37 Modelling and experimental evidence for two separate steady states in the photosynthetic Calvin cycle

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Introduction

We have recently been investigating the behaviour of a detailed kinetic model of the Calvin cycle [1–3]. The model was based on that described by Pettersson and Ryde-Pettersson [4], with the major modification that no assumption was made that fast reversible reaction would remain at equilibrium ; all reactions were assigned explicit rate equations. The model was implemented using the SCAMP program [5], and its behaviour investigated with the Scampi application programmers interface [1]. A schematic of the model is shown in figure 37.1. Full details of the model can be found in [1] (available free from http://bms-mudshark.brookes.ac.uk/mark/thesis.pdf).

The major findings (relevant here) from this investigation were as follows:

1. The model can exhibit two steady states, and may be induced switch reversibly between the two, by adjusting the parameters representing the external P_i concentration, or the activity of the light reactions[1] . The two states were dubbed “Fast” and “Slow” reflecting the relative CO_2 assimilation rates. We assume that under most *in vivo* circumstances, the fast state is the more likely, as this attains a higher assimilation rate for the same protein investment.

2. In the fast steady state, control of assimilation, $C^{J_{Assim.}}$, is dominated by either rubisco or sedoheptulose bisphosphatase (SBPase), depending on the

[1]The light reactions were considerably simplified in the model, and assumed to simply regenerate ATP from ADP and P_i . In the context of quantitative discussion in this paper “Light” refers to the activity of this reaction.

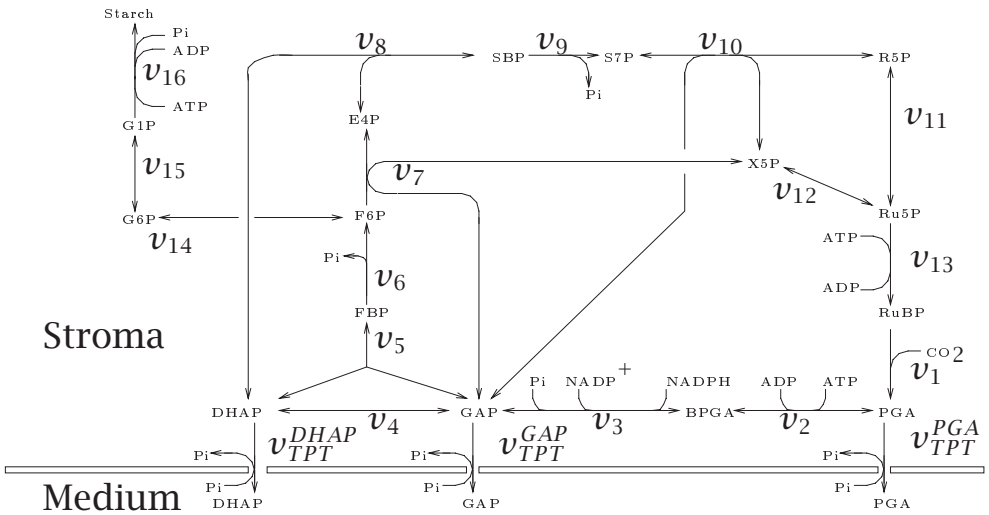

Fig. 37.1 Reaction schematic of the Calvin Cycle model. NADP/H and H^+ were regarded as fixed, and the light reactions (not shown) regenerate ATP from ADP and P_i.

Table 37.1 Reaction subscripts, abbreviations, and names, used in the Calvin cycle model of figure 37.1.

Subscript	Abbreviation	Name
1	Rubisco	Ribulose bisphosphate carboxylase-oxidase
2	PGK	Phosphoglycerate kinase
3	G3Pdh	Glyceraldehyde-3-phosphate dehydrogenase
4	TPI	Triose phosphate isomerase
5	-	Aldolase (FBP reaction)
6	FBPase	Fructose-1,6-bisphosphatase
7	TKL	Transketolase (F6P reaction)
8	-	Aldolase (SBP reaction)
9	SBPase	Sedoheptulose bisphosphatase
10	TKL	Transketolase (S7P reaction)
11	R5Piso	Ribose-5-phosphate isomerase
12	X5Pepi	Xylose-5-phosphate epimerase
13	Ru5Pk	Ribulose-5-phosphate kinase
14	PGI	Phosphoglucose isomerase
15	PGM	Phosphoglucose mutase
16	StSyn	Starch Synthase
TPT	TPT	Triose phosphate translocator (Superscript indicates metabolite)

ratio of their activities. In the model as described, no other step can exert significant control over assimilation.

