Modelling photosynthesis and its control

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Abstract

The dynamic and steady-state behaviour of a computer simulation of the Calvin cycle reactions of the chloroplast, including starch synthesis and degradation, and triose phosphate export have been investigated. A major difference compared with previous models is that none of the reversible reactions are assumed to be at equilibrium. The model can exhibit alternate steady states of low or high carbon assimilation flux, with hysteresis in the transitions between the steady states induced by environmental factors such as phosphate and light intensity. The enzymes which have the greatest influence on the flux have been investigated by calculation of their flux control coefficients. Different patterns of control are exhibited over the assimilation flux, the flux to starch and the flux to cytosolic triose phosphate. The assimilation flux is mostly sensitive to sedoheptulose bisphosphatase and Rubisco, with the exact distribution depending on their relative activities. Other enzymes, particularly the triose phosphate translocator, become more influential when other fluxes are considered. These results are shown to be broadly consistent with observations on transgenic plants.

Key words: Computer modelling, photosynthesis, genetic manipulation techniques.

Introduction

Historically, the goal of increasing crop productivity has been achieved by repeated selection and cross-breeding of the most productive strains. More recently, the advent of genetic manipulation (GM) technology has offered the potential of meeting this goal by increasing carbon assimilation more rapidly through genetic engineering technology, rather than by selective breeding.

However, despite the fact that the mechanisms of carbon assimilation, and the techniques to modify them, are well known, there have been no reports to date of useful increases in carbon assimilation brought about by GM techniques. Progress appears hampered not by technological constraints, but by the absence of a coherent body of theory that allows the a priori prediction of the effect of changes in enzyme activities on the behaviour of a given biochemical system, or that, more usefully, can identify the enzyme(s) which must be modified in order to bring about a particular change in behaviour.

A useful first step in developing such an all-embracing body of knowledge is the field of Metabolic Control Analysis (MCA) first proposed in the early 1970s (Kacser and Burns, 1973; Heinrich and Rapoport, 1974; for current reviews and introductory material see Fell 1992, 1997; Kacser et al., 1995). For the purposes of this paper, the use of MCA lies in the fact that it provides a precise, quantitative definition of the amount of influence that a given enzyme has over the properties of the system of which it is a part: the control coefficient.

An important consequence of MCA is that the value of any particular control coefficient for a given enzyme is a function not of the kinetic characteristics of that enzyme alone, but of those of all reactions in the system (see previous citations). From this it follows that the influence that a particular enzyme has, cannot be inferred solely from the properties of that enzyme; all enzymes in the system must be considered.

How control analysis models can be applied in the prediction and interpretation of the effects of enzyme over-expression in potatoes has been described previously (Thomas et al., 1997a, b). Another approach (Fell et al., 1999) is the computer simulation of metabolic models. Here the effects of arbitrary changes in enzyme activities can be determined directly, although MCA is still a useful tool in the analysis and interpretation of the behaviour of the model.

A well-known problem, faced by workers involved in the quantitative investigation of biochemical systems is
that, although the rate equations for individual reactions are generally known (or at worst readily determinable) and it is therefore straightforward to define systems as sets of ordinary differential equations, algebraic solutions to all but the most trivial of such systems are effectively impossible to obtain. Happily, the recent spectacular increases in the power of computer hardware, coupled with appropriate software, means that this problem can, for most purposes, be circumvented. Steady-state values of fluxes and metabolite concentrations can be determined from a description of the system in terms of its individual rate equations (i.e., by computer modelling).

In this paper, results obtained from a computer model of the Calvin cycle are compared with those obtained by experimental, principally GM, techniques. By such comparison, it is possible to test hypotheses explaining experimental observations, in a manner not possible by the consideration of individual experimental data in isolation.

In reporting the response of the model to changes in environmental parameters, this study is restricted to external (i.e., extra-stromal) phosphate, $P_{\text{ext}}$, and light. The response to CO$_2$ is not reported for several reasons: (1) The model is less sensitive to CO$_2$ than to $P_{\text{ext}}$ or light. (2) Under eutrophic conditions, and over short to medium (seconds to hours) time scales, CO$_2$ has much less potential to vary than $P_{\text{ext}}$ or light. (3) Although the impact of a long-term increase in global CO$_2$ concentrations is of considerable interest, response of plants to this would presumably include changes in gene expression. As this is not included in the model, it is not possible to use it to investigate such long-term change. (4) If the sensitivity of an isolated enzyme towards a given chemical species is known, then the impact that changes in that species will have upon the system may be calculated, without explicitly including it in the model, as shown in equation 4 below.

