STARCH ACCUMULATION IN PHOTOSYNTHETIC CELLS

I. INTRODUCTION

The classic equation for photosynthesis is often written as

$$6CO_2 + 6H_2O \rightarrow C_6H_{12}O_6 + 6O_2 \quad (1)$$

The most likely end product, designated in the equation by $C_6H_{12}O_6$, is not actually hexose. Originally, starch seemed the most likely end product, and the historic observations of Sachs, Pfeffer, and Godlewski established an intimate relationship between photosynthesis and the process of starch accumulation in green leaves (see Rabinowitch, 1945). However, even in the time of Sachs, it was known that some plants do not accumulate starch within their leaves under any circumstances and, in addition, an almost insoluble polysaccharide could clearly not be moved about the plant. Eventually, it became increasingly accepted that sucrose was the real end product of photosynthesis (Rabinowitch, 1956), whereas starch was relegated to the role of a temporary storage compound. Even when it became evident that sugar phosphates played a central role in photosynthetic carbon metabolism (Benson *et al.*, 1950, 1952; Benson and Calvin, 1950; Bassham and Calvin, 1957), sugar phosphates were regarded as intermediates. Conversely, the percentage of radioactive carbon in sucrose extrapolated to zero at zero time, and the percentage increased thereafter in a way that might have been predicted for an end product awaiting movement to other parts of the plant.

The rate of photosynthesis does not depend on the amount of a single component (e.g., the activity of a particular enzyme). There is a wide range of possible regulatory factors, proven to exist in vitro, but the importance of which in vivo has still to be determined. In particular, there is a multitude of factors affecting the activity of the enzymes involved, with pH, ions, coenzymes, and metabolite effectors modulating the activity of every enzyme studied thus far. Compartmentation is the other key factor. The role of metabolite transport in the cell, particularly between chloroplast and cytosol, but also to and from mitochondria, vacuole, and other organelles, is now considered to be fundamental to the regulation of photosynthesis. In this chapter, we look at the factors considered to be of major importance

in the determination of the nature of the products of photosynthesis, and we look at the partition of carbon and energy between sucrose and starch.

II. THE REDUCTIVE PENTOSE PHOSPHATE PATHWAY

The reductive pentose phosphate pathway (RPPP), also called the Benson-Calvin cycle, is the only pathway in plants that can catalyze the net fixation of CO_2. The entire cycle can be divided into three phases (Fig. 1):

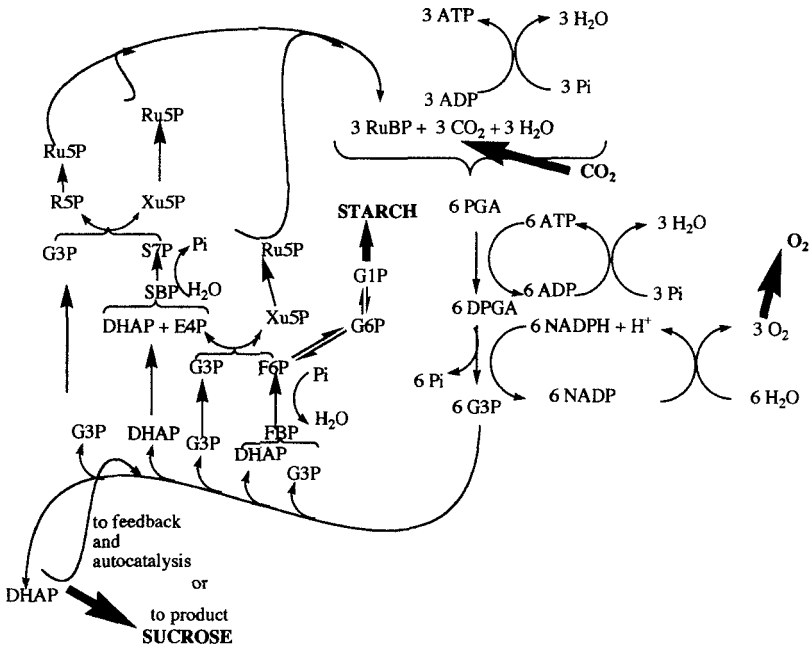
1. Carboxylation of ribulose-1,5-bisphosphate by ribulose 1,5-bisphosphate carboxylase-oxygenase (Rubisco) with the formation of two molecules of glycerate-3-P.
2. Reduction of the two molecules of glycerate-3-P to triose phosphate at the expense of 2 ATP and 2 NADPH.
3. Regeneration of the primary acceptor, RuBP, from triose phosphate in the sugar phosphate shuffle.