3. The behaviour of the model in the slow steady-state is entirely different to that in the fast. In particular $C^{J_{Assim.}}_{SBPase}$ and $C^{J_{Assim.}}_{rubisco}$ become negative in the slow steady-state.

4. If transaldolase, more commonly considered a part of the oxidative pentose phosphate pathway but never the less present in the stroma [6], is included in the model, the effect is to reduce $C^{J_{Assim.}}_{SBPase}$, without significantly effecting $C^{J_{Assim.}}_{rubisco}$.

The results obtained from the model in the fast steady-state are in reasonable agreement with experimental observations of both whole plants and isolated chloroplasts (see [1] for detailed comparison and discussion), but there has been no[2] direct experimental evidence for the existence of two steady-states in the Calvin cycle, until now.

Method

Experimental

Harrison *et al.* [8] introduced an anti-sense SBPase gene into *N. tabacum* to produce plants with SBPase activities ranging from ~ 30 to ~ 90% wild type (wt). Although these authors did not use metabolic control analysis, calculating the slope of a log-log plot of SBPase activity vs. assimilation in fully expanded leaves, under light saturating conditions, yields a value of $C^{J_{Assim.}}_{SBPase}$ close to 1.0 (> 0.9). More recently these workers (personal communication) have extended their work to investigate the effect of reduced SBPase activity on assimilation in leaves at various stages of development. Plants were grown in the greenhouse until 23 leaves were produced. At this point in vivo measurements of CO_2 assimilation rates were made on alternate leaves, including young expanding leaves (Leaves 16 and 14) new fully expanded leaves (12 and 10) and a mature, fully expanded leaf (8). Immediately following photosynthetic measurements, leaf tissue was harvested directly into liquid N_2 for SBPase activity assays, chlorophyll and carbohydrate measurements. Other experimental details are as reported in [8].

Data analysis

Confidence limits were calculated using a Monte Carlo approach, similar to that advocated by Ainscow and Brand [9] by repeatedly calculating $C^{J_{Assim.}}_{SBPase}$, drawing

[2]Laisk *et al.* [7] cite a Russian language publication, which reports a discontinuous response of photosynthesis to light intensity in lilac leaves.

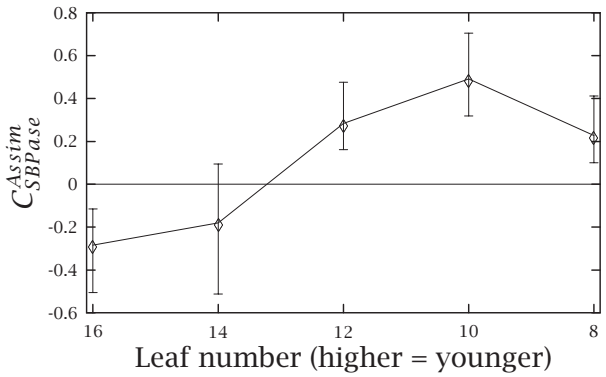

Fig. 37.2 Median $C^{J_{Assim.}}_{SBPase}$ as a function of leaf position, in a series of transgenic plants. Leaves were counted from the base of the plant, and so younger leaves have higher position numbers. The limits of the error bars represent the upper and lower 2.5 %ile limits, calculated by Monte-Carlo simulation, as described in the body of the text.