### The model

#### Model definition

A model of the Calvin cycle was defined as shown in Fig. 1 and Table 1, based on that described previously (Pettersson and Ryde-Pettersson, 1988). The major difference between the structure of model described here, and that described previously (Pettersson and Ryde-Pettersson, 1988) is that no assumption is made that any reversible reactions attain equilibrium, rather each (reversible) reaction is assumed to have simple, reversible, mass-action kinetics:

$$V = K \left( \prod_{i=1}^{n_s} S_i \right) - \frac{\prod_{j=1}^{n_p} P_j}{k_{\text{eq}}}$$  

where $V$ is the reaction velocity, $k_{\text{eq}}$ the equilibrium constant, $S$ and $P$ substrate and product concentration(s), respectively, and $K$ is a rate constant with dimensions determined by the molecularity of the reaction. Unless stated otherwise, $K$ was set to $5 \times 10^6$ for all reversible reactions, and values for $k_{\text{eq}}$ as described previously (Pettersson and Ryde-Pettersson, 1988). Irreversible reactions, were assigned the same reaction kinetics and parameters as previously (Pettersson and Ryde-Pettersson, 1988).

As is common in the field of plant physiology, Pettersson expressed reaction rates in dimensions of $\mu$mol h$^{-1}$ mg$^{-1}$ Chl$^{-1}$, and these are the units used here. When equation 1 is scaled (using Pettersson’s estimate of stromal volume as 30 $\mu$l mg$^{-1}$ Chl$^{-1}$ to yield $V$ in units of M$^{-1}$ s$^{-1}$, $K$ (for a bimolecular reaction) becomes equal to $5.4 \times 10^{7}$ M$^{-1}$ s$^{-1}$. This compares with substrate enzyme binding (forward) rate constants (reported by Fersht, 1984), falling within the range $10^6–10^9$ M$^{-1}$ s$^{-1}$, and well below the diffusion controlled rate limit of $10^9$ M$^{-1}$ s$^{-1}$.

A second difference in the structure of this model is that a starch degradation step, catalysed by starch phosphorylase, was included. The effect of including this step is to stabilise the model in the face of high carbon demand, abolishing the ‘overload breakdown’ observed at high $P_{\text{ext}}$ in the model system when this step is absent (Pettersson and Ryde-Pettersson, 1988; Poolman, 1999), and observed in dark-harvested (and therefore starch-less) chloroplasts (Flügge et al., 1980). Although this step does introduce a futile cycle, investigation shows that less than 1% of ATP turnover in the model was thus accounted for (Poolman, 1999).

It should be noted that although the model describes the Calvin cycle in considerable detail, the light reactions are greatly simplified, being assumed to simply regenerate ATP from ADP and Pi, NADP and NADPH are assumed to be fixed at concentrations of 0.29 and 0.21 mM, respectively. Changes in light intensity were thus modelled by altering $V_{\text{max}}$ of this ATP synthesis step and, within the context of this paper, ‘light’ refers to this value.

Although fixing the NADP/NADPH ratio in this way is a simplification, it is predicted that the inclusion of NADPH oxidation and the concomitant reduction by the light reactions will have little effect on the qualitative behaviour of this model for two reasons: Firstly, the only reaction in the cycle sensitive to NADP/H is the G3Pdh reaction ($v_8$) in Fig. 1. This reaction immediately follows the PGK reaction, driven by ATP generated from the light reactions. Hence, the sense of the response of the G3Pdh reactions to changes in the light reaction activity will be the same regardless of whether the NADP/H ratio is fixed or floating. Secondly, varying the ratio of (fixed) NADP:NADPH appears to have little
Fig. 1. Reactions and topology of the Calvin cycle as used in the model. See Table 1 for enzyme names.

Table 1. Reaction subscripts, abbreviations, names, and kinetics types, as used in the Calvin cycle model of Fig. 1

