PHYSIOL. PLANTARUM 69: 387–394. Copenhagen 1987

Regulation and roles for alternative pathways of hexose metabolism in plants

Clanton C. Black, Laszlo Mustardy, S. S. Sung, P. P. Kormanik, D.-P. Xu and Nachman Paz

Black, C. C., Mustardy, L., Sung, S. S., Kormanik, P. P., Xu, D.-P. and Paz, N. 1987. Regulation and roles for alternative pathways of hexose metabolism in plants. – Physiol. Plantarum 69: 387–394.

Plant cells have two cytoplasmic pathways of glycolysis and gluconeogenesis for the reversible interconversion of fructose 6-phosphate (F-6-P) and fructose 1,6-bisphosphate (F-1,6-P_2). One pathway is described as a maintenance pathway that is catalyzed by a nucleotide triphosphate-dependent phosphofructokinase (EC 2.7.1.11; ATP-PFK) glycolytically and a F-1,6 bisphosphatase (EC 3.1.3.11) gluconeogenically. These are non-equilibrium reactions that are energy consuming. The second pathway, described as an adaptive pathway, is catalyzed by a readily reversible pyrophosphate-dependent phosphofructokinase (EC 2.7.1.90; PP_i-PFK) in an equilibrium reaction that conserves energy through the utilization and the synthesis of pyrophosphate. A constitutive regulator cycle is also present for the synthesis and hydrolysis of fructose 2,6-bisphosphate (F-2,6-P_2) via a 2-kinase and a 2-phosphatase, respectively. The pathway catalyzed by the ATP-PFK and F-1,6-bisphosphatase, the maintenance pathway, is fairly constant in maximum activity in various plant tissues and shows less regulation by F-2,6-P_2. Plants use F-2,6-P_2 initially to regulate the adaptive pathway at the reversible PP_i-PFK step. The adaptive pathway, catalyzed by PP_i-PFK, varies in maximum activity with a variety of phenomena such as plant development or changing biological and physical environments. Plants can change F-2,6-P_2 levels rapidly, in less than 1 min when subjected to rapid environmental change, or change levels slowly over periods of hours and days as tissues develop. Both types of change enable plants to cope with the environmental and developmental changes that occur during their lifetimes. The two pathways of sugar metabolism can be efficiently linked by the cycling of uridylates and pyrophosphate required for sucrose breakdown via a proposed sucrose synthase pathway. The breakdown of sucrose via the sucrose synthase pathway requires half the net energy of breakdown via the invertase pathway. Pyrophosphate occurs in plant tissues as a substrate pool for biosynthetic reactions such as the PP_i-PFK or uridine diphosphate glucose pyrophosphorylase (EC 2.7.7.9; UDPG pyrophosphorylase) that function in the breakdown of imported sucrose. Also, pyrophosphate links the two glycolytic/gluconeogenic pathways; and in a reciprocal manner pyrophosphate is produced as an energy source during gluconeogenic carbon flow from F-1,6-P_2 toward sucrose synthesis.

Additional key words – F-6-P, F-2,6-P_2, F-1,6-P_2, glycolysis, gluconeogenesis, invertase, pyrophosphate, sucrose breakdown, sucrose synthase, UDP-glucose pyrophosphorylase.

C. C. Black (reprint requests), S. S. Sung, D.-P. Xu and N. Paz, Biochemistry Dept, Univ. of Georgia, Athens, GA 30602 USA; Laszlo Mustardy, Institute of Plant Physiology, Biological Research Center, Hungarian Academy of Sciences, Szeged, Hungary; P. P. Kormanik, Institute for Mycorrhizal Research and Development, U.S. Forest Service, Athens, GA, USA.

This paper is part of the contributions to the 1986 Annual Symposium of the Southern Section of the American Society of Plant Physiologists, held in Charleston, SC, USA, 10–11 March 1986.

Received 9 June, 1986; revised 21 October, 1986

Introduction

Our earliest understanding of how plants function metabolically involved carbohydrates. For example, Sachs literally watched the accumulation and removal of starch in various plant tissues via iodine staining (Sachs 1862) and Haberlandt described "migrating carbohydrate materials" and "transitory starch" in various plant cell types (Haberlandt 1914). Even with the literature to date on synthesis, structure, circulation, utilization, storage and mobilization of carbohydrates, we still search for understanding of how carbohydrates function. Indeed, we are in the midst of a resurgence in research on sugar metabolism stimulated first by the recognition of pyrophosphate as an energy source, particularly in plant sugar metabolism (Carnal and Black 1979), and second by the detection of a new sugar, fructose 2,6-bisphosphate, which is an effective regulator of sugar metabolism in both plants and animals (Furuya and Uyeda 1980, Van Schaftingen and Hers 1980, Sabularse and Anderson 1981).

These discoveries led us to realize that plants have two cytoplasmic pathways of glycolysis and gluconeogenesis (Carnal and Black 1979, Smyth and Black 1984b). Of course, we have long recognized that the chloroplast also duplicates many enzyme activities found in the cytoplasm. In addition, in the cytoplasm of each plant cell, evidence exists now for two pathways of sugar metabolism, with much of the accumulated data concerning the F-6-P interconversion with F-1,6-P_2. The present work will concentrate on plant metabolism with emphasis on (1) the roles of alternative pathways in hexose metabolism, (2) the regulatory roles of F-2,6-P_2, and (3) the contributions of PP_i to the energetics of plant sugar metabolism.

