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Metabolites controlling the rate of starch synthesis in the chloroplast of C_3 plants

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The extent to which different stromal metabolites affecting ADPglucose pyrophosphorylase control the rate of photosynthetic starch production in the chloroplast of C_3 plants has been examined by kinetic model studies. The results indicate that ATP, glucose 1-phosphate, 3-phosphoglycerate, fructose 6-phosphate, and orthophosphate may provide significant contributions to the starch synthesis rate changes induced by variation of the external concentration of orthophosphate, the detailed control situation being dependent on the actual concentration of the external metabolite.

Evidence has accumulated to show that the activity of the Calvin photosynthesis cycle, as well as the partitioning of photosynthate between different metabolic pathways, is regulated in response to the physiological needs of photosynthesizing cells [1–3]. A possible mechanism for such regulation is indicated by results obtained in experimental studies of isolated chloroplasts, which establish that the rates of carbon dioxide fixation and starch production within the chloroplast are dependent on the concentration in the external reaction medium of central metabolites, such as triose phosphates and inorganic (ortho)phosphate [4–6]. This dependence has been attributed to modification by the external metabolites of the photosynthate export capacity of the phosphate translocator of the chloroplast envelope [7], resulting in modified stromal levels of the metabolites that participate in the Calvin cycle reactions and affect the biosynthesis of starch. The basic validity of that explanation is strongly supported by model studies showing that experimentally observed effects of external orthophosphate on the rates of photosynthetic carbon dioxide fixation and starch production can be most satisfactorily reproduced using reported kinetic data for the influence of the external metabolite on the export capacity of the phosphate translocator [8].

Starch production in the chloroplast of C_3 plants is initiated by the action of ADPglucose pyrophosphorylase on glucose 1-phosphate and ATP [2, 9], a catalytic step which is known to be affected by several Calvin cycle metabolites (inhibited by ADP and orthophosphate; activated by 3-phosphoglycerate, fructose 6-phosphate, and fructose 1,6-bisphosphate, [10]). This complicates the detailed analysis and description of the regulatory effect of external orthophosphate on the rate of starch biosynthesis. Preiss and collaborators have proposed that such regulation is mediated mainly by changes in the stromal concentrations of orthophosphate and phosphoglycerate [11]. The available experimental evidence appears to be largely in favour of this idea [2], but attention has been drawn also to the control possibly exerted by the substrates glucose 1-phosphate [5] and ATP [12], or by the allosteric activators fructose 6-phosphate [4] and fructose 1,6-bisphosphate [5]. Actually, each one of the seven metabolites that are known to affect the ADPglucose pyrophosphorylase reaction must be assumed to contribute to the control of starch synthesis. The descriptive and regulatory problem one faces is to establish quantitatively the magnitude of the different contributions.

In the present investigation, that problem is tackled by control analysis, based on our previously described kinetic model for the operation of the Calvin cycle and ancillary pathway of starch production under conditions of light and carbon dioxide saturation [8]. Relationships are derived which make it possible to estimate quantitatively the extent to which substrates and reported effectors of the ADPglucose pyrophosphorylase reaction contribute to the starch synthesis rate changes induced by changes in the external concentration of orthophosphate. Some additional model data relating to the control of the photosynthetic process of starch production are reported and discussed.

THEORY

We have recently described a detailed kinetic model for photosynthetic carbohydrate formation in the chloroplast of C_3 plants under conditions of light and CO_2 saturation [8]. The 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 photosynthate export to the external reaction medium are included as output processes. The model defines the steady-state concentrations of eighteen stromal metabolites (the thirteen Calvin cycle intermediates, glucose 6-phosphate, glucose 1-phosphate, orthophosphate, ATP and ADP) and the corresponding rates of CO_2 fixation (v) and starch production (v_{st}) as a function of various parameters, including the external concentration of orthophosphate, $[P_{ext}]$. For given values of these parameters, application of the model provides estimates of all of the concentration and rate variables and makes it possible to calculate control coefficients, **C**, defined [13] by

$$\mathbf{C}^{\text{variable}}_{\text{parameter}} = \frac{\partial \ln \text{variable}}{\partial \ln \text{parameter}} . \qquad (1)$$

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Enzyme. ADPglucose pyrophosphorylase (EC 2.7.7.27).