<table>
<thead>
<tr>
<th>Subscript</th>
<th>Abbreviation</th>
<th>Name</th>
<th>Kinetics</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Abbreviation</td>
<td>Ribulose bisphosphate carboxylase-oxidase</td>
<td>Irreversible</td>
</tr>
<tr>
<td>2</td>
<td>PGK</td>
<td>Phosphoglycerate kinase</td>
<td>Reversible</td>
</tr>
<tr>
<td>3</td>
<td>G3Pdh</td>
<td>Glyceraldehyde-3-phosphate dehydrogenase</td>
<td>Reversible</td>
</tr>
<tr>
<td>4</td>
<td>TPI</td>
<td>Triose phosphate isomerase</td>
<td>Reversible</td>
</tr>
<tr>
<td>5</td>
<td>F-Aldo</td>
<td>Aldolase (FBP reaction)</td>
<td>Reversible</td>
</tr>
<tr>
<td>6</td>
<td>FBPase</td>
<td>Fructose-1,6-bisphosphatase</td>
<td>Irreversible</td>
</tr>
<tr>
<td>7</td>
<td>F.TKL</td>
<td>Transketolase (F6P reaction)</td>
<td>Reversible</td>
</tr>
<tr>
<td>8</td>
<td>S.Aldo</td>
<td>Aldolase (SBP reaction)</td>
<td>Reversible</td>
</tr>
<tr>
<td>9</td>
<td>SBPase</td>
<td>Sedoheptulose bisphosphatase</td>
<td>Irreversible</td>
</tr>
<tr>
<td>10</td>
<td>S.TKL</td>
<td>Transketolase (S7P reaction)</td>
<td>Reversible</td>
</tr>
<tr>
<td>11</td>
<td>R3Pso</td>
<td>Ribose-5-phosphate isomerase</td>
<td>Reversible</td>
</tr>
<tr>
<td>12</td>
<td>XS5Pepi</td>
<td>Xylose-5-phosphate epimerase</td>
<td>Reversible</td>
</tr>
<tr>
<td>13</td>
<td>Ru5Pk</td>
<td>Ribulose-5-phosphate kinase</td>
<td>Irreversible</td>
</tr>
<tr>
<td>14</td>
<td>PGI</td>
<td>Phosphoglucose isomerase</td>
<td>Reversible</td>
</tr>
<tr>
<td>15</td>
<td>PGM</td>
<td>Phosphoglucose mutase</td>
<td>Reversible</td>
</tr>
<tr>
<td>16</td>
<td>StSyn</td>
<td>Starch synthase</td>
<td>Irreversible</td>
</tr>
<tr>
<td>TPT</td>
<td>TPT</td>
<td>Triose phosphate translocator (subscript indicates metabolite)</td>
<td>Irreversible</td>
</tr>
<tr>
<td>17</td>
<td>StPase</td>
<td>Starch phosphorylase</td>
<td>Irreversible</td>
</tr>
</tbody>
</table>

effect on the behaviour of the model, other than varying the ratio of TP:PGA export via the TPT.

For reasons of time and space, the thioredoxin system in this model has not been included, although a simple representation of it has been included elsewhere (Poolman, 1999). The effect of doing this was to alter the response of the model to light (see below), but the general conclusions drawn in this paper remain unaffected.

Model control

Initial work on the model described above was undertaken with the SCAMP modelling package (Sauro, 1993). It
was found that although SCAMP provides a very convenient platform for defining large models, difficulty was encountered (amongst other things) in reliably obtaining steady-state solutions with a model of this complexity. In order to address such problems, a new interface package was developed for SCAMP, Scampi (= SCAMP application programmer’s Interface).

Scampi allows individual models, defined with SCAMP, to be placed in the context of a programming language and made the subject of arbitrary, user-defined algorithms. In this way Scampi provides a system for the investigation of large models that is more reliable, more flexible, and faster than other metabolic modelling packages currently available. The disadvantage is that the user must have some expertise in the use of the ‘C’ programming language.

All results presented in this paper were obtained using Scampi, and further details of this, the Calvin cycle model, and the input used to obtain these results can be found in Poolman (Poolman, 1999) or via the Internet from http://bms-mudshark.brookes.ac.uk ftp://bmshuxley.brookes.ac.uk/pub/mcasoftware/Scampi ftp://bmshuxley.brookes.ac.uk/pub/mcamodels/ CalvinCycle.

Model analysis

Although the Calvin cycle model as described is quite complex, its analysis is made simpler here by only considering the behaviour of the external fluxes: the CO₂ assimilation flux (\(J_{\text{Assim}}\)), the flux to starch (\(J_{\text{Starch}}\)), which can become negative, indicating net starch degradation, and the TP flux to cytosol via TPT (\(J_{\text{TPT}}\)). The third of these can be divided into three components, but in the context of this paper it is only the net carbon flux that is of interest. Unless stated otherwise these will be reported as net carbon flux (\(\mu\text{mol C h}^{-1}\ \text{mg}^{-1}\ \text{Chl}^{-1}\)).

The influence of the activity of a given enzyme over these fluxes was determined as the MCA flux control coefficient, \(C_i^a\), which for an alteration in the activity, \(v_i\), of an enzyme, \(i\), upon the steady-state flux, \(J\), through a given step \(a\), is defined:

\[
C_i^a = \frac{\partial J}{\partial v_i} \frac{v_i}{J_a} \tag{2}
\]

This may be rewritten as

\[
C_i^a = \frac{\partial \ln J}{\partial \ln v_i} \tag{3}
\]

which has the practical implication that if \(v_i\) can be varied independently, then \(C_i^a\) is the slope of the plot of \(\ln J\) versus \(\ln v_i\).

Metabolic control analysis defines an analogous concentration control coefficient, \(C_i^c\), for the influence of the activity of enzyme \(i\) over the concentration of metabolite \(M\).