Abbreviations – DHAP, dihydroxyacetone phosphate; NTP, nucleotide triphosphate; PP_i, inorganic pyrophosphate; UTP, uridine triphosphate.

Alternative reactions at the F-6-P interconversion with F-1,6-P_2 step

First, the question of whether plants have developed alternative glycolytic and gluconeogenic pathways in their cytoplasm will be addressed. This question is asked in the knowledge that plants not only synthesize sugars during photosynthesis (which the photosynthetic cell uses), but plants also export massive amounts of sucrose and other sugars or sugar alcohols, which recipient cells then convert into cellular components through glycolysis and gluconeogenesis. To answer this question we will present support for the following hypotheses. First, plants developed a maintenance pathway of sugar metabolism involving an ATP-dependent phosphofructokinase for glycolysis and F-1,6,-bisphosphatase for gluconeogenesis, with both enzyme reactions being physiologically irreversible i.e., non-equilibrium reactions. Second, plants developed an adaptive pathway of sugar metabolism involving a readily reversible PP_i-dependent phosphofructokinase, i.e., an equilibrium reaction. Third, a regulator cycle developed to control these pathways by changing the level of F-2,6-P_2, which serves as a regulator of both pathways. Fourth, PP_i is the energy source that links these alternative pathways of sugar metabolism. Finally, we will illustrate an efficient cooperation by portions of these pathways in the breakdown of sucrose by a new sucrose synthase pathway.

In these alternative pathways of F-6-P metabolism the overall biochemical reactions that will be considered are outlined for each pathway or cycle in Tab. 1. In brief, the maintenance pathway is catalyzed by two distinct enzymes functioning as irreversible, non-equilibrium-reaction enzymes. Note that the plant ATP-PFK readily uses other nucleotide triphosphates, and this non-specificity will be evident later in its use of UTP in sucrose breakdown.

In contrast, the adaptive pathway is catalyzed by a reversible activity functioning in an equilibrium reaction. We are not yet certain whether the regulator cycle requires one protein or two for the two activities in plants, but the reaction catalyzed by the 2-kinase and 2-phosphatase are probably both non-equilibrium ones.

Tab. 1. Reactions in the alternative pathways of glycolysis and gluconeogenesis and the cycle for producing their regulator, fructose 2,6-bisphosphate. FBPase, F-1,6-bisphosphatase.

Cytoplasmic, pathway or cycle	Reactions
Maintenance pathway	F-6-P + ATP $\xrightarrow{\text{ATP-PFK}}$ F-1, 6-P_2 + ADP (glycolysis)
	F-6-P + P_i $\xleftarrow{\text{FBPase}}$ F-1, 6-P_2 (gluconeogenesis)
Adaptive pathway	F-6-P + PP_i $\xrightleftharpoons{\text{PP_i-PFK}}$ F-1, 6-P_2 + P_i (both glycolysis and gluconeogenesis)
Regulator cycle	F-6-P + ATP $\xrightarrow{\text{2-kinase}}$ F-2, 6-P_2 + ADP
	F-6-P + P_i $\xleftarrow{\text{2-phosphatase}}$ F-2, 6-P_2

The maintenance pathway of glycolysis and gluconeogenesis

The standard textbook pathways for glycolysis and gluconeogenesis comprise the maintenance pathway in Tab. 1. This route for the reversible conversion of hexoses to pyruvate has been recognized and extensively studied in plants for several decades (Turner and Turner 1980). However, these studies need to be re-evaluated in view of current knowledge. Indeed the phosphofructokinase step is of such recognized importance that it has been called "the first commited step," "the first unique step," and the "allosteric controlling enzyme step" of glycolysis. Though recently made, such phrases are outdated and are certainly incomplete in view of today's knowledge regarding the ubiquitous presence of the reversible PP_i-PFK in the cytoplasm of plant cells in addition to these maintenance pathway enzymes. For example, when several hundred plants were assayed (Black et al. 1982, Carnal and Black 1983, Kowalczyk et al. 1984), both the ATP- and the PP_i-dependent PFK activities were readily found, with the PP_i-PFK generally several-fold more active than the ATP-PFK.

The logic for ascribing functional roles to the maintenance and adaptive pathways (Tab. 1) can be seen from a more holistic examination of plants at the phosphofructokinase step (Figs 1 and 2). In these studies, the ATP-PFK activity was quite steady on a protein ba-

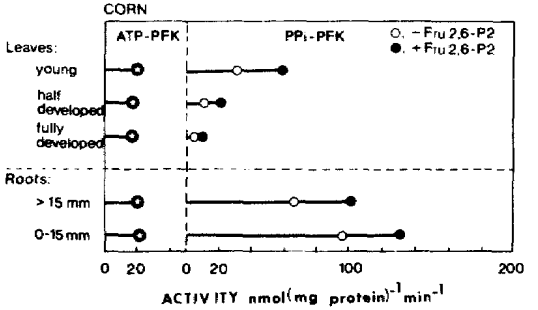

Fig. 1. ATP- and PP_i-dependent phosphofructokinase activities in leaves and roots of 7- to 8-day-old corn seedlings. "Young leaves", shoot with leaves still enclosed in the sheath. The "0–15 mm" root section included the root tip. 10 μM F-2,6-P_2.

