycerate transport out of the chloroplasts, whereas in the dark 3-phosphoglycerate was found to be transported about equally in either direction [6]. This suggested that the influence of light on the transport is related to a pH gradient across the envelope, resulting from an alkalinization of the stroma due to light-driven proton transport into the thylakoid space. This was supported by the observation that the effect of light on 3-phosphoglycerate transport was abolished when the proton gradient across the envelope was made to collapse [6]. Although this hypothesis was feasible, it had to be proven. In addition, the precise manner in which such a proton gradient could affect 3-phosphoglycerate transport remained to be solved. In order to elucidate this matter, the characteristics of the countertransport of 3-phosphoglycerate and the other metabolites in either direction of the gradient were studied. With intact chloroplasts this is not possible, since the stroma always contains a mixture of all these metabolites. Such difficulties can be overcome when using liposomes. We have recently achieved the isolation of the phosphate translocator from spinach chloroplasts and its incorporation into liposomes in a functional state [7]. By loading these liposomes with a certain metabolite and a buffer of a certain pH value, it is now possible to measure the activity of the phosphate translocator under strictly defined conditions. In this study, the effect of pH gradients on the function of the phosphate translocator in liposomes is examined.

## Materials and Methods

Triton X-100, soybean phospholipids and nigericin were obtained from Sigma Chemical Co., the radiochemicals from Amersham Buchler (Braunschweig, F.R.G.). Sephadex G-75 was purchased from Pharmacia (Uppsala, Sweden), AG 1-X8 (acetate form, 100–200 mesh) and hydroxyapatite (Bio-Gel HTP) from Bio-Rad Laboratories. All other reagents were of the highest purity available.

*Preparation 3-phospho[$^{14}$C]glycerate*

1 U of ribulosebisphosphate carboxylase (gift from Dr. W. Laing) was incubated in 2 ml buffer containing 100 mM Tris-HCl, pH 8.2, 20 mM $MgCl_2$, and 0.4 mCi $NaH^{14}CO_3$ (spec. act. 50 Ci/mol). After 20 min at room temperature, 300 $\mu$l of a reaction mixture were added containing 50 U phosphoriboisomerase (Sigma), 8 U ribulose-5-phosphate kinase (Sigma), 12.5 mmol dithiothreitol, 380 U creatine kinase (Boehringer), 60 $\mu$mol phosphocreatine, 5 $\mu$mol ATP, 20 mM $MgCl_2$ and 100 mM Tris-HCl, pH 8.2. The formation of 3-phospho[$^{14}$C]glycerate was started after another 30 min by adding 20-$\mu$l aliquots of 0.3 M ribose-5-phosphate at 20-min intervals. The time course of 3-phospho[$^{14}$C]glycerate formation was followed by acidifying and counting of 5-$\mu$l aliquots. The enzymatic reaction was stopped by acidification with 250 $\mu$l of 40% $HClO_4$. After centrifugation at $10000 \times g$ and 4°C for 15 min, the supernatant was reneutralized, applied to an anion-exchange column ($1 \times 10$ cm, AG 1-X8, acetate form) and eluted at 4°C with a linear gradient of HCl (0–0.35 M). Each fraction was tested for 3-phosphoglycerate and radioactivity. The active fractions were pooled, reneutralized, lyophilized, resuspended in a volume of 3 ml of 30% (v/v) ethanol and stored at −30°C.

*Preparation of [$^{14}$C]triose phosphate*

2 $\mu$mol 3-phospho[$^{14}$C]glycerate were added to a reaction mixture (total volume 4.3 ml) containing 0.15 mM ATP, 2 mM phosphocreatine, 1 mM NADH, 1000 U creatine kinase, 50 U triosephosphate isomerase, 5 U glyceraldehyde-3-phosphate dehydrogenase and 5 U phosphoglycerate kinase (all enzymes from Boehringer). After 30 min the reaction was stopped by the addition of 120 $\mu$l of 70% $HClO_4$. The treatment of the mixture was the same as described above for the preparation of 3-phospho[$^{14}$C]glycerate except that the linear gradient of HCl was 0–0.2 M.

