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Update on Metabolism

Role of Sucrose-Phosphate Synthase in Sucrose Metabolism in Leaves[1]

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ABSTRACT

Sucrose is formed in the cytoplasm of leaf cells from triose phosphates exported from the chloroplast. Flux control is shared among key enzymes of the pathway, one of which is sucrose-phosphate synthase (SPS). Regulation of SPS by protein phosphorylation is important *in vivo* and may explain diurnal changes in SPS activity and carbon partitioning. The signal transduction pathway mediating the light activation of SPS *in vivo* appears to involve metabolites and novel "coarse" control of the protein phosphatase that dephosphorylates and activates SPS. Regulation of the phosphorylation of SPS may provide a general mechanism whereby sucrose formation is coordinated with the rate of photosynthesis and the rate of nitrate assimilation. There are apparent differences among species in the properties of SPS that may reflect different strategies for the control of carbon partitioning. The SPS gene has recently been cloned from maize; results of preliminary studies with transgenic tomato plants expressing high levels of maize SPS support the postulate that SPS activity can influence the partitioning of carbon between starch and sucrose.

The major functions of a mature leaf include the assimilation of inorganic carbon (CO_2) and nitrogen (usually nitrate) into forms that can be exported from leaves (sucrose and certain amino acids, respectively). Compartmentation of C and N metabolism between the chloroplast and cytoplasm has long been recognized. In terms of carbohydrate biosynthesis, starch is synthesized within the chloroplast, whereas sucrose is synthesized in the cytosol from triose-P[2] exported on the phosphate translocator (3). In broad terms, several generalizations have emerged during the last decade. First, analysis of mutants and inhibitor studies have indicated that alterations in sucrose formation can affect starch synthesis, whereas the reverse is not necessarily true (16). Thus, carbon partitioning can be controlled somewhat independently of photosynthetic rate, and primary control appears to reside in the cytosol. Second, control in any pathway involves the interplay among various enzymes (*i.e.* control is shared), and distribution of control can vary depending upon conditions (*e.g.* high *versus* low light). The principles of flux control analysis were initially described by Kacser and Burns (9) and have been applied to the study of photosynthetic sucrose formation by Stitt and colleagues (14).

With respect to sucrose synthesis, the process and enzymes involved have been studied most extensively in spinach. The cytosolic sucrose formation pathway starts with triose-P exported from the chloroplast, which are converted to hexose-P and ultimately to sucrose. There are at least two key aspects of the regulation of the pathway: (a) control of cytosolic Fru1,6Pase by the regulatory metabolite Fru2,6P_2 and (b) control of SPS activity by allosteric effectors and protein phosphorylation. The complex regulation of cytosolic Fru1,6Pase by Fru2,6P_2 and other factors has been reviewed recently by Stitt (15). In this brief review, we will focus on recent developments concerning the role and regulation of SPS. Molecular genetic manipulations have confirmed that SPS plays a key role in carbon partitioning (22), and a new emerging concept is that regulation of SPS and certain other key cytoplasmic enzymes by protein phosphorylation (*e.g.* NR; refs. 6 and 10) may be critical to the control of carbon flux between sucrose and amino acids. At least for SPS, some of the elements of the signal transduction pathway that mediate the activation/dephosphorylation of the enzyme in response to a light signal have been tentatively identified.

ROLE OF SPS IN PARTITIONING

It must be emphasized that control of sucrose synthesis is shared between cytosolic Fru1,6Pase and SPS. In this review, we will focus on SPS and how information about levels of control of this enzyme relate to regulation of flux through the pathway under different conditions. It is known that SPS is minimally regulated at three levels. First, the steady-state level of SPS enzyme protein is regulated, *e.g.* developmentally during leaf expansion (20). There are two distinct mechanisms to control the enzymic activity of the SPS protein: (a) allosteric control by Glc6P (activator) and Pi (inhibitor), and (b) protein phosphorylation (covalent modification). These

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[2] Abbreviations: P, phosphates; SPS, sucrose-phosphate synthase; Fru1,6Pase, fructose-1,6-bisphosphatase; Fru2,6P_2, fructose-2,6-bisphosphate; Glc6P, glucose-6-P; PP, protein phosphatase; NR, nitrate reductase; CHX, cycloheximide.

mechanisms are often referred to as "fine" and "coarse" control, respectively.

How do these different mechanisms for regulation of SPS activity contribute to the control of flux through the pathway? Neuhaus *et al.* (11) have addressed this by studying the rate of carbon flux into sucrose when photosynthetic rate is manipulated by controlling irradiance (feedforward control) and in leaves that have low or high levels of endogenous sucrose (feedback control). Results of the "dual-modulation method" indicated that sucrose synthesis increased with irradiance, but absolute rates were always lower when leaves contained high concentrations of endogenous sucrose.