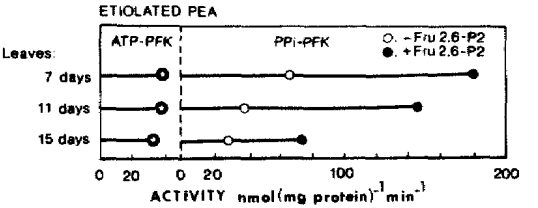

Fig. 2. ATP- and PP_i-dependent phosphofructokinase activities in pea leaves given various etiolation periods after planting.

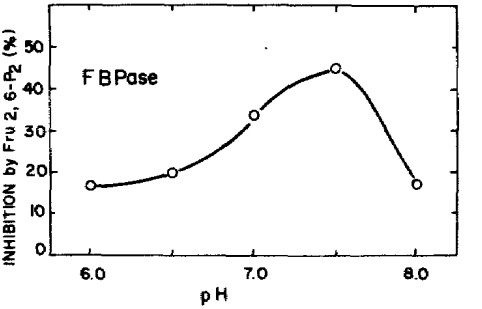

Fig. 3. Fructose 2,6-P_2 inhibition of rabbit muscle fructose 1,6-bisphosphatase activity vs pH. 10 μM F-2,6-P_2. FBPase, F-1,6-bisphosphatase.

sis, regardless of the developmental age of the organ, the degree of photoautotrophy, or species (Figs 1 and 2). We have similar data with corn, pea, wheat and sweetgum for these two enzyme activities. It is important to note that the PP_i-PFK varied, but in a predictable manner, a point that will be considered later.

The maximum catalytic activity of the ATP-PFK was maintained near a constant level in all of these tissues. This observation is remarkable when one considers the range of tissue development and the presence of cytoplasmic and plastid forms of the enzyme. These activities were not influenced by the absence or presence of F-2,6-P_2. Thus, the "maintenance glycolysis" pathway is designated in Tab. 1, as that catalyzed by the classical cytoplasmic ATP-PFK. As work on the ATP-PFK reaction proceeded, we also became aware of numerous underlying questions regarding the functional roles and regulation of F-1,6-bisphosphatase.

The research history on this enzyme in plant cells indicates cytoplasmic and plastid forms (Turner and Turner 1980), their role being to reverse the PFK step, i.e., to function in gluconeogenesis (Tab. 1). These activities have different pH optima and sensitivities to effectors such as AMP, DHAP and P_i; and even light activation. But even with this research history, in the last few years the regulation and roles of the cytoplasmic F-1,6-bisphosphatase has become more uncertain. A partial inhibition by F-2,6-P_2 of the cytoplasmic F-1,6-bisphosphatase caused a re-evaluation of that enzyme (Cseke et al. 1982, Stitt et al. 1982) because it also was learned that plants contain changing levels of F-2,6-P_2. In brief, this work on F-1,6-bisphosphatase shows there is a partial inhibition by F-2,6-P_2 at about 10 μM, which can be modified by AMP, DHAP and P_i (Stitt and Heldt 1985). A typical partial inhibition by F-2,6-P_2 of a F-1,6-bisphosphatase can be seen in Fig. 3, which also illustrates the pH dependence of the inhibition. Thus, current theories (Black et al. 1985a,b; see also Stitt et al. this volume, 1987) claim that the cytoplasmic F-1,6-bisphosphatase is inhibited by F-2,6-P_2 and that relief of this inhibition, by lowering the F-2,6-P_2 level, favors

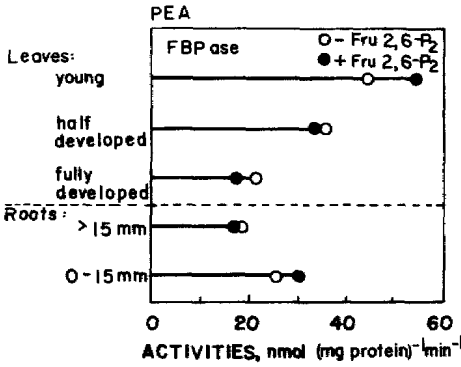

Fig. 4. "Apparent FBPase" activity in leaves and roots of 14 to 18 day-old pea plants. "Young leaves", are those still enclosed in stipules. The "0–15 mm" root section included the root tip. pH 7.5, 0.1 m*M* Fru 1,6-P_2, 5 m*M* $MgCl_2$, 10 μ*M* Fru 2,6-P_2.

gluconeogenesis. Thus gluconeogenically the maintenance pathway with F-1,6-bisphosphatase (Tab. 1) can be partially regulated by F-2,6-P_2.

When crude protein extracts of various plant tissues, as illustrated in Figs 1 and 2, were assayed, an "apparent F-1,6-bisphosphatase" activity was either stimulated or weakly inhibited by F-2,6-P_2, an observation that depends upon the developmental stage of the tissue (Fig. 4). This rather surprising stimulation by F-2,6-P_2 in young leaf and root tip tissues led us to a more detailed evaluation of the activity in total protein extracts. In total tissue extracts, we made the assumption that we were extracting soluble proteins equally and assaying for them under optimum in vitro conditions (e.g. saturating substrate concentration). Because we were aware that the reaction catalyzed by the ubiquitous plant PP_i-PFK is readily reversible and stimulated by F-2,6-P_2 (N.W. Carnal, 1984. Thesis, Univ. of Georgia, Athens, GA, USA; Smyth and Black 1984b), we immediately suspected that the stimulation by F-2,6-P_2 of "F-1,6-bisphosphatase" activity (Fig. 4) was due to the PP_i-PFK activity in the reverse, i.e. gluconeogenic direction. This reaction, however, requires P_i (Tab. 1, adaptive pathway), which had not been intentionally added exogenously to the assay cocktail, and as is well known (Fig. 3, Carnal and Black 1979, Carnal 1984) both enzymes are pH dependent. Therefore, to distinguish the gluconeogenic, (or "F-1,6-bisphosphatase"), portions of the pathways, we studied total protein extracts of young pea leaves under various pH values and levels of exogenous P_i. "Young pea leaves" (Figs 2, 4) designate the shoot tip still folded in the stipules. The shoot tip or bud, which is a sucrose-importing tissue, was completely harvested, extracted and assayed for "F-1,6-bisphosphatase" activity (Fig. 5).

