BOT 4503
3 Hours
Photosynthate utilization, including phloem transport

Objectives

1. Summarize generally the fate of photosynthate.

2. Discuss, in general, the relative permeabilities of the inner and outer envelope of the chloroplast.

3. Write a brief essay on how the functioning of the phosphate translocator serves to maintain, at least on a short-term basis, the overall level of elemental P in the chloroplast.

4. Describe in detail how the two ionic changes that occur in the stroma favor the retention of 3-PGA in the illuminated chloroplast. Briefly, on energetic grounds, why would you expect that PGA^{2-} would not be exported at appreciable rates from the illuminated chloroplast?

5. Describe how the phosphate translocator could serve as an energy shuttle.

6. Describe how the dicarboxylate translocator could serve as an energy shuttle. How could this carrier shuttle assimilated N?

7. Discuss generally the role of glycolate and glycerate carriers in the operation of the PCOP.

8. Is there a direct method for import into the plastid of ATP? . . . during which developmental stage is it presumed to function?

9. Discuss the role of the hexose-P translocator. Where is it found? Why would the phosphate translocator not serve to import carbohydrate destined for starch biosynthesis in these plastids?

10. Briefly describe starch.

11. Write an outline of the starch biosynthetic pathway. What is the name of the enzyme that catalyzes the regulated step? What are the products and substrates? How is this enzyme regulated? Explain how, in detail, the regulatory properties of this enzyme "fit" the conditions that prevail in the plastid when starch biosynthesis is indicated. Describe experiments that show that the properties of the isolated enzyme are really regulating metabolism in the plant.

12. In general terms, explain starch degradation.

13. Explain "biochemical pathway." Contrast "regulated step" with "equilibrium step."

14. Write out all the enzymes that catalyze the interconversion of fru 6-P and fru 1,6 P_2. Which is unique among eukaryotes in plants? Explain how subsets of these enzymes could catalyze a futile cycle. On the other hand, describe how two of them may function to synthesize PPi (at the cost of an ATP) and how another pair of them could serve to remove PPi.

15. Describe how the regulatory molecule fru 2,6-P_2 could facilitate metabolism in one direction or the other. How does this effector differently regulate plant and animal enzymes?

16. Discuss the regulatory mechanisms that affect the concentration of fru 1,6 P_2. Explain how the conditions that indicate the need to increase (decrease) the rate of sucrose synthesis are "signals" from pathway intermediates.

17. What is a common substrate for an enzyme that catalyzes the extension of a carbohydrate chain?

18. How is sucrose synthesized? How is the regulated enzyme in this "terminal" pathway regulated?

19. Briefly describe sucrose degradation.

20. Describe the mass flow mechanism of phloem transport and supplement this description with the requisite details of anatomy and plant water potential.

Lecture

Our attention turns to the utilization of photosynthate. The first overhead provides a general introduction to the subject, and provides a description of how we will proceed.

Overhead: Summary: fate of photosynthate

The processes that we will cover and emphasize are (1) synthesis of starch. As mentioned previously, starch (an α 1$\rightarrow$4, α 1$\rightarrow$6 glucan) is the prototypical carbohydrate storage product of higher plants. As mentioned before, starch is stored in plastids. In leaves, the plastids are generally differentiated as chloroplasts, whereas in storage organs, such as tubers, the plastids are differentiated as amyloplasts. (2) synthesis of sucrose. Also as mentioned often, sucrose is the prototypical carbon translocate. Thus, recently assimilated carbon is partitioned either into starch or sucrose. Synthesis and utilization of sucrose will be further divided into several sections, namely, (a) export of reduced carbon out of the chloroplast [along with a summary of the export of other metabolites], (b) synthesis in the cytosol of hexose from the

exported triose [which is regulated by the signal substance fru-2,6-P_2], and (c) translocation of the sucrose via the phloem to sinks.

