Plant Physiol. (1983) 71, 936–943
0032-0889/83/71/0936/08/$00.50/0

Analysis of the Role of the Phosphate Translocator and External Metabolites in Steady-State Chloroplast Photosynthesis

Received for publication August 24, 1982 and in revised form December 15, 1982

ARCHIE R. PORTIS, JR.
United States Department of Agriculture, Agricultural Research Service and Department of Agronomy, University of Illinois, Urbana, Illinois 61801

ABSTRACT

The role of the phosphate translocator and the importance of the extrachloroplastic concentrations of phosphate, 3-phosphoglycerate, and dihydroxyacetone phosphate in steady-state photosynthesis is examined with a kinetic model. The steady-state stromal concentrations of these compounds are calculated as a function of the rate of the various partial reactions of photosynthesis, at various external concentrations which span those likely to occur *in vivo*. It is shown how the net transport requirements of the various reactions necessitate different adjustments in the stromal concentrations of these compounds, away from the equilibrium values expected in the absence of metabolism. Under most circumstances, the high exchange capacity of the phosphate translocator relative to the transport requirements of CO_2 fixation limits the extent of these displacements, but conditions when the phosphate translocator is limiting photosynthesis are observed and discussed. The model provides a basis for a more quantitative understanding of the role of the phosphate translocator and the external concentrations of phosphate, 3-phosphoglycerate, and dihydroxyacetone phosphate in photosynthesis.

In the presence of illumination, the intact chloroplast utilizes ATP and NADPH generated by the light reactions to convert CO_2, Pi, and H_2O into triose-P and O_2. The triose-P is either converted to starch inside the chloroplast, with a recycling of the Pi in the stroma, or is exported to the cytoplasm in exchange for external Pi. In the cytoplasm, the triose-P is converted to sucrose for export to the rest of the plant and the Pi released is recycled back into the chloroplast. During darkness, the starch formed in the light is converted back into triose-P and exported to the cytoplasm permitting a decreased, but continued, export of sucrose (*e.g.* see Ref. 4). In this manner, starch formation buffers the otherwise large oscillations in the availability of photosynthetic energy. It is also possible to export ATP and NADH indirectly and presumably at the expense of CO_2 fixation by a shuttle system involving the movement of DAP[1] out of the chloroplast in exchange for PGA, rather than Pi (16). DAP is then converted back to PGA in the cytoplasm with the formation of ATP and NADH for use in metabolism.

The utilization of photosynthetic energy by the chloroplast for sucrose formation, or starch formation, or for the generation of ATP and NADH in the cytoplasm therefore requires the appropriate rates of Pi-DAP, PGA-DAP, and Pi-PGA exchange. The movement of these compounds is controlled by a specific transport protein which has been named the phosphate translocator (9). The protein catalyzes a relatively strict 1:1 counterexchange of Pi, PGA, and DAP. Studies with isolated spinach chloroplasts have provided experimental values for the rates of transport and the relative affinities of the protein for these compounds (2). Many other closely related compounds may also be transported, but at greatly decreased rates or with a much lower affinity. The counterexchange property of the translocator is in accord with the continuous recycling of Pi/organic phosphate that occurs in the stroma and cytoplasm.

Knowledge of the kinetic properties of this protein have been very useful in providing a qualitative understanding of such phenomena as the lag period required before attaining maximal rates of photosynthesis and the sharp Pi optimum for photosynthesis in isolated chloroplasts (11). The properties of this transport system would also appear to be important in a full understanding of the regulation of starch synthesis (21) and the effects of cytoplasmic conditions on photosynthesis, particularly in relation to source-sink effects (10).

Further progress will be aided by a more quantitative understanding of the role of the phosphate translocator in photosynthesis. A kinetic model for translocator-mediated diffusion was developed by Giersch (5) and applied to the phosphate translocator such that the parameters of the model could be directly related to the experimental kinetic constants obtained for spinach (9). More recently aspects of this model were incorporated into a computer simulation of the effects of light-dark transients on metabolite concentrations in the chloroplast and cytoplasm of leaf cells and the efficiency of indirect ATP transfer between chloroplasts and leaf cells (6).

In this report, I have used Giersch's model for a quantitative analysis of how metabolic reactions occurring in the chloroplast and the external or cytoplasmic concentrations of Pi, PGA, and DAP should determine the stromal concentrations of these compounds and their relative rates of movement into and out of the chloroplast. In this manner, the properties of the phosphate translocator can strongly influence the allocation of photosynthetic energy for starch and sucrose synthesis and for metabolic processes in the cytoplasm. The establishment of the relationship between transport properties of the translocator, concentrations of exchangeable compounds, and the requirements for exchange permit a quantitative analysis of the role of the phosphate translocator in the regulation of photosynthesis. The results considerably extend previous efforts in this direction (5, 19). Emphasis is placed on conditions that can be utilized in *in vitro* experiments with isolated chloroplasts and which should span those occurring in the cytoplasm *in vivo*. We plan to use the model in conjunction with experiments on *in vitro* measurements of the stromal concentrations of Pi, PGA, and DAP under a wide variety of conditions to help in understanding the role of these metabolites in the regulation of photosynthesis.

[1] Abbreviations: PGA, 3-phosphoglycerate; DAP, dihydroxyacetone phosphate; RuBP, ribulose 1,5-bisphosphate.

RESULTS AND DISCUSSION

The Kinetic Model for the Phosphate Translocator in Chloroplasts. In order to simplify the present analysis, the following assumptions will be incorporated into the more general model derived by Giersch. (a) Only movement of PGA, Pi, and DAP will be considered; (b) the maximal exchange velocity for these compounds will be considered equivalent; (c) the affinity of the transport system for each individual compound will be assumed to be the same on both sides of the membrane as expected for a simple passive system (1). Other compunds are known to be transported by the phosphate translocator (2), but the influence of these on the movement of Pi, PGA, and DAP between the stroma and the medium under most physiological conditions is calculated to be small. Differences in the apparent maximal exchange velocity of PGA relative to Pi or DAP have been observed experimentally but conclusive evidence on this point is not available (2). In any case, removing this simplification does not drastically alter the conclusion to be drawn from this analysis (A. R. Portis, unpublished).

Incorporating assumptions (a) and (b) into the rate equations derived by Giersch, the specific equations that describe the movement of Pi, PGA, and DAP across the chloroplast envelope are presented in Table I. Net transport of each metabolite is the difference between the rate of influx $\left[e.g.\text{ for Pi, } V_{A_{II}} = V_{max}\left(\frac{A_{II}(A_I + B_I + C_I)}{M}\right)\right]$ and the rate of efflux $\left[e.g.\text{ for Pi, } V_{A_I} = V_{max}\left(\frac{A_I(A_{II} + B_{II} + C_{II})}{M}\right)\right]$, and therefore is usually only a small fraction of the actual movement occurring in each direction (Table I). All movement is by exchange, so the sum of all uptake rates is equal to the sum of all efflux rates and the sum of all net transport rates is zero.

