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Dependence of the Calvin cycle activity on kinetic parameters for the interaction of non-equilibrium cycle enzymes with their substrates

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Kinetic model studies and control analyses of the Calvin photosynthesis cycle have been performed to characterize the dependence of the cycle activity on maximum velocities and K_m values for the interaction of the non-equilibrium cycle enzymes and ATP synthetase with their substrates under conditions of light and carbon dioxide saturation. The results show that K_m values have no major influence on the cycle activity at optimal concentrations of external orthophosphate. The maximum cycle activity is controlled mainly by the catalytic capacities of ATP synthetase and sedoheptulose-bisphosphatase, and is close to the maximum cycle flux that can be supported by these two enzymes.

The Calvin cycle for photosynthetic carbohydrate formation plays a critical role in agricultural productivity and is of outstanding importance for life on earth in general. Considerable research, therefore, has been directed towards the biological regulation of this metabolic pathway [1–4]. Such research has provided valuable information on a variety of factors that may control and ultimately limit the cycle activity, but a deeper insight into the regulatory mechanisms that actually apply has been hampered by the extreme kinetic complexity of the reaction system; the Calvin cycle involves 13 enzymes acting on 16 metabolites in an intricate network of reactions, and is dependent on input processes providing the system with ATP and NADPH as well as on output steps withdrawing photosynthetic products from the reaction cycle. The dynamic and regulatory properties of such a complex system cannot be reliably established by intuitive reasoning, but require detailed analysis and characterization by mathematical modelling.

We have recently presented a kinetic model for the Calvin photosynthesis cycle and ancillary pathway of starch production in the chloroplast of C_3 plants under conditions of light and carbon dioxide saturation [5]. This model is based on experimentally documented rate equations for all enzymically catalysed non-equilibrium steps in the reaction system and has been shown to provide a most satisfactory description of certain experimentally observed steady-state characteristics of the photosynthetic process of carbohydrate formation. Despite extensive use of simplifying approximations, the model is rather complex and describes the rate behaviour of the Calvin cycle as a function of more than 50 different reaction parameters. Such complexity may be a drawback in certain contexts, but increases the reliability and utility of the model from an analytical point of view. Examination of the parameter dependence of a model that is sufficiently elaborated to account for all major factors of potential regulatory interest should lead to a substantially deepened understanding of the actual operation and control of the modelled biological system.

Most of the parameters considered in our model represent kinetic or equilibrium constants for enzymically catalysed steps of the examined reaction system. In particular, the model includes kinetic parameters for the interaction of substrates, products, and inhibitors with the four non-equilibrium enzymes of the Calvin cycle (ribulose-bisphosphate carboxylase, fructose-bisphosphatase, sedoheptulose-bisphosphatase and ribulose-phosphate kinase). We have previously reported parameter-dependence data which indicate that effects of products and inhibitors on the activity of the latter enzymes are of no major regulatory significance under physiological conditions [6]. The present investigation was undertaken to characterize the dependence of the Calvin cycle activity on kinetic parameters for the interaction of the non-equilibrium cycle enzymes with their substrates. In particular, we have attempted to obtain information with bearing on the much-discussed question as to what enzymes and kinetic factors may limit the Calvin cycle activity under optimal environmental conditions.

METHODS

We have previously described in detail a kinetic model for photosynthetic carbohydrate formation in the chloroplast of C_3 plants [5]. This model considers the 13 enzymically catalysed steps of the reductive pentose phosphate pathway (the Calvin cycle) and treats ATP synthesis as a system-dependent input step. Starch production within the chloroplast and export of photosynthetic products to the external reaction medium are included as output processes. The model defines the time-dependence of the concentration of 18 stromal metabolites (13 phosphorylated carbohydrates representing Calvin cycle intermediates, glucose 6-phosphate, glucose 1-phosphate, orthophosphate, ATP, and ADP) as a function of various parameters, including maximum velocities (V) and K_m values for the interaction of different substrates with non-

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Enzymes. Fructose-bisphosphatase (EC 3.1.3.11); sedoheptulose bisphosphatase (EC 3.1.3.37); ribulose-5-phosphate kinase (EC 2.7.1.19); ribulose-bisphosphate carboxylase (EC 4.1.1.39).