In general, changes in the rate of sucrose synthesis were associated with large changes in Fru2,6P_2 and the activation state of SPS, whereas metabolites changed much less (11). Coarse control of SPS (via protein phosphorylation) was effective in regulating fluxes at low to moderate photosynthetic rates and maintaining relatively constant metabolite levels. However, at high photosynthetic rates (saturating CO_2 and light), sucrose synthesis responded dramatically to increasing metabolite levels (11). This could reflect the "signal amplification" that occurs by increasing hexose-P coupled with decreasing Pi because these metabolites act at two levels: (a) they are allosteric effectors of SPS, and (b) they are effectors of the interconverting enzymes that regulate the phosphorylation status of SPS (see below). It is also becoming apparent that, although control of the pathway is shared between Fru1,6Pase and SPS, SPS probably exerts more of a limitation to the maximal rate of sucrose synthesis than does the Fru1,6Pase (14).

The importance of SPS in the regulation of carbon partitioning in leaves has been recently confirmed using recombinant DNA technology. It was found that transgenic tomato plants expressing high levels of maize SPS had lower levels of leaf starch and increased concentrations of sucrose (22). This result is consistent with numerous physiological and biochemical studies.

CONTROL OF SPS BY PROTEIN PHOSPHORYLATION

The mechanism underlying covalent modification of SPS is protein phosphorylation. In the dark, SPS is phosphorylated and inactivated. Upon illumination, phospho-SPS is dephosphorylated/activated by a type 2A PP (SPS-PP) that is extremely sensitive to the specific inhibitor okadaic acid. Thus, light activation of SPS *in vivo* can be blocked by pretreating leaves in the dark with okadaic acid (5). Interconversion of spinach SPS between the dark (phospho-) and light (dephospho-) forms has no effect on maximum catalytic activity. Rather, the kinetic effect of phosphorylation is only apparent when assayed under more physiological conditions of limiting substrates in the presence of Pi (17). Kinetically distinct forms of SPS were partially purified from spinach leaves that presumably correspond to the phospho- and dephospho-forms (13). The SPS preparations showed less than twofold differences in affinities for substrates but about fourfold differences in affinities for allosteric effectors. Dephospho-SPS had an increased affinity for Glc6P (activator) and decreased affinity for Pi (inhibitor). Thus, the covalent modification primarily affects the allosteric site, where Glc6P and Pi are antagonistic effectors.

Spinach leaf SPS is phosphorylated *in vivo* when leaves are fed [^{32}P]Pi, and partially purified SPS can be phosphorylated and inactivated *in vitro* using [γ-^{32}P]ATP and an endogenous protein kinase (SPS-kinase) that copurifies with SPS (5). SPS is phosphorylated exclusively on seryl residues. There appear to be multiple sites of phosphorylation as evidenced by two-dimensional tryptic peptide maps of immunopurified SPS protein. One or two of the phosphorylation sites have been identified as regulatory (inhibitory) sites that play a role in light/dark regulation (5). Interestingly, other phosphorylation sites are not correlated with activation state of the enzyme and appear to be constitutively phosphorylated.

REGULATION OF THE PHOSPHORYLATION STATUS OF SPS *IN SITU*

SPS is one of several plant enzymes for which light signals have been demonstrated to alter the phosphorylation status and thereby enzymic activity (1). How does light regulate the phosphorylation status of cytosolic enzymes? With SPS we have identified some of the possible components of the signal transduction pathway, and it appears that elements of fine and coarse control may be involved. SPS-kinase is inhibited by Glc6P, and SPS-PP is inhibited by Pi. Coarse control involves an apparent light activation of SPS-PP that itself requires a protein synthesis step that is sensitive to the cytoplasmic protein synthesis inhibitor CHX (21). The apparent light activation of SPS-PP appears to be essential for rapid activation of SPS *in situ* in response to light because pretreatment of leaves with CHX in the dark dramatically slows the dephosphorylation/activation of SPS without a significant inhibition of photosynthetic rate. Because Glc6P and Pi are effectors of the interconverting enzymes and also allosteric effectors of SPS itself (Fig. 1), a small increase in