Phosphate is known to have an inhibitory effect on plant F-1,6-bisphosphatase (Turner and Turner 1980, Stitt and Heldt 1985) as we confirmed (Fig. 5) at pH 6.0 to 6.5. As the pH rises, however, further levels up to 1 m*M* of P_i result in a stimulation, then inhibition may occur. The overall pH optimum is near 7.5, which is similar to the PP_i-PFK optimum. (Experiments were not conducted above pH 8.0, because of contributions by the plastid F-1,6-bisphosphatase.) Our interpretation of these data is that two enzyme activities were being measured simultaneously in the total tissue protein extracts – one was the maintenance F-1,6-bisphosphatase, which is inhibited by P_i, and the other was the PP_i-PFK, which requires P_i in the gluconeogenic direction. The PP_i-PFK has a K_m (P_i) between 0.1 and 1 m*M* in the gluconeogenic pathway (Carnal 1984), which is consistent with the pH-dependent manner of the stimulation of P_i in Fig. 5.

Our test of this interpretation was to add 10 μ*M* F-2,6-P_2 to these reaction mixtures. (This concentration of F-2,6-P_2 is sufficient to cause either an inhibition of the cytoplasmic F-1,6-bisphosphatase or a stimulation of the PP_i-PFK). Indeed, below pH 6.5 and below 0.5 m*M* P_i, an inhibition occurred. At higher pH values and lower P_i levels, a stimulation occurred that peaked near pH 7.5, very near the pH optimum of the PP_i-PFK.

Thus, the "F-1,6-bisphosphatase" data in Fig. 4 are interpretable. We know the PP_i-PFK activity, measured glycolytically, is changing in various tissues (Figs 1 and 2). Therefore, PP_i-PFK measured gluconeogenically must change in a similar manner. Thus, the total F-1,6-bisphosphatase activity given in Fig. 4 is the sum of the

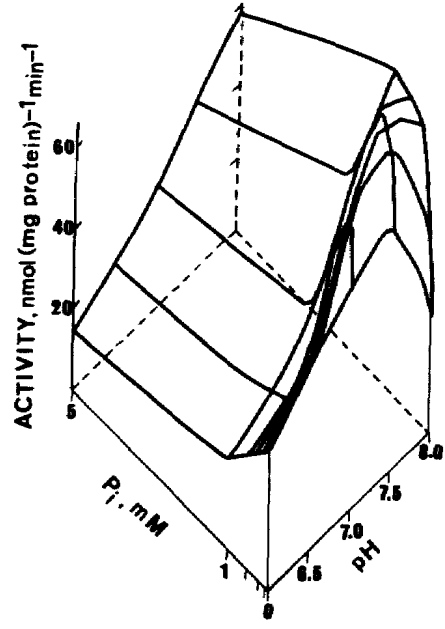

Fig. 5. "Apparent F-1,6-bisphosphatase" activity vs pH and inorganic phosphate concentration in young terminal leaf extracts of pea plants. 0.1 m*M* F-1,6-P_2, 5 m*M* $MgCl_2$.

maintenance F-1,6-bisphosphatase and the PP_i-PFK gluconeogenic activity (Tab. 1). Furthermore, we speculate that the F-1,6-bisphosphatase of the maintenance pathway is maintained fairly constant, as is the ATP-PFK in Figs 1 and 2. Therefore in tissues with high PP_i-PFK activity (e.g., terminal buds and root tips in Fig. 1), the F-2,6-P_2 stimulated "F-1,6-bisphosphatase" activity shown in Fig. 4 is due to PP_i-PFK activity in the gluconeogenic direction. In this reaction PP_i is produced; hence energy conservation occurs when F-1,6-P_2 is dephosphorylated by the PP_i-PFK rather than energy loss, which occurs during F-1,6-bisphosphatase hydrolysis (Tab. 1).

The adaptive pathway of glycolysis and gluconeogenesis

We designate the readily reversible PP_i-PFK catalyzed reactions as an adaptive pathway for both glycolysis and gluconeogenesis which is present in the cytoplasm of all plant cells (Tab. 1). Not only is the adaptive pathway a cytoplasmic alternative to the ATP-dependent/F-1,6-bisphosphatase maintenance pathway, but the adaptive pathway is a flexible, highly regulated, energy conserving pathway, which plants use to different extents in different developmental stages and changing environments throughout their lifetimes. In other words, as a plant changes developmentally or in response to an environmental perturbation, it adapts by regulating the PP_i-PFK catalyzed pathway. Two major regulatory mechanisms are evident. One is seen in Figs 1, 2 and 4 by a change in the maximum PP_i-PFK activities as a plant develops. The second is the regulation with F-2,6-P_2. Previously we presented the following hypothesis for this mechanism: "A fundamental molecular mechanism in plants for coping with physical and biological changes in their environment is to change the intracellular level of F-2,6-P_2. F-2,6-P_2 then activates or inhibits enzymes which regulate the interconversion of hexoses and trioses; thereby the flow of carbon is directed throughout a plant" (Black et al. 1985a,b).