Autocatalysis is a crucial property of the RPPP, and it refers to the fact that the product, triose phosphate, can be recycled, generating more substrate for carboxylation. If the cycle turns over five times, the amount of the primary acceptor, RuBP, doubles. During steady-state photosynthesis (after the induction is over), one-sixth of the triose phosphate generated from CO_2 is available for product synthesis. There is, however, a relatively major drain on fixed carbon because photorespiration results in the net loss of carbon and energy. Photorespiration refers to the fact that C_3 plants evolve CO_2 when illuminated in CO_2-free air (apparently, it is absent in C_4 plants). Glycollate is considered the primary substrate of this "light respiration"; Rubisco is a branch point between photorespiratory and photosynthetic metabolism. Oxygen, reacting with RuBP, leads to glycollate synthesis and photorespiration, whereas CO_2, reacting with RuBP, leads to photosynthesis. The glycollate formed by Rubisco when it oxygenates RuBP cannot be used directly in the RPPP, although it is salvaged to some extent by the photorespiratory pathway.

Some of the triose phosphate available for product synthesis will be transported into the cytosol and converted into sucrose (Fig. 2). In both photosynthetic and nonphotosynthetic cells, sucrose is synthesized via sucrose phosphate synthase, which catalyses the reaction

$$\text{UDP-glucose} + \text{fructose-6-phosphate} \rightarrow \text{sucrose-6-phosphate} + \text{UDP}.$$

The sucrose phosphate formed is hydrolyzed by a specific phosphatase to



$$3\,CO_2 + 2\,H_2O + Pi \longrightarrow CH_2O\text{-}CO\text{-}CH_2OPO(OH)_2 + 3\,O_2$$

FIG. 1. The reactions that lead to the regeneration of RuBP and the formation of triose phosphate. On the right, three molecules of RuBP combine with three molecules of CO_2 and three molecules of water to give six molecules of PGA. These are phosphorylated at the expense of ATP, and the resulting DPGA is reduced by NADPH to G3P. The major part of this is converted to its isomer DHAP. Aldol condensation of these two triose phosphates give a molecule of FBP, which undergoes hydrolysis to F6P. This hexose phosphate is also the precursor of G6P and G1P, which, after further transformation, give rise to starch. The F6P also enters the first transketolase reaction donating a 2-carbon unit to G3P to form Xu5P and E4P. The process of condensation, phosphorylation, and 2-carbon transfer is repeated, yielding SBP, S7P, and two more molecules of pentose phosphate, respectively. All three molecules of pentose monophosphate are finally converted to Ru5P, which is phosphorylated to RuBP.

give free sucrose. The formation of UDPglucose is analogous to the formation of ADPglucose in starch synthesis.

It is worth noting that the two sucrose metabolizing enzymes, sucrose synthase and sucrose-phosphate synthase, were both discovered by Leloir and Cardini (1955) (see "Preface").

There is yet another possible route for the triose phosphate formed in the RPPP, and that is starch synthesis within the chloroplast. Stromal starch