The behaviour of the model was investigated over a range of environmental conditions, namely \(P_i\) and light. The kinetics of Rubisco were insensitive to CO₂ concentration; although if it is assumed that Rubisco is not saturated to CO₂, then the qualitative effect of varying CO₂ can be determined by varying Rubisco \(V_{\text{max}}\). However, if the relative sensitivity (in MCA terms, the elasticity, \(\varepsilon\)) of Rubisco toward CO₂, \(v_{\text{rbc}}^{\text{CO}_2}\) is known, then the response coefficient, \(R_{\text{CO}_2}^v\), of any model variable, \(v\), toward CO₂ may be determined as

\[
R_{\text{CO}_2}^v = C_{\text{rbc}}^{\text{rbc}} v_{\text{rbc}}^{\text{CO}_2} \tag{4}
\]

Equation 4 can be generalized for any enzyme and any external effector, and the response of the model toward O₂ could thus be determined by substituting O₂ for CO₂.

An entirely different approach to model analysis, based only on a consideration of the system topology, elementary modes analysis, was also employed. Detailed description of the technique lies beyond the scope of this paper (but see Schuster et al., 1996, 1999); its purpose is to identify the number of independent steady-state fluxes that can exist in a system. If this is zero, the system cannot sustain any steady-state flux.

Two sets of model behaviour are considered here: response of the three external fluxes to environmental parameters, and the flux control coefficients of various steps toward these fluxes.

Results

Flux responses

The response of the external fluxes to \(P_i\) is shown in Fig. 2. At low concentrations of \(P_i\) (< ~0.1 mM) all three fluxes respond positively to \(P_i\), but undergo an abrupt transition, beyond which assimilation flux is insensitive to \(P_i\). \(J_{\text{TPT}}\) responds positively and starch

![Fig 2](image-url)

Fig. 2. Response of \(J_{\text{Assim}}\) (○), \(J_{\text{TPT}}\) (△) and \(J_{\text{Starch}}\) (□) to \(P_i\) in the Calvin cycle model.
synthesis flux negatively to $\text{Pi}_{\text{ext}}$. At about 0.4 mM $\text{Pi}_{\text{ext}}$, $J_{\text{TPT}}$ exceeds that of assimilation and, in consequence, $J_{\text{Starch}}$ becomes negative. The exact value of $\text{Pi}_{\text{ext}}$ at which this occurs is quite sensitive to other model parameters, notably the sensitivity of TPT towards PGA. In the absence of starch phosphorylase the model collapses in the face of high $\text{Pi}_{\text{ext}}$ values. The value at which this happens is also quite sensitive to other parameters, and is typically higher ($\sim 1.5$ mM) than the value at which this model moves into net starch degradation.

Figure 3 shows the response of $J_{\text{Assim}}$ to varying light. In this figure the model was evaluated at the maximum light, which was then reduced by a fixed increment to a minimum value, with $J_{\text{Assim}}$ recorded at each step. The light was then increased, by the same increments, again recording $J_{\text{Assim}}$ at each step. The figure shows that, at certain critical values of light intensity, $J_{\text{Assim}}$ undergoes an abrupt transition between a relatively fast and a relatively slow steady-state (the two will be referred to simply as the ‘fast’ and ‘slow’ steady-states), and that the fast-slow transition occurs at a light intensity below that of the slow-fast transition (i.e. the system exhibits hysteresis).

In the fast steady-state the $J_{\text{Assim}}$ is unaffected by changes in light. In the slow steady-state the $J_{\text{Assim}}$ responds positively to increases in light. A small region, seen as the slow–fast transition, is approached in which the model exhibits continuous oscillation, and for which no true (stationary) steady-state exists. The other two external fluxes show the same behaviour (data not shown). Subsequent analysis of the model will concentrate upon the fast steady-state.

**Metabolic control analysis**

One of the general conclusions of MCA is that control coefficients are variables, not constants, and therefore should, if possible, be determined over a range of conditions. As there are three fluxes of interest, three sets of flux control coefficients must be considered. Figure 4 shows flux control coefficients over $J_{\text{TPT}}$, determined for a range of $\text{Pi}_{\text{ext}}$ values. An equivalent curve for control over assimilation is not shown as the pattern of control for this flux was found to be simple: it is dominated by a single enzyme, SBPase. Over the range shown $C^J_{\text{SBPase}}$ was 1.0, and for all other enzymes $C^J_{\text{Assim}} < 0.01$.

