# Effects of metabolite binding to ribulosebisphosphate carboxylase on the activity of the Calvin photosynthesis cycle

Pettersson, Gösta; Ryde-Pettersson, Ulf

*Published in:*
European Journal of Biochemistry

*DOI:*
10.1111/j.1432-1033.1988.tb14382.x

1988

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

Eur. J. Biochem. 177, 351 – 355 (1988)


# Effects of metabolite binding to ribulosebisphosphate carboxylase on the activity of the Calvin photosynthesis cycle

Gösta PETTERSSON and Ulf RYDE-PETTERSSON
Avdelningen för Biokemi, Kemicentrum, Lunds Universitet, Lund

(Received May 11, 1988) – EJB 88 0539

1. The regulatory implications of the interaction of ribulosebisphosphate carboxylase with metabolites participating in the Calvin photosynthesis cycle has been examined by control analysis based on our recently described kinetic model for photosynthetic carbohydrate formation in the chloroplast of $C_3$ plants. The results provide clear evidence that the Calvin cycle activity under conditions of light and $CO_2$ saturation is insignificantly affected by the inhibition of ribulosebisphosphate carboxylase caused by metabolites such as 3-phosphoglycerate, fructose 1,6-bisphosphate, sedoheptulose 1,7-bisphosphate, NADPH, and inorganic orthophosphate.

2. Due to the exceptionally high stromal concentration of the carboxylase, metabolite binding to the enzyme affects the Calvin cycle activity indirectly by reducing the pool of free orthophosphate and phosphorylated metabolites available for the cyclic reactions. This pool reduction corresponds typically to about 5 mM total phosphate and derives mainly from the binding of ribulose bisphosphate and orthophosphate.

3. Substantial amounts of the metabolites interacting with ribulosebisphosphate carboxylase are present in an enzyme-bound form. The bound form of the Calvin cycle intermediates sedoheptulose bisphosphate, fructose bisphosphate, and ribulose bisphosphate typically accounts for about 70, 80, and 90%, respectively, of the total stromal concentration of the intermediate.

Ribulosebisphosphate carboxylase catalyses the $CO_2$ fixation step in the Calvin photosynthesis cycle [1, 2]. The enzyme is inhibited by several metabolites which participate in the Calvin cycle reactions [3], such as 3-phosphoglycerate, fructose 1,6-bisphosphate, sedoheptulose 1,7-bisphosphate, NADPH, and inorganic orthophosphate. These inhibitory interactions could have an obvious regulatory function attributable to their direct effect on the activity of one of the non-equilibrium cycle enzymes. They might also be of indirect regulatory significance because they involve an enzyme which comprises the major portion of soluble protein in the chloroplast [1]. The stromal active-site concentration of ribulosebisphosphate carboxylase has been estimated to about 4 mM [2, 4], which greatly exceeds the concentrations of most of the Calvin cycle intermediates. This means that substantial amounts of certain cycle intermediates may be enzyme-bound rather than free in solution, with consequent effects on the steady-state cycle activity and metabolite levels.

The potential regulatory importance of the above interactions between ribulosebisphosphate carboxylase and inhibitory Calvin cycle metabolites has been generally recognized and extensively discussed [5 – 9], but no evidence is available as to the actual influence of the interactions on the Calvin cycle activity. In particular, the regulatory implications of the indirect effects of metabolite binding to the enzyme have been difficult to assess [4]. This is hardly surprising; the enzyme-bound fraction of a certain Calvin cycle intermediate will be dependent on the concentrations of all metabolites interacting with the carboxylase and hence on the state of the reaction system as a whole. The state of the system, in its turn, is fundamentally dependent on the total concentration of phosphate in the system, including that of free and enzyme-bound phosphorylated cycle intermediates. The net outcome of this complex interdependence between the dynamic state of the system and metabolite binding to the carboxylase cannot be reliably established by intuitive reasoning, but requires detailed analysis by mathematical modelling.

The present investigation provides such an analysis based on the kinetic model that we recently have put forward for description of the operation of the Calvin cycle under conditions of light and $CO_2$ saturation [10]. The examination of the indirect dynamic effects of metabolite binding to ribulosebisphosphate carboxylase is complemented by a control analysis of the regulatory significance of the direct effects on the enzyme activity of the interacting inhibitory metabolites.