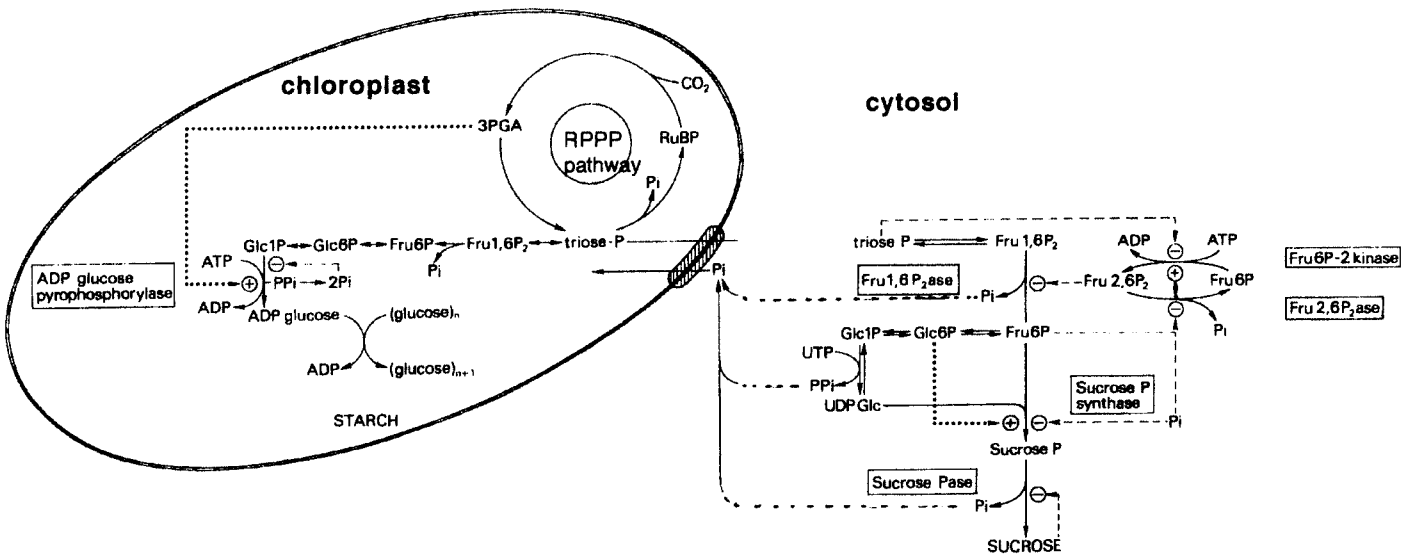


FIG. 2. The control of synthesis of sucrose and starch in photosynthetic cells, and the role of metabolite modulation, including that by fructose 2,6-bisphosphate.

is formed primarily from triose phosphate released from the RPPP. If triose phosphate is retained within the chloroplast, the initial reactions are the same as those involved in sucrose synthesis in the cytoplasm (i.e., a proportion of the triose phosphate undergoes aldol condensation to Fru1,$6P_2$, which is then hydrolyzed to Fru6P). This is then converted into its isomer by hexose phosphate isomerase. In the reaction catalyzed by phosphoglucomutase, Glc6P is converted into Glc1P. At equilibrium, a mixture of the enzymes mentioned previously would yield hexose phosphates in the proportions of approximately Fru6P (9) to Glc6P (17) to Glc1P (1). Although these reactions are considered freely reversible, the overall equilibrium may still be important in determining the distribution of carbon between starch and pentose monophosphate in the illuminated chloroplast. Both Fru6P and triose phosphate are substrates for the first transketolase reaction, and this, in turn, influences the amount of triose phosphate entering the second aldolase condensation and the second transketolase reaction. An active sink for Glc1P would therefore tend to deflect carbon toward starch. This sink could be provided by ADPGlc PPase, which catalyses the reaction

$$\text{glucose-1-P} + \text{ATP} \rightarrow \text{ADPglucose} + \text{PP}_i$$

when low external P_i decreases triose phosphate and PGA export.

Few studies on the localization of the starch biosynthetic enzymes were done before 1978, when it was found that ADPGlc PPase was located exclusively in the chloroplast fraction in both spinach (Mares *et al.,* 1978) and pea (Levi and Preiss, 1978). The first detailed study was done by Okita *et al.* (1979), in which spinach leaf chloroplasts were isolated either by differential centrifugation (Walker, 1971; see also later) or from protoplasts (Nishimura *et al.,* 1976). These plastid preparations contained essentially all of the activity of the starch biosynthetic enzymes, ADPGlc PPase, starch synthase, and branching enzyme. Subsequently, in guard cells of *Commelina communis,* Robinson and Preiss (1987) showed that the starch biosynthetic enzymes were present exclusively in the chloroplast fraction.

III. THE CHLOROPLAST AS A TRANSPORTING ORGANELLE

The chloroplast must operate its carbon cycle as an autocatalytic breeder reaction, but it must export elaborated carbon and chemical energy to its cellular environment. In order to export, it must produce more than it uses, but can only do this by returning newly synthesized intermediates to the cycle. In order to satisfy the needs of the cell, it must release newly made products to the cytoplasm. These competing processes can be accomplished

efficiently because the choloroplast keeps a delicate balance among recycling, export, and internal storage.