Control of $J_{\text{TPT}}$ is less simple, with several enzymes exerting both positive and negative control. Starch synthase and FBPase both have small negative control coefficients that show little variation in response to $\text{Pi}_{\text{ext}}$. The majority of positive control is held between four steps, and this control appears to be exchanged between two pairs as $\text{Pi}_{\text{ext}}$ varies. The two steps having most control are those catalysed by SBPase and TPT: at low $\text{Pi}_{\text{ext}}$, most of the control is by TPT itself, but this is lost to SBPase as $\text{Pi}_{\text{ext}}$ increases until high $\text{Pi}_{\text{ext}}$ concentrations are reached, when both of these steps have approximately equal control. The control coefficient of G3Pdh, and that of starch phosphorylase, is relatively small, not rising much above 0.2. The former decreases, and the latter increases, monotonically with increases in $\text{Pi}_{\text{ext}}$, leaving starch phosphorylase with more control in the range of $\text{Pi}_{\text{ext}}$ in which there is net starch degradation.

Interpretation of results for $C^J_{\text{Starch}}$ is complicated by the fact that $J_{\text{Starch}}$ becomes negative at high $\text{Pi}$, resulting in a discontinuity in the curve of $C^J_{\text{Starch}}$ versus $\text{Pi}_{\text{ext}}$, with $C^J_{\text{Starch}}$ approaching $\pm \infty$. However, the overall pattern of control is similar to that shown by $J_{\text{TPT}}$, with all steps holding the same proportion of total control (i.e. $\Sigma C^J$) for $J_{\text{Starch}}$ as for $J_{\text{TPT}}$.

As long as the model was maintained in the fast steady-state, control coefficients showed relatively little change in response to light. At light intensities below the transition, a marked rearrangement in control occurs. In particular, SBPase loses its dominance over assimilation,

![Figure 3](image-url)  
**Fig. 3.** Response of assimilation flux in the Calvin cycle model to changing light. Flux was initially calculated at light intensities of 1500 µmol h$^{-1}$ mg$^{-1}$ Chl and then recalculated, decrementing light down to 900 µmol h$^{-1}$ mg$^{-1}$ Chl, at which point light was increased incrementally back up to the initial value of 1500 µmol h$^{-1}$ mg$^{-1}$ Chl.

![Figure 4](image-url)  
**Fig. 4.** Effect of $\text{Pi}_{\text{ext}}$ on control of $J_{\text{TPT}}$ by SBPase (□), TPT (Δ) and G3Pdh (○).

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and in this region control is shared between several enzymes (data not shown).

Discussion

Response to \( \text{Pi}_{\text{ext}} \) and light

The response of external fluxes in the model to \( \text{Pi}_{\text{ext}} \) is in reasonable qualitative agreement with reported experimental results. The effects of external Pi, TP, and TPT inhibitors upon photosynthesis in isolated chloroplasts have been investigated (Flügge et al., 1980; Heldt et al., 1977; Portis, 1982); except at very low concentrations of \( \text{Pi}_{\text{ext}} \), \(< \sim 0.1 \text{ mM} \) the rate of carbon assimilation is either unaffected, or decreases in response to increased external demand. Rather, the Calvin cycle responds by altering the partitioning of carbon flux between \( J_{\text{TPT}} \) and \( J_{\text{Starch}} \). If demand is very high, and in the absence of (the ability to degrade) starch, the cycle is prone to collapse.

At low concentrations of \( \text{Pi}_{\text{ext}} \), the response of the model to increasing \( \text{Pi}_{\text{ext}} \) is an increase in assimilation, with concomitant increases both in starch synthesis and TP export, although this has not (to our knowledge) been reported experimentally.

Although the switching response of the model to changes in light seen in Fig. 3 is rather unexpected, consideration of Calvin cycle models of greatly simplified topology (Poolman, 1999), have led to the conclusion that (the potential for) this behaviour can be expected in any system with the following characteristics: (1) the presence of an auto-catalytic cycle; (2) the presence of a conserved moiety, Pi in the case of the Calvin cycle; and (3) the presence of saturable elements in the system. The absence of such behaviour implies the presence of specific mechanisms to prevent it. For example, when the effects of the thioredoxin system (Anderson, 1986) were incorporated into the model by coupling those reactions under its influence to the light reaction activity, the switching was abolished, and the model remained in the fast steady-state over a wide range of light intensities. There is, however, some experimental evidence to suggest that switching in response to changing light does occur in vivo, albeit rarely. A Russian language paper (Oja and Laisk 1976; cited by Laisk and Walker, 1986), claims that the response of assimilation to light in lilac leaves more closely resembled a discontinuous line than a rectangular hyperbola. Furthermore Elrifi et al. exposed nitrogen-starved Chlorella to \( \text{NH}_4^+ \), the resulting assimilation–light response data resemble the discontinuous curve shown in Fig. 3 at least as well as the smooth curve imposed by the authors (Elrifi et al., 1988).