Overhead: Chloroplast metabolite traffic I

As you infer from the previous overhead, sucrose is not synthesized in the chloroplast. Indeed, there is probably no sucrose in the chloroplast stroma, or the content there is vanishingly small. Carbohydrate is transported from the plastid via a so-called "phosphate translocator." This carrier protein is an antiport that provides for the strict counter exchange of PGA, PGal, Pi, and DHAP[1]. It transports only divalent species, the importance of which will be revealed later. This is the "most important" carrier (from the perspective of our discussions), and is found in the inner envelope[2] of all chloroplasts. One function of the phosphate translocator is in the export of triose-P, a RPPP product. [Please refer back to the metabolic chart on the RPPP. This chart, for ease, showed a hexose as the product of the RPPP, but please recall that any removed intermediate, be it an amino acid, a hexose, or a triose, is a product.] Thus, PGal is exported from the chloroplast at the same time as a Pi is imported. This strict exchange means that as photosynthesis proceeds on an even keel, PGal is used for sucrose biosynthesis, during which Pi is released. The direct uptake of Pi by the chloroplast maintains a constant concentration of elemental P in the chloroplast. (The form that elemental P takes, of course, is under metabolic control in the plastid. E.g., P may be Pi, or it may be in PGA, or fru 6-P, or whatnot.) In overall summary, CO_2 enters the plastid and carbohydrate leaves it. What if the rate of sucrose synthesis is too low? Triose-P can not be exported because the cytosolic [Pi] is exhausted. In this case, one would expect the chloroplastic Pi pool to be small (because elemental P is mostly as phosphate esters). This fluctuation of [Pi] is important to the regulation of partitioning, as you will see. A second function of the phosphate translocator is to act in the "shuttling" of energy to and from the chloroplast, as shown on the overhead. Imagine that exported PGal is exchanged for PGA, and that the PGal is oxidized in glycolysis (yielding an ATP and an NADH). That oxidation, as you

[1]Actually, the phosphate translocator will accept Pi and 3-carbon compounds that are phosphorylated in the #3 carbon position, so the above list is not exhaustive (e.g., glycerol 3-P could be added to it). The C_3 version of this translocator will not transport at appreciable rates 3-carbon compounds that are phosphorylated in the #2 carbon position, such as PEP and 2-PGA. (Our references to "PGA" have implied 3-PGA.)
[2]The outer envelope is non-specifically permeable to molecules Mr<10 KD; this permeability is facilitated by "porins."

know, results in the formation of PGA. Thus, the "circulation" of PGal→PGA (in the cytosol, glycolytic enzymes) and PGA→PGal (in the chloroplast, RPPP enzymes) is equivalent to the export of energy harvested by the light reactions. The opposite flow of carbon would be equivalent to the import of energy (from, say, glycolysis). This concept—that the chloroplast can be an energy source, independent of its role as a carbohydrate producer—is important. Some chloroplasts (like those of C_4 mesophyll cells) may act as an energy source without the complete complement of the RPPP enzymes.

The central tenet in the regulation of the phosphate translocator is tied to the light-harvesting machinery. As we mentioned before—e.g., during our discussions on the regulation of rubisco—the chloroplast stroma becomes more alkaline in the light. The pK of PGA is in the range of 6.8-7.1 (depending on conditions such as temperature and ionic strength). Thus, in the dark, PGA is about equally PGA^{2-} and PGA^{3-}. Because the phosphate translocator is selective for the divalent anion, PGA may be exported in darkness because of the "abundant" PGA^{2-} present. At pH 8.0 (a nominal value for the stroma under illumination), >90% of the PGA is in the non-translocatable form PGA^{3-}. As the K_Ms for the principal substrates (i.e., Pi, PGA, PGal, and DHAP) are all in the range 0.1-0.3 mM, and because Mg^{2+} (which, recall, is elevated in concentration in the illuminated stroma) blocks PGA export, PGA will not be exported from the illuminated chloroplast at appreciable rates even when its concentration is 10x that of PGal.[3] The lack of PGA export from illuminated chloroplasts has two consequences: (a) this energy-poor compound will not be exported when energy (resulting from light harvesting) is abundant. (b) the concentration of PGA will be high in illuminated chloroplasts (recall the reciprocal pool-size changes of RuBP and PGA that occur when the RPPP cranks up). Keep this important fact in mind, as it will come into play during our discussions on the regulation of starch biosynthesis.

[At much much slower rates, the phosphate translocator (like most proteins) will accept alternative substrates, in this case, such as Ru 5-P. At much much lower rates, the phosphate translocator will act as a uniport for Pi.]

Overhead: Chloroplast metabolite traffic II

[3]One could argue against the export of PGA^{2-} on energetic grounds also. The stroma is more alkaline than the cytosol in the illuminated plant. Thus, the export of PGA^{2-} and its subsequent dissociation in the cytosol to PGA^{3-} and a H^+ is equivalent to the export of a H^+ against a *p*H gradient.

