Spinach leaf sucrose phosphate synthase

Activation by glucose 6-phosphate and interaction with inorganic phosphate

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1. INTRODUCTION

Sucrose phosphate synthase (UDP-glucose:D-fructose-6-phosphate-2-glucosyl transferase, EC 2.4.2.14) is thought to be the principal sucrose synthesizing enzyme in leaves, yet little is known of its regulation. Spinach leaf SPS was inhibited by the end products, sucrose phosphate and UDP [1,2] as well as by P_i. In [3] an endogenous activator of SPS from *Vicia faba* cotyledons was reported, but this was apparently limited to only one variety and could not be found in other cultivars [4]. SPS was activated by the F6P analog 1,5-anhydroglucitol-6-phosphate, especially at low F6P concentration. To date, however, no metabolic activators have been identified.

This communication reports the activation of spinach leaf SPS by G6P and demonstrates the antagonism between G6P activation and P_i inhibition. The results suggest that cytoplasmic G6P/P_i ratio may be a mechanism for the metabolic control of SPS.

Abbreviations: SPS, sucrose phosphate synthase; F6P, fructose 6-phosphate; UDPG, uridinediphosphate glucose; G6P, glucose 6-phosphate; PGI, phosphoglucoisomerase; AH-4B, aminohexyl-Sepharose 4B; UDP, uridine diphosphate; P_i, inorganic phosphate

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2. MATERIALS AND METHODS

2.1. *Extraction and partial purification of SPS*

Spinach (*Spinacea oleracea* L.) leaves were homogenized with a Brinkman Polytron in ice-cold extraction buffer containing 50mM Hepes-NaOH (pH 7.0), 5 mM dithiothreitol, 5 mM $MgCl_2$ and 0.5 mM EDTA (4 ml extraction buffer/g fresh wt of leaves). The extract was filtered through 4 layers of cheese cloth and centrifuged at 10000 × *g* for 10 min. The supernatant was then applied to a column (6 ml bed vol.) of AH-4B, in a procedure

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similar to that in [1]. Up to 200 ml crude supernatant have been applied to the column with nearly complete retention of SPS activity. The column was then rinsed with 20 ml extraction buffer followed by 20 ml extraction buffer that contained 0.1 M KCl. SPS was then eluted in a concentrated peak by applying 20 ml extraction buffer that contained 0.5 M KCl. The resulting preparation was partially purified and concentrated, but contained significant PGI activity and was, therefore, unsuitable for kinetic analysis. PGI was removed by gel filtration on an Ultragel AcA 34 column (2 × 120 cm), equilibrated with a buffer containing 50 mM Hepes–NaOH (pH 7.0), 200 mM KCl, 5 mM $MgCl_2$, 2.5 mM dithiothreitol, 0.5 mM EDTA and 20% (v/v) ethylene glucol. Fractions containing SPS activity with no trace of PGI activity were pooled and stored at −80°C.

2.2. *Enzyme assays*

SPS was assayed by monitoring the production of sucrose phosphate (as sucrose) by a modification of the procedure in [5] or by monitoring UDP production [6]. Unless otherwise noted, the reaction mixture contained 7.5 mM F6P, 7.5 mM UDPG, 5 mM $MgCl_2$, 50 mM Hepes–NaOH (pH 7.0) and enzyme. Reactions were typically run for 10 min at 30°C and terminated by immersion in boiling water (for UDP detection) or by addition of 1 vol. 1 N NaOH (for sucrose detection). Color blanks were developed using the complete assay plus denatured enzyme. PGI was assayed in reaction mixtures containing 50 mM Hepes–NaOH (pH 7.0), 5 mM $MgCl_2$, 25 mM F6P, 1 unit/ml G6P dehydrogenase and 1 mM NADP. Phosphoglucomutase was assayed in an identical mixture, substituting 10 mM glucose 1-phosphate (containing 1% glucose 1,6-diphosphate) for F6P. UDPG-4-epimerase activity was measured in reaction mixtures containing 50 mM Hepes–NaOH (pH 7.0), 5 mM $MgCl_2$, 1 mM uridine-diphosphate–galactose, 0.2 units/ml UDPG dehydrogenase and 1 mM NAD^+. In the above assays, NAD(P) reduction was followed by monitoring the change at A_{340} with time. Phosphatase activity with G6P or F6P as substrates was measured in reactions containing 50 mM Hepes–NaOH (pH 7.0) and 5 mM substrate. Release of P_i after 10 min incubation was determined as in [7].