Chloroplasts are enclosed by two membranes. The outer membrane is freely permeable to small molecules (up to about 10 kDa) due to the presence of a *porin* and the inner membrane is the osmotic barrier and the site where specific transport occurs. The specificity of envelope permeability is strikingly highlighted by the contrast between P_i and PP_i, the former being among the most rapidly translocated molecules and the latter among those to which the envelope is relatively impermeable. Carrier-mediated anion transport can be classified as:

1. Electroneutral, involving exchange of one anion with another of equal charge
2. Electroneutral proton compensated, in which the different charge is compensated by cotransport of a proton
3. Electrogenic, involving an exchange between anions of different charges (which requires energy as membrane potential or proton electrochemical gradient)

The study of transport by isolated chloroplasts requires the use of "good" organelles, and the criteria for this are photosynthetic rate and chloroplast intactness. If isolated chloroplasts are capable of rapid electron transport and photophosphorylation, but have lost the ability to assimilate CO_2 when illuminated in a suitable reaction mixture, they have then been damaged or have been irreversibly inhibited during isolation. Because there is often a clear correlation between envelope integrity and function, results obtained with relatively inactive chloroplasts are unlikely to reflect the behavior of chloroplasts in situ.

Techniques developed with the aim of separating intact chloroplasts from leaf tissues (Walker, 1971) yield preparations containing, on average, some 70 to 80% (or higher, depending on the species and quality of the material) of class A chloroplasts (Hall, 1972).

IV. CONTROL OF CARBOHYDRATE METABOLISM

During the day, the rates of starch and sucrose synthesis and the rate of photosynthetic carbon assimilation must be coordinated. There is a clear need to determine how much assimilated carbon can be diverted into sucrose and starch synthesis without decreasing too much the amount that returns to the RPPP. Conversely, when sucrose accumulates in the cytosol because the rate of export diminishes (and/or photosynthesis increases), starch begins to accumulate inside the chloroplast. During the night, the

sucrose accumulated in the vacuole during the day and the starch accumulated in the chloroplast are remobilized to be used to support the metabolism of the leaf itself or to be exported as sucrose (Stitt *et al.,* 1987a). Stromal amylases and phosphorylases degrade starch, and Glc1P is then converted into triose phosphate, which can be exported from the chloroplast.

In this way, photosynthates are constantly available. The importance of these remobilization mechanisms is highlighted when they are disturbed. For example, mutants of the crucifer *Arabidopsis thaliana,* which are unable to synthesize starch but can still synthesize sucrose, grow at the same rate as the wild type under continuous light, but the growth rate is drastically diminished if placed in a day–night regime (Caspar *et al.,* 1986).

V. REGULATION OF THE ADPGlc PATHWAY IN THE CHLOROPLAST

As discussed elsewhere (see the chapter, "Synthesis of the Glucosyl Donor: ADPglucose Pyrophosphorylase"), for every leaf system studied, whether the leaf source is from a plant using the C_3 or C_4 pathway or Crassulacean metabolism, the major activator is still 3PGA and the inhibitor is P_i. There is much evidence obtained in vitro suggesting that ADPGlc synthesis is regulated by activation of the plant ADPGlc PPase by 3-phosphoglycerate (3PGA) and inhibition by P_i. In vivo and in situ experiments showed a correlation between the concentrations of 3PGA and starch, and inverse correlations between P_i and starch levels (see the chapter, "Regulation of the Starch Synthesis Pathway: Targets for Biotechnology").

The increasing availability of mutant and transgenic plants now facilitates the study of how plant metabolism is controlled. In particular, control analysis involves asking how much a flux changes for a given change in enzyme activity, such that the flux control coefficient.

$$C_{JE} = \frac{dJ/J}{dE/E}$$

In this equation, E is the original amount of enzyme, J is the original pathway flux, and dJ is the change that results from a relatively small change in the amount of the enzyme dE. For an enzyme in a simple, unbranched pathway, if $C_{JE} = 1$, then C_{JE} can vary between 0 (no control) to 1 (total control), that particular enzyme limits the rate of the overall pathway (see Kacser and Burns, 1973; Heinrich and Rapoport, 1974).