In addition to the phosphate translocator, had by all plastids apparently, there are a number of other proteins that facilitate metabolite traffic across the inner envelope of the chloroplast. As implied, the phosphate translocator in C_3 plants does not facilitate transfer of PEP to any important extent. PEP plays a central role in C_4 plants, as you know. In C_4 plants, a modified version of the phosphate translocator is expressed; this C_4 carrier will transport PEP.

Chloroplasts have a generic dicarboxylic translocator. It can function in the exchange of malate for OAA. This shuttle is equivalent to importing (or exporting, depending on the direction of the transport) NADH ("reducing power"). This carrier can also function n the exchange of glutamate for α-keto glutarate (aka 2-oxy-glutarate). Glutamate is the transamination product of α-keto glutarate and glutamate is the usual amine donor, recall, for the synthesis of other amino acids from α-keto acids. This latter shuttle serves to exchange assimilated N.

There is kinetic evidence for glycolate and glycerate carriers. Recall that glycolate, the dephosphorylated product of RuBP oxygenation, leaves the plastid in an early event of the PCOP. As glycerate, up to three-fourths of the carbon exported as glycolate is "recovered" by the PCOP and this glycerate is imported into the chloroplast, where it is acted on by a kinase, to yield PGA, which "feeds" into the RPPP. The protein(s) responsible for the described transport has not been isolated. Is there a single antiport with a 2:1 (glycolate:glycerate) stoichiometry? Do different proteins transport the distinctive molecular species? We do not know.

An ATP carrier serves to import, but not export, ATP. This carrier may be only present or functional during chloroplast biogenesis. It is instructive to contrast this carrier to a similar one on the mitochondrial membrane that preferentially imports ADP[4].

The final carrier that we will discuss in any detail is the hexose-P translocator that facilitates transport of starch precursors into the amyloplast. This type of plastid also has a phosphate translocator, but it is unlikely that PGal can function as the precursor for starch for the simple reason that amyloplasts lack the gluconeogenic enzyme fru 1,6 P_2 phosphatase (FBPase). [Utilization of PGal would involve the condensation of two triose-Ps to form one hexosebisphosphate—see the RPPP. This hexosebisphosphate would have to be dephosphorylated, to yield hexosemonophosphate, a suitable substrate for starch biosynthesis.]

[4]Our description of this carrier is abbreviated. Another important metabolite, PEP, can be moved by it.

Listed as item #6 are slews of other carriers and postulated carriers that time will not permit our discussion of.

Back to the central issue, in brief summary, up to this point, the illuminated plant has made a "decision:" assimilated carbon, if destined for conversion to translocatable form, is exported from the plastid as a triose-P, prototypically PGal, whereas assimilated carbon destined for temporary storage in the chloroplast is not exported. Thus, recently fixed carbon is partitioned between starch and sucrose.

Overhead: Starch metabolism

A synopsis of starch biosynthesis is given on this overhead. A starch grain comprises two types of molecules that are composed entirely of glucose. First, amylose, which is a linear α 1,4 glucan, is relatively small, containing from a few hundred to a few thousand glucosyl residues. Amylopectin is highly branched (α 1,6 bonds form branches on the α 1,4 "backbone") and is much larger, from 2,000 to 500,000 glucosyl moieties.

A starting point for starch biosynthesis is the hexosemonophosphate fru 6-P, which is formed as an intermediate of the RPPP. Else, it may be formed from triose-P that is imported (via the phosphate translocator), and by subsequent condensation of 2 triose-Ps to form fru 1,6 P_2, which, finally, is hydrolyzed to Pi and fru 6-P. Recall, also, that plastids specialized for starch storage have a hexose-P importer. The hexosemonophosphates (fru 6-P, glc 6-P, glc 1-P) are readily interconvertible. The enzyme ADP-glucose pyrophosphorylase catalyzes the regulated step in starch biosynthesis, *viz*., the formation of ADP-glucose. This enzyme is regulated in a number of ways, particularly by the ratio [PGA]/[Pi]. A high ratio activates the enzyme; by way of example—with the extracted spinach enzyme—the presence of 1mM PGA (compared with its absence) lowers the K_MATP by 10x and increases the V_{max} by 7x, whereas Pi inhibits the enzyme by 50% at concentrations as low as 20 (to 400) μM. To put these data into a physiological perspective, the roughly 50% decrease in $[Pi]_{chlpt}$ (to 5 mM) and the 2-10x increase in $[PGA]_{chlpt}$ are calculated to increase v by ~23x. Notwithstanding, is starch synthesis really regulated *in vivo* by the ratio [PGA]/[Pi]? Several lines of evidence suggest that it is: (1) fungus-infected leaves are low in Pi, and they accumulate more starch; (2) incubation of chloroplasts in media containing different ratios of PGA/Pi (which will distribute into the chloroplast by the phosphate translocator) predictably controls the