*Reconstitution of transport, measurement of substrate uptake and adjustment of $\Delta pH$*

The purification of the phosphate translocator

was essentially as described in Ref. 7. For the preparation of liposomes, acetone-washed soybean phospholipids were used. The sonication buffer with a high buffering capacity contained 120 mM phosphate or 3-phosphoglycerate, 50 mM potassium gluconate and 100 mM Tricine-NaOH, pH 7.8, or 100 mM Pipes-NaOH, pH 6.8, depending on the intended direction of the $H^+$ gradient. A high internal metabolite concentration was employed in order to avoid a limitation of the counterexchange by outward transport. Incorporation of the phosphate translocator and the assay of the transport activity in the forward direction by the pyridoxal 5′-phosphate inhibitor stop method were performed as described earlier [7]. To remove the external medium, the liposomes with the incorporated phosphate translocator were passed over a Sephadex G-75 column which had been equilibrated with a medium containing 300 mM sodium gluconate, 50 mM potassium gluconate and 20 mM Tricine-NaOH or Pipes-NaOH at the same pH as the liposome suspension. The liposomes in the eluant were used for the uptake experiments.

An $H^+$ gradient between the liposomal compartment with a prefixed pH and the external space was adjusted by addition of 5–10 $\mu$l external buffer solution (total volume 220 $\mu$l). The dissipation of this gradient was achieved by adding the ionophore nigericin (5 $\mu$M) which induced an $H^+$-$K^+$ exchange allowing the internal pH of the liposome to equilibrate with the pH in the external medium. For monitoring the pH gradient between the external and the liposomal space, the liposomes were prepared in the presence of the impermeable dye phenol red, and the absorbancy was measured using a dual-wavelength spectrophotometer [8]. Fig. 1 (left) illustrates this for the case of an inward directed proton gradient. To the liposomes with an internal pH of 7.9 a buffer is added to change the external medium to pH 6.8. After the application of the pH jump, the light absorption of the dye remained almost constant for several minutes, indicating that the $H^+$ gradient is maintained during this time. The subsequent addition of nigericin leads to an instantaneous collapse of the pH gradient, as monitored by phenol red absorption. When an outward directed pH gradient was to be applied, the liposomes were prepared in a medium of pH 6.8 and the $H^+$ gradient was

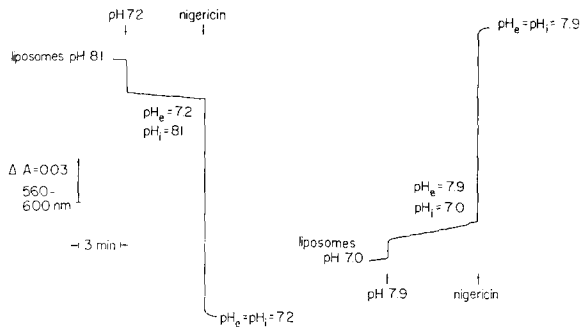


Fig. 1. Measurement of the internal liposomal pH using phenol red by dual-wavelength spectrophotometry at the indicated wavelength couple. Liposomes with internal phenol red (0.3 mM) at pH 8.1 (left) or 7.0 (right) were prepared as described in Materials and Methods. After a 6-fold dilution, the pH jump is adjusted by addition of acidic or basic buffer solution. When indicated 5 $\mu$M nigericin was added. Downward (upward) deflection indicates acidication (alkalinization) of the internal liposome volume.

adjusted by adding alkaline buffer solution which changes the external pH to 7.8. Here again the pH gradient appears to be maintained for several minutes (Fig. 1, right).

## Results and Discussion

### *Influence of an inward directed $H^+$ gradient on the uptake of substrates*

Fig. 2 shows the influence of an applied inward directed $H^+$ gradient on the time course of phosphate uptake. Transport was always measured under two conditions: (i) in the homoexchange mode, i.e., with also phosphate as the internal substrate and (ii) in the heteroexchange mode, i.e., with 3-phosphoglycerate as counterion in the internal liposome volume. In the homoexchange mode phosphate uptake compared to control is slightly inhibited by the pH gradient (Fig. 2, left). This inhibition is even increased if 3-phosphoglycerate is the internal substrate (Fig. 2, right). The same is true for the transport of triose phosphate, which under the conditions of our experiments behaved like the transport of phosphate. Fig. 3 shows the influence of an inward directed $H^+$ gradient on the triose phosphate transport with phosphate or 3-phosphoglycerate as the counterion. In contrast to the above transport of phosphate and triose phosphate, the transport of 3-phosphoglycerate