## THEORY

We have previously described in detail a kinetic model for photosynthetic carbohydrate formation in the chloroplast of $C_3$ plants under conditions of light and $CO_2$ saturation [10]. The model considered the 13 enzymically catalysed steps of the reductive pentose phosphate pathway (the Calvin cycle), and treated ATP synthesis as a system-dependent input step. Starch production within the chloroplast and photosynthate export to the external reaction medium were included as output processes. The export of photosynthate was assumed to be mediated by the phosphate translocator of the chloroplast

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

*Enzyme.* Ribulosebisphosphate carboxylase (EC 4.1.1.39).

envelope [11] and to be strictly associated with a counter-import of inorganic (ortho)phosphate, such that the total concentration of phosphate within the chloroplast remains constant. Consequently, a fixed phosphate moiety conservation parameter $c_P$ was defined as the sum of concentrations of free orthophosphate, ATP, and phosphorylated carbohydrates (bisphosphates being counted twice) in the reaction system.

The phosphate moiety conservation equation thus applied to define the state of the reaction system was based on the simplifying assumption that the dynamics of the system are negligibly affected by changes in the concentrations of enzyme-bound orthophosphate and phosphorylated metabolites. In this investigation, we will examine an extended model which redefines the parameter $c_P$ as

$$c_P = c_{P\text{free}} + c_{P\text{bound}} \tag{1}$$

where $c_{P\text{bound}}$ denotes the total concentration of phosphate bound to ribulosebisphosphate carboxylase (E) in the form of binary complexes ($EL_j$) formed with ribulose 1,5-bisphosphate ($L_0$), 3-phosphoglycerate ($L_1$), fructose 1,6-bisphosphate ($L_2$), sedoheptulose 1,7-bisphosphate ($L_3$), orthophosphate ($L_4$), and NADPH ($L_5$; the latter five ligands are those of the reaction system considered which have been reported to cause significant inhibition of ribulosebisphosphate carboxylase [3]. $c_{P\text{free}}$ in Eqn (1) represents the phosphate moiety conservation parameter as defined previously [10], and $c_{P\text{bound}}$ is given by

$$\begin{aligned} c_{P\text{bound}} = {} & 2[EL_0] + [EL_1] + 2[EL_2] \\ & + 2[EL_3] + [EL_4] + [EL_5]. \end{aligned} \tag{2}$$

Assuming that the interaction of the enzyme with its substrate $L_0$ conforms to Michaelis-Menten kinetics and is competitively affected by the inhibitors $L_1, \ldots, L_5$ [3], steady-state concentrations of the different enzymic complexes will be given by

$$[EL_j] = \frac{c_E \cdot \dfrac{[L_j]}{K_j}}{1 + \sum_{n=0}^{5} \dfrac{[L_n]}{K_n}} \tag{3}$$

where $c_E$ represents the total active-site concentration of enzyme. $K_j$ stands for the apparent steady-state kinetic dissociation constant for the respectively complex, i.e. $K_0 = K_{m1}$ and $K_j = K_{i1j}, j = 1, \ldots, 5$, in the terminology of our previous paper [10].

Eqns (1–3) can be used in conjunction with the previously described kinetic model [10] to calculate, under different external conditions, the steady-state Calvin cycle activity and the corresponding concentrations of free and enzyme-bound metabolites in the reaction system. Unless otherwise stated, parameter values used for these calculations were as detailed previously [10]. The value of 3.6 mM reported by Lilley et al. [4] was chosen for the parameter $c_E$.

Control coefficients [12] providing information on the direct regulatory influence of the different inhibitors on the steady-state Calvin cycle activity ($v$) can be defined as

$$C^v_{K_j} = \frac{\delta v}{\delta K_j} \cdot \frac{K_j}{v} = \frac{\delta \ln v}{\delta \ln K_j}; j = 1, \ldots, 5 \tag{4}$$

and were calculated by numerical differentiation [10] of the steady-state solutions predicted by the kinetic models.