3. RESULTS AND DISCUSSION

3.1. *Enzyme preparation*

The two major problems encountered in enzyme preparation for this study involved the lability of SPS activity and contamination of preparations with PGI. Although authors in [1] report the removal of all PGI by chromatography on AH-4B, we found that SPS and PGI elute from AH-4B at nearly identical salt concentrations (not shown). Hence, gradient elution was not performed typi-

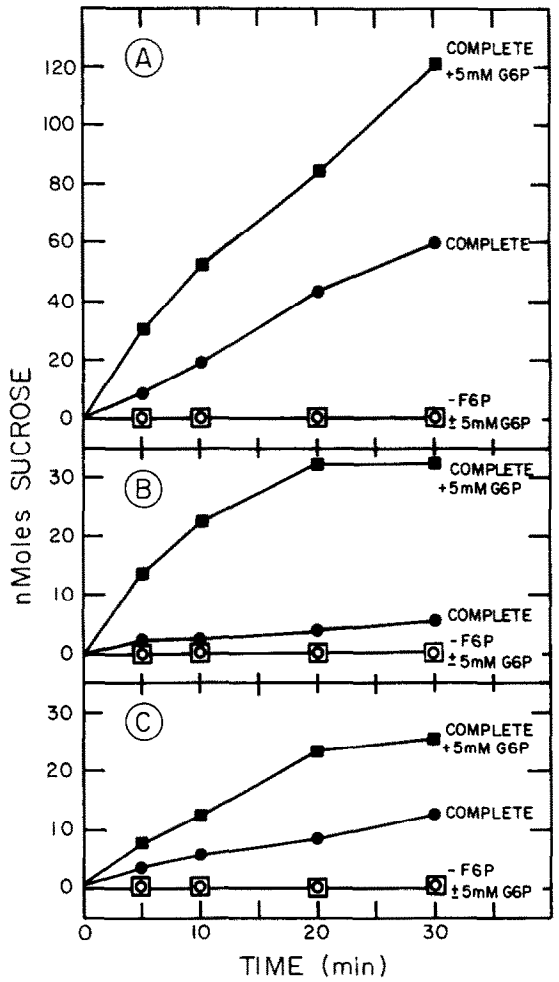

Fig. 1. Time course of sucrose production by spinach leaf SPS at different substrate levels with and without 5 mM G6P: (A) complete assay contains 7.5 mM F6P, 7.5 mM UDPG; (B) complete assay contains 1 mM F6P, 7.5 mM UDPG; (C) complete assay contains 1 mM UDPG, 7.5 mM F6P.

cally. Rather, protein bound to the hydrophobic resin was eluted with a 0.5 M salt wash. Subsequent gel filtration resulted in the separation of SPS and PGI activities. It was necessary to add 20% ethylene glycol and 0.2 M KCl to the elution buffer to retain activity during this step. The resulting preparation was free of PGI, phosphatase (with F6P or G6P), phosphoglucomutase and UDPG-4-epimerase.

