A rapid-equilibrium model for the control of the Calvin photosynthesis cycle by cytosolic orthophosphate

Pettersson, Gösta; Ryde-Pettersson, Ulf

Published in:
European Journal of Biochemistry

DOI:
10.1111/j.1432-1033.1987.tb13629.x

1987

Document Version:
Publisher's PDF, also known as Version of record

Eur. J. Biochem. *169*, 423–429 (1987)

A rapid-equilibrium model for the control of the Calvin photosynthesis cycle by cytosolic orthophosphate

Gösta PETTERSSON and Ulf RYDE-PETTERSSON

Avdelningen för Biokemi, Kemicentrum, Lunds Universitet

(Received March 27/June 23, 1987) – EJB 87 0359

1. A simple model based on rapid-equilibrium assumptions is derived which relates the steady-state activity of the Calvin cycle for photosynthetic carbohydrate formation in C_3 plants to the kinetic properties of a single cycle enzyme (fructose bisphosphatase) and of the phosphate translocator which accounts for the export of photosynthate from the chloroplast. Depending on the kinetic interplay of these two catalysts, the model system may exhibit a single or two distinct modes of steady-state operation, or may be unable to reach a steady state.

2. The predictions of the model are analysed with regard to the effect of external orthophosphate on the steady-state rate of photosynthesis in isolated chloroplasts under conditions of saturating light and CO_2. Due to the possible existence of two distinct steady states, the model may account for the stimulatory as well as the inhibitory effects of external phosphate observed in experiments with intact chloroplasts. Stability arguments indicate, however, that only the steady-state case corresponding to phosphate inhibition of the rate of photosynthesis could be of physiological interest.

3. It is concluded that chloroplasts under physiological conditions most likely operate in a high-velocity steady state characterized by a negative Calvin cycle flux control coefficient for the phosphate translocator. This means that any factor enhancing the export capacity of the phosphate translocator can be anticipated to decrease the actual steady-state rate of photosynthate export due to a decreased steady-state rate of cyclic photosynthate production.

Photosynthetic carbohydrate formation by the reductive pentose phosphate pathway (the Calvin cycle) in C_3 plants takes place in the stromal solution of the chloroplast. The process requires an import of carbon dioxide and orthophosphate from the cytosol, and yields triose phosphate and 3-phosphoglycerate as principal products exported from the chloroplast [1, 2]. Such photosynthate export, which is mediated by the phosphate translocator of the chloroplast membrane, is strictly linked with a counter-transport of orthophosphate from the cytosol to the stromal solution [3]. The rate of photosynthesis in isolated chloroplasts is enhanced by low, but inhibited by high, concentrations of orthophosphate in the reaction medium [4–6]. These effects have been convincingly demonstrated to reflect the kinetics of action of the phosphate translocator [6, 7]. A dependence of the Calvin cycle activity on the cytosolic concentration of orthophosphate has been observed also in experiments carried out with intact leaves [8–10].

The above results provide compelling evidence that cytosolic orthophosphate may play a major role in the regulation of stromal photosynthesis by affecting the transport capacity of the phosphate translocator. An enhancement of that capacity has been proposed to stimulate photosynthesis by facilitating the export of photosynthate and the import of orthophosphate required to sustain photosynthesis at a steady-state rate [6, 10–13]. The opposite view has also been expressed that enhancement of the export capacity of the phosphate translocator might inhibit photosynthesis by depleting the stromal solution of Calvin cycle intermediates [6, 14, 15]. No theoretical work is available, however, which could support either (or both) of these views by establishing the actual relationship between the transport capacity of the phosphate translocator and the steady-state Calvin cycle activity. That lack of knowledge about the basic control properties of the reaction system has made it difficult to draw any firm conclusions with regard to the detailed mechanisms underlying the experimentally documented coordination between stromal and cytosolic carbohydrate metabolism [9–12, 16, 17].

In this investigation, we draw attention to some implications of the kinetic interplay of the export processes mediated by the phosphate translocator and the Calvin cycle enzymes which act on the exported metabolites. A rapid-equilibrium model is presented which relates the steady-state Calvin cycle activity primarily to the kinetic properties of the phosphate translocator and one of the stromal cycle enzymes (fructose bisphosphatase). Despite its simplicity, the model would seem to significantly deepen our understanding of the dynamic principles according to which orthophosphate and other cytosolic metabolites may control the rate of stromal photosynthesis.

