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Apoplastic Phloem Loading of Photoassimilate

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ABSTRACT

Photoassimilates translocate from regions of carbohydrate synthesis(source) to regions of carbohydrate utilization or storage(sink). In the source, assimilate loads into the phloem for long-distance transport. Current evidence suggests that there are two loading mechanisms : one involves assimilate transfer via the apoplasm and then load into the phloem by carrier-mediated proton-sucrose cotransport, while the other involves movement through the continuous symplastic connections between the mesophyll cells and the phloem. Inspite of problems associated with the interpretation of experiments, the evidence for apoplastic loading remains convincing because the apoplastic loading systems explains well the observed accumulation capacity and the selectivity of assimilate uptake by the phloem.

Abbreviations : $\Delta\Psi$, membrane potential ; 3-O-MeG, 3-O-methylglucose ; FC, fusicoccin ; IAA, indol-3-yl acetic acid ; kDa, kilodalton ; Km, Michaelis-Menten constant ; MW, molecular weight ; NEM, N-ethyl-maleimide ; PCMBS, parachlromercuri-benzene sulfonic acid ; PMF, proton motive force ; SE-CCC, sieve element-companion cell complex ; Vmax, maximum rate of reaction.

Key words : Proton-sucrose cotransport, sucrose carrier, mechanism of translocation.

INTRODUTION

For translocation from source to sink, photoassimilate in the source leaves should load into the phloem. This process, phloem loading is defined by Geiger[35] as the "process by which the major translocate substances are selectively and actively delivered to the sieve tubes in the source region prior to translocation". From this point of view, phloem loading may be envisioned either as occurring directly in the apoplast through membrane transport or as occurring from the symplast of the cells surrounding the SE-

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CCC.

In the early 1980s, a model(Fig. 1) emerged which appeared to describe satisfactorily sucrose transport from the photosynthetic cells into the leaf vascular tissue[41]. This model suggested that (a)the assimilates are released in the free space at some point between the mesophyll and the veins, (b)that the veins are able to take up sucrose selectively and actively from the apoplast against a large concentration difference by a proton-sucrose transport. The resulting osmotic potential generated within the phloem is responsible for long distance transport through the positive hydrostatic pressure produced within the conducting cells, as water moves in down its potential energy gradient.

Until recent years, lots of data have been considered as favoring apoplastic phloem loading with a proton-sucrose cotransporter. Evidence for the proton-sucrose transporter can be summarized as follows : firstly, H^+ ions are removed from the medium surrounding the cell(alkalization of the medium) following addition of exogenous sucrose[53], secondly, membrane depolarization occurs temporarily after the addition of sucrose to the medium surrounding the cells, followed by re-polarization as the H^+-ATPase re-establishing the proton gradient[54], thirdly, sucrose transport ceases when H^+ is in equilibrium across the membrane and reverses if the H^+ concentration