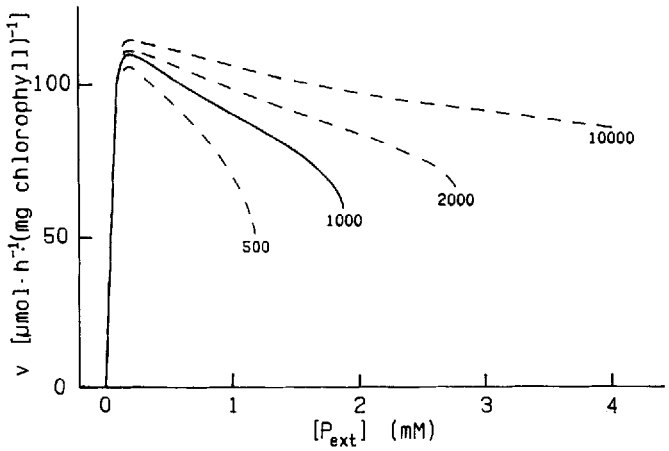

Fig. 1. *Effect of enzyme maximum velocities on the orthophosphate load characteristic.* Calvin cycle activity (v) as a function of the concentration of external orthophosphate, [P_{ext}], calculated using previously reported [5] realistic parameter estimates (full curve). Dashed curves were obtained following modification of the maximum velocity of ribulose-phosphate kinase to the values (μmol substrate consumed · mg chlorophyll^{-1} · h^{-1}) indicated in the figure

equilibrium enzymes in the reaction system. For given values of these parameters, steady-state concentrations of all concentration variables and the corresponding rate of photosynthetic carbon dioxide fixation (v) become determined by the model, which can be applied also for calculation of flux control coefficients C defined [7] by

$$C_V^v = \frac{\partial \ln v}{\partial \ln V} \quad (1)$$

$$C_{K_m}^v = \frac{\partial \ln v}{\partial \ln K_m}. \quad (2)$$

Such control coefficients provide dimensionless measures of the change in cycle activity caused by variation of the magnitude of a certain kinetic parameter (V or K_m) and may be used for quantitative evaluation of the control exerted by individual enzymes and substrates in the reaction system under specified conditions. They were determined by numerical differentiation of v with regard to one parameter at fixed values of the others.

Unless otherwise stated, all data reported below were calculated by application of the above kinetic model using our previously detailed estimates of kinetic constants and other parameters of the model [5]. These parameter estimates have been selected to be representative for the operation of isolated chloroplasts under conditions of light and carbon dioxide saturation in a reaction medium, pH 7.6, containing orthophosphate as the only metabolite that may imported into the chloroplast in exchange for the export of photosynthetic products.

RESULTS

Effect of enzyme maximum velocities on the orthophosphate load characteristic

Fig. 1 (full curve) shows the dependence of the steady-state Calvin cycle activity (v) on the concentration of external orthophosphate (P_{ext}), as predicted by our kinetic model for photosynthetic carbohydrate formation in the chloroplast of

Table 1. *Enzyme control of the optimal Calvin cycle activity*
Flux control coefficients C_V^v for non-equilibrium enzymes considered in the model, referring to the external orthophosphate concentration (0.18 mM) yielding maximum cycle activity. Data calculated using the parameter estimates reported previously [5]

Enzyme	C_V^v
Ribulose-bisphosphate carboxylase	0.00
Fructose-bisphosphatase	−0.02
Sedoheptulose-bisphosphatase	0.33
Ribulose-phosphate kinase	0.04
ATP synthetase	0.66
Phosphate translocator	0.00
ADPglucose pyrophosphorylase	−0.02