rate of starch biosynthesis; (3) feeding leaves mannose (a hexose) results in the dropping of [Pi] because the mannose is phosphorylated, but mannose-P can not be further metabolized. This sequestration of orthophosphate results in elevated starch biosynthesis[5]. Let us back off now and look at the overall picture, albeit somewhat simplified: the RPPP is going at full-speed. Carboxylation of RuBP produces high levels of PGA, specifically of PGA^{3-}, because of the stromal alkalinization. [Pi] in the chloroplast drops because of the "expansion" of the phosphoester pool size. PGal is exported, but faster than the sucrose-synthesizing machinery (which releases Pi, but incorporates the carbon of PGal, as will be discussed below) can use it. The unavailability of cytosolic Pi for transport into the chloroplast prevents the replenishing of the chloroplastic pool of Pi. ATP concentration drops (because of the unavailability of Pi, a substrate for its synthesis). With low [ATP]s, PGA can not be phosphorylated efficiently (to form 1,3 diPGA), and for this reason also, [PGA] goes high. The resulting high ratio PGA/Pi turns on starch deposition. Whereas the foregoing explains the diverting of reduced C to starch, we will later examine how (under what conditions) sucrose biosynthesis is stimulated. As you will see, there is "competition" between the starch-synthesizing pathway and the sucrose-synthesizing pathway.

ADP-glc is the glucosyl donor for the extension of the glucan by, primarily, starch synthase and branching enzyme. The hydrolysis of the ADP-to-glucose bond and concomitant formation of the glucose-glucose bond (overall $\Delta G \approx -3$ kcal mol^{-1}) "drives" starch biosynthesis. We do not consider that the regulation of these "assembly" enzymes is typically of great importance, although under some conditions exertion of control by them is manifested[6]. [Please note that the entire process of starch biosynthesis is more involved than I have had the time to reveal; by way of example, there are interchain transfers of dextrins, &c.]

Starch degradation is accomplished through the agency of many different enzymes, and we have time only to touch on them. α-Amylase is a key enzyme; it accepts as a substrate glucose chains of $n>6$ glucosyl residues—it functions as an endohydrolase, and depending on the exact site of cleavage releases free glucose or glucose chains of up to 6 glucosyl residues. β-Amylase releases glucose dimers. Several different glucosidases hydrolyze the terminal glucose from an α 1,4 chain. Another activity, transglycolase, combines two shorter residues to form a longer chain that can be used by other enzymes.

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[5]As a technique, mannose feeding has been called into question recently because it is difficult to assess to what extent Pi pools in various leaf compartments have been affected.

[6]Starch synthase is one of many enzymes that requires K^+. Plants nutritionally deficient in K^+ produce primarily sucrose as an endproduct of photosynthesis and produce only minor amounts of starch.

Finally, phosphorylase[7] degrades starch with the conservation of part of the bond energy in the glucose-glucose bond—the released product is not simply glucose, but instead is glc 1-P.

That reduced carbon that is not assimilated into starch is exported and used for sucrose biosynthesis, a process that we will now seek an understanding of. Let us start with PGal in the cytoplasm to which it has been transported from the chloroplast via the phosphate translocator. A portion of the glycolytic sequence (fru 1,6 P_2↔DHAP + PGal↔1,3diPGA↔3PGA↔2PGA↔PEP) is in equilibrium. In other words, these intermediates will be present generally[8] at a fixed ratio, one to the others. Thus, if fru 1,6 P_2 is removed (for sucrose biosynthesis), the effect will be to inhibit glycolysis (because less, e.g., PGal will be present). On the other hand, if PEP is removed (e.g., by its regulated-step hydrolysis to pyruvate, or its regulated-step carboxylation to OAA), the effect will be to inhibit sucrose biosynthesis because fru 1,6 P_2 is "drained away" from that direction. Our focus will be restricted to fru 1,6 P_2 interconversion with fru 6-P because whatever causes phosphorylation of fru 6-P will be in the direction of carbon oxidation (energy yielding) via glycolysis and whatever causes the hydrolysis of fru 1,6 P_2 will be in the direction of carbon translocation (energy "banking") via the synthesis of sucrose.