The transport system is passive, and thus in the absence of reactions requiring the net movement of Pi, PGA, and DAP across the chloroplast envelope, an equilibrium condition will be established by the phosphate translocator in which there is rapid exchange but no net movement of these compounds (*i.e.* $V_a = V_b = V_c = 0$). It can be shown that this occurs when $\frac{A_I}{A_{II}} = \frac{B_I}{B_{II}} = \frac{C_I}{C_{II}}$, or in terms of the experimental parameters:

$$\frac{[Pi]_{in}}{[Pi]_{out}} \cdot \frac{K_{out}^{Pi}}{K_{in}^{Pi}} = \frac{[PGA]_{in}}{[PGA]_{out}} \cdot \frac{K_{out}^{PGA}}{K_{in}^{PGA}} = \frac{[DAP]_{in}}{[DAP]_{out}} \cdot \frac{K_{out}^{DAP}}{K_{in}^{DAP}} = \frac{A_I + B_I + C_I}{A_{II} + B_{II} + C_{II}} = C \quad (4)$$

Examination of Eq. 4 indicates that the equilibrium concentration ratio of each compound depends only on the ratio of the internal to the external affinity of the phosphate translocator for each compound. Differences between the affinity of the phosphate translocator for Pi, PGA, and DAP have no effect on the equilibrium position, although they do affect the relative equilibrium exchange rates.

Recently, it was found that the phosphate translocator catalyzes the exchange of only the doubly ionized forms of Pi, PGA, and DAP (2). Therefore, the affinity and concentration terms in the original model derived by Giersch and in the equations presented in Table I are applicable to these forms. However, it is more convenient to have expressions that utilize the total concentration of each compound. At physiological pH, only two ionized forms of each compound need be considered (A^- and A^{2-} for DAP and Pi, A^{2-} and A^{3-} for PGA), and the relative amounts of these are determined by the appropriate pK_a. The expressions relating the apparent affinitives K^T and the total concentration $[A^T]$ of both forms of each compound to that of the doubly ionized species above (K^{2-} and $[A^{2-}]$) are of the form:

$$K^T = (1 + 10^{(pK_a - pH)})(K^{2-}) \quad (5)$$

$$[A^T] = (1 + 10^{(pK_a - pH)})[A^{2-}] \quad (6)$$

Table I. *Rate Equations Describing the Movement of Pi, PGA, and DAP across the Chloroplast Envelope in Terms of Experimental Parameters*

Let:

$K_{in}^{Pi}, K_{in}^{PGA}, K_{in}^{DAP}, K_{out}^{Pi}, K_{out}^{PGA}, K_{out}^{DAP}$ = apparent transport affinities

V_{max} = maximal exchange rate (μmol mg^{-1} Chl h^{-1})

$$A_I = \frac{[Pi]_{in}}{K_{in}^{Pi}} \qquad B_I = \frac{[PGA]_{in}}{K_{in}^{PGA}} \qquad C_I = \frac{[DAP]_{in}}{K_{in}^{DAP}}$$

$$A_{II} = \frac{[Pi]_{out}}{K_{out}^{Pi}} \qquad B_{II} = \frac{[PGA]_{out}}{K_{out}^{PGA}} \qquad C_{II} = \frac{[DAP]_{out}}{K_{out}^{DAP}}$$

$V_{A_I}, V_{B_I}, V_{C_I}$ = rates of efflux (μmol mg^{-1} Chl h^{-1})

$V_{A_{II}}, V_{B_{II}}, V_{C_{II}}$ = rates of influx (μmol mg^{-1} Chl h^{-1})

V_a, V_b, V_c = net exchange rates for Pi, PGA, and DAP, respectively

$$M = (A_I + B_I + C_I) + (A_{II} + B_{II} + C_{II}) + (A_I + B_I + C_I)(A_{II} + B_{II} + C_{II})$$

Then:

$$V_a = V_{A_{II}} - V_{A_I} = V_{max}\left[\frac{A_{II}(A_I + B_I + C_I)}{M} - \frac{A_I(A_{II} + B_{II} + C_{II})}{M}\right] \text{ for Pi} \quad (1)$$

$$V_b = V_{B_{II}} - V_{B_I} = V_{max}\left[\frac{B_{II}(A_I + B_I + C_I)}{M} - \frac{B_I(A_{II} + B_{II} + C_{II})}{M}\right] \text{ for PGA} \quad (2)$$

$$V_c = V_{C_{II}} - V_{C_I} = V_{max}\left[\frac{C_{II}(A_I + B_I + C_I)}{M} - \frac{C_I(A_{II} + B_{II} + C_{II})}{M}\right] \text{ for DAP} \quad (3)$$

From Eqs. 5 and 6, the following relationship is derived: $\frac{K^T}{[A^T]} = \frac{K^{2-}}{[A^{2-}]}$, etc. Since only $K^{2-}/[A^{2-}]$ terms appear in the expressions for V/V_{max} in Table I, the equations in Table I may also be used when the total concentration and the apparent affinity based on the total concentration of each compound are utilized, although the apparent affinities utilized in the equations are now pH dependent.

With respect to the equilibrium situation (Eq. 4), substitution of Eqs. 5 and 6 gives for Pi:

$$\frac{[Pi_{in}^T]}{[Pi_{out}^T]} = \frac{(1 + 10^{pK_a - pH_{in}})K_{in}^{2-}}{(1 + 10^{pK_a - pH_{out}})K_{out}^{2-}} \cdot C \quad (7)$$

Similar equations can be derived for DAP and PGA. The equilibrium ratio between the total internal and external concentration (*i.e.* all ionized forms) of each compound is therefore dependent on the pK_a of the compound and the pH in the stroma and external medium.