C_3 plants. This theoretical 'load characteristic' [8] of the reaction system adequately reproduces that observed experimentally [5] and illustrates that the cycle activity exhibits a maximum of slightly more than 100 rate units (μmol carbon dioxide fixed · mg chlorophyll^{-1} · h^{-1}) at an external orthophosphate concentration of about 0.2 mM. When [P_{ext}] exceeds a critical value of about 1.9 mM (corresponding mathematically to a bifurcation point [9]), the reaction system cannot operate at a steady state according to the model but will exhibit overload breakdown; due to the stimulatory effect of external orthophosphate on the transport capacity of the phosphate translocator [10], an excessive rate of export of photosynthetic products will develop such that the chloroplast becomes deprived of Calvin cycle intermediates and the cycle activity approaches zero without reaching a steady state.

Dashed curves in Fig. 1 indicate how the orthophosphate load characteristic depends on the maximum velocity (V_{13}) for ribulose-5-phosphate kinase, one of the four non-equilibrium cycle enzymes. A decrease in magnitude of V_{13} by a factor of two destabilizes the reaction system such that the value of [P_{ext}] at the bifurcation point is lowered from 1.9 mM to 1.2 mM. Increases in magnitude of V_{13} have the opposite effect of stabilizing the reaction system and raising the value of [P_{ext}] at the bifurcation point. Analogous dependences of the load characteristic on the maximum velocity were observed for the remaining three non-equilibrium cycle enzymes, as well as for ATP synthetase. In the case of ribulose-1,5-bisphosphate carboxylase, effects caused by a certain change of the maximum velocity were found to be much less pronounced than with the other enzymes.

The parameter-dependence pattern indicated by data in Fig. 1 can be well understood in view of our previous analyses of the interplay between output and recycling of metabolites in reaction cycles leading to net synthesis of a cycle intermediate [5, 11, 12]. Overload breakdown in the Calvin cycle may occur when the catalytic capacity of the system for photosynthetic product output exceeds that for cyclic generation of these products. Since the latter capacity will be decreased on lowering of the maximum velocity for one of the cycle enzymes or for the enzyme providing the reaction system with ATP, such parameter changes will render the system more susceptible to overload breakdown.

Effect of enzyme maximum velocities on the maximum cycle activity

Table 1 lists flux control coefficients C_V^v for all non-equilibrium enzymes considered in the model, referring to the concentration (0.2 mM) of external orthophosphate where the Calvin

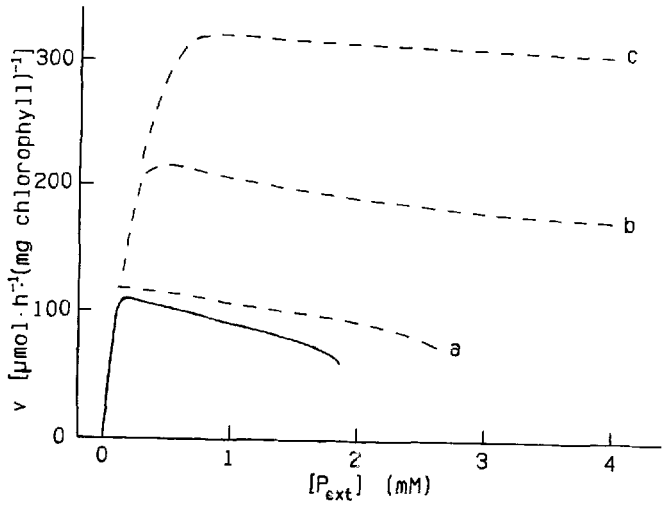

Fig. 2. *Effect of enzyme maximum velocities on the orthophosphate load characteristic.* Calvin cycle activity (v) as a function of the concentration of external orthophosphate, $[P_{ext}]$, calculated using previously reported [5] realistic parameter estimates (full curve) and estimates modified as described in Table 3, a–c (dashed curves)