These points notwithstanding, there are four lines of reasoning to suggest that the fast steady-state is the one which most plants will exhibit most of the time. Firstly, in the fast steady-state the redistribution of \( J_{\text{TPT}} \) and \( J_{\text{Starch}} \) in response to \( \text{Pi}_{\text{ext}} \) (Fig. 2) is in good qualitative agreement with the previously cited experimental evidence. Secondly the distribution of metabolite concentrations in the model in the fast steady-state (data not shown) is in much better agreement with those experimentally reported, than in the slow state. Thirdly, the MCA results from the model in the fast state are also in much better agreement with experimental data than in the slow. Finally, at least over some of the range of light values, the slow state is inefficient: the system can, in the fast steady-state, assimilate more carbon with the same investment in enzyme activity, and under the same environmental conditions. One would, therefore, expect a positive selection pressure to act in favour of the fast state.

Metabolic control analysis

The influences of all the irreversible enzymes of the Calvin cycle have been investigated by the use of GM techniques, typically by use of anti-sense technology to produce organisms with reduced expression of the target enzyme. The general (but not universal, see below) finding is that reduction of activity of an individual enzyme has little, if any, detectable effect on assimilation rate, until relatively low (~30–40% wild type) activities are reached. Furthermore, a common observation is that the levels of expression that result in appreciable reduction in assimilation are close to, if not coincident with, the onset of various pleiotropic effects and, in particular, reduction in chlorophyll content. It is, therefore, frequently not possible to attribute observed reductions in \( J_{\text{Assim}} \) solely to the further reduction of the target enzyme activity, even when expression is much decreased. In particular, the anti-sensing of FBPase (Kossman et al., 1994) and Ru5Pk (Gray et al., 1995) resulted in no reported change in fluxes in the absence of pleiotropic effects, and is not considered further.

Rubisco

Stitt and co-workers (Fichtner et al., 1993; Quick et al., 1991; Stitt et al., 1991) investigated the properties of tobacco plants transformed with the anti-sense gene for the Rubisco small subunit (rbcS). The resulting transformants exhibited Rubisco activities ranging from ~20–100% of wild type activity. Under ambient growth conditions very little impact on \( J_{\text{Assim}} \) was observed until Rubisco activity fell to ~40% of the wild type (wt). Using the plot of Rubisco activity versus \( J_{\text{Assim}} \) an approximate value for \( C_{\text{R rubisco}} \) of 0.1 at ambient conditions was calculated (Stitt et al. 1991). This varied with environment, and a value as high as 0.7 was calculated with saturating light. Even the lower of these values is substantially greater than the typical values of ~2 × 10^{-3} obtained from the model described here.

Photosynthetic assimilation was also recorded in these
plants under a range of non-ambient conditions, including high light intensities and saturating CO₂. The latter is particularly relevant here as the concentrations of CO₂ were high enough to abolish photorespiration. As photorespiration is not included in the model described here, the structure of the model more closely resembles these plants than those under ambient CO₂ concentrations.

By varying these environmental parameters Stitt et al. (Stitt et al., 1991) were able to demonstrate that C₃/SBPase can vary considerably. At high light and ambient CO₂, C₃/SBPase was estimated as ~0.7, increasing to ~1.0 at ~20%wt. At high CO₂ and ambient light C₃/SBPase remains low (~0.2), again until Rubisco has declined to ~30%wt at which point C₃/SBPase increases abruptly to a value close to unity. However, the behaviour of the plants at high light and high CO₂ to which the model would be a still better representation, were not reported.

**Glyceraldehyde-3-phosphate dehydrogenase**

Price et al. anti-sensed G3Pdh in N. tabacum, producing organisms with G3Pdh activity ranging from 7–100% of the wild type (Price et al., 1995). No effect on J₃assim was observed until activity had been reduced to 35% of the wild type, and this coincided with a reduction in chlorophyll concentration and other pleiotropic effects. Interestingly, it was observed that RuBP concentration fell before the onset of diminished assimilation, with G3Pdh activity; a mean value of ~0.2 can be calculated for C₃RuBP over this range. This compares with average C₃GaPdh = 0.51 over the same proportionate range in the model described here. It should be pointed out that the kinetic equation of G3Pdh used in the model, defined by equation 1, is likely to be a considerable simplification of the situation in vivo, and quantitative comparisons should be treated with caution.

**The triose phosphate translocator**

The behaviour of potato plants has been reported in which TPT activity was reduced to between 70% and 100% of the wild type, again by anti-sense transformation (Riesmeier et al., 1993). Plants showed marked growth retardation at 4 weeks, but there was little difference in the gross phenotypes of mature plants. In particular, there was no reduction in tuber yield. The effect of the transformation upon photosynthetic metabolism was examined in extracted chloroplasts. Reduction of TPT activity resulted in the maximum assimilation rate, suggesting an approximate value for C₃ TPT of ~0.5, although the wide confidence limits on the data (Riesmeier et al., 1993) mean that C₃ TPT could be much greater, and that these particular data should be interpreted with some caution.