From the equations in Table I and Eqs. 4 and 7, with $K_{in}^{2-} = K_{out}^{2-}$ (assumption 3) for each compound, letting

$$R'_{Pi} = \frac{(1 + 10^{pK_a - pH_{out}})}{(1 + 10^{pK_a - pH_{in}})}, \quad R'_{PGA} = \frac{(1 + 10^{pH_{out} - pK_a})}{(1 + 10^{pH_{in} - pK_a})},$$

$$R'_{DAP} = \frac{(1 + 10^{pK_a - pH_{out}})}{(1 + 10^{pK_a - pH_{in}})}$$

and $T = [Pi_{in}^T] + [PGA_{in}^T] + [DAP_{in}^T]$, the following expressions for the concentration of all ionized forms of Pi, PGA, and DAP in the stroma can be derived:

$$[PO_{4in}^T] = \frac{T}{1 + \frac{R'_{Pi}}{[PO_{4out}^T]}\left(\frac{[DAP_{out}^T]}{R'_{DAP}} + \frac{[PGA_{out}^T]}{R'_{PGA}}\right)} \quad (8)$$

$$[PGA_{in}^T] = \frac{T}{1 + \frac{R'_{PGA}}{[PGA_{out}^T]}\left(\frac{[DAP_{out}^T]}{R'_{DAP}} + \frac{[Pi_{out}^T]}{R'_{Pi}}\right)} \quad (9)$$

$$[DAP_{in}^T] = T - [PO_{4in}^T] - [PGA_{in}^T]$$

$$= \frac{T}{1 + \frac{R'_{DAP}}{[DAP_{out}^T]}\left(\frac{[Pi_{out}^T]}{R'_{Pi}} + \frac{[PGA_{out}^T]}{R'_{PGA}}\right)} \quad (10)$$

For given external and internal pH values, and the total internal concentration of Pi, PGA, and DAP, Eqs. 8, 9, and 10 permit the direction calculation of the internal concentrations of these compounds at equilibrium from a given set of external concentrations.

Relationships between Photosynthetic Reactions Occurring in the Chloroplast and the Requisite Exchange Reactions Catalyzed by the Phosphate Translocator. In the preceding, we have established the calculated relationships between the equilibrium concentrations of PGA, DAP, and Pi in the stroma and the external medium (cytoplasm) when the pH in the stroma and medium are known. Before exploring the effects of metabolism and net metabolite exchange on the equilibrium concentrations, it is important to examine the stoichiometric relationships between the rates of the different partial reactions of photosynthesis and exchange of metabolites by the phosphate translocator. The differing requirements for metabolite exchange by the alternative reactions possible with chloroplasts are summarized in Table II.

In the first reaction, starch is the only product of photosynthetic CO_2 fixation. Phosphate uptake is not required since no carbon leaves the chloroplast, and the phosphate cycles between the inorganic and organic pools in the stroma. Although starch formation does not require transport, the characteristics of the phosphate translocator are a very important factor in the regulation of starch synthesis because of their role in determining the PGA/Pi ratio in the stroma (21). The 2nd and 3rd reactions in Table II are the two other alternative end-products of CO_2 fixation in chloroplasts: DAP and PGA, respectively. The formation of these two compounds requires an equimolar uptake of Pi from the medium. While one might expect little net formation and export of PGA to the cytoplasm by CO_2 fixation *in vivo*, where sucrose is the predominate final product, PGA is normally observed to be a major end-product of photosynthesis by isolated chloroplasts with only Pi in the external medium. These three reactions complete the normal CO_2 fixation reactions possible with chloroplasts. The latter two reactions require a net exchange rate by the phosphate translocator of one-third the rate of CO_2 fixation.

The 4th reaction in Table II is the reduction of external PGA to DAP, and provides a means of making ATP and NADH available in the cytoplasm, using photosynthetic energy (7, 16). When normalized with respect to O_2 evolution (*i.e.* energy utilization), this reaction requires a 6-fold greater net exchange rate by the phosphate translocator than does CO_2 fixation. In *in vitro* experiments, PGA-dependent O_2 evolution is usually significantly less than that possible with CO_2 (17), an observation which sometimes could be due in part to the greater transport requirements. However, *in vivo*, less than 10% of the available photosynthetic energy would be expected to be utilized for PGA reduction in order to make ATP available in the cytoplasm for other biosynthetic and transport reactions (7).

The 5th reaction in Table II which I refer to as DAP-supported CO_2 fixation, includes all the reactions of the reductive pentose phosphate pathway except those comprising reaction 4, PGA reduction. Therefore, when combined in the appropriate ratios, reactions 4 and 5 will result in reactions 2 or 3. Reaction 5 utilizes only ATP for CO_2 fixation and therefore O_2 evolution is not required. This reaction has been examined in *in vitro* experiments (14, 15) and rates of CO_2 fixation of up to one-third that found under optimal conditions have been observed. This reaction also places a great demand on rapid exchange by the phosphate translocator, which may again be a factor in the decreased rates observed relative to CO_2 fixation with Pi alone. Previously, this was suggested to be due either to a limitation on ATP synthesis in the absence of linear electron transport or a limitation in the capacity of this section of the Calvin cycle when operating alone (14). With appropriate experiments, use of the model of the phosphate translocator may allow this question to be answered more definitively.

Finally, the 6th reaction in Table II accounts for the oxygenase activity of RuBPase-ribulose, 1,5 bis, in which the products are PGA and P-glycolate, and completes the table. The P-glycolate is hydrolyzed inside the chloroplast to glycolate which is the final product of this reaction with chloroplasts. In terms of metabolite transport, two alternative possibilities are listed (reactions 6a and 6b). Reaction 6b includes the complete reduction of the PGA produced in the oxygenase reaction to DAP, which can be recycled to RuBP inside the chloroplasts and therefore decreases the overall transport demands by 60%. Certain aspects of this reaction under conditions where the oxygenase activity is predominate have also been studied *in vitro* (18) with isolated chloroplasts. One interesting feature of this partial reaction is that there is a stoichiometric release of Pi inside the chloroplast with O_2 uptake by the oxygenase reaction, in contrast to a stoichiometry of one Pi taken up for every three CO_2 fixed in the carboxylase reaction.

Effects of Varying the Net Rates of Pi-PGA, PGA-DAP, and Pi-DAP Exchange on the Steady-State Stromal Concentration of These Metabolites. The phosphate translocator catalyzes the passive exchange of Pi, PGA, and DAP at rates determined by the internal and external concentration of each compound and the affinity of the translocator for each (*i.e.* the apparent K_a) and the

Table II. *Relationships between Metabolite Fluxes through the Phosphate Translocator and the Partial Reactions of Photosynthesis with Isolated Chloroplasts*

Fluxes and rates are μmol mg^{-1} Chl h^{-1} and have been normalized for comparative purposes. Fluxes are: (−), for uptake; (+), for export.