Correspondence to G. Pettersson, Avdelningen för Biokemi, Kemicentrum, Lunds Universitet, Box 124, S-221 00 Lund, Sweden

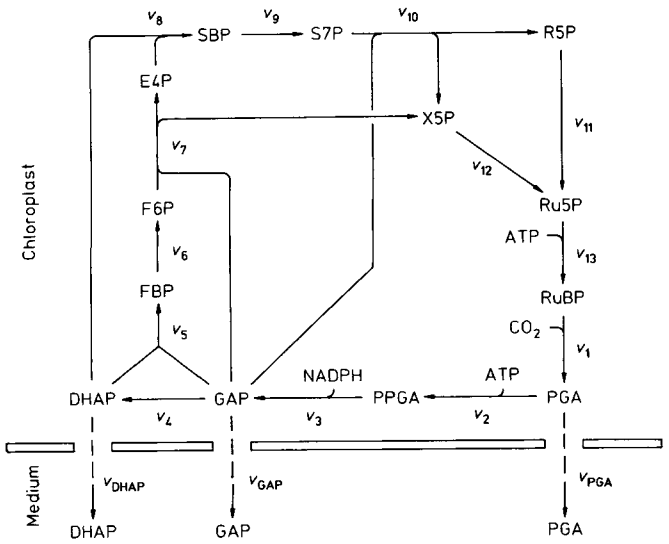
Abbreviations. FBP, fructose 1,6-bisphosphate; DHAP, dihydroxyacetone phosphate; GAP, glyceraldehyde 3-phosphate; PGA, 3-phosphoglyceric acid.

Enzyme. Fructose bisphosphatase or D-fructose-1,6-bisphosphate 1-phosphohydrolase (EC 3.1.3.11).

THEORY

Steady-state relationships for the Calvin cycle

Scheme 1 shows the Calvin cycle for photosynthetic carbohydrate formation in C_3 plants. The reactions involving CO_2, ATP, and NADPH represent input steps which supply



Scheme 1. *The Calvin photosynthesis cycle and major steps of photosynthate export.* Notations: PGA, 3-phosphoglycerate; PPGA, 2,3-diphosphoglycerate; GAP, glyceraldehyde 3-phosphate; DHAP, dihydroxyacetone phosphate; FBP, fructose 1,6-bisphosphate; F6P, fructose 6-phosphate; E4P, erythrose 4-phosphate; SBP, sedoheptulose 1,7-bisphosphate; S7P, sedoheptulose 7-phosphate; R5P, ribose 5-phosphate; X5P xylulose 5-phosphate; Ru5P, ribulose 5-phosphate; RuBP, ribulose 1,5-bisphosphate

the cycle with reactants for net synthesis of triose phosphates according to the stoichiometric relationship

$$3CO_2 + 6NADPH + 9ATP \rightarrow \text{triose phosphate} + 6NADP^+ + 9ADP + 8P_i \quad (1)$$

where P_i represents inorganic phosphate (orthophosphate). The transport processes leading to export of 3-phosphoglycerate, glyceraldehyde 3-phosphate, and dihydroxyacetone phosphate from the chloroplast are included as output steps in Scheme 1.

Assuming that other output processes (e.g. starch production within the chloroplast) may be neglected, the steady-state rate v of cyclic triose phosphate synthesis must equal the net rate v_{ex} of photosynthate export. Denoting rates of individual reaction steps as in Scheme 1, v_{ex} is given by

$$v_{ex} = v_{DHAP} + v_{GAP} + v_{PGA}. \quad (2)$$

Since each cycle intermediate in Scheme 1 under steady-state conditions must be produced at the same rate as it is consumed, the requirements for such conditions to obtain then may be expressed as

$$v_{ex} = v_5 = v_6 = v_7 = v_8 = v_9 = v_{10} = v_{11} = v \quad (3)$$

$$v_{12} = 2v \quad (4)$$

$$v_{13} = v_1 = 3v \quad (5)$$

$$v_2 = v_3 = 6v - v_{PGA} \quad (6)$$

$$v_4 = 3v - v_{PGA} - v_{GAP}. \quad (7)$$

In particular, it follows from Eqn (3) that one must have

$$v = v_{ex} = v_6 \quad (8)$$

where v_6 denotes the steady-state rate of enzymic hydrolysis of fructose 1,6-bisphosphate (FBP). Under light conditions, the stromal fructose bisphosphatase accounting for that reaction can be assumed (as a satisfactory approximation) to conform to Michaelis-Menten kinetics [18–20] such that

$$v_6 = \frac{V_6[FBP]}{K_6 + [FBP]}. \quad (9)$$

Kinetics of the export steps

The kinetic behaviour of the reaction system in Scheme 1 will be analysed with the assumption that the reaction medium outside the chloroplast contains orthophosphate as the only transferable ligand. According to the model described by Giersch [21] for the action of the phosphate translocator, steady-state rates of the three export steps in Scheme 1 then conform to the relationships

$$v_{DHAP} = \frac{V_t[DHAP]}{N \cdot K_{DHAP}} \quad (10)$$

$$v_{GAP} = \frac{V_t[GAP]}{N \cdot K_{GAP}} \quad (11)$$

$$v_{PGA} = \frac{V_t[PGA]}{N \cdot K_{PGA}} \quad (12)$$

where

$$N = 1 + \left(1 + \frac{K'_{P_i}}{[P'_i]}\right) \cdot \left(\frac{[DHAP]}{K_{DHAP}} + \frac{[GAP]}{K_{GAP}} + \frac{[PGA]}{K_{PGA}} + \frac{[P_i]}{K_{P_i}}\right). \quad (13)$$

V_t denotes the maximum transport velocity for the translocator and is assumed to differ insignificantly for different ligands [22]. K_{DHAP}, K_{GAP}, K_{PGA}, and K_{P_i} represent the apparent dissociation constants of the translocator complexes formed with the respective stromal ligands. $[P'_i]$ refers to the concentration of orthophosphate outside the chloroplast and K'_{P_i} to the apparent dissociation constant of the corresponding translocator complex.