Overhead: Bi-directional carbon flow is facilitated by unique enzymes that are differentially regulated

This overhead examines in more detail the interconversion of fru 6-P and fru 1,6 P_2. The triose-Ps combine to form fru 1,6 P_2. The latter may be hydrolyzed by frutosebisphosphatase (FBPase) to form fru 6-P, which leads to sucrose biosynthesis, a process that will be examined later. Fru 6-P (which can be formed from sucrose breakdown in a sink tissue or in a source tissue at a time that photosynthesis is not meeting energy demands) can be phosphorylated to form fru 1,6 P_2, which as mentioned "feeds" into glycolysis. Three important facts emerge: (a) bi-directional flow of carbon is facilitated by different enzymes, (b) these enzymes are differentially regulated, to avoid futile cycling with its attendant energy loss, and (c) only some steps in a biochemical pathway need to be regulated. We will focus on two enzymes: (a) as

[7] *In vitro*, phosphorylase catalyzes the reverse reaction also. Several lines of evidence indicate that *in vivo* its role is degradative (particularly, the energetics and pool-size ratios). It is of interest to note that this enzyme, unlike the Cori-Cori enzyme of mammals, is not regulated by phosphorylation.

mentioned, FBPase is a gluconeogenic ("new making of glucose"), and (b) pyrophosphate-dependent phosphofructotransferase (PFT, goes also by other trivial names, most commonly, PFP), which is glycolytic.[9,10] For present purposes, we will ignore the fact that plants also have two versions (cytosolic and chloroplastic) of the classical ATP-dependent phosphofructokinase, which is unquestionably glycolytic in all organisms, and the regulation of which we thought we understood following the detailed investigations of Lowry and his co-workers in the 1960's. In the mid-80's, E. Van Shaftingen and G. Hers made a landmark discovery: the endogenous novel regulatory metabolite, fru 2,6 P_2. The substance, at very low concentrations—reminiscent of those of hormones—is a very powerful activator of animal PFK and an inhibitor of FBPase. As it has turned out, fru 2,6 P_2 has no effect on plant PFK, but it is an even more powerful activator of PFT, found only in plants and some bacteria. Referring to the overhead, then, note that fru 2,6-P_2 stimulates glycolysis and inhibits metabolite flux toward sucrose biosynthesis. The bottom line is that fru 2,6 P_2 must be low, when sucrose is being made because this metabolite inhibits FBPase, and it also must be low in concentration, otherwise glycolysis would be stimulated.

F 2,6 P_2 is formed by the phosphorylation of fru 6-P though the agency of a specific ATP-dependent PF 2-kinase (the "2" is used colloquially to distinguish this activity from the glycolytic PFK). It is removed by a specific phosphatase[11]. The kinase is stimulated by Pi [12]and inhibited by 3-carbon compounds, particularly PGA. So far, then, abundant Pi means that sucrose synthesis is not limiting triose-P export from the chloroplast, so a brake is put on FBPase, and thus the velocity of formation of fru 6-P, which is used for sucrose synthesis, is slowed. PGA (at high concentration because it is in equilibrium with triose-P) signals the abundance of "feedstock" for sucrose biosynthesis--PGA tends to lower fru 2,6-P_2

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[8]Obviously, the concentrations of requisite cofactors (ATP, ADP, NAD^+, and NADH) will affect the balance, and, in extreme cases, whether the compounds are in equilibrium.

[9]This reaction is readily reversible, but, in most tissues, it is considered glycolytic. In all (?) non-plant eukaryotic organisms and most prokaryotes, the glycolytic enzyme that phosphorylates fru 6-P is the classical **P**hospho**f**ructo**k**inase (PFK). This enzyme behaves "as expected" —PEP (a down-stream glycolytic product) and citrate (a down-stream TCA product) inhibit it. Simply put, PFK is throttled back by product inhibition.

[10]It is interesting to consider that the classical ATP-dependent PFK (ATP + fru 6-P$\rightarrow$fru 1,6 P_2) operating at the same time as PFT (operating gluconeogenically, fru 1,6-P_2 + Pi$\rightarrow$fru 6-P + PPi) constitutes a means of synthesizing PPi at the expense of ATP. Similarly, the operation of FBPase operating concomitantly with PFT operating glycolytically provides a means of PPi removal. Looked at from this standpoint, the simultaneous interconversions of fru 6-P and fru 1,6 P_2 would not necessarily be a futile cycle.

[11]Interestingly, a bifunctional enzyme catalyzes both these reactions, at different active sites and by different mechanisms. This bizarre situation is known only for a handful of enzymes. In addition to the bifunctional enzyme, there are also other nonfunctional and specific enzymes for these tasks. We do not know which of these alternative activities are most "important" or whether one or the other may serve a special role at some time or in some cells.