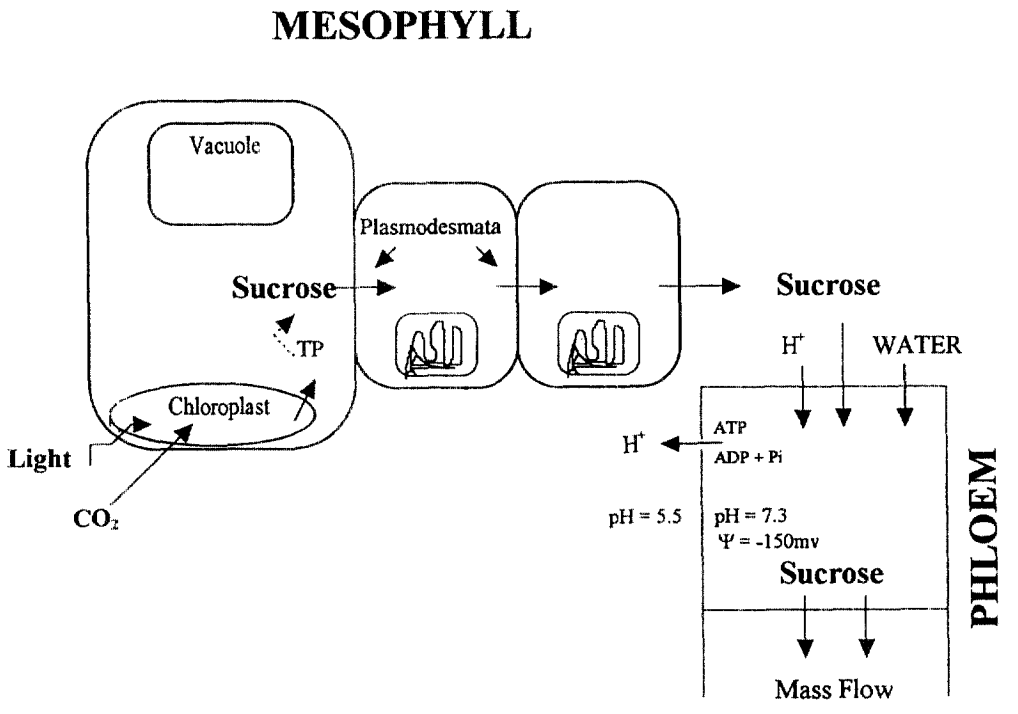

Fig. 1. The apoplastic phloem loading model. Carbon dioxide is reductively assimilated in the chloroplasts. Triose phosphates transport out of the chloroplast by the phosphate translocator (a)and, through a series of enzymic reaction, sucrose is synthesized in the cytoplasm. Sucrose is stored in the vacuole or it moves from cell to cell via plasmodesmata(symplastic phloem loading). Sucrose is released from the mesophyll in the vicinity of the vascular tissue. Apoplastic sucrose is transported into the phloem against a large concentration difference by the proton-sucrose cotransporter (b)(apoplastic phloem loading). The thermodynamic driving force for proton-sucrose cotransporter is a proton motive force. Redrawn from D.R. Bush[11].

gradient is reversed[70], and fourthly, transgenic plants expressing the *suc2* gene, encoding a yeast-derived invertase targeted to the apoplast of tobacco[90], *Arabidopsis*[90], tomato[22,59] and potato[44], showed decreased phloem loading of sucrose.

However, the variety in the plant kingdom which shows different structural arrangements of the leaf as well as experimental processes make a universal pathway of sugar transport in the leaf unlikely. Recent evidence is beginning to change to see phloem loading as a process which is different in various plant species. Good evidence for symplastic phloem loading was obtained with microautoradiography of minor veins of *Cucumis*[88], *Ipomoea*[67], and *Coleus*[96,97]. The results imply an absence of apoplastic loading in plants with a symplastic minor-vein configuration. However, one of the disadvantage for any symplastic loading mechanism is that the hypothesis does not explain well the thermodynamics of symplastic sucrose transfer against its concentration gradient. In support of symplastic phloem loading, several mechanisms have been proposed[94].

The objective of this study is to understand the mechanism of apoplastic phloem loading of assimilates. In this review, I focused my discussion on the universality of apoplastic loading in plant kingdom, the energetic feasibility of proton-sucrose cotransport system, the kinetic and biochemical characteristics of sucrose carrier, the control mechanism of sucrose carrier, and the reactivation of sucrose carrier in plasma membrane. The knowledge on the phloem transport of assimilates can be used to understand the mechanism of phloem transport of herbicide since the phloem-loading of herbicide is dependent on that of assimilate[21,51].

MECHANISM OF PHLOEM LOADING

Universality of apoplastic loading in plant kingdom

The concept of apoplastic loading of sucrose claims that photosynthates produced in the mesophyll move to the apoplast prior to active accumulation by the SE-CCC. This concept has been supported from the study of anatomy and cytology, and presence of sugars in the leaf apoplast.

The results of anatomical and cytological studies are that there are only a few symplastic connections(plasmodesmata) between the mesophyll and the SE-CCC in the monocots[99] and dicots[29]. Such symplastic constrictions in the sucrose loading path are believed to be strong anatomical support for apoplastic transfer. In many cases, the minor vein ultrastructure seems to fulfill the prerequisite for apoplastic transfer i.e. that number of plasmodesmatal connections between mesophyll symplast and sieve tube is very much restricted.