	Fluxes			Partial Reaction	Rates (Evolution)	
	Pi	PGA	DAP		CO_2	O_2
(1)	0	0	0	Starch synthesis from CO_2	−60	60
(2)	−20	0	+20	DAP from CO_2	−60	60
(3)	−20	+20	0	PGA from CO_2	−60	50
(4)	0	−120	+120	PGA reduction	0	60
(5)	−20	+120	−100	DAP-supported CO_2 fixation	−60	0
(6a)	+40	+60	−100	DAP-supported photorespiration	0	−60
(6b)	+40		−40	With PGA reduction	0	−30

maximal capacity of the system (*i.e.* V_{max}) as shown in Table I. In the absence of metabolic reactions resulting in the formation or disappearance of these compounds, Eqs. 8, 9, and 10 describing the equilibrium state have been presented. The partial reactions of photosynthesis place varying demands on the phosphate translocator for the appropriate exchanges (Table II). In the following, we examine how these exchange requirements result in new steady-state stromal concentrations of these compounds displaced from their equilibrium position.

Both the internal and external concentrations of PGA, DAP, and Pi are possible variables in determining the net exchange rate for each compound and therefore different combinations of these can give nearly equivalent exchange rates. In the following analysis, the external concentrations will be held constant, as a simplification. This assumption would not necessarily be true for chloroplasts *in vivo*, but would generally apply to *in vitro* experiments with isolated chloroplasts, if all three compounds are initially present in the medium at adequate levels. The sum of the concentrations of PGA, Pi, and DAP in the stroma will also be kept constant at 10 mM. This assumption is unlikely to be valid either *in vivo* or *in vitro*, as variable amounts of the total stromal Pi pool could be localized in the pentose, hexose, and heptulose, mono-, and biphosphates. However, the factors that might control the amount of these sugar phosphates are not known as yet, and therefore cannot be incorporated into the model at this time. The apparent external affinities based on the total concentration of each compound that are utilized are the average values obtained over many experiments with spinach chloroplasts (2) (external pH of 7.6 assumed) and the corresponding apparent internal affinities were calculated from Eq. 5 (see legend to Fig. 1).

Attempts to derive explicit expressions for $[Pi_{in}^T]$, $[PGA_{in}^T]$, and $[DAP_{in}^T]$ as a function of the exchange rates (V_a, V_b, V_c) were only partially successful and too complex for easy analysis. Therefore, the equations in Table I were utilized to obtain the approximate solutions for the steady-state stromal concentrations of Pi, PGA, and DAP recursively using a small time interval (Δt).

For example, if Δt is sufficiently small, one can start with any initial set of concentrations ($[P^T]_{t_o}$, $[PGA^T]_{t_o}$, $[DAP^T]_{t_o}$) inside the chloroplast, calculate the corresponding initial rates of net exchange, V_{a_o}, V_{b_o}, V_{c_o}. These are then used to calculate the new concentrations, $[P^T]_{t_1}$, $[PGA^T]_{t_1}$, $[DAP^T]_{t_1}$ at time $t_1 (t_1 - t_o = \Delta t)$. This iterative process may be repeated until after a sufficiently long time $V_a \simeq V_b \simeq V_c \simeq 0$, and the corresponding concentrations $[PGA^T]_{V_a=0}$, $[P^T]_{V_b=0}$, $[DAP^T]_{V_c=0}$ are then found to be equal (<1% error) to the equilibrium concentrations, whose explicit solution was derived previously (Eqs.8, 9, and 10) if Δt is made sufficiently small. To obtain the steady-state concentrations of Pi, PGA, and DAP at non-zero values of V_a, V_b, and V_c, the same process is used except that the desired values of V_a, V_b, and V_c are used in the equations and the quantity $[V_a^{desired} - V_{a_i}]$ approaches zero, after a sufficient number of iterations.

Since the net result of photosynthesis in chloroplasts can be resolved into various combinations of the basic partial reactions given in Table II, the effects of each reaction on the stromal concentrations will be examined separately. The data can be presented in several different forms: (a) concentration *versus* exchange rate; (b) concentration *versus* CO_2 fixation rate; (c) concentration *versus* O_2 evolution rate. Of these, a comparison *versus* the rate of O_2 evolution accompanying the reaction appeared to be the most useful since it reflects the extent to which the available energy provided by the light reactions is utilized for the alternative reactions.

The results of the calculations for the case when the external concentrations are 1.0 mM for Pi, 1.5 mM for PGA, and 1.5 mM for DAP and a maximal capacity of 500 μmol mg^{-1} Chl h^{-1} is chosen for the phosphate translocator is shown in Figure 1A. The hatched areas encompass the effects of CO_2 fixation with one edge (solid line) as the limit when only DAP is the product (reaction 2), and the other edge (broken line) as the limit when only PGA is the product (reaction 3). It should be remembered that while the $O_2 : CO_2$ ratio is 1 : 1 with DAP, it is only 5 : 6 for PGA as the sole product. Starch synthesis (reaction 1) does not affect stromal concentrations and therefore is not shown. However, from the high PGA : Pi ratio, a relatively high rate of starch synthesis would be expected. The effect of PGA reduction (reaction 4) as the only reaction is indicated by the solid lines. One example of the effect of a fixed rate of CO_2 fixation and increasing the rate of PGA reduction (*i.e.* reaction 2 plus increasing reaction 4) in excess of that required by CO_2 fixation itself is indicated by the dashed lines (PGA′, DAP′, Pi′). The close parallels between these lines and those given by PGA reduction alone indicate that under the chosen conditions the effects of these reactions on the stromal metabolite concentrations can be considered to be nearly independent. The effects of reactions 5 and 6 will be discussed below.

The following conclusions can be drawn from the results. With a high exchange capacity (V_{max} = 500), CO_2 fixation does not cause PGA or DAP to deviate markedly from the equilibrium position expected with no net exchange. Increasing rates of CO_2 fixation (excluding starch synthesis) lowers the stromal [Pi] and under the conditions chosen, CO_2 fixation could not exceed a rate of ~145 μmol O_2 mg^{-1} Chl h^{-1} (off scale and not shown). PGA reduction has the opposite effect on the stromal concentrations, with there being little change in stromal Pi and very dramatic changes in PGA and DAP with an increasing rate. Rates of PGA reduction in excess of 100 μmol O_2 mg^{-1} Chl h^{-1} are not possible, for lack of [PGA] inside. Actually, a rate much less than this can be expected due to the rapid change in $\frac{[PGA]}{[DAP][Pi]}$ limiting the