Rapid-equilibrium model

Examination of mass action ratios for Calvin cycle intermediates in illuminated chloroplasts have indicated that the enzymic reaction steps converting PGA into FBP (Scheme 2) are close to equilibrium [23–26]. This may justify application of the rapid-equilibrium assumptions

$$[GAP] = m[PGA] \quad (14)$$

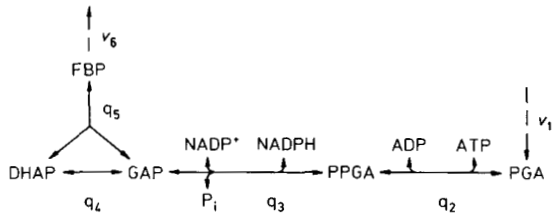
$$m = q_2 q_3 \cdot \frac{[NADPH][ATP][H^+]}{[NADP^+][ADP][P_i]} \quad (15)$$

$$[DHAP] = q_4[GAP] \quad (16)$$

$$[FBP] = q_5[GAP][DHAP] \quad (17)$$

where q_i denote equilibrium constants for the respective reaction steps in Scheme 2. With these assumptions, it follows from Eqns (10–13) that Eqn (2) may be written as

$$v_{ex} = \frac{V_{ex}[DHAP]}{K_{ex} + [DHAP]} \quad (18)$$



Scheme 2. *Rapidly equilibrating part of the Calvin cycle.* q_i denote equilibrium constants for the respective reaction steps. Other notations as in Scheme 1

where

$$V_{ex} = \frac{V_t[P_i']}{K_{P_i}' + [P_i']} \tag{19}$$

$$K_{ex} = \frac{\dfrac{[P_i]}{K_{P_i}} + \dfrac{[P_i']}{K_{P_i}' + [P_i']}}{\dfrac{1}{K_{DHAP}} + \dfrac{1}{q_4 K_{GAP}} + \dfrac{1}{mq_4 K_{PGA}}}. \tag{20}$$

According to Eqns (16) and (17) we, further, have

$$[FBP] = \frac{q_5[DHAP]^2}{q_4} \tag{21}$$

and insertion of this into Eqn (9) gives

$$v_6 = \frac{q_5 V_6[DHAP]^2}{q_4 K_6 + q_5[DHAP]^2}. \tag{22}$$

It may now be recalled that steady-state conditions cannot obtain unless Eqn (8) is satisfied. According to Eqns (18) and (22), this means that the steady-state concentration of DHAP must satisfy the relationship

$$\frac{V_{ex}[DHAP]}{K_{ex} + [DHAP]} = \frac{q_5 V_6[DHAP]^2}{q_4 K_6 + q_5[DHAP]^2}. \tag{23}$$

Introducing the dimensionless parameter α defined by

$$\alpha = \frac{V_6}{V_{ex}} \tag{24}$$

Eqn (23) may be written as

$$q_5(1-\alpha)[DHAP]^2 - \alpha q_5 K_{ex}[DHAP] + q_4 K_6 = 0 \tag{25}$$

and two cases may obtain depending on the magnitude of α. If $\alpha > 1$, Eqn (25) will have a single positive root given by

$$[DHAP] = \frac{\alpha K_{ex}(R-1)}{2(\alpha-1)} \tag{26}$$

where

$$R = \sqrt{1 + \frac{4(\alpha-1)}{\alpha^2\beta}} \tag{27}$$

$$\beta = \frac{q_5 K_{ex}^2}{q_4 K_6}. \tag{28}$$

The corresponding steady-state cycle activity can be obtained from Eqn (18) or (22), which gives

$$v = \frac{V_{ex}[2 + \alpha\beta(1-R)]}{2(1+\beta)}. \tag{29}$$

Table 1. *Parameter values used for calculation of data in Figs 1–4*
Estimates given refer to conditions considered representative for the operation of isolated chloroplasts (chl denotes chlorophyll) at saturating light and CO_2, 25°C, and an external pH of 7.6. The estimate of K_6 has been corrected for inhibition caused by fructose 6-phosphate at an assumed concentration of 1 mM [20, 26]