The availability of chloroplast mutants of phosphoglucose isomerase of *Clarkia xantiana* (Kruckeberg *et al.*, 1989; Neuhaus *et al.*, 1989), of phosphoglucomutase (Caspar *et al.*, 1986), and of ADPGlc PPase of *A. thaliana* (Lin *et al.*, 1988a,b; Neuhaus and Stitt, 1990), has allowed the analysis of the extent of control that these enzymes exert on chloroplast starch synthesis. Mutant plants with reduced activity of both cytosolic (64%, 36%, 18% of wild type) and chloroplastic (75%, 50% of wild type) phosphoglucoisomerase were used to determine the effect of these enzymes on fluxes toward starch and sucrose synthesis as well as on photosynthetic rate and control coefficients (Kacser and Burns, 1973; Kruckeberg *et al.*, 1989). The plastid phosphoglucoisomerase exerted little control over starch or sucrose synthesis in low light, but did exert control of starch synthesis in saturating light. Lowering the cytosolic enzyme activity had little effect on either starch or sucrose synthesis in saturating light, but increased starch synthetic rate and decreased sucrose synthesis in low light. Thus variation of the cytosolic phosphoglucoisomerase affected the partitioning of carbon between sucrose and starch. Further studies (Neuhaus *et al.*, 1989) confirmed that reduction of plastid phosphoglucoisomerase had little effect in low light, but reduced starch synthesis by 50% in saturating light with no corresponding increase in sucrose synthesis. Reduced levels of cytosolic enzyme (18% of wild type) lowered the sucrose synthetic rates and increased the rate of starch synthesis. Metabolite levels were also affected in these mutants. In the mutant containing only 18% of the wild type cytosolic phosphoglucoisomerase activity, both fructose-2,6-bisphosphate and 3PGA levels increased approximately 100%. Neuhaus *et al.* (1989) suggested that the lower rate of sucrose synthesis rate is due to the increased Fru-2,6-P_2 concentration, which causes increased inhibition of cytosolic fructose-1,6-bisphosphatase (for reviews on sucrose synthesis and its regulation, see ap Rees, 1987; Stitt *et al.*, 1987b), which is on the pathway toward sucrose synthesis (Fig. 2). Their data strongly support the view that increased starch synthesis in the mutants with reduced levels of phosphoglucoisomerase is due to activation of the ADPGlc PPase by the increased 3PGA concentration and 3PGA/P_i ratio.

These experiments have been extended to the null chloroplast phosphoglucomutase (Caspar *et al.*, 1986) and the low activity (7% of wild type) ADPGlc PPase mutants (Lin *et al.*, 1988a,b) of *A. thaliana*. Neuhaus and Stitt (1990) used the alleles to construct hybrid plants containing, respectively, 50% of wild-type phosphoglucomutase activity and 50% of wild-type ADPGlc PPase activity. The effects of these reduced activities on starch and sucrose fluxes and on CO_2 fixation in low-light and high-light intensities were measured. In low light, a 50% decrease in phosphoglucomutase activity had no significant effect on the fluxes

mentioned previously. However, a 50% and 93% decrease of ADPGlc PPase activity resulted in a 23% and 74% decrease in flux of starch synthesis, with a concomitant increase of a 17% and 42% increase in sucrose synthetic rate. Thus, a decrease in the synthesis of ADPGlc not only affected starch synthesis but also affected the partitioning of photosynthetic carbon, causing more to be directed toward sucrose biosynthesis. In high light a 50% decrease in phosphoglucomutase activity resulted in a 20% decrease in starch synthesis with little effect on the sucrose synthesis rate. However, reduction of the the ADPGlc synthesizing activity by 50% and 93% resulted in a 39% and 90% decrease in starch synthesis flux. The flux of photosynthetic carbon under these conditions was not redirected toward sucrose synthesis but rather the photosynthetic rate was inhibited approximately 46%. The flux control coefficients (Burns *et al.,* 1985) for the enzymes for starch synthesis were calculated to determine the distribution of control and were compared with previous results obtained with the *C. xantiana* phosphoglucoisomerase.

A kinetic model was developed by Petersson and Ryde-Petersson (1989) that was consistent with the metabolite concentrations and mass action ratios measured in vivo and with enzyme properties and equilibria. These authors reached the conclusion that 3PGA and P_i play important roles in regulating starch synthesis with significant contributions made by ATP, glucose-1-P, and fructose-6-P. Since these metabolites are either substrates or effectors of the ADPGlc PPase, the analysis is consistent with the view that 3PGA is a positive effector and P_i is a negative effector of ADPGlc synthesis and, therefore, that the 3PGA/P_i ratio regulates starch synthesis via regulation of ADPGlc PPase.