The rate of starch production from glucose 1-phosphate and ATP is assumed in the model to equal the velocity of the ADPglucose pyrophosphorylase step and to be given by

$$v_{st} = \frac{V_{st} S_1 S_2}{(S_1 + K_{mst1})\left[\left(1 + \frac{S_3}{K_{ist}}\right)(S_2 + K_{mst2}) + \frac{S_4 K_{mst2}}{S_5 K_{ast1} + S_6 K_{ast2} + S_7 K_{ast3}}\right]} \quad (2)$$

where $S_1 - S_7$ denote the stromal concentrations of, respectively, glucose 1-phosphate, ATP, ADP, orthophosphate, 3-phosphoglycerate, fructose 6-phosphate, and fructose 1,6-bisphosphate; V and K (with subscripts) represent standard steady-state kinetic parameters defined by Eqn (2). Differentiation of Eqn (2) with regard to the concentration of external orthophosphate, P_{ext}, followed by rearrangement and introduction of control coefficients according to the definition in Eqn (1), yields

$$\mathbf{C}^{v_{st}}_{P_{ext}} = \sum_{i=1}^{7} \alpha_i \cdot \mathbf{C}^{S_i}_{P_{ext}} \quad (3)$$

where

$$\alpha_1 = \frac{K_{mst1}}{S_1 + K_{mst1}} \quad (4)$$

$$\alpha_2 = 1 - \frac{S_2(1+\beta)}{\delta} \quad (5)$$

$$\alpha_3 = -\frac{\beta(S_2 + K_{mst2})}{\delta} \quad (6)$$

$$\alpha_4 = \frac{S_4 K_{mst2}}{\gamma\delta} \quad (7)$$

$$\alpha_5 = \frac{S_5 K_{ast1} \alpha_4}{\gamma} \quad (8)$$

$$\alpha_6 = \frac{S_6 K_{ast2} \alpha_4}{\gamma} \quad (9)$$

$$\alpha_7 = \frac{S_7 K_{ast3} \alpha_4}{\gamma} \quad (10)$$

$$\beta = \frac{S_3}{K_{ist}} \quad (11)$$

$$\gamma = -(K_{ast1}S_5 + K_{ast2}S_6 + K_{ast3}S_7) \quad (12)$$

$$\delta = (1+\beta)(S + K_{mst2}) + \frac{S_4 K_{mst2}}{\gamma}. \quad (13)$$

Control coefficients $\mathbf{C}^{S_i}_{P_{ext}}$ in Eqn (3) provide dimensionless measures of the change of each stromal concentration variable S_i (i = 1, ..., 7) induced by a change in concentration of external orthophosphate. Eqn (3), therefore, expresses how the changes in concentration of the different stromal metabolites contribute to the change of the starch production rate induced by a change in concentration of external orthophosphate. Since these contributions may be of different sign and unlimited magnitude, we will use normalized quantities p_i defined by

$$p_i = \frac{\alpha_i \cdot \mathbf{C}^{S_i}_{P_{ext}}}{\sum_{i=1}^{7} |\alpha_i \cdot \mathbf{C}^{S_i}_{P_{ext}}|} \quad (14)$$

as a measure of the relative magnitude of the control contribution provided by a certain stromal metabolite. It follows from Eqn (14) that $-1 < p_i < 1$ and that

$$\sum_{i=1}^{7} |p_i| = 1. \quad (15)$$

A p_i value of 0.4 or -0.4 indicates that the metabolite provides a 40% contribution to the control of the starch production rate, the sign indicating in which direction the contribution tends to change the rate. Metabolites exhibiting p_i values with an absolute magnitude close to unity (or zero) exert virtually exclusive (or no) rate control.

RESULTS

Our kinetic model for carbohydrate formation in the chloroplast of C_3 plants has been shown [8] to account satisfactorily for experimentally observed effects of external orthophosphate (P_{ext}) on the Calvin cycle activity (v) and rate of starch production (v_{st}) over the range of $[P_{ext}]$ values for which the reaction system may attain a stable steady state. This is indicated by curves A and B in Fig. 1, which were calculated using the previously reported realistic values for parameters in the model (e.g. for the kinetic constants in Eqn 2). Curve C in Fig. 1 shows the v versus $[P_{ext}]$ profile predicted by the model in the hypothetical case that V_{st} in Eqn (2) equals zero, i.e. when there is no production of starch but only an export of photosynthate from the chloroplast to the external reaction medium. The close similarity between the latter activity profile and that (Fig. 1, curve B) obtained in the realistic case provides the inference that the main dynamic behaviour of the Calvin cycle is determined by factors that are essentially independent of the process of starch synthesis. The latter process may be considered to reflect, rather than affect, the Calvin cycle activity.