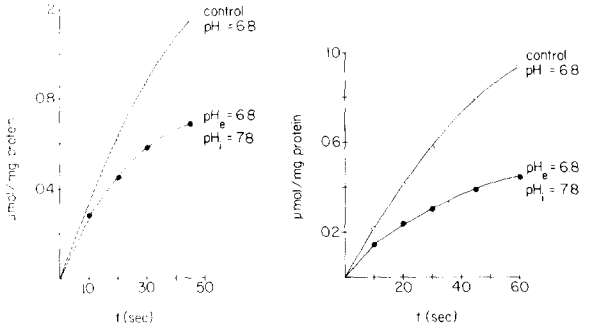


Fig. 2. Effect of an inward directed $H^+$ gradient on the time course of [$^{32}$P]phosphate uptake into reconstituted liposomes. The liposomes had been preloaded with phosphate (left, homexchange) or 3-phosphoglycerate (right, heteroexchange). The external [$^{32}$P]phosphate concentration was 0.5 mM. For details see text.

compared to control is stimulated (Fig. 4). With phosphate as the internal substrate (Fig. 4, right) the uptake of 3-phosphoglycerate is greatly enhanced, but this stimulation is reduced when the transport is measured in the homoexchange mode (Fig. 4, left).

In order to determine whether these observed effects are associated with changes in substrate affinities and/or changes of the mobilities of the substrate-loaded carrier molecules involved, the concentration dependence of phosphate and 3-phosphoglycerate transport in both the homoexchange and the heteroexchange mode was examined by varying the external substrate concentrations. In the case of phosphate transport, the application of a pH gradient results in an increase

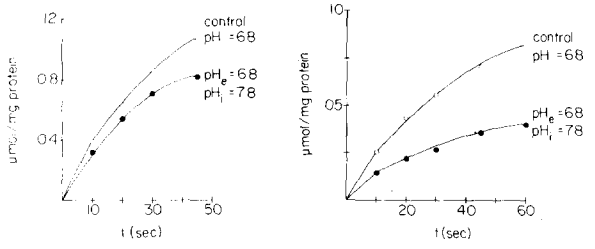


Fig. 3. Effect of an inward directed $H^+$ gradient on the time course of [$^{14}$C]triose phosphate uptake into reconstituted liposomes preloaded with phosphate (left) or 3-phosphoglycerate (right). The external [$^{14}$C]triose phosphate concentration was 0.5 mM. For details see text.

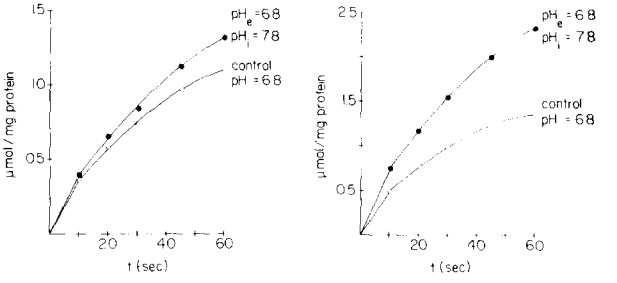


Fig. 4. Effect of an inward directed $H^+$ gradient on the time course of 3-phospho[$^{14}$C]glycerate uptake into reconstituted liposomes. Left, homoexchange; right, heteroexchange mode. The external 3-phospho[$^{14}$C]glycerate concentration was 0.5 mM. For details see text.

of $K_m$, with the $V_{max}$ being virtually unaltered (Fig. 5, left). It appears from these finding, that the inhibition of the phosphate transport is associated with a decrease of the apparent affinity for phosphate under the influence of an inward directed $H^+$ gradient. The homoexchanges are also influenced by the pH gradient in a similar manner although the increase of the $K_m$ value is larger in the heteroexchange mode (see also Table I). In the case of 3-phosphoglycerate, the inward directed $H^+$ gradient does not affect the $K_m$, but is linked to an increase of $V_{max}$. This increase, however, is evident only in the heteroexchange mode (Fig. 5, right). Thus, phosphate and 3-phosphoglycerate transport is influenced by a $\Delta$pH in a different manner. In both cases the observed effects are