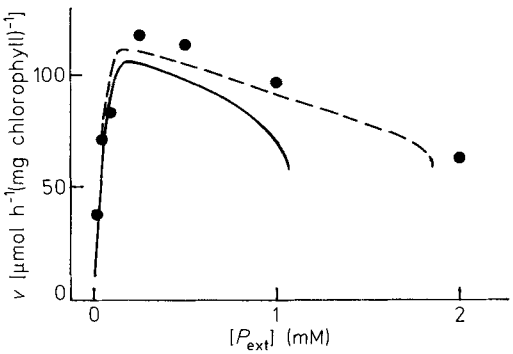


Fig. 1. *Load characteristic for the Calvin cycle.* Calvin cycle activities ($v$) calculated as a function of the concentration of external orthophosphate, $[P_{ext}]$, using our original kinetic model (dashed curve) and the extended one (full curve) based on Eqns (1–3). Reported [14] experimental data (●) are included for comparison

## RESULTS

### *Load characteristics predicted by the kinetic models*

Experiments performed with isolated chloroplasts [13–17] have established that the Calvin cycle reactions occurring in the stromal solution of the chloroplast are stimulated by low, and inhibited by high, concentrations of orthophosphate in the external reaction medium (Fig. 1). This load characteristic [18] of the reaction system has been shown to be most satisfactorily accounted for (dashed curve in Fig. 1) by our previously described kinetic model [10] when a value of 15 mM is chosen for the phosphate moiety conservation parameter $c_P$ as originally defined ($c_{P\text{bound}} = 0$). Using the same estimate of $c_P$, application of the extended model based on Eqns (1–3) gives the load characteristic indicated by the full curve in Fig. 1. Inspection of the model data shows that metabolite binding to ribulosebisphosphate carboxylase does affect the kinetic behaviour of the reaction system; the steady-state Calvin cycle activity is generally attenuated and the operation of the system destabilized such that overload breakdown [10. 18] occurs at lower external orthophosphate concentrations than in the assumed absence of metabolite binding to the enzyme. These effects must be primarily of stoichiometric origin, because the direct inhibitory action of the metabolites is equivalently considered in both models.

Fig. 2 shows the effect of the parameter $c_P$ on the load characteristic as predicted by the original kinetic model ($c_{P\text{bound}} = 0$). Increasing values of $c_P$ (i.e. increasing total concentrations of stromal phosphate) tend to stabilize the operation of the Calvin cycle such that progressively higher concentrations of external orthophosphate are required to cause overload breakdown of the reaction system. When $c_P$ exceeds about 30 mM, the system gains unlimited stability and cannot be brought to overload breakdown by increases in the concentration of external orthophosphate.

Corresponding data for the extended model are given in Fig. 3. Comparison with the results in Fig. 2 shows that load characteristics for the reaction system are analogously affected by variation of the parameter $c_P$ whether or not the system is assumed to involve a stoichiometrically significant binding of metabolites to the carboxylase; curves obtained for a certain value of $c_P$ in Fig. 3 essentially agree with those obtained for an about 5 mM lower value of $c_P$ in Fig. 2. In particular, the experimentally observed load characteristic in Fig. 1 is satisfactorily reproduced by the extended model when a value

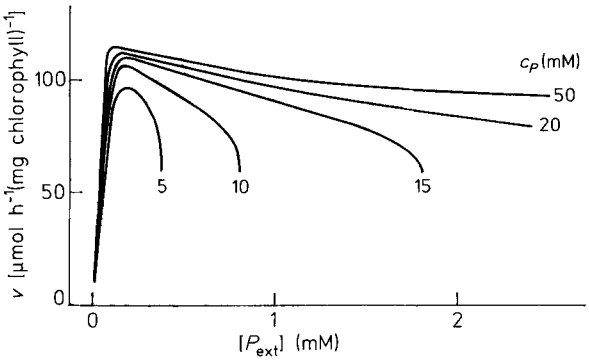


Fig. 2. *Effect of the internal phosphate pool size on the load characteristic for the Calvin cycle.* Calvin cycle activities ($v$) calculated as a function of the concentration of external orthophosphate, $[P_{ext}]$, for different values of the phosphate moiety conservation parameter $c_P$, using our original kinetic model ($c_{Pbound} = 0$)