In summary, analysis of the starch biosynthetic system in a number of plants or using data obtained in vivo from different plants and applying the control analysis method of Kacser and Burns (1973; see also Kacser, 1987) show that the major site of regulation of starch synthesis is at ADPGlc PPase and that 3PGA and P_i are important regulatory metabolites of that enzyme. A decisive proof that this regulatory mechanism is functional in vivo would be the isolation of a plant containing an ADPGlc PPase with altered allosteric properties that would correlate with its starch content. So far, such mutations have not been found in higher plants but have been reported for *E. coli* and *Salmonella typhimurium* (reviewed in Preiss and Romeo, 1989), and for *Chlamydomonas reinhardtii.* Ball and his collaborators at Lille have obtained mutants of this unicellular green algae that have an ADPGlc PPase with a low sensitivity to PGA activation; these mutants display a low starch content. The genetic manipulation of either the structural or regulatory genes of the starch biosynthetic enzymes may provide means for alteration of the starch levels in a plant, and a significant advance

is described in the chapter, "Regulation of the Starch Synthesis Pathway: Targets for Biotechnology."

VI. STARCH SYNTHESIS IN YOUNG LEAVES

In young developing leaves that still behave as sinks (rather than sources), sucrose is first hydrolyzed in the cytosol by the action of the invertase or by the sucrose synthase followed by UDPGlc pyrophosphorylase. The hexose sugars formed are then metabolized via glycolysis into C_3 intermediates that are then transported into the chloroplast via the P_i translocator, where they can be used or stored as starch.

VII. SYNTHESIS OF STARCH AND SUCROSE IN C_4 PLANTS

The C_4 cycle can be viewed as an ATP-dependent CO_2 pump that delivers CO_2 from the mesophyll cells to the bundle-sheath cells, thereby suppressing photorespiration (Hatch and Osmond, 1976). The development of the C_4 syndrome has resulted in considerable modifications of inter- and intracellular transport processes. Perhaps the most striking development with regard to the formation of assimilates is that sucrose and starch formation are not only compartmented within cells, but in C_4 plants also may be largely compartmented between mesophyll and bundle-sheath cells. This has been achieved together with a profound alteration of the Benson-Calvin cycle function, in that 3PGA reduction is shared between the bundle-sheath and mesophyll chloroplasts in all the C_4 subtypes. Moreover, since C_4 plants are polyphyletic in origin, several different metabolic and structural answers have arisen in response to the same problem of how to concentrate CO_2. C_4 plants have three distinct mechanisms based on decarboxylation by NADP+-malic enzyme, by NAD+-malic enzyme, or by phosphoenolpyruvate (PEP) carboxykinase in the bundle-sheath (Hatch and Osmond, 1976).

Downton and Hawker (1973), showed that starch, starch synthase, and ADPGlc PPase were much higher in bundle-sheath cells than in mesophyll cells on a protein basis or on a chlorophyll basis. The mesophyll cell is able to synthesize starch on exposure of the leaf to continuous light for approximately 2.5 days. Under these conditions, starch synthase levels in the mesophyll cell increased. Thus the mesophyll cell is capable of starch synthesis under certain conditions. Later reports on other C_4 plants (e.g., nutsledge leaves, Chen *et al.*, 1974; *Digitaria pentzii,* Mbaku *et al.*, 1978) also indicate that both tissues are capable of starch synthesis.

Although starch synthase is present in both tissues, in *Digitaria pentzii* activity of the starch synthase is 10 times higher (calculated on a chlorophyll basis) in the bundle sheath. The studies on the maize leaf have been confirmed by more recent research, which is also more comprehensive since it also includes the measurement of branching enzyme activity (Preiss *et al.*, 1985; Echeverria and Boyer, 1986; Spilatro and Preiss, 1987). Their results are in agreement with the earlier conclusion that starch and the starch biosynthetic enzymes are primarily in the bundle sheath. Moreover, the exclusive localization of the starch biosynthetic enzymes in the bundle sheath chloroplast has been reported (Echeverria and Boyer, 1986). Thus, the discovery that the leaf starch biosynthetic is located solely in the chloroplast is in keeping with the finding that starch in higher plants is found exclusively in the chloroplast.