Parameter	Unit	Estimate	Reference
q_2		3.1×10^{-4}	[26]
q_3		1.6×10^{7}	[26]
q_4		22	[26]
q_5	M^{-1}	7100	[26]
K_{DHAP} (pH 8.1)	μM	124	[3, 22]
K_{GAP} (pH 8.1)	μM	73	[3, 22]
K_{PGA} (pH 8.1)	μM	370	[3, 22]
K_{P_i} (pH 8.1)	μM	241	[3, 22]
K_{P_i}' (pH 7.6)	μM	300	[3, 22]
K_6	μM	80	[18–20]
V_t	μmol (mg chl)$^{-1}$ h^{-1}	300	[15]
V_6	μmol (mg chl)$^{-1}$ h^{-1}	150	[18–20]
$[P_i]$ (stroma)	mM	6	[2]
pH (stroma)		8.1	[27]
[ATP]/[ADP]		2.0	[27]
[NADPH]/[NADP$^+$]		0.7	[27]

If $\alpha < 1$, Eqn (25) may have an additional positive root

$$[DHAP] = \frac{\alpha K_{ex}(1+R)}{2(1-\alpha)} \tag{30}$$

corresponding to a higher concentration of DHAP and hence to a higher steady-state cycle activity given by

$$v = \frac{V_{ex}[2 + \alpha\beta(1+R)]}{2(1+\beta)}. \tag{31}$$

In this case, however, the condition $\alpha^2\beta \geqslant 4(1-\alpha)$ must be fulfilled to ensure that real roots are obtained. When $\alpha^2\beta < 4(1-\alpha)$, Eqn (25) lacks real solutions and the reaction system in Scheme 1 cannot reach a steady-state.

If $\alpha = 1$ one obtains

$$[DHAP] = \frac{K_{ex}}{\beta} \tag{32}$$

$$v = \frac{V_{ex}}{1+\beta} \tag{33}$$

which may be considered as a limiting case of Eqns (26–31) when α approaches unity.

Effect of external orthophosphate on the cycle activity

The above rapid-equilibrium model relates the steady-state behaviour of the reaction system in Scheme 1 to kinetic parameters for the stromal fructose bisphosphatase and the phosphate translocator. Estimates of these parameters are given in Table 1, together with estimates of equilibrium constants for reactions in Scheme 2 and reactant concentrations required for calculation of the parameter m defined by Eqn (15). Values in Table 1 are based on available literature data and have been chosen such that they should be relevant for the operation of isolated chloroplasts under conditions of saturating light and CO_2 in a reaction medium (pH 7.6)

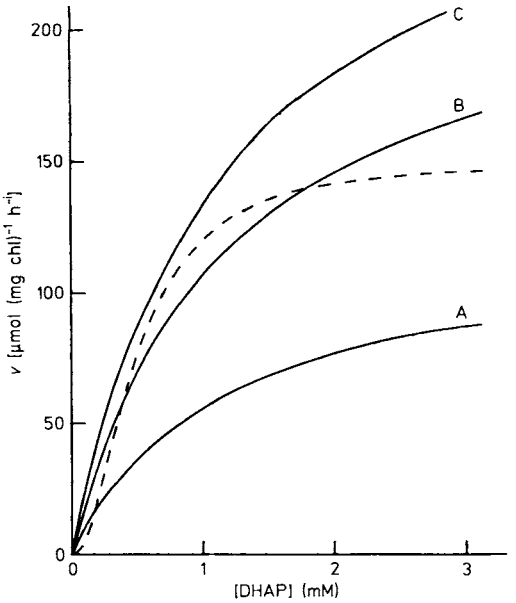


Fig. 1. *Dependence of the rate of enzymic fructose 1,6-bisphosphate hydrolysis (v_6) and net rate of photosynthate export (v_{ex}) on the stromal concentration of dihydroxyacetone phosphate [DHAP].* Graphs of v_6 (dashed curve) and v_{ex} (full curves) calculated from Eqns (22) and (18–20), respectively, for three different concentrations of external orthophosphate (0.2 mM, A; 1 mM, B; 10 mM, C). Crossover points between v_6 and v_{ex} define the [DHAP] and cycle activities that obtain under steady-state conditions

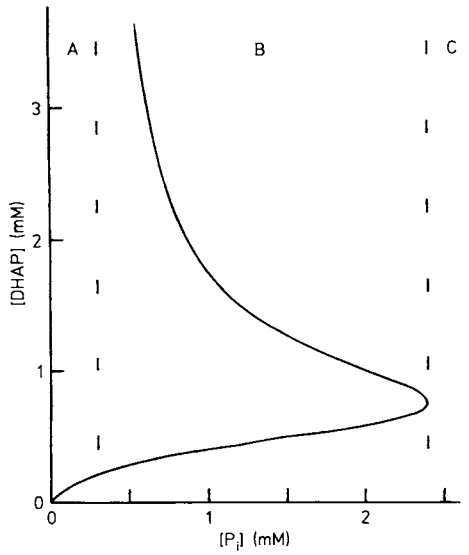


Fig. 2. *Dependence of the steady-state concentrations of dihydroxyacetone phosphate [DHAP] on the concentration of external orthophosphate.* Graphs calculated from Eqns (26) and (30) using data in Table 1. A, B, and C denote phosphate concentration regions where the reaction system in Scheme 1 may operate in, respectively, one, two, or no steady states

containing orthophosphate as the only transferable metabolite.