This makes it reasonable to relate the observed effects of external orthophosphate on v_{st} (Fig. 1, curve A) to the orthophosphate-induced changes in stromal concentrations of metabolites participating in the Calvin cycle and starch production reactions. The kinetic model, therefore, was applied for calculation of the control-characterizing quantities p_i, defined by Eqns (3 – 14). The results established that fructose 1,6-bisphosphate and ADP contribute insignificantly ($p_3, p_7 < 0.01$) to the starch production rate changes induced by changes in $[P_{ext}]$. Relative control contributions (p_i) provided by other metabolites affecting v_{st} are given as a function of $[P_{ext}]$ in Fig. 2 and Fig. 3 shows the effect of $[P_{ext}]$ on the stromal concentrations of these metabolites as indicated by the kinetic model.

The detailed information provided by data in Figs 2 and 3 on the internal control of the reaction flux leading to starch synthesis may be summarized as follows. The increase in v_{st} caused by increasing concentrations of P_{ext} in the range 0 – 0.1 mM (Fig. 1) can be attributed mainly to the increasing level of ATP, one of the two substrates for ADPglucose pyrophosphorylase; the positive ATP contribution ($p_2 > 0.4$) overcomes the large negative control contribution provided by the increasing level of stromal orthophosphate (an inhibitor of ADPglucose pyrophosphorylase). Control contributions from other metabolites are rather insignificant in this $[P_{ext}]$ range, except for the minor positive contribution ($p_5 < 0.2$) reflecting the increasing concentration of the activator 3-phosphoglycerate. The latter concentration variable passes through a maximum when $[P_{ext}] \approx 0.1$ mM (Fig. 3) and gains relatively strong negative control at higher concentrations of P_{ext}. The combined effects of stromal orthophosphate and

3-phosphoglycerate then become sufficient to overcome the positive control contribution from ATP, such that v_{st} starts decreasing when $[P_{ext}]$ exceeds 0.12 mM. This control situation is largely maintained up to about 1 mM P_{ext}. At higher concentrations of external orthophosphate, stromal orthophosphate and ATP become of minor importance as regulators of the starch production rate. 3-Phosphoglycerate remains the predominant regulator ($p_5 \approx -0.4$), but significant negative control is gained also by the ADPglucose pyrophosphorylase activator fructose 6-phosphate and the substrate glucose 1-phosphate. The decreasing concentrations of the latter three metabolites account for the attenuation of the rate of starch synthesis at high concentrations of external orthophosphate.

The Calvin cycle activity (v) and rate of starch synthesis (v_{st}) both tend towards zero when $[P_{ext}]$ does so (Fig. 1). As indicated by Fig. 4, however, the quotient v_{st}/v exhibits an almost steady increase with decreasing external orthophosphate concentrations, tending towards unity when $[P_{ext}]$ approaches zero. On the other hand, the quotient remains below 0.3 as long as $[P_{ext}]$ exceeds 1 µM. This means that starch production, in all likelihood, represents a minor output process during photosynthesis under all conditions of physiological interest.

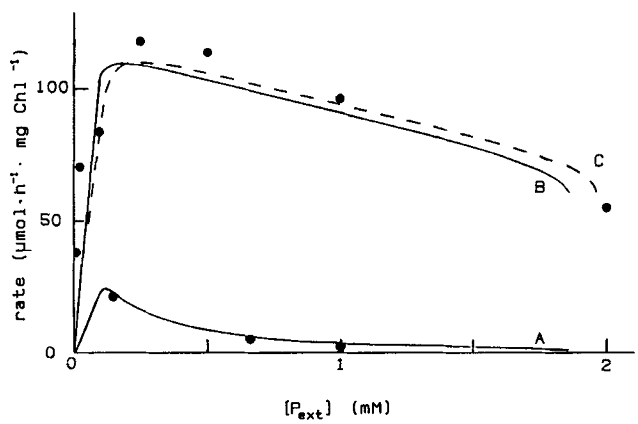
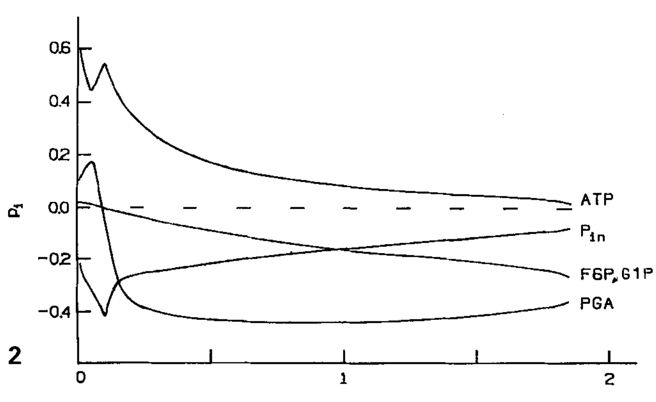