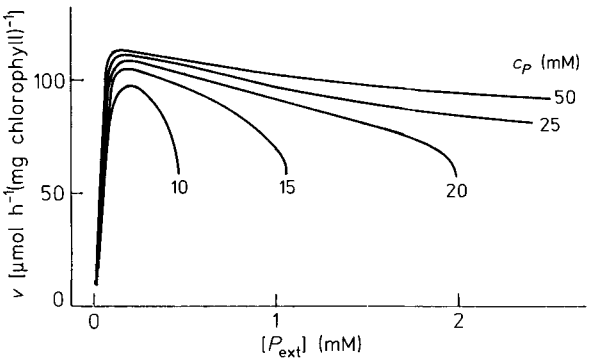


Fig. 3. *Effect of the internal phosphate pool size on the load characteristic for the Calvin cycle.* Calvin cycle activities ($v$) calculated as a function of the concentration of external orthophosphate, $[P_{ext}]$, for different values of the phosphate moiety conservation parameter $c_P$, using the extended kinetic model based on Eqns (1 – 3)

of about 20 mM is chosen for the parameter $c_P$. The latter value agrees well with that (18 – 19 mM) indicated by experimentally determined total metabolite levels in the stromal solution of illuminated chloroplasts [4]. That fact and the results in Figs 2 and 3 justify the use of a lower estimate ($c_P$ = 15 mM) on application of the original kinetic model which does not consider enzyme-bound metabolites as state variables.

*Concentrations of enzyme-bound metabolites*

Using $c_P$ = 20 mM, the extended kinetic model was applied for calculation of the steady-state concentrations of enzyme-metabolite complexes formed through the interaction of ribulosebisphosphate carboxylase with its substrate and inhibitors at different external orthophosphate concentrations. Typical results are given in Table 1. They provide clear evidence that substantial amounts of the metabolites interacting with the carboxylase are present as enzymic complexes. The substrate and most of the inhibitors are actually found to be present predominantly in the enzyme-bound form.

Table 1. *Metabolite binding to ribulosebisphosphate carboxylase*
Steady-state concentrations of enzyme-metabolite complexes predicted by the extended kinetic model based on Eqns (1 – 3), calculated for different concentrations of external orthophosphate, $[P_{ext}]$, using $c_P$ = 20 mM and $c_E$ = 3.6 mM. Figures within brackets indicate the molar fraction of the carboxylase-bound form of each metabolite

| Metabolite | Conc. at $[P_{ext}]$ = | | | |
|---|---|---|---|---|
| | 0.05 mM | 0.2 mM | 0.5 mM | 2 mM |
| | mM | | | |
| Ribulose bisphosphate | 0.59 (0.96) | 1.16 (0.89) | 1.09 (0.89) | 0.70 (0.88) |
| Phosphoglycerate | 0.94 (0.34) | 0.31 (0.16) | 0.11 (0.16) | 0.02 (0.15) |
| Fructose bisphosphate | 0.11 (0.92) | 0.12 (0.80) | 0.09 (0.80) | 0.04 (0.79) |
| Sedoheptulose bisphosphatase | 0.07 (0.85) | 0.42 (0.69) | 0.28 (0.67) | 0.07 (0.67) |
| Orthophosphate | 0.13 (0.33) | 0.92 (0.15) | 1.41 (0.15) | 2.16 (0.15) |
| NADPH | 1.32 (0.86) | 0.49 (0.70) | 0.47 (0.69) | 0.46 (0.69) |

It may, further, be concluded from data in Table 1 that the enzyme-bound fraction of the metabolites remains fairly constant over a wide range of concentrations of external orthophosphate. This means that the relative concentrations of free metabolites (and hence the fundamental dynamic behaviour of the reaction system) will remain essentially unaffected by metabolite binding to the enzyme. Such binding will provide mainly an approximately constant contribution to the total stromal content of phosphate; results in Table 1 indicate that the latter contribution corresponds to about 5 mM under conditions where the cycle activity is relatively high. This explains why load characteristics predicted by the extended model for a certain value of $c_P$ are closely similar to those predicted by the original model for about 5 mM lower values of the parameter $c_P$ (Figs 2 and 3).