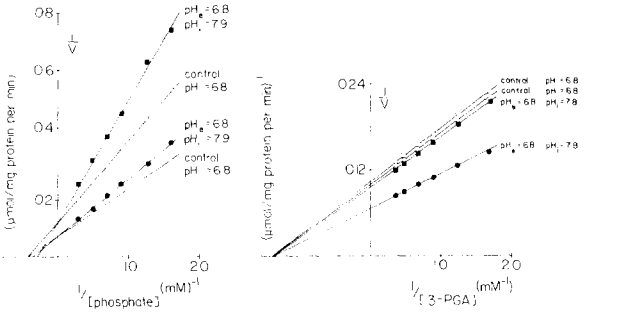


Fig. 5. Effect of an inward directed $H^+$ gradient on the concentration dependence of the uptake of [$^{32}$P]phosphate (left) and 3-phospho[$^{14}$C]glycerate (right) into the reconstituted liposomes which had been preloaded with either phosphate (circles) or 3-phosphoglycerate (3-PGA) (squares). For details see text.

more pronounced if the substrates are transported in a heteroexchange mode which is consistent with the earlier observation that the transport of 3-phosphoglycerate in intact chloroplasts is accompanied by a direct or indirect cotransport of a proton [1]. Under conditions of an applied inward directed $H^+$ gradient, the export of 3-phosphoglycerate in exchange for the inward transport of phosphate is directed against the $H^+$ gradient. This might explain why the inhibition of phosphate uptake and the stimulation of 3-phosphoglycerate transport are more pronounced under conditions of heteroexchange compared to homoexchange.

*Influence of an outward directed $H^+$ gradient on the uptake of substrates*

Uptake can be also followed if an $H^+$ gradient is applied in the opposite direction. In this case the substrates are transported against an $H^+$ gradient into a more acidic liposome space. Fig. 6 shows the effect of an outward directed $H^+$ gradient on phosphate and 3-phosphoglycerate uptake in both the homoexchange and heteroexchange modes. The double-reciprocal plots show that the observed stimulation of phosphate transport is also linked to a change in the apparent $K_m$ for phosphate (Fig. 6, left) as was shown for phosphate transport under an inward directed $H^+$ gradient. Again the alteration of the $K_m$ is greater in liposomes with internal 3-phosphoglycerate used for the transport studies. The transport of triose phosphate is influenced similarly to the phosphate transport

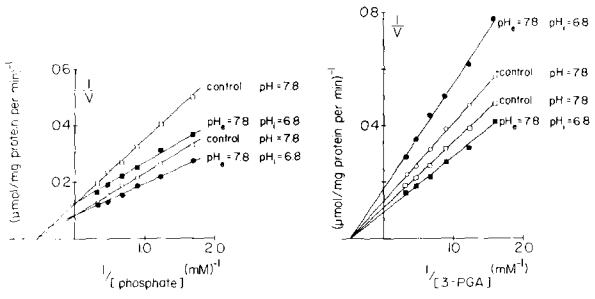


Fig. 6. Effect of an outward directed $H^+$ gradient on the concentration dependence of the uptake of [$^{32}$P]phosphate (left) and 3-phospho[$^{14}$C]glycerate (right) into the reconstituted liposomes. The liposomes had been preloaded with phosphate (circles) or 3-phosphoglycerate (3-PGA) (squares)

(experiments not shown).

Fig. 6 (right) shows that the effect of an outward directed $H^+$ gradient on the 3-phosphoglycerate transport results in a change of the $V_{max}$. In the heteroexchange mode, the $V_{max}$ is decreased by the outward directed $H^+$ gradient. This is consistent with the results shown in Fig. 5 (right), since the 3-phosphoglycerate uptake in exchange with phosphate, involving presumably the transfer of a proton [1], is now directed against the applied $H^+$ gradient. In the homoexchange mode, the $V_{max}$ for 3-phosphoglycerate is slightly increased by the application of the outward directed $H^+$ gradient. The reason for this increase is not fully understood, but appears to reflect an enhanced limitation of the outward transport of 3-phosphoglycerate under an increase of pH.