Fig. 1 shows the dependence of v_{ex} and v_6 on [DHAP], as calculated from Eqns (18) and (22) using data in Table 1. Graphs of v_{ex} have been calculated for three different values of the external concentration of orthophosphate ($[P_i'] = 0.2$, 1, and 10 mM) to illustrate the effect of this concentration variable on the export process and hence on the dynamic behaviour of the reaction system. Depending on the magnitude of $[P_i']$, the v_{ex} graph may exhibit a single (curve A), two distinct (curve B), or no (curve C) crossover points with the graph of v_6. The crossover points for v_{ex} and v_6 are of interest because they define the DHAP concentrations for which the steady-state condition $v_{ex} = v_6$ is fulfilled.

The variation with $[P_i']$ of the steady-state concentration(s) of DHAP is shown in Fig. 2. For $[P_i'] < 0.3$ mM (region A) we have $\alpha > 1$ and the steady-state level of DHAP will be uniquely defined. For 0.3 mM $< [P_i'] <$ 2.4 mM (region B) the reaction system may reach a steady state at two distinct concentrations of DHAP. For $[P_i'] > 2.4$ mM (region C) $\alpha^2\beta$ will exceed $4(1-\alpha)$ and steady-state conditions cannot obtain.

Fig. 3 shows the corresponding variation with $[P_i']$ of the steady-state cycle activity (v). It illustrates that v in region B may increase or decrease with increasing concentrations of external orthophosphate depending on whether the reaction system operates in the high-velocity or the low-velocity steady state. This characteristic of the system reflects a fundamental difference between the control properties of the phosphate translocator in the two steady states. Thus, it can be shown by differentiation of Eqn (29) with respect to V_{ex} that

$$\frac{d\ln v}{d\ln V_{ex}} = 1 + \frac{1}{R} > 0 \qquad (34)$$

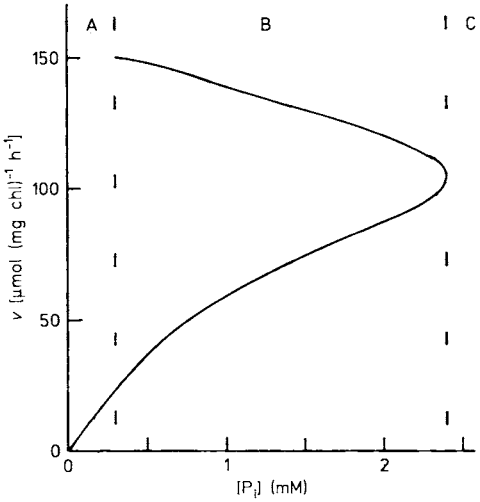


Fig. 3. *Dependence of the steady-state Calvin cycle activity on the concentration of external orthophosphate.* Graphs calculated from Eqns (29) and (31) using data in Table 1

which means that the effect of the phosphate translocator on the cycle activity is characterised by a positive flux control coefficient in the low-velocity case. Differentiation of Eqn (31) for the high-velocity case ($R < 1$), however, gives

$$\frac{d\ln v}{d\ln V_{ex}} = 1 - \frac{1}{R} \qquad (35)$$

showing that the flux control coefficient in the latter case becomes negative. Since it can be readily verified that

$$\frac{d\ln v}{d\ln V_6} = 1 - \frac{d\ln v}{d\ln V_{ex}} \qquad (36)$$

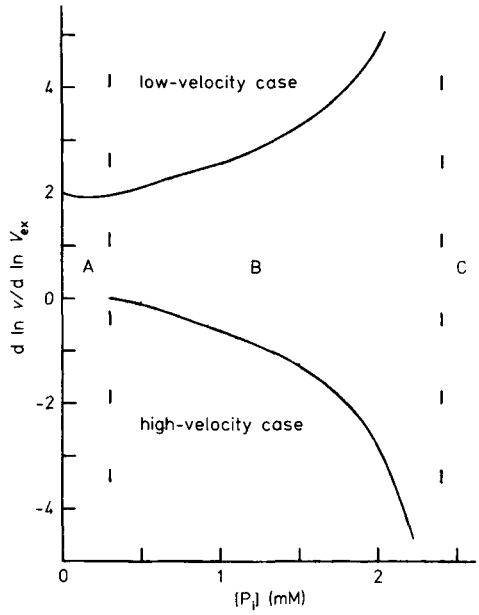


Fig. 4. *Dependence of Calvin cycle flux control coefficient for the phosphate translocator on the concentration of external orthophosphate.* Graphs calculated from Eqns (34) and (35) using data in Table 1

the flux control coefficient for fructose bisphosphatase must exhibit a converse dependence on the dynamic state of the reaction system, being negative in the low-velocity case and positive in the high-velocity case.