Fig. 1. *Effect of external orthophosphate on the Calvin cycle activity and rate of starch synthesis.* Rate profiles predicted by the kinetic model [8] for starch production (A) and CO_2 fixation (B), compared with reported [5, 6] experimental data (●); chl denotes chlorophyll. Curve C indicates the cycle activity profile calculated for the hypothetical case when there is no production of starch

DISCUSSION

Photosynthetic starch production in isolated chloroplasts is optimal at low (about 0.1 mM) concentrations of orthophosphate in the external reaction medium and strongly inhibited at higher concentrations where the Calvin cycle shows optimal or appreciable activity (see Fig. 1). As was mentioned in the Introduction, contemporary thinking attributes this control of the synthesis of starch to effects of external orthophosphate on the phosphate translocator of the chloroplast envelope. Increased levels of the external metabolite have been envisaged to inhibit starch formation by increasing the rates of photosynthate export and orthophosphate import, such that the stromal concentrations of glucose 1-phosphate and ADPglucose pyrophosphorylase activators decrease at the same time as the stromal concentration of the inhibitor orthophosphate increases [1–3, 14, 15]. The internal orthophosphate/3-phosphoglycerate ratio has received particular attention and is widely considered to exert the main control of the rate of starch synthesis [2–5, 11, 16].

The above explanation for the regulation of starch production by external orthophosphate may seem attractive due to its conceptual simplicity, but is open to criticism in two fundamental respects. Firstly, it does not lead to any understanding of the basic observation [4] that external orthophosphate may have a stimulatory (below 0.1 mM) as well as an inhibitory (above 0.1 mM) effect, such that the starch production rate passes through a maximum, as a function of the external concentration variable (Fig. 1). Secondly, there is no justification for an attribution of the inhibitory effect of external orthophosphate to an increased rate of photosynthate export. Model studies [8] have provided evidence showing that the export rate increases monotonously when the external orthophosphate concentration is raised from zero to about 0.3 mM, i.e. over a concentration range where starch production may be either stimulated or inhibited. Further increase of the external orthophosphate concen-



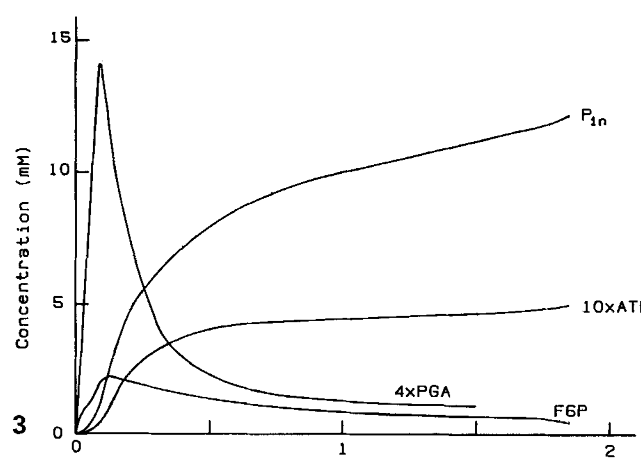


Fig. 2. *Control of starch synthesis by stromal metabolites.* Relative control contributions (p_i) defined by Eqn (14) calculated as a function of the external concentration of orthophosphate. PGA, F6P, G1P, and P_{in} denote, respectively, 3-phosphoglycerate, fructose 6-phosphate, glucose 1-phosphate, and stromal orthophosphate

Fig. 3. *Calculated effect of external orthophosphate on stromal metabolite levels.* Notations as in Fig. 2. The concentration of glucose 1-phosphate (not shown) is proportional to that of fructose 6-phosphate