*Influence of an $H^+$ gradient under competitive conditions*

Under physiological conditions the transport of phosphate or 3-phosphoglycerate occurs in the presence of other metabolites. The different substrates will then compete for binding and transport. Therefore, the concentration dependence of the uptake of one substrate (e.g., 3-phosphoglycerate) in the presence of the second substrate (i.e., phosphate) was studied with and without an applied $H^+$ gradient.

Fig. 7 shows the effect of an inward directed

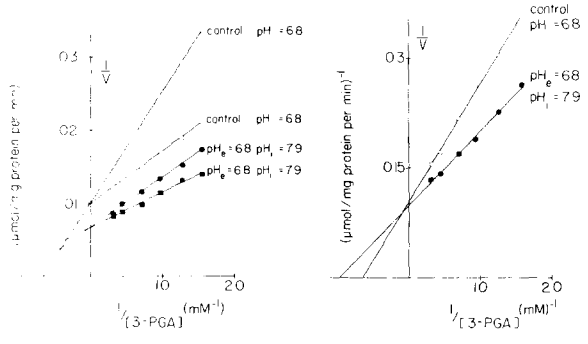


Fig. 7. Effect of an inward directed $H^+$ gradient on the concentration dependence of the uptake of 3-phospho [$^{14}$C]glycerate under competitive condition. During the uptake, 3 mM phosphate was also present (circles). Left, internal substrate: phosphate. The squares refer to the uptake of 3-phospho[$^{14}$C]glycerate without added phosphate. Right, internal substrate: 3-phosphoglycerate (3-PGA).

TABLE I

INFLUENCE OF THE $H^+$ GRADIENT ON THE $K_m$ AND $K_i$ VALUES WITH AND WITHOUT COMPETITIVE CONDITIONS

| | Internal substrate | $K_m(P_i)$ (mM) | | $K_i$(PGA) (mM) | |
|---|---|---|---|---|---|
| | | Without ΔpH | With ΔpH | Without ΔpH | With ΔpH |
| Phosphate transport | | | | | |
| inward directed $H^+$ gradient | phosphate | 2.34 | 2.83 | 0.99 | 0.88 |
| | 3-phosphoglycerate | 2.34 | 3.49 | 0.77 | 0.65 |
| outward directed $H^+$ gradient | phosphate | 1.80 | 1.33 | 2.38 | 2.10 |
| | 3-phosphoglycerate | 1.80 | 1.13 | 2.10 | 2.19 |
| | | $K_m$(PGA) (mM) | | $K_i(P_i)$ (mM) | |
| | | Without ΔpH | With ΔpH | Without ΔpH | With ΔpH |
| 3-Phosphoglycerate transport | | | | | |
| inward directed $H^+$ gradient | phosphate | 0.72 | 0.72 | 2.66 | 7.00 |
| | 3-phosphoglycerate | 0.72 | 0.72 | 2.66 | 5.90 |
| outward directed $H^+$ gradient | phosphate | 2.17 | 2.17 | 2.31 | 0.97 |
| | 3-phosphoglycerate | 2.17 | 2.17 | 2.55 | 1.62 |

$H^+$ gradient on 3-phosphoglycerate transport in the presence of phosphate. Under such competitive conditions, the observed stimulation of the transport of 3-phosphoglycerate into the liposomes loaded with either phosphate (Fig. 7, left) or 3-phosphoglycerate (Fig. 7, right) by an inward directed $H^+$ gradient is much greater than the uptake observed when 3-phosphoglycerate alone is present in the external volume as shown in Fig. 5 (right). The same holds, if the $H^+$ gradient is applied in the opposite direction. These results are summarized in Table I.