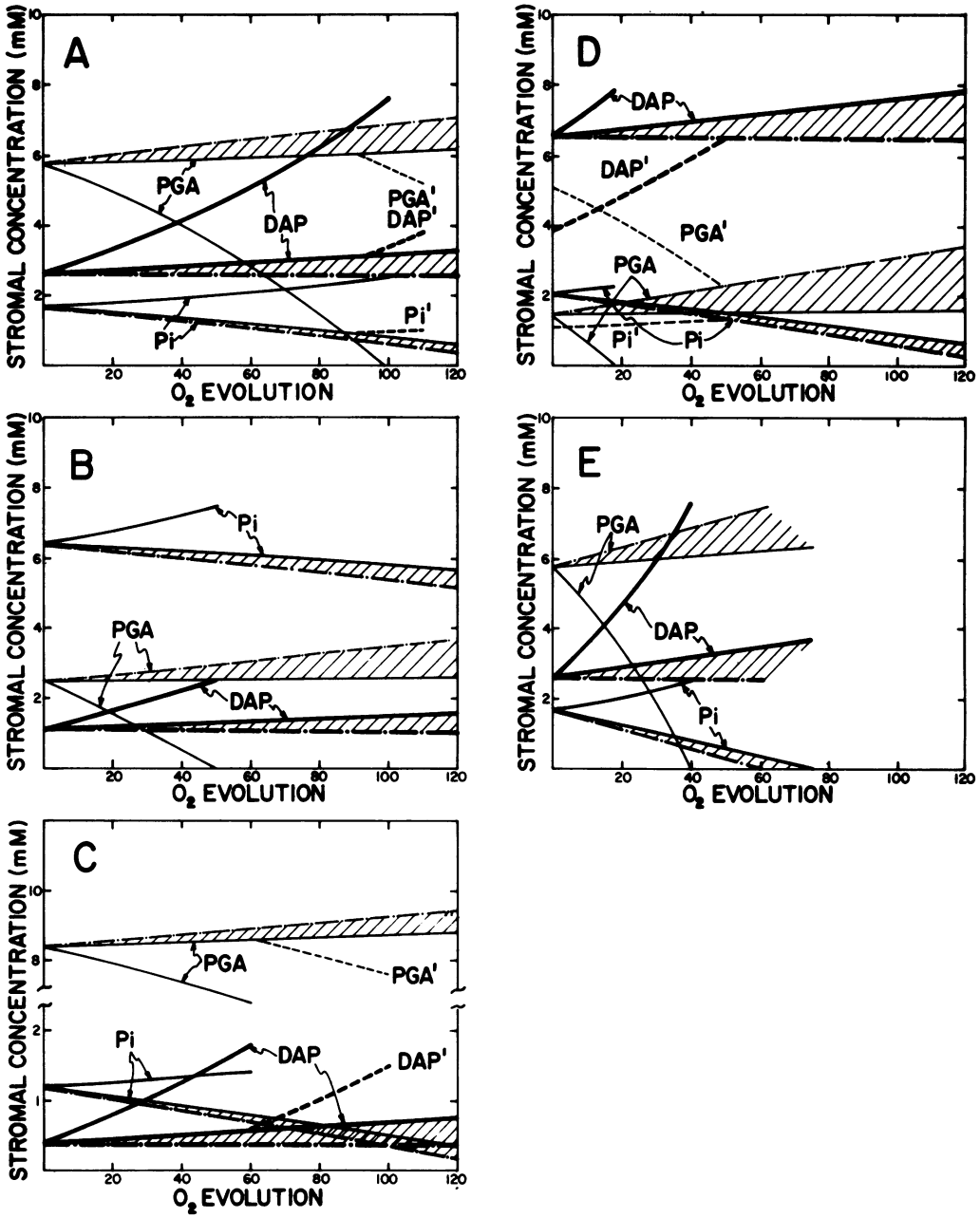

FIG. 1. Calculated effects of the partial reactions of photosynthesis on the stromal concentrations of Pi, PGA, and DAP. The apparent external K_m values (pH 7.6) used are 0.3 mM for Pi, 0.14 mM for PGA, and 0.13 mM for DAP (see Ref. 1). The internal (stromal) K_m values (pH 8.0) were then calculated from these values using Eq. 4 and are 0.275 mM for Pi, 0.301 mM for PGA, and 0.125 mM for DAP. V_{max} is 500 μmol mg^{-1} Chl h^{-1} for A to D and 200 μmol mg^{-1} Chl h^{-1} for E. External metabolite concentrations are as follows: A and E, Pi = 1 mM, PGA = 1.5 mM, DAP = 1.5 mM; B, Pi = 9.0 mM, PGA = 1.5 mM, DAP = 1.5 mM; C, Pi = 1 mM, PGA = 3.0 mM, DAP = 0.3 mM; D, Pi = 1 mM, PGA = 0.3 mM, DAP = 3.0 mM. The total internal concentration of Pi + PGA + DAP was kept constant at 10 mM. The lines corresponding to the concentrations of Pi, PGA, and DAP are indicated in each figure. The effect of CO_2 fixation is indicated by the shaded areas with PGA as the only product (reaction 3) being one boundary (- · - · -) and with DAP as the only product (reaction 2) being the other boundary (—). The effect of PGA reduction (reaction 4) is indicated by (——). For a determination of the rate of CO_2 fixation (no starch) or the actual net exchange rate required for each compound see Table II. For the meaning of the lines Pi′, PGA′, and DAP′ in Sections A, C, and D and a complete discussion of the calculations, see the text. The reaction numbers referred to above correspond to those listed in Table II. For the initial equilibrium exchange rates, *i.e.* no metabolism and no net exchange occurring, see Table III.

reaction by mass action. PGA reduction in excess of that required for CO_2 fixation would be increasingly inhibitory for starch formation as it decreases the PGA : Pi ratio.

The effect of decreasing the maximal capacity of the translocator for exchange with all other parameters being the same as in Figure 1A is shown in Figure 1E. All displacements from the equilibrium situation occur at proportionally lower rates of exchange (or O_2 evolution as shown). Therefore, the displacements in [PGA] and [DAP] by CO_2 fixation are now considerably more significant. A comparison of PGA reduction with CO_2 fixation indicates that the phosphate translocator is much more limiting for the former. With adequate information on the dependence of PGA reduction (total

rate due to both CO_2 fixation and PGA reduction) on the $\frac{[PGA]}{[DAP][Pi]}$ ratio, it may be possible to calculate the expected rates of CO_2 fixation and PGA reduction by use of the equations.

As phosphate might be limiting for CO_2 fixation under the conditions of Figure 1A, the effects of increasing the external Pi to 9 mM were examined and the results shown in Figure 1B. Under these conditions, it is unlikely that stromal Pi could ever be limiting for CO_2 fixation. However, the low initial equilibrium concentrations of PGA and DAP do not permit very rapid rates of PGA reduction (a maximal rate of <50 μmol mg^{-1} Chl h^{-1}), in the absence of CO_2 fixation. Starch synthesis under these conditions would be expected to be small because of the high Pi : PGA ratio in the stroma. Experimental data on the phosphate dependence of starch synthesis in the presence of mM concentrations of DAP and PGA (20) are generally consistent with the model.