*Inhibitor control of the Calvin cycle activity*

Calvin cycle flux control coefficients defined by Eqn (5) were calculated for representative concentrations of external orthophosphate using the extended kinetic model with $c_P$ = 20 mM. The typical results in Table 2 illustrate that values thus obtained are of insignificant magnitude (much less than unity) for all of the inhibition parameters considered. This means that none of the corresponding inhibitors has any significant regulatory influence on the Calvin cycle activity according to the model tested. Similar results were obtained by application of our kinetic model as originally defined ($c_P$ = 15 mM and $c_{Pbound} = 0$).

## DISCUSSION

Control coefficients defined by Eqn (4) provide standard dimensionless measures [12] of the sensitivity of the Calvin cycle activity to changes in magnitude of the inhibition constants $K_j$. Since such changes are formally equivalent to a

Table 2. *Control of the Calvin cycle activity by inhibitors interacting with ribulosebisphosphate carboxylase*
Calvin cycle flux control coefficients (Eqn 4) with regard to the inhibition parameters $K_j$, calculated for different concentrations of external orthophosphate, $[P_{ext}]$, by application of the extended kinetic model based on Eqns (1–3) using $c_P$ = 20 mM

| Parameter | Coefficients at $[P_{ext}]$ = | | | |
|---|---|---|---|---|
| | 0.05 mM | 0.2 mM | 0.5 mM | 2 mM |
| $K_1$ | 0.000 | 0.000 | 0.000 | 0.000 |
| $K_2$ | 0.000 | 0.001 | 0.001 | 0.008 |
| $K_3$ | 0.000 | 0.002 | 0.002 | 0.008 |
| $K_4$ | 0.000 | 0.001 | 0.002 | 0.049 |
| $K_5$ | 0.000 | 0.000 | 0.001 | 0.010 |

change in concentration of the respective inhibitor, it may be concluded from the results in Table 2 that the direct inhibitory effects of Calvin cycle metabolites on the catalytic activity of ribulosebisphosphate carboxylase are of insignificant interest with regard to the regulation of the Calvin cycle under conditions of light and $CO_2$ saturation. This is consistent with previous analytical results indicating that the carboxylase under such conditions contributes negligibly to the control of the steady-state cycle activity [10].

Data in Table 1 and Fig. 1, on the other hand, establish that metabolite binding to ribulosebisphosphate carboxylase affects the Calvin cycle activity indirectly by contributing significantly to the pool of total phosphate in the stromal solution of the chloroplast. Since the latter pool can be anticipated to remain constant during photosynthetic $CO_2$ fixation [11], the accumulation of enzyme-bound metabolites will tend to lower the levels of free metabolites and hence lead to Calvin cycle activities lower than those which would have been obtained if no significant amounts of enzyme-metabolite complexes were formed.

It would appear from the results in Figs 2 and 3, however, that these stoichiometric effects of metabolite binding to the carboxylase have no major influence on the basic dynamic behaviour of the reaction system. The kinetic effects indicated by data in Figs 1–3 can be rationalized in terms of an approximately constant contribution of about 5 mM from enzymic complexes to the total concentration of stromal phosphate. Provided that the magnitude of the phosphate moiety conservation parameter $c_P$ is chosen with adequate consideration of that contribution, the main dynamic features of the Calvin cycle would seem to be equally well described by our original kinetic model [10] as by the extended one now examined.

Metabolite levels in chloroplast compartments are usually determined by methods that do not distinguish between free and enzyme-bound forms of the metabolites. Lilley et al. [4] discussed the implications of this fact in relation to their measurement of the exceptionally high stromal concentration of ribulosebisphosphate carboxylase. They proposed that most of the enzyme could be present as the complex formed with its product, phosphoglycerate, such that experimentally determined total concentrations of phosphoglycerate greatly exceed those of the free product. Results in Table 1 confirm that most (typically more than 90%) of the carboxylase is in a complexed form, but also indicate that orthophosphate and the substrate ribulose bisphosphate are the stoichiometrically predominant ligands. There is certainly reason to believe that a substantial molar fraction of phosphoglycerate may be in the enzyme-bound form, but it follows from data in Table 1 that there should be even stronger reason to make a clear distinction between total and free metabolite concentrations in the case of ribulose bisphosphate, fructose bisphosphate, and sedoheptulose bisphosphate. The latter conclusion has obvious implications with regard to the mechanistic interpretation of experimentally determined (total) metabolite levels. For example, it supports the proposal of Dietz and Heber [19] that measured mass action ratios for reaction steps involving fructose bisphosphate may be biased and require correction for the carboxylase-bound amount of the metabolite.