Table I shows that under control conditions (i.e., without an $H^+$ gradient) the $K_i(P_i)$ values for 3-phosphoglycerate transport correspond to their respective $K_m(P_i)$ values for phosphate transport as one would expect when both substrates are transported by the same translocator. On the other hand, the application of an $H^+$ gradient results in large changes between the $K_i(P_i)$ values for 3-phosphoglycerate transport and the corresponding $K_m(P_i)$ values for phosphate transport. In the case of an inward directed $H^+$ gradient, the $K_i(P_i)$ values are increased, while in the case of an outward directed $H^+$ gradient these values are decreased with respect to the corresponding $K_m(P_i)$ values. This suggest that the competitive effect of phosphate on 3-phosphoglycerate transport is decreased under conditions of an inward directed $H^+$ gradient but is enhanced under conditions of an outward directed $H^+$ gradient. Consequently, the stimulation of 3-phosphoglycerate transport under conditions of an inward directed $H^+$ gradient (Fig. 7) as well as its inhibition with an $H^+$ gradient in the opposite direction are enhanced in the presence of phosphate (Table I).

Table I also shows the effects of an $H^+$ gradient

TABLE II

INFLUENCE OF THE INTERNAL LIPOSOME SUBSTRATE ON THE MAXIMAL VELOCITY OF PHOSPHATE- AND 3-PHOSPHOGLYCERATE TRANSPORT

Measurements were performed using liposomes preloaded with either phosphate or 3-phosphoglycerate at pH 7.4 as described in Materials and Methods.

| Internal liposome substrate | Transported substrate | $V_{max}$ (μmol/mg protein per min) |
|---|---|---|
| Phosphate | [$^{32}$P]phosphate | 14.0 |
| 3-Phosphoglycerate | [$^{32}$P]phosphate | 8.2 |
| Phosphate | [$^{14}$C]triose phosphate | 13.5 |
| 3-Phosphoglycerate | [$^{14}$C]triose phosphate | 9.7 |
| Phosphate | phospho[$^{14}$C]glycerate | 10.0 |
| 3-Phosphoglycerate | phospho[$^{14}$C]glycerate | 8.2 |

on phosphate uptake with 3-phosphoglycerate as the competitive substrate. As shown in Figs. 5 and 6 an $H^+$ gradient affects the $V_{max}$ for 3-phosphoglycerate transport without changing the $K_m$(PGA) values. This finding is supported by the fact that $K_i$(PGA) values for phosphate transport, reflecting the ability of 3-phosphoglycerate to inhibit phosphate transport as a competitive substrate, are not changed under the influence of an $H^+$ gradient (Table I). The above evidence indicates that the effect of phosphate as a competitive inhibitor of 3-phosphoglycerate transport is substantially influenced by $\Delta$pH. On the other hand, the effect of 3-phosphoglycerate on phosphate transport does not appear to be affected by a pH gradient.

*Influence of the internal substrate on the uptake of phosphate and 3-phosphoglycerate*

Table II shows the dependence of $V_{max}$ for the transport of phosphate, triose phosphate and 3-phosphoglycerate on the internal liposomal substrate in the absence of $\Delta$pH. Using liposomes loaded with phosphate, the $V_{max}$ values for the transport of phosphate and triose phosphate are higher than that of 3-phosphoglycerate. This is similar to observations made in intact chloroplasts where $V_{max}$ values ($\mu$mol/h per mg Chl, 4°C) of 57 for phosphate transport, 51 for triose phosphate transport and 36 for 3-phosphoglycerate transport have been reported [1]. However, the $V_{max}$ values for phosphate and triose phosphate transport measured with liposomes loaded with 3-phosphoglycerate are similar to the maximal transport rates for 3-phosphoglycerate which appear to be relatively independent of the internal substrate used. These relative differences in $V_{max}$ values for phosphate, triose phosphate and 3-phosphoglycerate transport into the liposomes are maintained over the pH range 6.5–8.0. Furthermore, as was observed in intact chloroplasts [1], these respective $V_{max}$ values remain constant over this pH range (data not shown). This indicates that the internal substrate concentrations used in liposome studies are high enough to ensure full saturation of the internal site of the carrier. However, the lower $V_{max}$ value obtained for 3-phosphoglycerate transport suggests that the latter might be the rate-limiting step for strictly coupled exchanges via the phosphate translocator under conditions where substrate concentrations are not limiting.