Returning to the situation where 1 mM Pi is present in the external medium, the effect of varying PGA : DAP ratio is of interest. Figures 1C and 1D present the results for PGA : DAP ratios of 10 : 1 and 1 : 10, respectively, with a total concentration of 3.3 mM and therefore can be compared to Figure 1A. Comparing Figures 1C (PGA : DAP = 10 : 1) and 1A (PGA : DAP = 1 : 1) with respect to CO_2 fixation, little effect is seen on the rate of depletion of stromal Pi accompanying increasing rates of this reaction, but all Pi concentrations are almost 30% lower than when PGA and DAP ratio is 1 : 1. The initial DAP concentration is 10-fold less and CO_2 fixation may become limited by the stromal DAP concentration. This would be expected to favor increased PGA reduction such that proportionately more energy is devoted to PGA reduction as shown by the lines (PGA', DAP') illustrating the effects of increasing PGA reduction at a given rate of CO_2 fixation. Comparing Figures 1D and 1A, increasing the DAP : PGA ratio has much less effect on the equilibrium Pi concentration and also little effect on the decreasing Pi concentration accompanying increasing rates of CO_2 fixation. However, it should be remembered that with intact chloroplasts the stromal DAP concentration may not rise to levels as high as calculated since the total Pi content of the stroma may be increasingly tied up in the form of fructose 1,6-bisphosphate and sedoheptulose 1,7-bisphosphate due to the action of aldolase, thereby decreasing the total stromal concentration of Pi + PGA + DAP. In any case, PGA reduction alone (solid lines) or in combination with CO_2 fixation is clearly not favored. These conditions, however, are favorable for reaction 5, DAP-supported CO_2 fixation. The effect of increasing rates of this reaction with a constant rate of CO_2 fixation of 60, starting with PGA as the sole product (*i.e.* an O_2 evolution rate of 50), are indicated by the dotted lines (Pi', DAP', and PGA'). The rate of DAP-supported CO_2 fixation can increase to a maximum such that the rate of O_2 evolution ceases and PGA is no longer being reduced to DAP inside the chloroplast. The net exchange rates at this point are Pi uptake at 20 μmol mg^{-1} Chl h^{-1}, PGA efflux at 120 μmol mg^{-1} Chl h^{-1}, and DAP uptake at 100 μmol mg^{-1} Chl h^{-1}. Increasing the rate of DAP-supported CO_2 fixation (reaction 5) results in an increase in the stromal concentration of PGA, at the expense of DAP, with little effect on stromal Pi, which would result in higher rates of starch synthesis than otherwise would be the case.

The effects of photorespiration (reaction 6) are not shown in Figure 1, because O_2 evolution is no longer representative of energy utilization. However, the calculated effect of this reaction on stromal metabolite levels is basically to reduce the Pi and DAP transport rates needed for a given rate of CO_2 fixation (compare reactions 2 and 6b in Table II), since now part of the fixed carbon is leaving the chloroplast as glycolate, rather than DAP.

The effects of the different reactions on the stromal concentrations of PGA, Pi, and DAP as illustrated in Figure 1 serve to emphasize further the importance of elucidating the regulatory features of Pi : PGA and Pi : DAP ratios on reactions other than starch synthesis. It seems that the most important unknown parameter is the effect of the stromal [PGA]/[Pi][DAP] ratio on the absolute rate of PGA reduction occurring inside the chloroplast. In any case, it is clear that given flexibility in combining the partial reactions indicated in Table II and sufficient transport capacity, relatively constant CO_2 fixation rates are possible over a fairly wide range of external metabolite concentrations, although the portion of the fixed carbon devoted to starch synthesis and the observed rate of O_2 evolution would vary greatly.

A More Simplified Analysis of the Effects of Net Exchange on the Stromal Concentrations of Pi, PGA, and DAP. In general, except for PGA-DAP exchange at high rates of PGA reduction, the steady-state stromal concentrations of Pi, PGA, and DAP were nearly linear functions of the net exchange rate in spite of the fact that the equations relating these parameters are complex. This linearity suggested that the displacements from the equilibrium concentrations expected in the absence of net transport could be approximately described with reference to the equilibrium exchange rates. In Table III, these equilibrium exchange rates are summarized for the different conditions analyzed in Figure 1, as calculated using Eqs. 1, 2, and 3. Using these rates and with reference to Figure 1, it can be seen that the displacement of the stromal metabolite concentrations from their equilibrium position is approximately the same percentage as the ratio of the steady-state net exchange rates required by the reaction to the equilibrium exchange rates. For example, in Figure 1A, a CO_2 fixation rate of 90 (O_2 evolution rate of 90) with DAP as the only product, causes a decrease in the stromal Pi concentration from 1.64 to 0.88 mM, a decrease of 46%. The ratio of the steady-state net Pi exchange rate to the equilibrium exchange rate is 30 : 61 (see Tables I and III) or 49%. Thus, the equilibrium exchange rates associated with any given set of conditions provide a good estimate of the extent to which stromal metabolites will be affected by the various partial reactions of photosynthesis.

Analysis of the Phosphate Dependence of CO_2 Fixation. Although the results of Figure 1 suggests that, in principle, chloroplasts could adjust to relatively wide variations in the extrachloroplastic concentrations of Pi, PGA, and DAP by a sufficient flexibility in utilizing the various partial reactions of photosynthesis outlined in Table II, either very high or very low concentrations of phosphate relative to the extrachloroplastic concentrations of PGA and DAP are known to inhibit CO_2 fixation with isolated chloroplasts (11). The model provides a quantitative basis to analyze these effects.

The effect of increasing the rate of CO_2 fixation (DAP as the only product) on the calculated stromal Pi concentration in the presence of 1 mM Pi and several external concentrations of PGA and DAP (present at a 1 : 1 ratio) is shown in Figure 2. A Pi : (PGA + DAP) ratio of 1 : 3 could theoretically allow a maximal CO_2 fixation rate of ~185. Decreasing the ratio of 1 : 6 and 1 : 12 would allow maximal rates of 100 and 50, respectively. Variation in the PGA : DAP ratio did not markedly affect the results (not shown). However, at external concentrations of Pi, PGA, and DAP near

Table III. *Equilibrium Exchange Rates of Pi, PGA, and DAP at the Various External Concentrations of These Compounds Utilized in Figure 1*

Figure 3 Section	Metabolite Exchange Rate			
	Pi	PGA	DAP	V_{max}
		$\mu mol\ mg^{-1}\ Chl\ h^{-1}$		
A	61	197	212	500
B	275	98	105	500
C	58	372	40	500
D	56	36	385	500
E	24	79	85	200