Our model for the regulation of PP_i-PFK by changing F-2,6-P_2 levels is presented in Fig. 6 (Smyth and Black 1984b, Black et al. 1985a,b). In earlier work we demonstrated that two molecular forms of the protein exist, one a dimer and the other a tetramer; and that F-2,6-P_2 causes an association into the larger, most active form (Wu et al. 1983, 1984). This interconversion is reversible depending upon the presence of F-2,6-P_2. Hence interconverting two molecular forms of PP_i-PFK is a glycolytic/gluconeogenic regulatory mechanism (Wu et al. 1984). The model in Fig. 6 shows that as the levels of F-2,6-P_2 rise in plant cells, the PP_i-PFK associates and glycolysis is favored. Simultaneously in the reverse direction the F-1,6-bisphosphatase may be inhibited. These processes reverse when F-2,6-P_2 levels fall and gluconeogenesis is favored. In the next section changing levels of F-2,6-P_2 in plants will be considered.

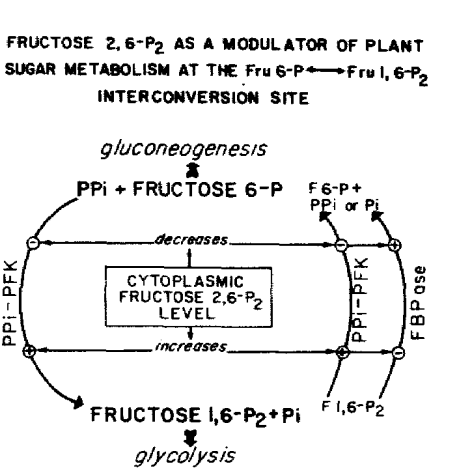

Fig. 6. Model for the modulation of glycolysis and gluconeogenesis by fructose 2,6-bisphosphate. FBPase, F-1,6-bisphosphatase.

The regulator cycle for glycolysis and gluconeogenesis via F-2,6-P_2

Our understanding of glycolysis and gluconeogenesis regulation has changed markedly since the discovery of F-2,6-P_2 (Furuya and Uyeda 1980, Van Schaftingen and Hers 1980) and the recognition that it activates the plant PP_i-PFK, but has no influence on the plant ATP-PFK (Smyth and Black 1984b). F-2,6-P_2 was originally discovered in animals along with a PFK acting as a 2-kinase and as a F-1,6-bisphosphatase, a 2-phosphatase (Tab. 1); apparently, a single protein has both the 2-kinase and 2-phosphatase activity in animals (El-Maghrabi and Pilkis 1984). F-2,6-P_2 and similar metabolic reactions were soon found in plants, although the single-protein theory has yet to be validated with plants (Smyth and Black 1984b).

With the PP_i-PFK, F-2,6-P_2 is a very effective activator at picomolar amounts. Presumably, for F-2,6-P_2 to be a regulator, plant tissues must be able to change its concentration. Indeed, such changes have been shown to occur over periods of hours or days (Hers et al. 1982, Stitt et al. 1982, Huber and Bickett 1984, Black et al. 1985a,b). However, for it to be effective in immediately coping metabolically with rapid environmental changes, plants must be able to change F-2,6-P_2 levels over minutes. Consistent with this requirement, very rapid changes in plant tissue levels of F-2,6-P_2 were demonstrated recently (Paz et al. 1985). In this later work, we flooded the roots of pea plants and measured responses in F-2,6-P_2 levels within 0.25 to 0.5 min, even in leaves. Clearly the intact plant was holistically responding to environmental change. Within similar time frames we also measured changes in the extractable activities of PP_i-PFK. Hence our thesis is that the enzyme activities to form and hydrolyze F-2,6-P_2 are ever-present in plant

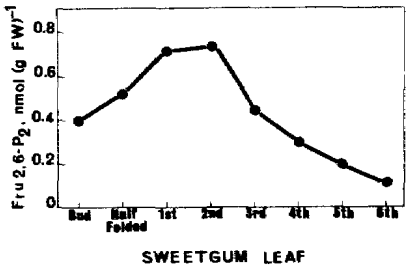

Fig. 7. Fructose 2,6-bisphosphate content vs developmental stage in sweetgum leaves. All extractions performed between 1000 and 1200 h each day. The numbered leaves are expanded below the shoot apex.

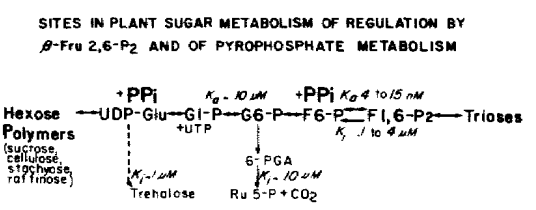

Fig. 8. Multiple sites of fructose 2,6-bisphosphate action in plant sugar metabolism and sites of PP_i metabolism.

cells. When environmental or developmental changes occur, these constitutive enzymes can change the levels of F-2,6-P_2. In other words, the regulator cycle in Tab. 1 is present and can rapidly modify the level of F-2,6-P_2 as plants experience sudden physical and biological changes.