3.2. *Time course for G6P activation*

The time course for activation of SPS by 5 mM G6P at different substrate concentrations is shown in fig.1. In all cases, there was no apparent lag period for activation. When neither substrate was limiting (7.5 mM each substrate; fig.1A), 5 mM G6P doubled the rate of sucrose synthesis. With limiting F6P (1 mM; fig.1B), 5 mM G6P caused a 16-fold increase in rate and with limiting UDPG (1 mM; fig.1C), there was a 4.6-fold increase in rate. In both cases of limiting substrate (fig.1B,C), the decrease in rate observed after 10 min was attributed to depletion of substrate. The elimination of F6P from the assays resulted in no sucrose synthesis either in the presence or absence of G6P (fig.1). When PGI was present in an SPS preparation, G6P-dependent sucrose synthesis was observed (not shown). Thus, the absence of sucrose synthesis with 5 mM G6P and no F6P was used as a secondary control to guard against PGI contamination of SPS preparations. Activation of SPS by G6P was observed whether enzyme activity was monitored by following production of sucrose (fig.1) or UDP (not shown).

3.3. *Substrate saturation kinetics and G6P activation*

A Woolf plot of F6P saturation kinetics clearly showed hyperbolic kinetics (fig.2), in agreement with results in [1], and were not sigmoidal as sug-

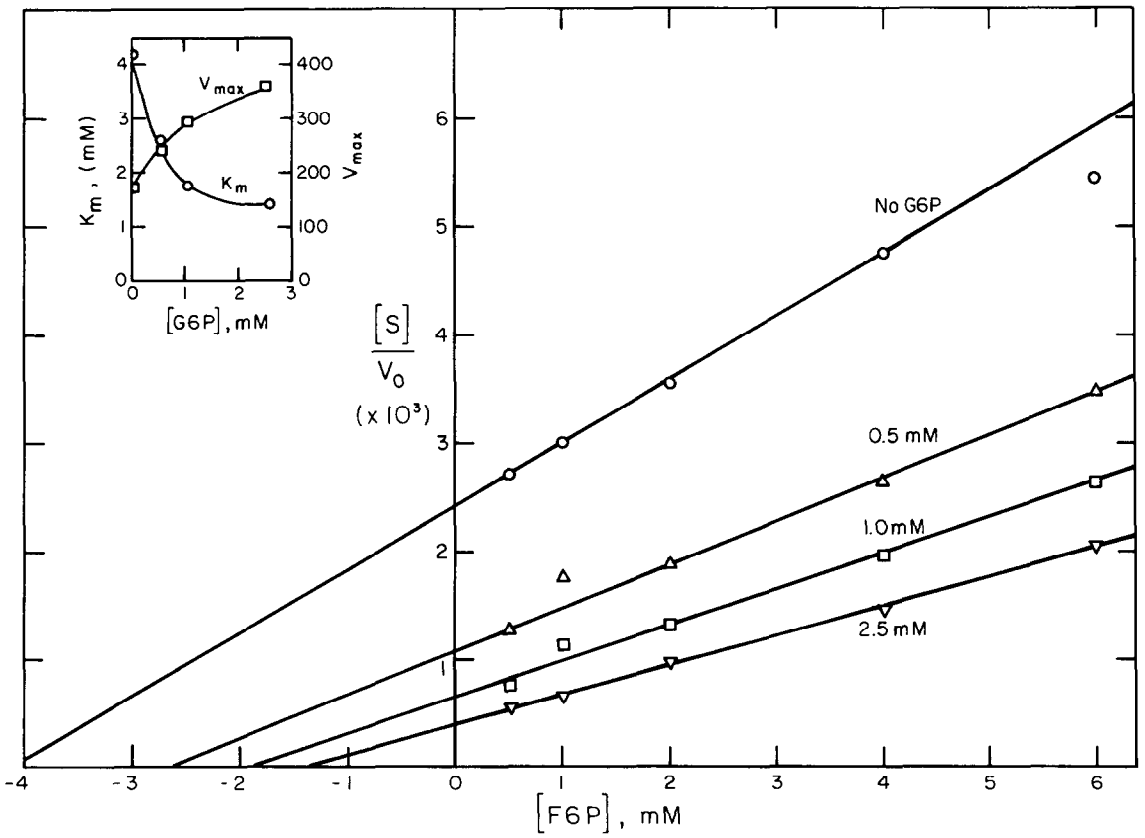

Fig.2. Woolf plots of F6P saturation curves for spinach leaf SPS with 8 mM UDPG and increasing concentrations of G6P. Inset: effect of G6P on K_m and V_{max}.