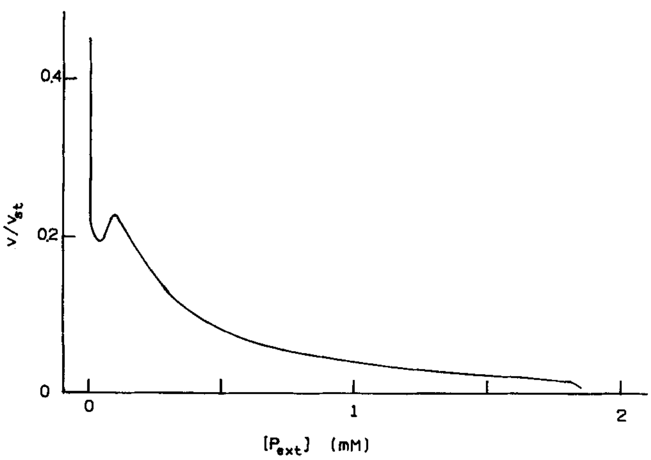


Fig. 4. *Utilization of photosynthate for starch synthesis.* Quotient of the rate of starch production (v_{st}) and CO_2 fixation (v), calculated from data in Fig. 1, curves A and B

tration in the range where starch production is strongly inhibited actually leads to a decreased rate of photosynthate export.

This criticism draws attention to the fact that the Calvin cycle is an extremely complex reaction system from a dynamic point of view [8]; it may not be feasible to describe its behaviour in terms that provide a simple explanation for the effects of external metabolites on stromal metabolite concentrations and reaction fluxes. In the present investigation, we have refrained from trying to explain why variations of the external orthophosphate concentration cause certain changes in the concentration of different stromal metabolites. The treatment is confined to a description of the consequences of these concentration changes with regard to the control of starch synthesis and, in this respect, the results in Fig. 2 provide a detailed answer. A main conclusion which can be drawn from the latter data is that the internal control of starch production is exerted by several metabolites with strengths that may vary drastically depending on the actual concentration of external orthophosphate, P_{ext}. Stromal orthophosphate and 3-phosphoglycerate are certainly of regulatory importance, providing major control contributions in the [P_{ext}] range 0.2 – 1.0 mM. Above that range, however, orthophosphate becomes of minor regulatory interest compared to glucose 1-phosphate and fructose 6-phosphate and, below 0.2 mM P_{ext}, ATP gains much stronger control than phosphoglycerate. It would not seem generally justified, therefore, to single out a certain metabolite or pair of metabolites as being of particular importance in the control of starch synthesis. In any case, it seems obvious that ATP should be included among the metabolites of main regulatory interest. It is due to the increased level of ATP that starch synthesis is stimulated by increasing low concentrations of external orthophosphate and, without consideration of that effect, one cannot understand why the rate of starch production passes through a maximum.

The effect of external orthophosphate on the Calvin cycle activity and rate of starch production originates from the phosphate translocator step. Photosynthate export requires a counter-import of orthophosphate, and the export capacity of the translocator steadily increases with increasing concentrations of the external metabolite [17, 18]. This arrangement has been proposed to serve the purpose of adjusting the export capacity of the phosphate translocator to the physiological needs of the cell; a high external orthophosphate level can be anticipated to correspond to low external levels of triose phosphates and hence to a strong need for photosynthate export [1 – 3]. The partitioning of photosynthate between export and starch production would seem to be governed primarily by a similar mechanistic principle. In the absence of external orthophosphate, the export capacity of the phosphate translocator becomes zero and starch production (according to the kinetic model now used) represents the only output process for withdrawal of Calvin cycle intermediates. With increasing concentrations of external orthophosphate, starch production proceeds in competition with photosynthate export catalysed by the increasingly efficient phosphate translocator and would hence be expected to account for a steadily decreasing part of the total Calvin cycle flux. The results in Fig. 4 confirm that such is generally the case, except for minor irregularities in the region where the rate of starch production exhibits its maximum.

The effect of external orthophosphate on the rate of starch synthesis, therefore, appears to serve the simple purpose of favouring starch production when there is little need for photosynthate export. The present investigation clarifies how this effect is mediated by different stromal metabolites, but does not consider the factors that account for the dependence of the stromal metabolite levels on the external orthophosphate concentration. Data in Fig. 1 indicate that the latter factors are identical with those controlling the Calvin cycle activity in the hypothetical absence of starch production and their elucidation represents a separate regulatory problem that will be addressed in future studies.

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