Not only do such quick changes occur, but plants also modify their levels of F-2,6-P_2 with development. Figure 7 illustrates that the steady state level of F-2,6-P_2 in sweetgum leaves differs depending on age. A pattern of maximum levels in the most recently expanded sweetgum leaves is evident. We made similar observations with wheat, pea and corn tissues. It is noteworthy that younger, sucrose importing, tissues have lower levels of F-2,6-P_2. Extractable F-2,6-P_2 levels (Fig. 7) do not exhibit the same pattern as PP_i-PFK or ATP-PFK activities in our sweetgum leaf data (unpublished) which are similar to the corn leaf data in Fig. 1. However, the levels of F-2,6-P_2 do change with development, and we also know they change over the course of a day (Black et al. 1985a,b). Although the values in Fig. 7 are from a single daily time point, we will soon show that these can be related to overall plant processes (e.g., sucrose translocation into meristems).

As discussed earlier, F-2,6-P_2 is a highly active regulator of the PP_i-PFK (K_a = 4 to 15 nM) and the F-1,6-bisphosphatase (K_i = 0.1 to 4 μM). However, other enzymes of sugar metabolism also are responsive to F-2,6-P_2, but at different levels. Figure 8 summarizes the literature on potential sites of F-2,6-P_2 action in plant sugar metabolism (Black et al. 1985a,b). Without doubt, as the levels of F-2,6-P_2 change, several potential regulatory sites in plant sugar metabolism are affected. Here we have concentrated on only the two most sensitive (viz. above) because of the key roles they play in glycolysis and gluconeogenesis (Tab. 1).

Energetic linking of metabolic pathways through pyrophosphate

A comparison of the energy relationships in the pathways and cycle in Tab. 1 shows the adaptive pathway to be an energy consuming reaction glycolytically; although F-1,6-P_2 conserves part of the PP_i bond energy. Gluconeogenically this pathway conserves this bond energy in producing PP_i. Considered from the viewpoint either of hydrolyzing PP_i or of using PP_i in biosynthetic reactions instead of using ATP, the adaptive pathway clearly conserves energy in the overall for plants. In contrast, the maintenance pathway is energy consuming in both directions, as is the regulator cycle.

With the discovery of the PP_i-dependent PFK we realized that during glycolysis plants could use PP_i as an energy source (Carnal and Black 1979). It also was evident that in the gluconeogenic direction PP_i would be produced as an energy source. At that time the only known function of PP_i was its hydrolysis to furnish a favorable thermodynamic environment for polymer synthesis. No evidence existed for a PP_i pool in plants or for a useful biosynthetic function of PP_i. We have assayed a PP_i pool in plant tissues at levels near 20 to 30% of the ATP pool (Smyth and Black 1984a), and at least eight other laboratories have reported similar PP_i levels in recent work. Moreover, PP_i has been shown to serve as an energy source to drive glycolysis from F-6-P to pyruvate in soluble plant extracts (Smyth et al. 1984b). We conclude from this evidence that PP_i is present as an energy source in plants, PP_i can drive glycolysis, and is produced during gluconeogenesis (as via the adaptive pathway in Tab. 1).

How can PP_i link metabolic pathways? Most obviously when PP_i is formed in polymer lengthening, e.g., in starch, cellulose, sucrose, stachyose, protein or lipid synthesis, it could be added to an intracellular soluble pool of PP_i. Indeed any reaction or process producing PP_i, e.g., the ethylene cycle or adenosine utilization, could link directly to glycolysis through the PP_i-PFK adaptive pathway (Tab. 1) or UDP-glucose pyrophosphorylase as shown in Fig. 8.

The gluconeogenic reaction of PP_i-PFK opens a route by which to combine directly the maintenance pathway and the adaptive pathway both to produce and to consume PP_i. For example, PP_i can be used to form hexose phosphates as modeled in Fig. 9B during sucrose breakdown in cells. Figure 9 also compares two potential alternative routes for sucrose breakdown as sucrose enters a plant cell. Viewed broadly, sucrose in a cell can be broken down by either invertase or sucrose synthase. Even though both beginning reaction sequences in Fig.

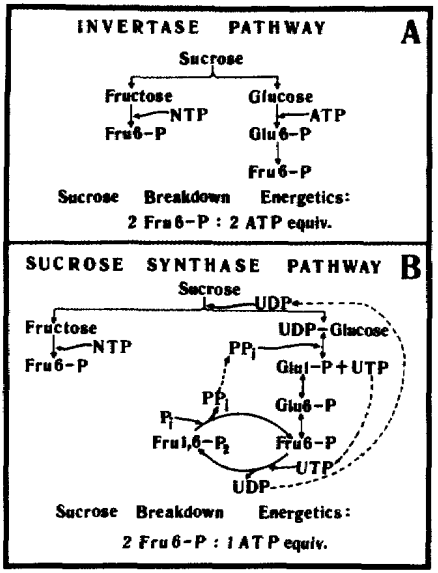

Fig. 9. Alternative routes for the breakdown of sucrose in plant cells and the roles of pyrophosphate in linking the maintenance and adaptive pathways for glycolysis/gluconeogenesis in the breakdown of sucrose. The two routes of sucrose breakdown modeled are: (a), Sucrose breakdown via the invertase pathway; (b), sucrose breakdown via the sucrose synthase pathway; NTP, nucleotide triphosphate. Note that the nucleotide-dependent PFK uses UTP in this pathway.