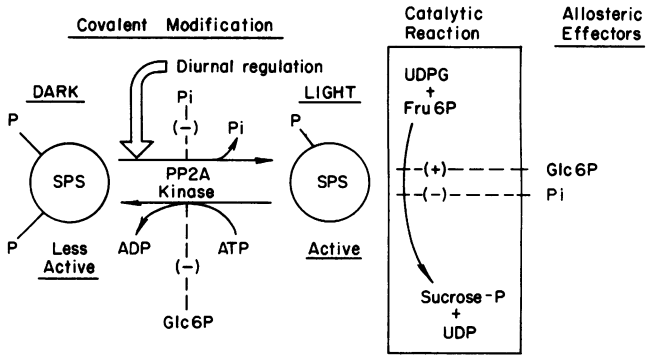

Figure 1. Simplified scheme showing effects of Glc6P and Pi on the interconverting enzymes that regulate SPS phosphorylation status and also act as allosteric effectors of SPS. An increase in Glc6P and a decrease in Pi (as might occur during a dark to light transition) would favor dephosphorylation/activation of SPS and would also increase catalytic activity *in situ* as a result of allosteric regulation. Another important factor is light activation (diurnal regulation) of the SPS-PP that is shown schematically with the open arrow.

Glc6P coupled with a small decrease in Pi will have a much larger effect on the activity of SPS *in situ*, which has been observed (14). The coarse control of SPS-PP appears to be regulated diurnally and may partially explain why SPS activation state is high early in the photoperiod and declines progressively through the day. In contrast, total SPS-kinase activity remains relatively constant throughout a day/night cycle.

SPS REGULATION VARIES AMONG SPECIES

One might expect that the regulatory properties of SPS would be highly conserved, but this appears not to be the case. We recently suggested (8) that there are three classes of SPS, which differ in allosteric regulation and the potential for activation/inactivation *in vivo*. Class I SPS (*e.g.* from *Zea mays*) is allosterically regulated, and dephosphorylation/activation affects V_{max} (two- to threefold increase) as well as sensitivity to effectors. Class II SPS (*e.g.* from spinach) is also allosterically regulated, but dephosphorylation has no effect on V_{max}, only sensitivity to substrates and effectors. Class III SPS (*e.g.* from soybean), in contrast, appears to be weakly regulated by metabolites, and there is no evidence for covalent modification *in vivo* (or *in vitro*) in response to short-term light/dark signals. These differences among plant species in metabolite regulation can be seen with partially purified enzyme preparations and are also apparent as differences in Pi sensitivity in crude desalted leaf extracts (2, 8). Species with class I and II SPS tend to accumulate sucrose in leaves as an end product of photosynthesis, whereas class III species tend to accumulate starch. The class III species contain soluble acid invertase in leaf cell vacuoles that apparently prevents sucrose accumulation during the light period (7). These plants are also more sensitive compared with class I and II species, to feedback inhibition of photosynthesis (loss of maximum photosynthetic capacity) in response to assimilate build-up (4). Thus, excessive "cycling" of sucrose and hexose sugars in the cytoplasm may result in down-regulation of the Calvin cycle and photosynthesis.

RELATION TO NITRATE ASSIMILATION

Another important process that occurs in leaf cells is the reduction of nitrate to ammonium and incorporation of C skeletons to form amino acids. Like sucrose biosynthesis, the process is dependent on (or stimulated by) light. Both processes occur simultaneously in leaves and must be coordinated with one another (19) and with the rate of photosynthesis. It has recently become apparent that several key cytosolic enzymes of these pathways, such as SPS (see above), NR (6, 10), and phosphoenolpyruvate carboxylase (18), are regulated by protein phosphorylation. It has been suggested that nitrate may be one of the key metabolic factors that modulates the activities of the protein kinases and PPs that act on each of the target enzymes and thereby can influence C flow between sucrose and amino acids (18).

OUTLOOK

SPS has long been thought to play an important role in control of sucrose biosynthesis. SPS is not the only determinant of the rate, but recent results from transgenic plants expressing high levels of SPS have confirmed the important role of this enzyme (22). Genetic manipulations of this sort are important from a fundamental standpoint, as well as a practical standpoint, because in some cases, whole plant growth rate is correlated closely with SPS activity in leaves (12). Much remains to be done to investigate the impact of increasing or decreasing SPS activity on photosynthesis and plant growth.

Of course, sucrose synthesis must not be viewed as the only metabolic process regulated by covalent modification of enzymes in the cytoplasm. Protein phosphorylation is emerging as a major mechanism for the control of cytoplasmic enzyme activity, and much remains to be learned about the protein kinases and PPs that act on important target enzymes such as SPS and NR.

With specific regard to SPS, the cloning of the maize SPS gene makes utilization of recombinant DNA technology a reality. It will allow for an understanding of factors regulating SPS gene expression during leaf development and in response to stress. Cloning of SPS from other species will undoubtedly follow rapidly. Comparisons of deduced amino acid sequences, in conjunction with other approaches, will identify important domains of the protein. Such studies may also provide the basis for the apparent differences in properties among species, resulting in further basic information and practical applications.

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