*On the mode of regulation of the transport*

We have previously studied the influence of the pH in the medium on the substrate uptake catalyzed by the phosphate translocator in intact chloroplasts [1]. The $V_{max}$ values for all substrates were found to be unaffected by changes in medium pH whereas the $K_m$ of phosphate increased and that of 3-phosphoglycerate decreased with a more alkaline pH. This led us to conclude that the different substrate species are transported preferentially as twice negatively charged ions. Table I shows the effect of the medium pH on the apparent $K_m$ values for phosphate and 3-phosphoglycerate at two different pH values in the reconstituted system. It can be seen that by changing the medium pH from 6.8 to 7.8 the apparent $K_m$ for phosphate is increased by a factor of 1.3 whereas the $K_m$ for 3-phosphoglycerate is decreased by a factor of 3. However, when these concentrations are expressed in terms of twice negatively charged species, the $K_m$ for 3-phosphoglycerate$^{2-}$ remains nearly constant while the $K_m$ for $P_i^{2-}$ increased by a factor of 2.2. Although there is no doubt that phosphate and 3-phosphoglycerate are transported by the same carrier [1], these observations suggest that these two substrates are recognized and bound to the carrier differently.

A transport of these different substrates as divalent anions implies that the phosphate ion in the transported phosphate and triose phosphate is differently charged than in the 3-phosphoglycerate. It contains two negative charges in the case of phosphate and triose phosphate, but only one in the case of 3-phosphoglycerate, where the other negative charge is contributed by the carboxylic group ($pK = 3.42$). Since both negative charges appear to be involved in the binding of substrate to carrier [9], the interactions between phosphate and 3-phosphoglycerate with the translocator may be different after all. These differences may partly account for the different responses to an applied $H^+$ gradient observed between phosphate and 3-phosphoglycerate. In the case of phosphate and triose phosphate transport, the modulation of the apparent $K_m$ values by $\Delta$pH may be due to an alteration in the conformation of the carrier and/or

to a coupled proton-substrate binding, since the apparent binding constants for the substrates are expected to be dependent on the proton concentration in the medium. On the other hand, the changes in $V_{max}$ for the transport of 3-phosphoglycerate by $\Delta$pH could probably be linked to a mobility effect of the substrate-loaded translocator. In this case, an inward directed $H^+$ gradient would lead to a faster, and an outward directed $H^+$ gradient to a slower reorientation of the translocator. However, at the present stage it is still uncertain whether the affinity and/or the mobility effect are responsible for the apparent modulation of phosphate and 3-phosphoglycerate transport by an $H^+$ gradient.

*Physiological significance of the regulation of the phosphate translocator by $\Delta$pH*

In intact chloroplasts illumination causes a large increase of the stromal pH leading to a $\Delta$pH across the envelope (inside alkaline) [10]. Although the concentration of 3-phosphoglycerate in the light is much higher than that of triose phosphate, the efflux of 3-phosphoglycerate is largely restricted in favor of triose phosphate efflux [5]. Under these conditions, the efflux of substrates out of the stroma space across the envelope is directed against the $H^+$ gradient and corresponds to the experiments reported in the present study in which an outward directed $H^+$ gradient was applied across the reconstituted liposome membrane. The influx of substrates into illuminated chloroplasts, however, is directed with the $H^+$ gradient and is analogous to the transport of substrates under the influence of an inward directed $H^+$ gradient.

The question arises as to whether the effects of $\Delta$pH observed in the reconstituted liposome system can explain the considerable changes in the transport of triose phosphate and 3-phosphoglycerate between illumination and darkness as observed with protoplasts or intact chloroplasts.