Although in a species such as maize the synthesis of sucrose appears to occur largely in the mesophyll cells: whereas the synthesis of starch occurs largely in the bundle-sheath cells, it is clear that there is a good deal of flexibility both within maize and between C_4 plants in general. For example, although the mesophyll tissue of maize grown under normal conditions contains no detectable starch, growth of plants in continuous light induces starch formation in the mesophyll (Downton and Hawker, 1973). However, *Digitaria* spp. (which, like maize, are also NADP+-malic enzyme–type C_4 plants) synthesize both sucrose and starch in the mesophyll compartment (Mbaku *et al.*, 1978; Hallberg and Larsson, 1983). A number of pieces of evidence support the contention that sucrose synthesis is confined to the mesophyll cells in leaves of at least some C_4 plants. Bucke and Oliver (1975) and Furbank *et al.* (1985) found that the majority of the sucrose–phosphate synthesis is located in the mesophyll of maize, *Pennisetum purpureum,* and *Muhlenbergia montana.* Other studies have shown the cytosolic fructose bisphosphatase to be confined largely to the mesophyll (Furbank *et al.*, 1985) as well as Fru6P, 2-kinase, fructose-2,6 bisphosphatase (Soll *et al.*, 1983), and fructose-2,6-bisphosphate itself (Stitt and Heldt, 1985). However, Ohsugi and Huber (1987) have shown that sucrose–phosphate synthase activity is present in both mesophyll and bundle-sheath cells in all C_4 subtypes, including maize. In addition, the response of the enzyme to light was different in the two compartments, with the bundle-sheath enzyme requiring higher irradiance for activation. Ohsugi and Huber (1987) suggest that sucrose–phosphate synthase may function in both mesophyll cells and bundle-sheath cells for sucrose synthesis in the light, particularly at high light intensity, whereas in the dark the major function of bundle-sheath cell sucrose–phosphate synthetase may be in sucrose formation following starch degradation, which is a function that has been largely overlooked. Perhaps the safest conclusion is that starch and sucrose synthesis may predominate in one or the other compartment in

maize, but that this compartmentation may readily be overridden when environmental conditions (e.g., high light intensity) require it.

VIII. THE REGULATION OF STARCH SYNTHESIS IN C_4 PLANTS

A number of studies have shown that the activities of starch synthase, branching enzyme, and ADPGlc PPase are higher in the bundle-sheath cells than in the mesophyll cells. However, the enzymes of starch degradation, starch phosphorylase, and amylase are more evenly distributed and are slightly higher in the mesophyll (Huber *et al.*, 1969; Downton and Hawker, 1973; Echeverria and Boyer, 1986; Spilatro and Preiss, 1987). ADPGlc PPase from spinach is activated by 3PGA and inhibited by P_i, with ratios of 3PGA/P_i for half-maximal activation typically being less than 1.5 (Ghosh and Preiss, 1966). ADPGlc PPase from maize leaves requires much higher ratios of 3PGA to P_i for half-maximal activation, with the enzyme from the mesophyll cells requiring a higher ratio (9 to 16) than the enzyme from the bundle-sheath cells (7 to 10) (Spilatro and Preiss, 1987). Measurements show that 3PGA may be as high as 15 to 16 mol · m^{-3} in the bundle sheath and 5 to 7 mol · m^{-3} in the mesophyll (Leegood, 1985; Stitt and Heldt, 1985) due to the requirement for metabolite gradients during photosynthesis. Thus 3PGA/P_i ratios in the mesophyll are likely to be considerably lower than in the bundle sheath. This factor, and the relatively low activities of the enzymes of starch synthesis in the mesophyll, would appear to limit synthesis of starch in the mesophyll relative to the bundle sheath, but this factor is a relationship that could be modified readily with fluctuations in physiologic and developmental conditions, and could therefore account for variations in the capacity of the mesophyll to make starch.