Microinjection of the fluorescent dye Lucifer-yellow[68] supported the anatomical work of plasmodesmata. However, there is no simple evaluation of the function of plasmodesmata from different tissues because : (1)the functional diameter of the plasmodesmata may vary. Burnell[8] reported that plasmodesmata of the bundle-sheath cells of C_4 plants were somewhat broader than those of other cells, (2)the diverse structure of the plasmodesmata[84,85] suggests diverse functioning, and (3)the method of functioning of plasmodesmata is unknown.

Since the number and structure of the plasmodesmata varies with the kind of bundle, the stage of development of a leaf and the cell type considered, no generalization concerning the number of plasmodesmata at the boundary of the conducting complex can be made. Besides, the

presence of these plasmodesmata does not necessarily imply that the symplastic pathway is open. Researchers have argued that plasmodesmata in the conducting bundles of mature leaves may not function in the early stages of leaf development when the young leaf may import assimilates by the symplastic pathway.

The leaves of *Commelina benghalensis*[99] and *Amaranthus retroflexus*[29] showed a low plasmodesmatal frequency between vascular parenchyma-mesotome parenchyma and the SE-CCC.

However, in some species the anatomy does not fulfill this requirement for apoplastic transfer. In *Populus deltoides*[86], the plasmodesmatal frequency does not decrease at all in the sieve area, as this appears to be the case for *Triticum aestivum*[55].

Anatomically C_3 and C_4 plants are different. One difference is that C_4 plants have a suberized mesotome sheath. If apoplastic loading theory says that sucrose loads into SE-CCC from the apoplast, then what is the role of the impermeable suberized sheath? On this issue, Robinson-Beers and Evert[85] reported that the walls of bundle-sheath cells of sugar cane contain continuous suberin lamellae like those of other NADP-malic enzyme C_4 grasses[6,43]. Free-space marker studies[5] showed that apoplastic movement of water and solutes does occur along this pathway, the impermeable suberine lamellae channelizing the flow of the transpiration stream there. Peterson et al.[78] and Eastman et al.[25] have also demonstrated that neither suberized mesotome sheath nor parenchymatous bundle sheaths function in a restricted sense but allow the free space passage of apoplastic water and solutes from the veins to the mesophyll. Ultrastructure and plasmodesmata frequency study[85] also support that phloem loading in the large bundles involves an apoplastic step.

Mature leaves of *Moricandia arvensis*, a C_3-C_4 intermediate, shows that all possible cell combinations of the leaf contain plasmodesmata except the bundle-sheath cell and sieve tube member interface where plasmodesmata are either rare or absent. On the basis of plasmodesmatal distribution and concentration gradient which exist along the mesophyll/bundle-sheath/vascular parenchyma/phloem parenchyma. Beebe and Evert[4] concluded that sucrose moves symplastically to the region of the sieve tube-companion cell complex and then phloem loading occurs from the apoplast.

As to the mechanism of phloem loading in source leaves[18,100], it has been reported to be apoplastic in some species[26,41,85], symplastic in other species[50,56,62,84] and both symplastic and apoplastic in still others[28,30,100,101].

Structural diversity among plant species precludes a universal pathway for assimilate movement from the photosynthetic tissue to the phloem. The distribution of plasmodesmata, the cellular composition of the veins involved in phloem loading, and the structural relationships between these veins and the adjacent photosynthetic tissues have been shown to be species-specific[6,29,33,34,72,85,86,88,98].

Study on the distribution of plasmodesmata shows that not all the plants load photoassimilate via an apoplastic route. This might be due to the structural diversity among plant species. Concerning the apoplastic route, the structural diversity raises many questions such as :

(1) Is the function of plasmodesmata determined by the number of plasmodesmata? The functional diameter and structure of plasmodesmata are open to question.

(2) The structure of plants is diverse. Will plants in one category, such as C_3, C_4, and CAM, show the same loading pattern?