Table 2. *Effect of enzyme maximum velocities on the optimal Calvin cycle activity*
Peak cycle activities predicted by the kinetic model following an increase of the maximum velocity for the indicated enzymes by a factor of two

Modified enzyme	Peak activity
	μmol CO_2 fixed · mg chlorophyll^{-1} · h^{-1}
None	110
Ribulose-bisphosphate carboxylase	112
Fructose-bisphosphatase	108
Sedoheptulose-bisphosphatase	116
Ribulose-phosphate kinase	111
ATP synthetase	119

Table 3. *Enzyme control of the optimal Calvin cycle activity*
Flux control coefficients C_V^v for non-equilibrium enzymes considered in the model, referring to the external orthophosphate concentration (a, 0.05 mM; b, 0.45 mM; c, 0.88 mM) yielding maximum cycle activity. Data calculated using parameter estimates as in Table 1, except that maximum velocities of ATP synthetase and sedoheptulose bisphosphatase were multiplied by factors of (a) 2 and 1, (b) 2 and 2, (c) 4 and 4, respectively

Enzyme	C_V^v		
	a	b	c
Ribulose-bisphosphate carboxylase	0.00	0.04	0.84
Fructose-bisphosphatase	0.00	−0.02	0.00
Sedoheptulose-bisphosphatase	0.90	0.28	0.03
Ribulose-phosphate kinase	0.00	0.06	0.07
ATP synthetase	0.05	0.65	0.07
Phosphate translocator	0.02	0.00	0.00
ADPglucose pyrophosphorylase	0.03	−0.01	0.00

Table 4. *Catalytic capacities of non-equilibrium enzymes in the reaction system*
Maximum velocity parameters (V) for non-equilibrium enzymes considered in the model and the maximum Calvin cycle flux in C_1 equivalents that the respective enzyme can support according to previously derived [5] relationships

Enzyme	V	Maximum flux
	μmol · mg chlorophyll^{-1} · h^{-1}	
Ribulose-bisphosphate carboxylase	340	340
Fructose-bisphosphatase	200	600
Sedoheptulose-bisphosphatase	40	120
Ribulose-phosphate kinase	1000	1000
ATP synthetase	350	117
Phosphate translocator	250	
ADPglucose pyrophosphorylase	40	990

cycle shows maximum activity. Inspection of the data shows that the control coefficient for ribulose-phosphate kinase is much less than unity, which means that the latter enzyme does not exert any significant flux control under the examined conditions. This explains the observation in Fig. 1 that even a 10-fold increase of the maximum velocity for ribulose-phosphate kinase has no major effect on the maximum Calvin cycle activity that may obtain.

According to data in Table 1, the optimal Calvin cycle flux is controlled mainly by ATP synthetase. The magnitude (0.66) of the corresponding control coefficient might seem to suggest that the peak value of the Calvin cycle activity should increase considerably when the maximum velocity for ATP synthetase is doubled. Results in Fig. 2 (curve a) establish that such is not the case. The reaction fluxes actually predicted by the model following a twofold increase of the maximum velocity for different non-equilibrium enzymes in the reaction system are given in Table 2 and invariably correspond to peak activity changes less than 10%. The maximum Calvin cycle activity obviously cannot be drastically enhanced by increasing the catalytic capacity of anyone of the individual enzymes participating in the cyclic process of photosynthetic carbohydrate production. Data in Table 1 point to a plausible explanation for that somewhat unexpected observation by showing that significant control of the maximum Calvin cycle activity is exerted not only by ATP synthetase, but also by sedoheptulose-bisphosphatase; following an increase of the maximum velocity for the former enzyme by a factor of two, the peak cycle activity might remain essentially unaffected due the rate-limitation contributed by the latter enzyme. Control data given in Table 3 (column a) confirm that such is actually the case by establishing that main flux control under such conditions is shifted from ATP synthetase to sedoheptulose bisphosphatase. The reason for this becomes evident on examination of Table 4, which lists the estimates of enzyme maximum velocities used in the model calculations, as well as the maximum cycle activities that can be supported by the individual enzymes according to these estimates and the steady-state kinetic solution for the reaction system [5]. The maximum velocities assigned to ATP synthetase ($V_{16} = 350$ rate units) and sedoheptulose-bisphosphatase ($V_9 = 40$ rate units) imply that these enzymes cannot support cyclic carbon dioxide fixation at a steady-state rate higher than $350/3 = 117$ and $3 \times 40 = 120$ rate units, respectively. Elimination of the former rate limitation is not sufficient to drastically change the rate behaviour of the reaction system, but merely shifts