In earlier experiments with illuminated protoplasts from spinach [11] we have found in the stroma 7.0 mM phosphate, 2.1 mM 3-phosphoglycerate and 0.6 mM triose phosphate. The cytosolic concentrations were 2.3 mM 3-phosphoglycerate and 2.8 mM triose phosphate (based on an estimated cytosolic volume of 12.5 $\mu$l/mg Chl and a chloroplast volume of 25.0 $\mu$l/mg Chl); phosphate may be estimated as 10 mM. Using these concentrations and the values for apparent $K_m$ and $V_{max}$ obtained in Tables I and II, and assuming a simple Michaelis-Menten characteristic for the transport, the rates of transport for each single metabolite can be calculated, e.g.:

$$V(\mathrm{DHAP}) = \frac{V_{max}(\mathrm{DHAP})\dfrac{[\mathrm{DHAP}]}{K(\mathrm{DHAP})}}{1 + \dfrac{[\mathrm{DHAP}]}{K(\mathrm{DHAP})} + \dfrac{[\mathrm{P_i}]}{K(\mathrm{P_i})} + \dfrac{[\mathrm{PGA}]}{K(\mathrm{PGA})}}$$

where DHAP represents dihydroxyacetone phosphate and PGA, 3-phosphoglycerate. The $K_m$ values of triose phosphate at pH 6.8 and 7.8 are 1.30 and 1.18 mM, respectively. Since the transport of triose phosphate is influenced by $\Delta$pH to the same extent as phosphate transport, the following constants have been taken from Tables I and II (inward directed $H^+$ gradient, control; heteroexchange) for the transport from the cytosol into the stroma space under dark conditions: $K$(triose phosphate) = 1.3 mM, $K(P_i)$ = 2.3 mM, $K$(PGA = 0.77 mM, $V_{max}$ = 9.7 $\mu$mol/mg protein per min.

This calculation yields for triose phosphate transport a rate of 2.0 $\mu$mol/mg protein per min, and the corresponding rate for 3-phosphoglycerate transport is 3.2. Thus, in the dark due to the corresponding subcellular substrate concentrations the two substrates would be transported into the chloroplasts in a ratio of triose phosphate/3-phosphoglycerate = 0.62. For the transport in the other direction, the same calculation yields a transport ratio of triose phosphate/3-phosphoglycerate = 0.15. In order to eliminate the influence of substrate concentration changes on the calculated fluxes, the same subcellular substrate concentrations have been employed in a model calculation for the illuminated state. For the transport from the cytosol into the stroma (inward directed $H^+$ gradient, heteroexchange) and for the transport in the opposite direction (efflux of substrates in the light, outward directed $H^+$ gradient, heteroexchange) the kinetic constants of Table I have been employed. Table III shows that in the light the ratio of triose phosphate/3-phosphoglycerate transported is more than 8-fold higher for outward transport and 3-fold lower for inward transport

TABLE III

MODEL CALCULATION OF THE INFLUENCE OF THE LIGHT-INDUCED pH GRADIENT ON THE FLUXES OF TRIOSE PHOSPHATE AND 3-PHOSPHOGLYCERATE

The kinetic constants have been derived from Tables I and II. The $V_{max}$ values ($\mu$mol/mg protein per min) for 3-phosphoglycerate transport employed were 10.0 without a pH gradient and 7.5 and 14.9 in the case of an outward and an inward directed $H^+$ gradient, respectively. DHAP, dihydroxyacetone phosphate; 3-PGA, 3-phosphoglycerate.

| | Transport (DHAP/3-PGA) | | Outward/inward |
|---|---|---|---|
| | Outward | Inward | |
| Light | 1.2 | 0.21 | 5.9 |
| Dark | 0.15 | 0.62 | 0.24 |

than the corresponding ratios obtained in the dark. If values of subcellular metabolite concentrations measured in the dark [11] were used, the ratio of triose phosphate/3-phosphoglycerate transported in the light is 12-fold higher with respect to outward transport and more than 5-fold lower with respect to inward transport than the corresponding values in the dark. These results clearly show that the observed effects of a pH gradient across the envelope on the kinetic constants of the phosphate translocator results in a considerable change in the ratio of the substrates transported. In the case where there is no pH gradient, the species transported out would be mainly 3-phosphoglycerate, and the species transported in would be mainly triose phosphate. These fluxes are reversed when there is a pH gradient (light conditions). It can be shown from further model calculations (not presented here) that the direct effect of the medium pH on the changes of the $K_m$ values contributes only a small part of the overall pH effect. Thus, our data demonstrate that the increase of the stromal pH during illumination leading to a pH across the envelope would indeed enhance the export of triose phosphate and the import of 3-phosphoglycerate.

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