At ambient conditions, decreased TPT expression resulted in a large increase in the rate of starch synthesis, without a significant impact on assimilation, from which a value for C₃ TPT of ~2.0 can be calculated. This compares with values ~1.0 for positive J₃SBPase in the model. Both sense and anti-sense transformation has been used on tobacco plants to obtain plants with TPT activity ranging between 20% and 300% of the wild type (Gray et al., 1995). Quantitatively the results were much less dramatic than those of Riesmeier (Riesmeier et al., 1993). Qualitatively, their results are consistent with both with Riesmeier (Riesmeier et al., 1993) and this model: under ambient conditions C₃ TPT is negligible, C₃ SBPase positive and C₃ TPT negative.

**Sedoheptulose-1,7-bisphosphatase**

One of the more unexpected results from the model is the very high control of J₃assim exerted by SBPase. It is an enzyme rarely mentioned in the research literature, and so the domination of assimilation by such an obscure enzyme would appear, to be anomalous. Recent work (Harrison et al., 1998; Raines et al., personal communication) provides strong evidence that SBPase does indeed have high control over J₃assim. The SBPase gene was anti-sensed, and introduced into Nicotiana to produce plants with levels of SBPase activity ranging from as low as 7% to 100% of wild type. In common with the anti-sense work described above, plants with small (in this case ~40%wt) activity had rather severe phenotypes, typified by low content of chlorophyll and stunted growth. Much more unusual is the fact that there were detectable reductions in J₃assim in plants with only a modest reduction in SBPase activity. The authors (Harrison et al., 1998) were kind enough to give us access to some prepublication results which were used here. This data set comes from a different set of plants than described (Harrison et al., 1998) but all experimental details are the same. The results refer to mature leaves in ambient conditions from glasshouse-grown plants. Calculating from the results reported previously (Harrison et al., 1998) leads to a slightly higher value than that described here.

If, as the model predicts, C₃SBPase really is equal to one, then the plot of J₃assim, versus SBPase will yield a curve of the form $y = mx + c$, $m = 3$ (from stoichiometric considerations $J_{3assim} = 3J_{SBPase}$), $c = 0$. In fact fitting this results in a very poor fit to this data but a much improved fit is obtained if the offset, $c$, is taken into consideration (Fig. 5) yielding values of $m = 2.9 ± 1.4$; $c = 20 ± 12$ (95% confidence interval).

Although these confidence limits are wide, the offset is significantly greater than zero. This is surprising because it implies that, were the organism otherwise viable, assimilation flux could be sustained with no SBPase activity. The conventional topology of the Calvin cycle as illustrated in Fig. 1 is such that no assimilation flux can be
sustained in the absence of SBPase, and hence the experimental evidence suggests that at least one other reaction is present that acts to ‘bypass’ SBPase. A good candidate for this extra reaction would appear to be transaldolase, as it can utilize E4P as a substrate and produces S7P, which otherwise depend upon SBPase for their consumption and production, respectively. Elementary modes analysis revealed that an assimilation route through the Calvin cycle does indeed exist in the absence of SBPase, if transaldolase is present. Furthermore, although transaldolase, under the influence of the thioredoxin mechanism, is down-regulated in the light (Anderson, 1986), its activity is only reduced to ~50% of dark activity (Anderson, 1981). Figure 5 shows the effect of the presence of transaldolase on the original model, and the relationship to experimental data. No existing parameters in the model were altered. The version of the model with transaldolase provides a reasonable fit to the experimental data, and is clearly superior to the model without it. In the former case $C_{\text{Assim}}^{\text{SBPase}} = 0.52$, which compares well with the of $0.54 \pm 0.08$ as calculated from a ln-ln plot of the experimental data (not shown).

**Relationship between Rubisco and SBPase activities**

It is possible to extend the consideration of the metabolic control analysis results obtained from the work of Raines et al. to explain the results of Stitt et al. As shown by Fell et al. (Fell et al., 1999), if Rubisco activity in the model is varied, a clear exchange of control over $J_{\text{Assim}}$ between Rubisco and SBPase is seen at Rubisco activity of about 120 $\mu$mol h$^{-1}$ mg$^{-1}$ Chl$^{-1}$, three times the SBPase activity. The transition is sharp and hence small changes in either enzyme in the region of the transition can lead to large changes in $C_{\text{Assim}}^{\text{SBPase}}$. This observation is substantially the same in the presence of transaldolase, the only difference being that the maximum value of $C_{\text{Assim}}^{\text{SBPase}}$, limited to a value lower than 1.0, depending on the assumed activity of transaldolase.