It is of interest to note in this context that flux control coefficients defined by Eqns (34–36) become of unlimited magnitude when R approaches zero, i.e. when β approaches $4(1-\alpha)/\alpha^2$. This means that the cycle activity under certain conditions (those favouring close-lying steady states in region B of Figs 2 and 3) may be exceptionally sensitive to changes in the concentrations of the phosphate translocator and the stromal fructose bisphosphatase, as well as to changes in the concentration of activators or inhibitors which act upon these two catalysts. Fig. 4 shows the variation with $[P_i']$ of the flux control coefficient for the phosphate translocator, as calculated from Eqns (34) and (35) and data in Table 1.

DISCUSSION

The Calvin cycle for photosynthetic carbohydrate formation in C_3 plants (Scheme 1) is basically dependent on the input processes which supply the reaction system with CO_2, ATP, and NADPH. The cyclic reaction cannot reach a steady state, however, unless photosynthate is withdrawn at the same rate as it is cyclically produced. This means that the steady-state cycle activity must be controlled also by the output processes which withdraw photosynthate from the system.

The present attempt to characterise such output control analytically is based on the experimental evidence showing that phosphate-translocator-mediated export of photosynthate occurs at a rate reflecting the stromal concentrations of the exported cycle intermediates [3, 21]. Since these intermediates all form part of the rapidly equilibrating PGA/GAP/DHAP/FBP pool [23–26], the net rate (v_{ex}) of photosynthate export can be related to the metabolite level in this pool as indicated, for instance, by the concentration of DHAP. The cycle activity, also, must reflect the metabolite level in the PGA/GAP/DHAP/FBP pool. In particular, the rate (v_6) of enzymic FBP hydrolysis will be determined by the concentration of FBP and hence can be related to the concentration of DHAP by application of the rapid-equilibrium approximations in Eqns (16) and (17). The simplifying assumption that photosynthate export represents the only output process contributing significantly to net reaction flux then provides the inference that steady-state conditions cannot obtain unless metabolites in the PGA/GAP/DHAP/FBP pool may reach a level where $v_6 = v_{ex}$. The latter condition is not sufficient to ensure that a steady state may be reached (cf. Eqns 3–7), but it is sufficient to define the concentration(s) of DHAP required to support a steady-state operation of the system and hence defines also the steady-state rate(s) which may obtain.

According to that analytical approach, the dynamic behaviour of the Calvin cycle can be related primarily to the partitioning of reaction flux between photosynthate export and recycling, as determined by the kinetic interplay of the phosphate translocator and the stromal fructose bisphosphate. Depending on the relative magnitudes of kinetic parameters for these two catalysts, the reaction system in Scheme 1 may exhibit a single or two distinct steady-state levels of operation, or may be unable to reach a stationary state (cf. Fig. 1). In the case that steady-state conditions do obtain, the interplay of the two catalysts may allow for an extremely efficient control of the cycle activity characterised potentially by flux control coefficients of unlimited magnitude (Eqns 34–36). This is consistent with other evidence attributing a regulatory function to fructose bisphosphatase [23, 26] and lends strong support to the idea that cytosolic metabolites may control the stromal Calvin cycle activity by affecting the kinetics of action of the phosphate translocator.

Eqns (19) and (20) express the dependence of kinetic parameters for the phosphate translocator on the external concentration of orthophosphate (P_i') when the reaction medium contains no other transferable metabolites. Eqns (29) and (31) provide explicit relationship for the steady-state rates of photosynthesis which may obtain under such conditions and the kinetic model thus defined confirms that the steady-state Calvin cycle activity may be strongly regulated by external phosphate (Fig. 3). When $[P_i'] < 0.3$ mM (region A), the model system exhibits a uniquely defined steady state characterised by a positive flux control coefficient for the phosphate translocator (Eqn 34) and hence also for the regulatory external metabolite. This is consistent with the results obtained in experimental studies of isolated chloroplasts [4–7, 13] which show that the rate of photosynthesis is enhanced by increasing concentrations of external phosphate up to about 0.2 mM. The inhibitory effect of high phosphate concentrations observed in such experiments has been proposed to reflect an artificial exaggeration of the normal export process precluding that intermediates of the Calvin cycle may build up to sufficiently high stromal concentrations to sustain appreciable photosynthesis [5, 8]. That explanation, according to the present kinetic model, could hold true for phosphate concentrations exceeding 2.4 mM (region C in Fig. 3) where $v_{ex} > v_6$ for any level of metabolites in the PGA/GAP/DHAP/FBP pool such that steady-state conditions cannot obtain (cf. curve C in Fig. 1). The excessive rate of photosynthate export would then deprive the stroma of cycle intermediates and progressively depress the rate of cyclic photosynthate formation.