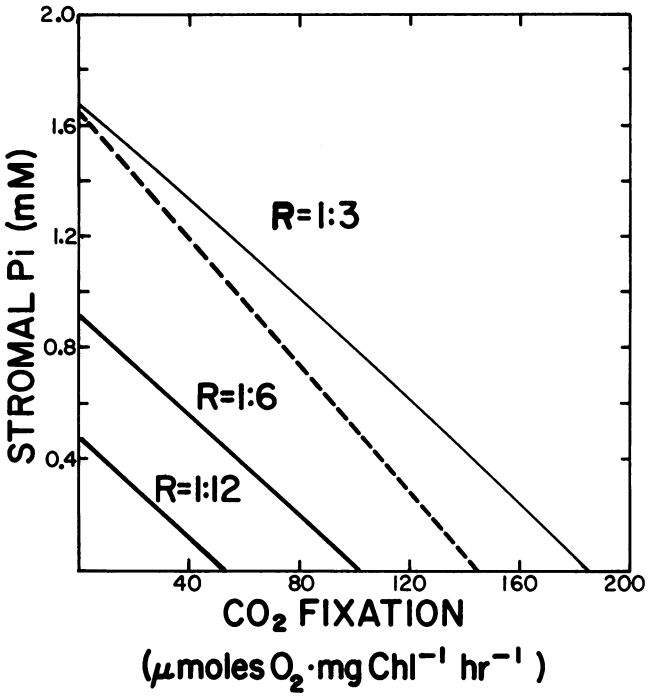

FIG. 2. Limitations to CO_2 fixation and stromal Pi imposed by low external Pi:(PGA + DAP) concentration ratios. R = [Pi]:([PGA] + [DAP]). The results are calculated for an external Pi concentration of 1 mM (——) and 0.1 mM (---), and equal external PGA and DAP concentrations.

the K_m of these compounds for the phosphate translocator (0.3 mM) and below, decreased rates were possible. A maximal rate of 145 is calculated with 0.1 mM Pi and 0.3 mM (PGA + DAP) as indicated by the dashed line. Of course, CO_2 fixation may be limited before a near zero stromal Pi concentration is reached, but the results clearly demonstrate that the phosphate translocator is a limiting factor in photosynthesis when high external (PGA + DAP):Pi ratios exist. It has previously been suggested that such inhibition of photosynthesis is due to inadequate cytoplasmic Pi (10). The model provides a means of quantitatively determining the stromal Pi dependence of CO_2 fixation in relation to the extrachloroplastic concentrations of Pi, PGA, and DAP. Furthermore, it should be noted that an inhibition of maximal transport capacity is calculated to result in the accelerated depletion of stromal Pi and an increased sensitivity to suboptimal Pi in the medium. Similarly, an inhibition of maximal CO_2 fixation capacity would cause a decreased sensitivity to low Pi since at each concentration, stromal Pi would be decreased to a lesser extent. These effects have recently been demonstrated in *in vitro* experiments with isolated chloroplasts (3).

The model also can be used to analyze quantitatively the inhibition of CO_2 fixation by high Pi concentrations relative to DAP and PGA in the medium. We have seen how increasing rates of CO_2 fixation are calculated to cause only a slight increase in the PGA and DAP concentrations (when both are products). The percent increase is somewhat greater relative to the equilibrium concentration, when the PGA and DAP concentrations are initially decreased to low levels by high external Pi concentrations (data not shown, but compare Figures 1A and 1B). A much greater increase in the PGA and DAP concentrations is calculated to occur when the maximal transport capacity of the phosphate translocator is decreased (not shown but compare Figures 1A and 1E), which readily accounts for the decreased sensitivity of CO_2 fixation by isolated chloroplasts to high Pi concentrations when the transport capacity is lowered by the presence of pyridoxal phosphate (3). However, slight increases in the external PGA and DAP concentrations are calculated to have as great an effect (not shown). Rapid changes in the external PGA and DAP relative to Pi generally occur in *in vitro* experiments when only Pi is initially present in the medium (19). The importance of some critical level of PGA and DAP is clearly indicated by recent experiments in which the presence of alkaline phosphatase in the medium greatly enhanced the Pi inhibition of CO_2 fixation (12). However, given that mM concentrations of PGA and DAP are present *in vivo* in the cytoplasm, the model suggests that a variation of external [Pi] over several mM would be expected to have only a minor effect on the total rate of CO_2 fixation. Experimental evidence for this has been obtained recently (20; Gruenewald and Portis, in preparation).

Table IV. *The Effect of Increasing or Decreasing the K_m Values of the Phosphate Translocator for Each of the Metabolites on the Equilibrium Exchange Rates*

External concentrations of 1.0 mM Pi, 1.5 mM PGA, and 1.5 mM DAP, V_{max} = 500 μmol mg^{-1} Chl h^{-1}, pH-stroma = 8.0, pH-medium = 7.6. Initial K_m values are in Figure 1.

Alteration	Exchange Rate		
	Pi	PGA	DAP
	μmol mg^{-1} Chl h^{-1}		
None	61	197	212
Increase K_m of Pi by 2	33	210	226
Decrease K_m of Pi by 2	109	176	189
Increase K_m of PGA by 2	76	123	265
Decrease K_m of PGA by 2	44	283	152
Increase K_m of DAP by 2	78	251	135
Decrease K_m of DAP by 2	43	138	298

Effects of Variations in the Transport Affinity for Pi, PGA, and DAP on the Response of the Stromal Concentrations to Net Exchange. The affinities used in the analysis presented thus far are the values reported for the phosphate translocator of spinach chloroplasts. It is possible that these parameters could vary substantially between plant species. I therefore briefly examined the effects of a variation in the transport affinities on the effects of net exchange on the steady-state stromal metabolite concentrations. As discussed above, the equilibrium stromal concentrations of Pi, PGA, and DAP are not affected by a variation in the apparent transport affinities. However, the equilibrium exchange rates are dependent on this parameter as shown in Table IV and it is relative magnitude of the equilibrium exchange rate which largely determines the response of the stromal concentrations to a requirement for net flux as outlined above.

In general, it was found that a variation in the affinities has little effect on the response of the stromal metabolite concentrations to a requirement for net flux as long as the associated equilibrium exchange rates are comparatively high. In any case, it is possible that if variations in affinity of the phosphate translocator exist they could easily be compensated for by an appropriate alteration in the 'normal' cytoplasmic concentrations of Pi, PGA, and DAP. Data on the kinetic properties of the phosphate translocator and the normal cytoplasmic concentrations of Pi, PGA, and DAP in various plant species are not available, but may prove to be very informative since wide variations in the response of ADP-glucose pyrophosphorylase to the Pi:PGA ratio have been found (21).