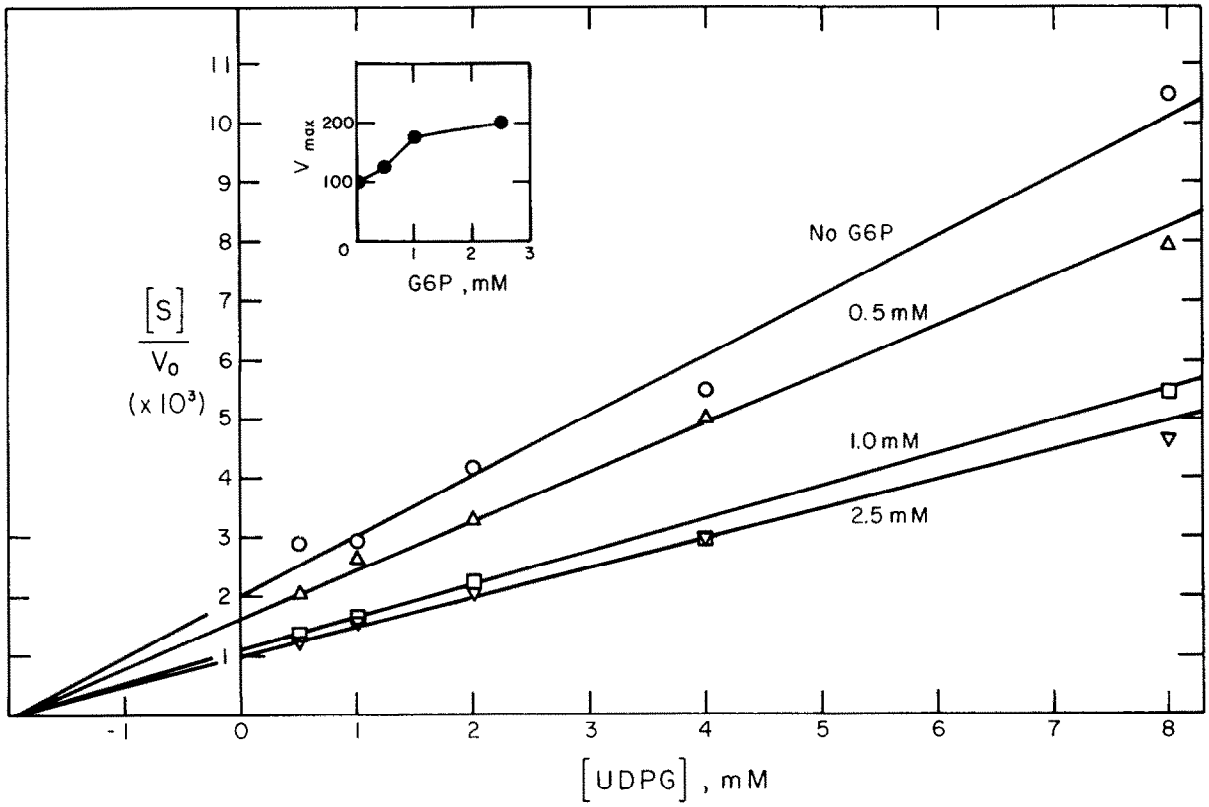

Fig.3. Woolf plots of UDPG saturation curves for SPS with 8 mM F6P and increasing concentrations of G6P. Inset: effect of G6P on V_{max}.

gested in [2]. Authors in [1] point out that contamination of SPS preparations with PGI can artifactually produce sigmoidal kinetics. Increasing the [G6P] to 2.5 mM caused a progressive increase in V_{max} and decrease in K_m (F6P) (fig.2, insert). In the absence of G6P, the K_m (F6P) was 4.1 mM and was reduced to 1.4 mM in the presence of 2.5 mM G6P.