9 have been known for several decades, uncertainties exist about both. In many plant tissues the invertase activity is low and may be bound; in others it is in the vacuole. This hydrolytic cleavage of sucrose yields glucose and fructose, which are phosphorylated by non-specific nucleotide triphosphate-dependent kinases (Turner et al. 1977, Turner and Turner 1980) to enter cellular sugar metabolism (Fig. 9A).

The entry of sucrose into sugar metabolism via sucrose synthase has a three decade long history; still until recently no evidence existed for how the uridylates were formed in substrate quantities or about PP_i as a substrate for the UDP-glucose pyrophosphorylase reaction (Fig. 9B). Echeverria and Humphreys (1984) and others in the last few months have reconsidered the sucrose synthase entry pathway. They proposed that PP_i is formed via an ATP pyrophosphohydrolase, i.e., ATP → AMP + PP_i. However, as already stated, several laboratories now have reported a pool of PP_i in plant tissues (Smyth et al. 1984a), and we recognize that the PP_i-PFK, as a readily reversible enzyme, also produces PP_i (Tab. 1).

Our hypothesis is that sucrose breakdown proceeds via the unified sucrose synthase pathway outlined in Fig. 9B. We arrived at this conclusion by unifying the data on the maintenance pathway and the adaptive PP_i-PFK pathway in plant growing points as illustrated in Figs 1, 2 and 4. Particularly during analysis of the data in Fig. 4, we realized that the apparent "F-1,6-bisphosphatase" activity is in fact, PP_i-PFK activity in the gluconeogenic direction (Tab. 1 and Fig. 5). The pathway shown in Fig. 9B is balanced and shows the formation of two molecules of F-6-P from sucrose breakdown. The key features of this scheme are: (1) the cycling of uridylates; (2) the cycling of PP_i; (3) the linking of the maintenance pathway i.e., the ATP- (now UTP) dependent PFK, and the adaptive pathway gluconeogenically to synthesize PP_i; and finally (4), the location of the primary site of regulation at the seemingly "futile cycle" interconverting F-6-P and F-1,6-P_2. The primary regulation here is via F-2,6-P_2, which influences the PP_i-PFK activity by facilitating the association of subunits (Fig. 6; Wu et al. 1983, 1984).

Therefore, the sucrose synthase pathway (Fig. 9B) is regulated by F-2,6-P_2, which enhances the PP_i-PFK reaction gluconeogenically. [Important to this conclusion is the fact that the F-2,6-P_2 levels in the sucrose importing shoot apex are low (Fig. 7)].

In addition to these key features the sucrose synthase pathway requires only half the energy of the invertase pathway to break sucrose down to 2 molecules of F-6-P (cf. Fig. 9).

Conclusions on the development of alternative pathways of glycolysis and gluconeogenesis by plants

As sessile organisms dependent upon the energy of sunlight, plants have evolved the ability to adapt to an ever-changing environment. Indeed, plants do vary their activities to suit environmental changes. Hence our hypothesis is that plants developed alternative pathways to accomplish similar tasks, e.g. glycolysis and gluconeogenesis, even in the cytoplasm of each single cell, that enable them to cope with change that could be either long-term as during a lifetime of development, or short-term as in a rapid environmental change.

In summary we propose that: (1) two cytoplasmic pathways of glycolysis and gluconeogenesis (Tab. 1) make the F-6-P interconversion with F-1,6-P_2 readily possible and even energetically conservative; (2) the two pathways are present in the cytoplasm of all plant cells; (3) the ATP-PFK/F-1,6-bisphosphatase or maintenance pathway is fairly uniform in maximum activity and shows less regulation; (4) the reversible PP_i-PFK or adaptive pathway is flexible in its maximum activity with a variety of phenomena such as plant development or changing biological and physical environments; (5) F-2,6-P_2 is a powerful regulator of PP_i-PFK activity in the adaptive pathway in both directions; (6) plants maintain a regulator cycle complement of enzyme activities to rapidly change the level of F-2,6-P_2, which facilitates plants in coping with sudden environmental and developmental changes; and (7) the two pathways also can be linked as in the cycling of uridylates and PP_i re-

quired for sucrose breakdown via the sucrose synthase pathway in sucrose-importing cells (Fig. 9B).

Acknowledgements – This work was supported in part by NSF grant DMB 84-06331, by the Dept of Energy and the U.S. Forest Service through grant 12-11-008-876, and by a U.S.-Hungarian Cooperative Research Program in photosynthesis through NSF INT-8403748.

The conference was generously supported by American Cyanamid, BASF Wyandotte, CIBA-GEIGY, DeKalb-Pfizer, DOW, duPont, FMC, ICI Americas, Monsanto, SDS Biotech, Union Carbide and Zoecon.

References

Black, C. C., Carnal, N. W. & Kenyon, W. K. 1982. Compartmentation and the regulation of CAM. – *In* Crassulacean Acid Metabolism (I. P. Ting and M. Gibbs, eds), pp. 51–68. American Society of Plant Physiology, Rockville, MD. ISBN 0-943088-00-3.

– , Paz, N., Morrell, S., Galloway, C. M. & Dugger, W. M. 1985a. Regulation of plant sugar metabolism. – Current Topics Plant Biochem. Physiol. 4: 66–73.

– , Smyth, D. A. & Wu, M.-X. 1985b. Pyrophosphate-dependent glycolysis and regulation by fructose 2,6-bisphosphate in plants. – *In* Nitrogen Fixation and CO_2 Metabolism (P. W. Ludden and J. E. Burris, eds), pp. 361–370. Elsevier Science Publishing Co. ISBN 0-444-0095 3-1.