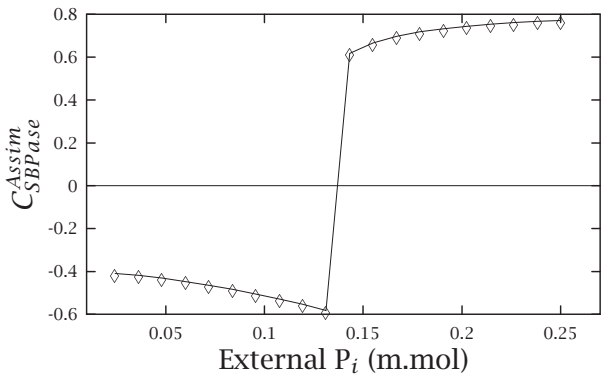

Fig. 37.3 $C^{J_{Assim.}}_{SBPase}$ as a function of $P_{i_{ext}}$ in a detailed kinetic model of the Calvin cycle.

values of $J_{\text{Assim.}}$ and SBPase from a set of normally distributed random numbers, $N(\mu, \sigma)$, with μ equal to the mean observation, and σ to the standard error of that observation. As it was not possible to take replicate measurements of assimilation flux in the transformed plants, we have assumed that the SE for these observations is equal to that of the wild type. 2500 repeated calculations were made, using a program constructed for the purpose.

Results and discussion

At levels of SBPase expression below 50% wt, no clear relationship between SBPase activity and carbon assimilation was seen. The reasons for this are not known at present, although it is possibly the result of multiple anti-sense insertions generating otherwise undetected pleiotropic effects. Regardless of the cause, data from plants whose SBPase activity was less than 50% wt is excluded from the analysis

presented here, as metabolic control analysis only considers the effects of small changes in enzyme activity, over ranges in which the response is, at least approximately, linear. Values of $C^{J_{Assim.}}_{SBPase}$ for the remaining data were calculated as the slope of a log – log plot of the data. Figure 37.2 shows $C^{J_{Assim.}}_{SBPase}$ as a function of leaf number. In the fully expanded leaves (8, 10 and 12) $C^{J_{Assim.}}_{SBPase}$ has a median value of ~ 0.5. Although this value is lower than that calculated from the results in [8], and from other unpublished data from those authors, it is still much greater than the value that Stitt *et al.* [10] obtained for $C^{J_{Assim.}}_{Rubisco}$ of ~ 0.1 (Rubisco being frequently described as the "rate limiting step" for CO_2 assimilation).

Figure 37.3 showed the effect of changing $P_{i_{ext}}$ upon $C^{J_{Assim.}}_{SBPase}$, in the model with some transaldolase activity (half that of the SBPase activity). At relatively high $P_{i_{ext}}$ $C^{J_{Assim.}}_{SBPase}$ falls into the range 0.7–0.8, but at a critical point (the exact value of which is sensitive to a number of other parameters) $C^{J_{Assim.}}_{SBPase}$ becomes negative, with values in the range -0.4– -0.5. As neither external TP or PGA were included in the model, $P_{i_{ext}}$ represents cytosolic carbon demand, and one can predict that, *in vivo,* increasing the concentration of either of these will have a qualitatively similar effect to decreasing $P_{i_{ext}}$ concentration. Now, the leaves in this study with negative $C^{J_{Assim.}}_{SBPase}$ were young, expanding leaves, presumably acting as carbon sinks. If this is the case, then the carbon demand (at least in the form of TP and PGA) on the chloroplast would be low. Thus, not only does an initial inspection of experimental results support the hypothesis that young leaves are in the slow steady state, while fully expanded leaves are in the fast, the conditions under which the two states prevail are also consistent with the behaviour of the model: i.e., the slow steady state is associated with low cytosolic carbon demand upon the Calvin cycle, and the fast with high.

Conclusion

We have presented evidence that the potential for the Calvin cycle to switch between two states observed in a detailed kinetic model, also exists *in vivo*, although more work is required to compare other model behaviour with that *in vivo* to confirm that the two effects are indeed the same. Although the precise physiological significance of the effect, should it be confirmed, is not clear, it remains interesting to observe that metabolic systems can exhibit behaviour far richer than the simple monotonic responses that we so often assume to be the case.

Acknowledgements

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