A Woolf plot of UDPG saturation kinetics (fig.3) also showed hyperbolic kinetics. Activation by G6P did not affect the K_m for UDPG, which remained constant at ~1.9 mM, but increased the V_{max} (fig.3, insert).

3.4. *G6P and P_i interaction*

Under conditions of high substrate concentrations (8 mM F6P, 8 mM UDPG), increasing [G6P] to 5.0 mM resulted in increased SPS activity (fig.4). Half-maximal activation occurred at about 0.4 mM G6P. P_i inhibited SPS activity in the absence of G6P. The inhibition was only partial and half-maximal inhibition required ~1.4 mM P_i. Increasing [P_i] to 20 mM antagonized the activation by G6P. A replot of the data in fig.4 shows that SPS activity was a hyperbolic function of the G6P/P_i ratio, irrespective of the absolute concentration of either effector (fig.4, insert).

Previously, no effect of G6P on SPS actvity from either wheat germ [4] or spinach leaf [1] was reported. We can offer no explanation for the difference between their results and ours as reported here.

3.5. *Metabolic considerations*

The possible significance of G6P and P_i as metabolic regulators of SPS activity is suggested by the position of these metabolites in the pathway of sucrose synthesis. P_i is released during sucrose synthesis while triose phosphates, exported from the chloroplast, are converted to neutral sugar molecules. The P_i released is taken up by the chloroplast in strict exchange for triose phosphates

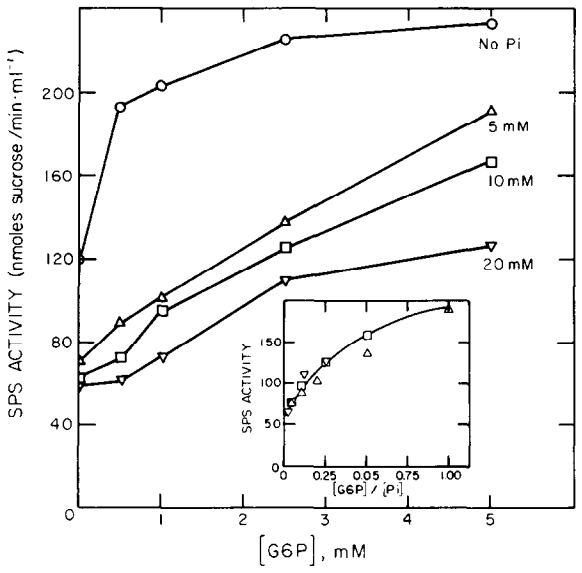

Fig.4. Effect of G6P concentration on SPS activity with 8 mM F6P, 8 mM UDPG and increasing [P_i]. Inset: replot of data showing the effect of G6P/P_i concentration ratio on SPS activity.

[8]. Thus, provision of triose phosphates to the cytosol is dependent both on chloroplast photosynthesis and P_i exchange across the envelope. An accumulation of G6P would occur when an abundance of carbon was available for synthesis of sucrose, whereas an accumulation of P_i may signal a reduced supply of triose phosphates from the chloroplast. The concentrations of G6P and P_i may vary inversely, and a small change in absolute concentrations would result in a large change in the concentration ratio. Thus, regulation of SPS activity by the G6P/P_i ratio may provide a mechanism for fine control of the rate of sucrose formation relative to the supply of available carbon.

The estimated cytosolic concentrations of UDPG (0.4–1.1 mM), and F6P (0.5–1.0 mM) [9] are in the range of the estimated K_m-values for SPS (fig.2,3). Under limiting substrate conditions, G6P (estimated to be 1.7–2.5 mM in the cytosol [9]) would be a potent activator, depending on [P_i] (fig.4).

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