[12]The fact that its own product Pi stimulates this enzyme should serve to alert you to the fact that this is unusual biochemistry.

concentration, and therefore relieve the bottleneck in the direction of sucrose biosynthesis. At the same time, lowered fru 2,6-P_2 removes the stimulation of the glycolytic direction of PFT. Breakdown of fru 2,6-P_2 is inhibited by Pi (as a reflection of its effect on the respective kinase). Fru 6-P has an effect on both enzymes as does Pi, but the role on the P'ase is thought to be most important: high fru 6-P indicates that there is abundant substrate for sucrose biosynthesis, and fru 6-P serves to maintain high fru 2,6-P_2, which blocks gluconeogenesis. In broad terms, high [PGA]/[Pi] stimulates sucrose biosynthesis in the cytosol, just as this high ratio stimulated starch biosynthesis in the plastid.

Overhead: Sucrose metabolism

As you have seen before in starch biosynthesis and cellulose biosynthesis, XDP-sugar is a donor for carbohydrate chain extension. Thus, UDP-glucose pyrophosphorylase catalyzes the formation of UDP-glucose, which is the substrate for the assimilative enzyme, sucrose-P synthase (SPS). SPS is activated by reversible post-translational modification (for sure, dephosphorylation activates the enzyme, but there may be other sites that are phosphorylated to activate the enzyme). Phosphorylation status of the enzyme is connected to photosynthetic carbon metabolism (CO_2, as well as light, is required for *in planta* activation). SPS, which exists as isoforms, is also regulated by intermediate metabolites, and is under developmental control (low SPS in sinks, like young leaves, and high SPS in sources, like mature leaves). Formed sucrose-P is readily hydrolyzed by specific enzyme to yield the endproduct sucrose.

Sucrose breakdown may proceed by several pathways. Invertase, of which there are cell-wall, cytosolic and vacuolar forms, simply hydrolyzes sucrose to the free hexoses. This reaction, which does not conserve the energy of the glucose-fructose bond is essentially irreversible. Sucrose synthetase (SS, a misnomer) catalyzes the breakdown of sucrose also--fructose and UDP-glucose are formed; thus, some bond energy is conserved as the UDP-glucose bond. This reaction is freely reversible. High SS is indicative of a sink.

Overhead: Phloem transport

With phloem anatomy, the concept of water potential and membrane transport of sucrose well in mind, the Pressure Flow hypothesis, first proposed by Münch in the 1930's and established as the universal mechanism of phloem transport by the early 1980's by scientists such as Swanson, Geiger, Fisher, Christy, Ferrier, et al., is easy to understand[13]. Sucrose is released into the apoplast by a photosynthesizing cell, from which it diffuses to the cell wall of the companion cell. (Recall the intimate relationship between the companion cell and the sieve tube--these cells essentially function as a single unit: they are derived from the same mother cell, the sieve tube element at maturity is enucleate and lacks a vacuole; these two cells types are connected at high frequency by plasmodesmata, but they do not have connections with other cells.) Sucrose is actively accumulated from the apoplast into the companion cell, where the concentration may be as high as 20%. The lowering of the Ψs by the concentrated sucrose causes the inward diffusive movement of water and, consequently, the internal hydrostatic pressure (Ψp) is high (because the overall water potential is about the same through out the leaf). As the sink end of the connected sieve-tube elements ($\Sigma \equiv$ sieve tube), sucrose leaves the companion cell-sieve tube element complex. Outside the complex, sucrose is metabolized (e.g., to starch) so that it does not contribute to the Ψs of the extra-phloem sink. Exit of sucrose from the complex raises the Ψs and decreases the Ψp. Therefore, there is a pressure gradient in the sieve tube from the sink to the source that is sufficient to drive mass or bulk flow of the solution from the source to the sink. (It is accurate enough for us to assert that any substance in the sieve tube will be transported at the same rate as any other substance; hence the phloem serves as the conduit for a variety of disparate compounds ranging from sucrose to the flowering stimulus.)

Note that an explanation of bulk flow from the source to the sink does not invoke the concept of water potential. Indeed, the water potential of source (e.g., a leaf) is typically lower than that of a sink (e.g., a root).

[13]There are some minor differences among plants. The two most important ones of these pertain to whether sucrose is delivered to the companion cell apoplastically (as I will show it), and whether export of sucrose from the companion

cell-sieve tube complex is an active process.