It should be noted, however, that external phosphate has been found to inhibit chloroplast photosynthesis already at moderately high concentrations (say 0.3–2.4 mM) that

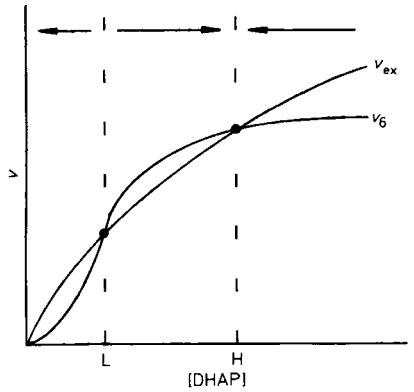


Fig. 5. *Stability properties of the reaction system in Scheme 1.* Schematic representation of the net rate of photosynthate export (v_{ex}) and the rate of enzymic fructose 1,6-bisphosphate hydrolysis (v_6) as a function of the dihydroxyacetone phosphate concentration [DHAP] in the case that distinct high-velocity (H) and low-velocity (L) steady states may obtain. Arrows indicate the expected direction of [DHAP] changes caused by the action of the reaction system at different non-steady-state levels of [DHAP] and illustrate that only the high-velocity steady state would be expected to be asymptotically stable

appear to sustain a steady-state rate of photosynthate formation and export. The present results provide a reasonable explanation for that observation by showing that the model system over that phosphate concentration range (region B in Fig. 3) may operate in a high-velocity steady state characterized by a negative flux control coefficient for the phosphate translocator (Eqn 35). Evidence that isolated chloroplasts do operate in a mode of negative translocator control at moderately high external phosphate concentrations has been presented by Heldt and coworkers [7], who found that partial inactivation of the phosphate translocator by reversible or irreversible inhibitors enhances the rate of photosynthesis in isolated chloroplasts. This raises the question of whether the dynamic structure of the reaction system in Scheme 1 could be such that the high-velocity case is favoured under conditions where two distinct steady states may obtain.

To answer that question, attention should be drawn to Fig. 5 which schematically illustrates some dynamic characteristics of the model system in the region of double steady states. The crossover points between graphs of v_{ex} and v_6 in Fig. 5 define the DHAP concentrations (marked L and H for, respectively, the low-velocity and high-velocity case) required to meet the steady-state condition $v_{ex} = v_6$. For [DHAP] < L or [DHAP] > H we have $v_{ex} > v_6$ and the system will be in a state where the excessive export rate will tend to decrease the level of DHAP and other cycle intermediates. For L < [DHAP] < H, however, v_6 exceeds v_{ex} and the system will tend to produce photosynthate at a higher rate than it is exported such that the level of DHAP will tend to increase. The implication of this is that small perturbations of the DHAP concentration from the steady-state value will bring the system into a state where the activity of the system tends to oppose the perturbation in the high-velocity case, but to reinforce the perturbation in the low-velocity case. In other words, there is strong reason to believe that only the high-velocity case may correspond to an asymptotically stable [28] steady state.

Similar considerations apply for the behaviour of the model system in the presence of low concentrations of external phosphate. Perturbations of the DHAP concentration from the single steady-state value defined under such conditions will bring the system into a state where the perturbation tends to be enhanced rather than reversed. This indicates that the steady state corresponding to Eqn (29) is dynamically unstable and suggests that the experimentally observed stimulation of photosynthesis by low phosphate concentrations may not refer to rate determinations performed with chloroplasts operating in a true steady state. Stability arguments, therefore, lead us to conclude that the inhibitory effect of moderately high concentrations of external phosphate most likely reflects the normal mode of steady-state operation of chloroplasts under physiological conditions.

Most previous attempts to explain observed correlations between stromal and cytosolic carbohydrate metabolism have been based on the (intuitively attractive) tacit assumption that the phosphate translocator exhibits a positive flux control coefficient, such that enhancement of its transport capacity also leads to an enhanced steady-state rate of metabolite transport across the chloroplast membrane [6, 10–13]. The present results provide the important inference, however, that the output regulation of the Calvin cycle is best described by the high-velocity case in Fig. 3 (Eqn 31) and characterised by a negative flux control coefficient for the phosphate translocator. This means that any factor tending to enhance the export capacity of the translocator would be expected to decrease the actual steady-state rate of photosynthate export. As previously suggested [14, 15], such an apparently paradoxical situation may be at hand because an increased capacity of the translocator can (and according to our analysis will) decrease the stromal concentrations of Calvin cycle intermediates and result in a decreased steady-state rate of photosynthate production and export.