IX. STARCH IN CAM PLANTS

Metabolism in CAM plants involves the transfer of large amounts of carbon between two storage pools: malic acid and storage carbohydrates (Fig. 3). Although malate is invariably stored in the vacuole, the carbohydrate store may be either chloroplastic (e.g., starch-storers such as *Bryophyllum tubiflorum, Kalanchoe diagremontiana*) or extrachloroplastic [as in *Ananas comosus* (pineapple) and *Aloe arborescens*]. This transfer of carbon involves glycolytic carbohydrate breakdown in the dark and gluconeogenic carbohydrate synthesis during the light. In starch-formers, large amounts of carbon must therefore enter the chloroplast during

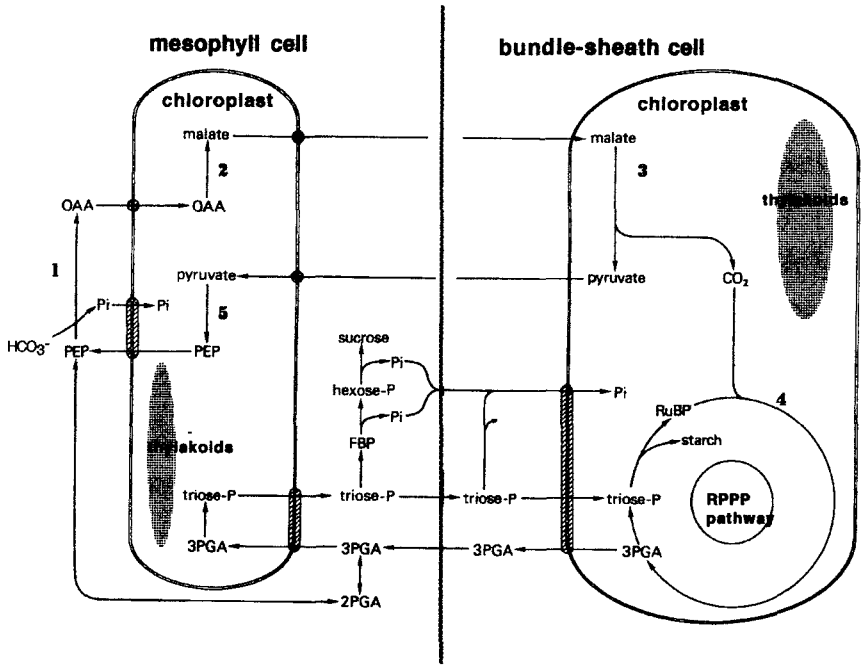


FIG. 3. Operation of the C_4 pathway in a NADP+-malic enzyme-type plant such as maize. The C_4 pathways, essentially CO_2 concentrating mechanisms, are classified according to the enzyme that decarboxylates the C_4 acid in the bundle-sheath chloroplast, decreasing the oxygenation reaction of Rubisco. In maize, the decarboxylating enzyme is a malic enzyme (3) that uses oxidized NADP (NADP+) as a cofactor. Hatched areas indicate carrier-mediated transporters of metabolites across the chloroplast envelope. Between cytosols of the two cell types, metabolites and phosphate move along diffusion-driven concentration gradients. For most reactions, cofactors and transaminations have been omitted for clarity. 1, phosphoenolpyruvate (PEP) carboxylase; 2, malate dehydrogenase; 3, malic enzyme; 4, Ribulose bisphosphate carboxylase/oxigenase (Rubisco); 5, pyruvate phosphate dikinase. In C_4 plants, the compartmentation and control of sucrose and starch synthesis is greatly modified with respect to the scheme shown in Fig. 2 in this chapter; for example, sucrose is synthesized mainly in the cytosol of mesophyll cells, and starch in the chloroplasts of bundle sheath cells.

deacidification. Fahrendorf *et al.* (1987) have proposed, as a working hypothesis, that in CAM plants (which are primarily starch-formers): 1. Malic enzyme is the principal decarboxylase, whereas pyruvate P_i dikinase is present in amounts sufficient to convert the pyruvate formed back into PEP; and 2. the capacity for conversion of FruBP into Fru6P in the cytosol is low and levels of Fru2, $6P_2$ are low during deacidification. It is suggested that this apparent paradox may be due to a very low affinity of the FruBPase for FruBP, as occurs in the CAM but not in

the C_3 form of *Mesembryanthemum crystallinum* (Keiller *et al.*, 1987) nor in C_4 plants such as maize (Stitt and Heldt, 1985).

FURTHER READINGS

These sources provide additional in-depth coverage of this topic. For complete reference, please see the Reference section at the end of the book.

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