Carnal, N. W. & Black, C. C. 1979. Pyrophosphate-dependent 6-phosphofructokinase, a new glycolytic enzyme in pineapple leaves. – *Biochem. Biophys. Res. Commun.* 86: 20–26.

– & Black, C. C. 1983. Phosphofructokinase activities in photosynthetic organisms: The occurrence of pyrophosphate-dependent 6-phosphofructokinase in plants and algae. – Plant Physiol. 71: 150–155.

Cseke, C., Weeden, N. F., Buchanan, B. B. & Uyeka, K. 1982. A special fructose bisphosphate functions as a cytoplasmic regulatory metabolite in green leaves. – Proc. Natl. Acad. Sci. USA 79: 4322–4326.

Echeverria, E. & Humphreys, T. 1984. Involvement of sucrose synthase in sucrose catabolism. – Phytochemistry 23: 2173–2178.

El-Maghrabi, M. R. & Pilkis, S. J. 1984. Rat liver 6-phosphofructo-2-kinase/fructose 2,6-bisphosphatase: A review of relationships between the two activities of the enzyme. – J. Cell Biochem. 26: 1–17.

Furuya, E. & Uyeda, K. 1980. An activation factor of liver phosphofructokinase. – Proc. Natl. Acad. Sci. USA 77: 5861–5864.

Haberlandt, G. 1914. Physiological Plant Anatomy. (Translation). pp. 276–302. Today & Tomorrow's Book Agency, New Delhi.

Hers, H.-G., Hue, L. & Van Schaftingen, E. 1982. Fructose 2,6-bisphosphate. – Trends Biochem. Sci. 7: 329–331.

Huber, S. C. & Bickett, D. M. 1984. Evidence for control of carbon partitioning by fructose 2,6-bisphosphate in spinach leaves. – Plant Physiol. 74: 445–447.

Kowalczyk, S., Januszewska, B., Cymerska, E. & Maslowski, P. 1984. The occurrence of inorganic pyrosphosphate D-fructose-6-phosphate 1-phosphotransferase in higher plants. I. Initial characterization of partially purified enzyme from *Sanseviera trifasciata* leaves. – Physiol. Plantarum 60: 31–37.

Paz, N., Xu, D.-P. & Black, C. C., Jr. 1985. Rapid oscillations in fructose 2,6-bisphosphate levels in plant tissues. – Plant Physiol. 79: 1133–1136.

Sabularse, D. C. & Anderson, R. L. 1981. D-fructose-2,6-bisphosphate: A naturally occurring activator for inorganic pyrophosphate: D-fructose-6-phosphate 1-phosphotransferase. – Biochem. Biophys. Res. Commun. 103: 848–855.

Sachs, J. 1862. Über den Einfluss des Lichtes auf die Bildung des Amylums in den Clorophyllkornern. – Bot. Z. 20: 365–373.

Smyth, D. A. & Black, C. C. 1984a. Measurement of the pyrophosphate content of plant tissues. – Plant Physiol. 75: 862–864.

– & Black, C. C. 1984b. The discovery of a new pathway of glycolysis in plants. – What's New Plant Physiol. 15: 13–16.

– , Wu, M.-X. & Black, C. C. 1984a. Phosphofructokinase and fructose 2,6-bisphosphatase activities in developing corn seedlings (*Zea mays* L.). – Plant Sci. Lett. 33: 61–70.

– , Wu, M.-X. & Black, C. C. 1984b. Pyrophosphate and fructose 2,6-bisphosphate effects on glycolysis in pea seed extracts. – Plant Physiol. 76: 316–320.

Stitt, M. & Heldt, H. W. 1985. Control of sucrose synthesis by fructose 2,6-bisphosphate. VI. Regulation of the cytosolic fructose 1,6-bisphosphatase levels by an interaction between metabolic intermediates and fructose 2,6-bisphosphate. – Plant Physiol. 79: 599–608.

– , Meiskes, G., Soling, H.-D. & Heldt, H. W. 1982. On a possible role for fructose 2,6-bisphosphate in regulating photosynthetic metabolism in leaves. – FEBS Lett. 145: 217–222.

Turner, J. F., Harrison, D. D. & Copeland, L. 1977. Fructokinase (Fraction IV) of pea seeds. – Plant Physiol. 60: 666–669.

– & Turner, D. H. 1980. The regulation of glycolysis and the pentose phosphate pathway. – *In* The Biochemistry of Plants. – Academic Press, New York. pp. 279–316.

Van Schaftingen, E. & Hers, H.-G. 1980. Synthesis of a stimulator of phosphofructokinase, most likely fructose 2,6-bisphosphate from phosphoric acid and fructose 6-phosphoric acid. – Biochem. Biophys. Res. Commun. 96: 1524–1531.

Wu, M.-X., Smyth, D. A. & Black, C. C. 1983. Fructose 2,6-bisphosphate and the regulation of pyrophosphate-dependent phosphofructokinase activity in germinating pea seeds. – Plant Physiol. 72: 188–191.

– , Smyth, D. A. & Black, C. C. 1984. Regulation of pea seed pyrophosphate-dependent phosphofructokinase. Evidence for the interconversion of two molecular forms as a glycolytic regulatory mechanism. – Proc. Natl. Acad. Sci. USA 81: 5051–5055.

Edited by G. Fritz, T. C. Vogelmann and J. F. Bornman