main flux control to an enzymic step showing a closely similar rate limitation.

Increasing both V_{16} and V_9 by a factor of two, one obtains the load characteristic indicated by curve b in Fig 2. The Calvin cycle peak activity is drastically enhanced (almost doubled), which indicates that rate limitations contributed by other enzyme maximum velocities still remain insignificant. This is confirmed by the control data given in Table 3 (column b) and suggests that a main picture of the enzyme control of the peak cycle activity can be obtained merely by examination of the maximum cycle fluxes that the different enzymes can support. According to that view and data in Table 4, an increase of V_{16} and V_9 by a factor of four (i.e. to values supporting a maximum flux exceeding 460 rate units) would be expected to cause additional enhancement of the peak cycle activity, but not to a value exceeding 340 rate units which represents the rate limitation provided by the maximum velocity of ribulose-bisphosphate carboxylase. Concomitantly, main flux control should be shifted to the carboxylase. The former expectations are confirmed by the results in Fig. 2 (curve c), and the latter by control data given in Table 3 (column c).

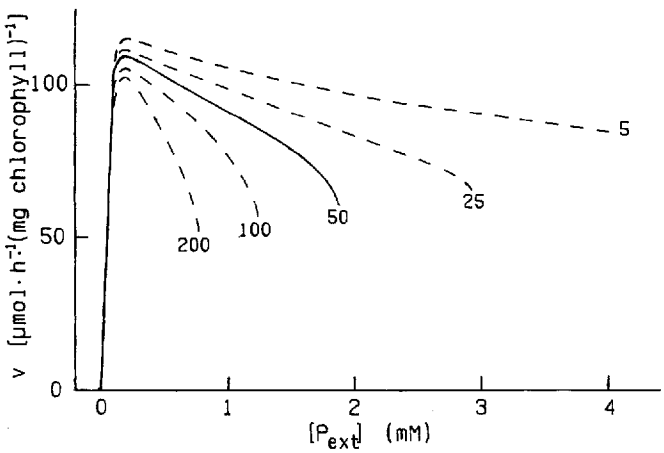

Fig. 3. *Effect of enzyme* K_m *values on the orthophosphate load characteristic.* Calvin cycle activity (v) as a function of the concentration of external orthophosphate, [P_{ext}], calculated using previously reported [5] realistic parameter estimates (full curve). Dashed curves were obtained following modification of K_m for the action of ribulose-phosphate kinase on ribulose 5-phosphate to the values (μM) indicated in the figure

Effect of K_m *values on the orthophosphate load characteristic*

The dependence of the orthophosphate load characteristic on $K_m$values for individual substrates of the non-equilibrium enzymes was found to be analogous to (and can be rationalized in analogous terms as) the parameter dependence indicated by data in Fig. 1. Decreasing $K_m$values (which increase the catalytic capacity of the respective enzyme at any fixed substrate level) stabilize the reaction system and extend the range of external orthophosphate concentrations over which the system may operate in a steady state, whereas increasing $K_m$values have the opposite effect. This is illustrated in Fig. 3 by example of the typical results obtained on variation of the $K_m$value for ribulose 5-phosphate in its interaction with ribulose-phosphate kinase.