The results of Stitt (Fichtner et al., 1993; Quick et al., 1991; Stitt et al., 1991) are consistent with wild-type Rubisco activity being slightly above this point under ambient conditions, but potentially able to gain control under altered environmental conditions. It is interesting to note that Rubisco increased control under high light conditions, when it might be predicted that SBPase is more highly activated as a result of the thioredoxin system. As noted previously, an abrupt transition in the curve of $C_{\text{Assim}}^{\text{Rubisco}}$ versus Rubisco activity has been reported (Stitt et al., 1991), and furthermore it has been shown that this coincides with the point at which the potential capacity to consume RuBP diverges from the rate at which RuBP is actually consumed, consistent with a transfer of control from Rubisco to the regenerative limb of the cycle. Raines’ previously unpublished results are also consistent with Rubisco activity lying slightly above the transition point. Here SBPase is close to the limiting value of its control coefficient, and therefore flux declines linearly with decreasing SBPase. Both groups used *N. tabacum* as their experimental subject, and so some consistency between the two is to be expected.

**Conclusions**

Investigation of a model of the Calvin cycle has revealed a range of behaviour that is generally consistent with experimental observation. The speed and flexibility of this technique allows the testing of hypotheses to explain experimental observation that would be difficult, if not impossible, to test experimentally (e.g. that transaldolase activity acts to reduce $C_{\text{Assim}}^{\text{SBPase}}$).

Consideration of the flux response to $P_{\text{ext}}$, and Metabolic Control Analysis with regard to Rubisco, SBPase and TPT, indicate that the Calvin cycle behaves opportunistically: as much carbon as possible is assimilated, and that not required for immediate use in the cytosol (as signalled by $P_{\text{ext}}$) is stored as starch. The response of the model to light indicates the potential for the Calvin cycle to switch between two steady states. Such behaviour has not been widely reported *in vivo*, and its physiological significance, if any, is not known. However, the model presented here would appear to offer a reasonable starting point for any experimental design to investigate this phenomenon. The major emphasis in this paper has been upon the control of assimilation flux by the Calvin cycle. From the consideration of experimental and model data it is concluded that only two enzymes, Rubisco and SBPase, have any significant influence over this flux. It should however be emphasized that other enzymes, notably TPT, exert considerable control over the fate of carbon once assimilated: control over assimilation does not confer control over destination. On
the basis of the material presented here, it would appear that it is SBPase that has the greater control over assimilation. However, given that a sharp exchange of control over assimilation between Rubisco and SBPase can exist, different experimental results indicating a large degree of control for the two enzymes are not necessarily inconsistent with one another.

Scope for 'improving' the Calvin cycle

A traditional view of metabolic control via rate-limiting steps, suggests that in order to increase flux through a pathway it is sufficient simply to increase the activity of the relevant rate limiting step. In contrast, MCA orthodoxy holds that, in general, rate-limiting steps do not exist, and that control of flux is shared amongst all enzymes on a pathway. If the modelling and experimental results discussed here are correct, it would appear that the Calvin cycle does in fact possess enzymes that behave as rate limiting (i.e. $C^I \approx 1.0$) for $J_{\text{Assim}}$. However, consideration of the same results leads to three lines of reasoning suggesting that simply increasing the activity of these steps is unlikely to be of any great benefit.

(1) The exchange of $C^{\text{Assim}}$ between Rubisco and SBPase, described previously (Fell et al., 1999), implies that a probable effect of increasing one of the rate-limiting steps over assimilation is simply to transfer control to the other with little or no net increase in assimilation.

(2) Any increase in potential carbon assimilation must be matched by a concomitant increase in the capacity of the light reactions to supply ATP, or the system will switch to the slow steady-state. If mechanisms do exist to prevent such switching, this can only be achieved by limiting ATP consumption, and hence assimilation.

(3) Even if an increase in assimilation could be brought about, it has been seen that those enzymes which have high $C^{\text{Assim}}$ also tend to have high $C^{\text{Starch}}$ and low, even negative, $C^{\text{VPR}}$. Thus the consequence of increasing assimilation flux might simply be to increase leaf starch. This point has been raised already (Geiger and Servaites, 1994), although on the basis of different reasoning.

Thus the results show that it is possible to build and investigate realistic models of the Calvin cycle, capable of predicting, at least semi-quantitatively, likely effects of GM intervention. It appears unlikely that the manipulation of any single enzyme will lead to useful improvement in the target organism.

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References


Fichtner K, Quick WP, Shulze ED, Mooney HA, Rodermel SR, Fichtner K, Quick WP, Shulze ED, Mooney HA, Rodermel SR, et al., 1999. Interactions of different regulatory metabolites in the Calvin cycle do in fact possess enzymes that behave as rate limiting (i.e. $C^I \approx 1.0$) for $J_{\text{Assim}}$. However, consideration of the same results leads to three lines of reasoning suggesting that simply increasing the activity of these steps is unlikely to be of any great benefit.

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