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Metabolites controlling the rate of starch synthesis in the chloroplast of C3 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 C3 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 (orthophosphate) [4–6]. This dependence has been attributed to modification by the external metabolites of the photosynthetic 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 C3 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 C3 plants under conditions of light and CO2 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 photosynthesize 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 CO2 fixation (v) and starch production (v) as a function of various parameters, including the external concentration of orthophosphate, [P0]. 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

\[ C_{\text{variable parameter}} = \frac{\delta \ln \text{variable}}{\delta \ln \text{parameter}}. \]
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

\[ v_{st} = \frac{V_{st} S_1 S_2}{(S_1 + K_{mat}) \left[ (1 + \frac{S_3}{K_{int}})(S_2 + K_{mat2}) + \frac{S_4 K_{mat2}}{S_6 K_{ext1} + S_6 K_{ext2} + S_7 K_{int3}} \right]} \]

(2)

where \( S_1 - S_2 \) 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

\[ C_{P_{ext}}^i = \sum_{j=1}^{7} x_i \cdot C_{P_{ext}}^j \]

(3)

where

\[ x_1 = \frac{K_{mat1}}{S_1 + K_{mat1}} \]

(4)

\[ x_2 = 1 - \frac{S_2 (1 + \beta)}{\delta} \]

(5)

\[ x_3 = -\frac{\beta (S_2 + K_{mat2})}{\delta} \]

(6)

\[ x_4 = \frac{S_4 K_{mat2}}{\gamma} \]

(7)

\[ x_5 = \frac{S_5 K_{int} x_4}{\gamma} \]

(8)

\[ x_6 = \frac{S_6 K_{mat2} x_4}{\gamma} \]

(9)

\[ x_7 = \frac{S_7 K_{mat3} x_4}{\gamma} \]

(10)

\[ \beta = \frac{S_3}{K_{int}} \]

(11)

\[ \gamma = -(K_{mat1} S_1 + K_{mat2} S_6 + K_{mat3} S_7) \]

(12)

\[ \delta = (1 + \beta)(S + K_{mat2}) + \frac{S_4 K_{mat2}}{\gamma} \]

(13)

Control coefficients \( C_{P_{ext}}^i \) in Eqn (3) provide dimensionless measures of the change of each stromal concentration variable \( S_i \) \((i = 1, \ldots, 7)\) 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

\[ p_i = \frac{x_i \cdot C_{P_{ext}}^i}{\sum_{j=1}^{7} x_i \cdot C_{P_{ext}}^j} \]

(14)

as a measure of the relative magnitude of the control contribution provided by a certain stromal metabolite. It follows from Eqn (14) that \(-1 < p_i < 0\) and that

\[ \sum_{i=1}^{7} |p_i| = 1. \]

(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 photosynthesis 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 \([P_{ext}] \approx 0.1 \text{ mM}\) (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 \( v_a \) starts decreasing when \( [P_{\text{ext}}] \) exceeds 0.12 mM. This control situation is largely maintained up to about 1 mM \( P_{\text{ext}} \). 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 \( (p_4 \approx -0.4) \), but significant negative control is gained also by the ADP-glucose 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 \( (v_a) \) both tend towards zero when \( [P_{\text{ext}}] \) does so (Fig. 1). As indicated by Fig. 4, however, the quotient \( v_a/v \) exhibits an almost steady increase with decreasing external orthophosphate concentrations, tending towards unity when \( [P_{\text{ext}}] \) approaches zero. On the other hand, the quotient remains below 0.3 as long as \( [P_{\text{ext}}] \) exceeds 1 \( \mu \)M. This means that starch production, in all likelihood, represents a minor output process during photosynthesis under all conditions of physiological interest.

**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 ADP-glucose 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.
Fig. 4. Utilization of photosynthate for starch synthesis. Quotient of the rate of starch production ($P_{starch}$) and CO$_2$ fixation ($v$), calculated from data in Fig.1, curves A and B.

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