Effects of $K_m$values for reactions catalysed by other non-equilibrium enzymes on the maximum value of the Calvin cycle activity were similar to, or less pronounced than, those indicated in Fig. 3. This is documented by the results in Table 5, column a, which show that control coefficients $C^v_{K_m}$ for the peak cycle flux are of insignificant magnitude for all of the enzyme-substrate interactions examined. Table 5 also includes control data which indicate that the lack of strong influence of K_m values on the peak cycle activity is a characteristic which is present irrespective of what enzyme maximum velocity is assumed to provide the main rate-limiting contribution.

DISCUSSION

Photosynthetic production at the highest possible rate permitted by the environmental conditions would appear to be a primary physiological function of the Calvin photosynthesis cycle. Fersht [13] and Crowley [14] have discussed the effect of natural selection on enzymic catalysis in systems where the achievement of high reaction fluxes has been a major evolutionary objective. The possibility was considered that evolution under such conditions may tend to increase both the maximum velocity and the K_m value for an enzyme conforming to Michaelis-Menten kinetics, so that K_m becomes large in comparison to the physiological substrate concentration. This would imply that enzymes participating in metabolic pathways such as the Calvin cycle should tend to exhibit maximum activities greatly exceeding the physiological reaction fluxes they are designed to support.

The present investigation lends no support to that idea, but indicates that the Calvin photosynthesis cycle under optimal environmental conditions operates at a rate close to the maximum one that can be supported by ATP synthetase and sedoheptulose-bisphosphatase. This is consistent with previously reported experimental [15–18] and theoretical [5] evidence showing that stromal concentrations of ATP, orthophosphate, and sedoheptulose 1,6-bisphosphate under conditions of saturating light greatly exceed the K_m values for their interaction with ATP synthetase and sedoheptulose-bisphosphatase. Furthermore, the typical data in Fig. 3 and Table 5 indicate that the parameter dependence of the examined reaction system is such that increasing K_m values (if changed independently of V values) will tend to decrease rather than increase the cycle activity.

As illustrated by the results in Figs 1 and 2, an evolutionary demand for higher cycle fluxes can best be met by increasing the maximum velocity (i.e. the turnover number and/or concentration) of the enzyme which exerts main control of the optimal cycle activity. Data in Tables 1 and 3 suggest that the latter enzyme may be identified simply as the one showing the lowest maximum velocity as expressed in terms of the maximum photosynthetic flux of one-carbon equivalents it can support. The present conclusion that ATP synthetase and sedoheptulose-bisphosphatase provide main rate-limiting contributions under optimal reaction conditions, therefore, is critically dependent on the validity of the maximum velocities assigned to different enzymes in Table 4 and might have to be modified if these estimates should turn out to be unrealistic.

Particular attention should be drawn in that respect to the maximum velocity of ATP synthetase; literature data are available [19, 20] which suggest that the light-saturation value of this parameter may be significantly higher than the value now used (350 rate units [21, 22]). The assumption that ATP synthetase exhibits a value of 500 rate units or higher would imply that the optimal Calvin cycle activity is regulated almost

exclusively by sedoheptulose-bisphosphatase ($C_V^v \approx 1$). However that may be, the low maximum-velocity estimate used for the latter enzyme appears to be well documented [17] and sufficiently precise to justify the conclusion that sedoheptulose-bisphosphatase represents the only Calvin cycle enzyme which may exert significant control of the cycle activity under optimal environmental conditions.

It should be emphasized, however, that the term optimal in this context not only refers to the external concentration of orthophosphate, but also to the concentration of carbon dioxide and the light intensity; all data now reported have been calculated by application of a model which has been designed to describe photosynthetic carbohydrate formation under conditions of saturating light and carbon dioxide. Ribulose-bisphosphate carboxylase may well gain predominant control at non-saturating levels of carbon dioxide, and low light intensities could drastically change the entire control situation by modification of the kinetic properties of ATP synthetase and the light-activated cycle enzymes. Characterization of such non-saturation effects would require more elaborate models than the one now applied and is beyond the scope of the present work.

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