The present investigation lends no support to the proposal of Dietz and Heber [19], however, that the Calvin cycle activity is limited mainly by ribulosebisphosphate carboxylase, even under conditions of light and $CO_2$ saturation. That proposal was based on evidence (obtained with intact leaves) showing that total stroma levels of ribulose bisphosphate are high and close to the active-site concentration of the carboxylase, observations which would seem to be of insufficient informative value for two main reasons. Firstly, it is the concentration of free substrate that is of primary interest from the point of view of regulation and rate limitation; at the observed high total levels of ribulose bisphosphate, only a small percentage (probably less than 5%) of the substrate would be expected to be free. Secondly, substrate concentrations or mass action ratios for a certain non-equilibrium step provide no reliable information on the control strength of the corresponding enzyme, particularly not in a reaction system of such dynamic complexity as the Calvin photosynthesis cycle; conclusions as to the regulatory properties of the system must be based on detailed analysis by, for instance, mathematical modelling. Our kinetic model as originally defined [10] may well be used for such purposes. In contexts where estimates of the amount of carboxylase-bound metabolites are required, the extended model now examined can be applied.

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

## REFERENCES

1. Akazawa, T. (1979) in *Enyclopaedia of plant physiology* (Gibbs, M. & Latzko, E., eds) vol. 6, pp. 208–229, Springer Verlag, Berlin.
2. Miziorko, H. M. & Lorimer, G. H. (1983) *Annu. Rev. Biochem. 52*, 507–535.
3. Badger, M. R. & Lorimer, G. H. (1981) *Biochemistry 20*, 2219–2225.
4. Lilley, R. McC., Chon, C. J., Mosbach, A. & Heldt, H. W. (1977) *Biochim. Biophys. Acta 460*, 259–272.
5. Paulsen, J. M. & Lane, M. D. (1966) *Biochemistry 5*, 2350–2357.
6. Bowes, G. & Ogren, W. L. (1972) *J. Biol. Chem. 247*, 2171–2176.
7. Rian, F.-J. & Tolbert, N. E. (1975) *J. Biol. Chem. 250*, 4234–4238.
8. McCurry, S. D., Pierce, J., Tolbert, N. E. & Orme-Johnson, W. N. (1981) *J. Biol. Chem. 256*, 6623–6628.
9. Gutteridge, S., Parry, M. A. J. & Schmidt, C. N. G. (1982) *Eur. J. Biochem. 126*, 597–602.
10. Pettersson, G. & Ryde-Pettersson, U. (1988) *Eur. J. Biochem. 175*, 661–672.
11. Fliege, R., Flügge, U.-I., Werdan, K. & Heldt, H. W. (1978) *Biochim. Biophys. Acta 502*, 232–247.

12. Burns, J. A., Cornish-Bowden, A., Groen, A. K., Heinrich, R., Kacser, H., Porteous, J. W., Rapoport, S. M., Rapoport, T. A., Stucki, J. W., Tager, J. M., Wanders, R. J. A. & Westerhoff, H. V. (1985) *Trends Biochem. Sci. 10*, 16.
13. Lilley, R. M., Schwenn, J. D. & Walker, D. A. (1973) *Biochim. Biophys. Acta 325*, 596–604.
14. Flügge, U.-I., Freisl, M. & Heldt, H. W. (1980) *Plant Physiol. 65*, 574–577.
15. Herold, A. (1980) *New Phytol. 86*, 131–144.
16. Preiss, J. (1984) *Trends Biochem. Sci. 9*, 24–27.
17. Walker, D. A. & Sivak, M. N. (1986) *Trends Biochem. Sci. 11*, 176–179.
18. Reich, J. G. & Selkov, E. E. (1981) *Energy metabolism of the cell*, Academic Press, London.
19. Dietz, K.-J. & Heber, U. (1984) *Biochim. Biophys. Acta 767*, 432–443.