CONCLUSIONS

The stromal metabolite concentrations of Pi, PGA, and DAP are determined by three factors: (a) stromal and external pH; (b)

the external concentrations of these metabolites; and (c) the rates of the various partial reactions of photosynthesis in relation to the properties of the phosphate translocator. A high transport capacity of the phosphate translocator relative to minimal requirements of CO_2 fixation assures that the stromal concentrations of Pi, PGA, and DAP are generally not displaced very far from their equilibrium concentrations, by metabolic reactions occurring in the stroma. Therefore, photosynthesis can occur over a wide range of conditions. However, the capacity of the phosphate translocator is an important factor in CO_2 fixation when the external Pi : (DAP + PGA) ratio is either very low or very high since either inadequate Pi or inadequate PGA + DAP, respectively, can be maintained in the stroma under these conditions. The analysis supports the suggestion that a high capacity of the Pi translocator exists for this reason (8).

At present, the steady-state stromal metabolite concentrations cannot be determined *a priori* because all of the factors that regulate the relative rates of the partial reactions of photosynthesis are not known, and an independent knowledge of these rates is required. However, the equations and approach developed in this analysis do provide a theoretical and quantitative basis for analyzing the role of the phosphate translocator and the extrachloroplastic metabolites (namely Pi, PGA, and DAP) in the regulation of CO_2 fixation. The ability to calculate the expected steady-state stromal concentrations of these metabolites under various conditions will allow the effects and limitations due to substrate levels on CO_2 fixation and starch synthesis to be determined, and possibly separated from any additional regulatory effects of these metabolites. Experiments with isolated intact chloroplasts are in progress in which the amounts of Pi, PGA, and DAP in the stroma are being measured directly and compared with various external concentrations of these metabolites and the rate of CO_2 fixation, O_2 evolution and starch synthesis. The results of these experiments will be compared to those predicted by the model. However, relating the measured amounts of these metabolites to their stromal concentrations may be severely hampered by the fact that a large proportion of these compounds could exist in a bound form, as appears to be the case for RuBP (13). Further verification of the kinetic characteristics of the phosphate translocator and appropriate alterations in the model such that it can be used expressly for this purpose may then be essential for further progress in achieving a detailed understanding of the regulation of CO_2 fixation.

LITERATURE CITED

1. Christensen HN 1975 Biological Transport, Ed 2. W. A. Benjamin, Inc., Reading, pp 158–159
2. Fliege R, U Flugge, K Werdan, HW Heldt 1978 Specific transport of inorganic phosphate, 3-phosphoglycerate and triosephosphates across the inner membrane of the envelope in spinach chloroplasts. Biochim Biophys Acta 502: 232–247
3. Flugge UI, M Freisl, HW Heldt 1980 Balance between metabolite accumulation and transport in relation to photosynthesis by isolated spinach chloroplasts. Plant Physiol 65: 574–577
4. Fondy BR, DR Geiger 1982 Diurnal pattern of translocation and carbohydrate metabolism in source leaves of *Beta vulgaris* L. Plant Physiol 70: 671–676
5. Giersch C 1977 A kinetic model for translocators in the chloroplast envelope as an element of computer simulation of the dark reaction of photosynthesis. Z Naturforsch 32c: 263–270
6. Giersch C, U Heber, GH Krause 1980 ATP transfer from chloroplasts to the cytosol of leaf cells during photosynthesis and its effect on leaf metabolism. *In* RM Spanswick, WJ Lucas, J Dainty, eds, Plant Membrane Transport: Current Conceptual Issues. Elsevier/North-Holland Biomedical Press, Amsterdam, pp 65–79
7. Heber U 1974 Energy transfer within leaf cells. *In* M Avron, ed, Proc 3rd Int Congr Photosynth., Elsevier/North-Holland, Amsterdam, pp 1335–1348
8. Heber U, HW Heldt 1981 The chloroplast envelope: structure, function, and role in leaf metabolism. Annu Rev Plant Physiol 32: 139–168
9. Heldt HW 1976 Metabolite transport in intact spinach chloroplasts. *In* J Barber, ed, The Intact Chloroplast. Elsevier/North-Holland Biomedical Press, Amsterdam, pp 215–234
10. Herold A 1980 Regulation of photosynthesis by sink activity—the missing link. New Phytol 86: 131–144
11. Herold A, DA Walker 1979 Transport across the chloroplast envelopes. The role of phosphate. *In* G. Giebisch, DC Tosteson, HH Ussing, eds, Membrane Transport in Biology. Springer-Verlag, Berlin, pp 411–439
12. Huber SC 1982 Photosynthetic carbon metabolism in chloroplasts. *In* LL Creasy, G Hrazdina, eds, Cellular and Subcellular Localization in Plant Metabolism. Plenum Publishing, New York, pp 151–184
13. Jensen RG, JT Bahr 1977 Ribulose 1,5-biphosphate carboxylase-oxygenase. Annu Rev Plant Physiol 28: 379–400
14. Kaiser W, W Urbach 1976 Rates and properties of endogenous cyclic photophosphorylation of isolated intact chloroplasts measured by CO_2 fixation in the presence of dihydroxyacetone phosphate. Biochim Biophys Acta 423: 91–102
15. Kaiser W, W Urbach 1977 The effect of dihydroxyacetone phosphate and 3-phosphoglycerate on O_2 evolution and on the levels of ATP, ADP and Pi in isolated intact chloroplasts. Biochim Biophys Acta 459: 337–346
16. Krause GH 1971 Indirect ATP transport between chloroplasts and cytoplasm during photosynthesis. Z Pflanzenphysiol 65: 13–23
17. Krause GH, U Heber 1976 Energetics of intact chloroplasts. *In* J Barber, ed, The Intact Chloroplast. Elsevier/North-Holland Biomedical Press, Amsterdam, pp 171–214
18. Krause GH, SW Thorne, GH Lorimer 1977 Glycolate synthesis by intact chloroplasts. Studies with inhibitors of photophosphorylation. Arch Biochim Biophys 183: 471–479
19. Lilley RMcC, CJ Chon, A Mosbach, HW Heldt 1977 The distribution of metabolites between spinach chloroplasts and medium during photosynthesis *in vitro*. Biochim Biophys Acta 460: 259–272
20. Portis AR 1982 Effects of the relative extrachloroplastic concentrations of inorganic phosphate, 3-phosphoglycerate and dihydroxyacetone phosphate on the rate of starch synthesis in isolated spinach chloroplasts. Plant Physiol 70: 393–396
21. Preiss J, C Levi 1979 Metabolism of starch in leaves. *In* M Gibbs, E Latzko, eds, Encyclopedia of Plant Physiology, New Series, Vol 6, Photosynthetic Carbon Metabolism and Related Processes. Springer-Verlag, Berlin