It seems evident from the above discussion that no realistic description of the dynamic behaviour and regulation of the Calvin photosynthesis cycle is possible without consideration of the flux control exerted by the phosphate translocator and by metabolites affecting the kinetic characteristics of the export process. It also seems obvious that such considerations preferably should be based on mathematical analysis or modelling of the kinetics and dynamics of the complex photosynthetic reaction system. This investigation has focused on the regulatory effects of the exported cycle intermediates and external orthophosphate on the action of the phosphate translocator; the simple model now put forward would appear to provide a qualitatively reliable (but quantitatively highly approximate) description of the main characteristics of such output control of the Calvin cycle activity. Much more elaborate models would be required to address analytically certain regulatory problems which have not been considered in the present work, e.g. the distribution of reaction flux between photosynthate export and starch synthesis, the role played by stromal orthophosphate, and the operation of the Calvin cycle under conditions of non-saturating light or CO_2.

This investigation was supported by grants from the Swedish Natural Science Research Council.

REFERENCES

1. Bassham, J. A., Kirk, M. & Jensen, R. G. (1968) *Biochim. Biophys. Acta 153*, 211–218.
2. Lilley, R. McC., Chon, C. J., Mosbach, A. & Heldt, H. W. (1977) *Biochim. Biophys. Acta 460*, 259–272.
3. Fliege, R., Flügge, U. I., Werdan, K. & Heldt, H. W. (1978) *Biochim. Biophys. Acta 502*, 232–247.
4. Cockburn, W., Baldry, C. W. & Walker, D. A. (1967) *Biochim. Biophys. Acta 143*, 614–624.

5. Lilley, R. McC., Schwenn, J. D. & Walker, D. A. (1973) *Biochim. Biophys. Acta 325*, 596–604.
6. Herold, A. & Walker, D. A. (1979) in *Membrane transport in biology* (Giebisch, G., Tosterson, D. & Ussing, H., eds) vol. 2, pp. 411–439, Springer-Verlag, Berlin.
7. Flügge, U. I., Freisl, M. & Heldt, H. W. (1980) *Plant Physiol. 65*, 574–577.
8. Chen-She, S.-H., Lewis, D. H. & Walker, D. A. (1978) *New Phytol. 74*, 383–392.
9. Sharkey, T. D. (1985) *Bot. Rev. 51*, 53–105.
10. Herold, A. (1980) *New Phytol. 86*, 131–144.
11. Preiss, J. (1984) *Trends Biochem. Sci. 9*, 24–27.
12. Walker, D. A. & Sivak, M. N. (1986) *Trends Biochem Sci. 11*, 176–179.
13. Heldt, H. W., Chon, C. J., Maronde, D., Herold, A., Stankovic, Z. S., Walker, D. A., Kraminer, A., Kirk, M. R. & Heber, U. (1977) *Plant Physiol. 59*, 1146–1155.
14. Walker, D. A. (1974) in *MTP International review of science* (Northcote, D. H., ed.) vol. 11, pp. 1–49, Butterworths, London.
15. Heber, U. & Heldt, H. W. (1981) *Annu. Rev. Plant Physiol. 32*, 139–168.
16. Leegood, R. C., Walker, D. A. & Foyer, C. (1985) in *Topics in photosynthesis* (Barber, J. & Baker, N. R., eds.) vol. 6, pp. 189–258, Elsevier, Amsterdam.
17. Cseke, C. & Buchanan, B. B. (1986) *Biochim. Biophys. Acta 853*, 43–63.
18. Charles, S. A. & Halliwell, B. (1980) *Biochem. J. 185*, 689–693.
19. Charles, S. A. & Halliwell, B. (1981) in *Photosynthesis* (Akoyunoglou, G., ed.) vol. 4, pp. 347–356, Balaban, Philadelphia.
20. Heldt, H. W., Gardemann, A., Gerhardt, R., Herzog, B., Stitt, M. & Wirtz, W. (1984) in *Advances in photosynthesis research* (Sybesma, C., ed.) vol. 3, pp. 617–624, Nijhoff/Junk, Haag.
21. Giersch, C. (1977) *Z. Naturforsch. 32c*, 263–270.
22. Portis, A. R. (1983) *Plant Physiol. 71*, 936–943.
23. Portis, A. R., Chon, C. J., Mosbach, A. & Heldt, H. W. (1977) *Biochim. Biophys. Acta 461*, 313–325.
24. Heber, U. (1984) in *Advances in photosynthesis research* (Sybesma, C., ed.) vol. 3, pp. 381–389, Nijhoff/Junk, Haag.
25. Dietz, K. & Heber, U. (1984) *Biochim. Biophys. Acta 767*, 432–443.
26. Bassham, J. A. & Krause, G. H. (1969) *Biochim. Biophys. Acta 189*, 207–221.
27. Giersch, C., Heber, U. & Krause, G. H. (1980) in *Plant membrane transport* (Sparswich, R. M., Lucas, W. J. & Daity, J., eds) pp. 65–83, Elsevier, Amsterdam.
28. Heinrich, R., Rapoport, S. M. & Rapoport, T. (1977) *Prog. Biophys. Mol. Biol. 32*, 1–82.