Energetic feasibility of proton-sucrose cotransport system

The accumulation of sucrose by the phloem has implicated the role of the proton-sucrose cotransport mechanism as the energetic basis for active sucrose transport into the sieve tubes. The marked proton gradient between the sieve tube contents and the apoplasts[39] strongly supports the concept that the PMF provides the energy for sucrose accumulation into sieve tubes.

Several investigators tested the electromotive force of proton-sucrose cotransport system by examining the effect of membrane potential on proton-coupled flux[7,10,49,58,102]. The results of these experiments showed that positive membrane potentials(positive inside) decreased and negative potentials increased transport activity. This electrogenic transport moves positive charge into the isolated vesicle, therefore it was concluded that the sucrose symport system is electromotive. The impact of the membrane potential on transport kinetics showed that changes in altered Vmax with little effect on apparent Km[10,11].

Proof of active uptake against electrochemical potential gradient has been observed in a variety of higher plant cells and tissues and plasma membrane vesicles[7,9,57,58]. The most widely favored hypothesis to account for active transport of sucrose at the present time proposes that the immediate source of energy driving the transport is an ion gradient[12,73]. Inward flux of sucrose is postulated to be coupled to the downhill flux of proton by means of a carrier system which binds both the proton and the sucrose. The proton gradient itself is generated by a membrane-sited energy transducing ATPase complex that transfers protons outward across the plasmalemma. Since positive charges(protons) are transferred, the pump is electrogenic and is believed to contribute substantially to the membrane potential $\Delta\Psi$. The PMF $\Delta\mu_{H^+}$ has electric and chemical components according to the relationship

$$\Delta\mu_{H^+} = \Delta\Psi - (2.3\ RT/F)\ \Delta pH$$

where R and F are constants and ΔpH is the difference between cytoplasmic and external pH. From the equation, the thermodynamic driving force for proton-sucrose cotransport consists of $\Delta\mu_{H^+}$, ΔpH and $\Delta\Psi$. It is also proved that plasma membrane vesicles were energized by artificial gradients(pH gradient [ΔpH], electrical gradient [$\Delta\Psi$], or both)[7,9,57,58].

Bush[9,10] showed that carrier-mediated sucrose transport was electrogenic which is driven by both ΔpH and $\Delta\Psi$, the binding affinity of the symport for protons(Km=0.7μM) was much higher than that for sucrose(Km=1), and the stoichiometry of the transport was 1 : 1. Considering the sucrose concentration of SE(0.8M) and mesophyll cells(0.02M) in sugar beet leaves[38,91] and osmotic pressure differences, the PMF(-198 mV at 25℃) is higher than the free energy needed to maintain the concentration difference(-94mV). This PMF is reported to produce a 100-fold difference between the rate constants for inward and outward migration of the charged species in the carrier model.

Lemoine et al.[57] showed that a high PMF (-200mV) was generated by plant plasma membrane vesicles and the PMF in the presence of $\Delta\Psi$ alone was higher than in the presence of ΔpH alone, whereas similar values of sucrose uptake were observed under both conditions[58]. This indicates that PMF consists of ΔpH and $\Delta\Psi$ and it is agrees well with the model of proton-sucrose symport implying that the proton has to bind to the sucrose carrier in order to give an overall positive values to the complex proton-sucrose-carrier[20].

Carrier specificity of apoplastic loading

The evidence supporting the carrier-mediated flux includes substrate specificity and sensitivity to inhibitors.

The loading of sucrose is selective, since sucrose is the major phloem translocating sugar in many species. However, the reasons for this are not clear but are suspected to involve its relative unreactive chemical structure.

From phloem exudation experiment, various translocating sugars are reported. Gamalei[32] reported the diverse mixture of translocating sugars such as mono-(glucose and fructose), di-(sucrose), and oligosaccharides(raffinose, stachyose, verbascose), sugar alcohols(mannitol, sorbitol) and other components. Translocation of oligosaccharides is interesting because raffinose is not transported by the sucrose carrier[19]. However, some data show that oligosaccharides are translocated[20]. Existence of oligosaccharides might be understand that from the cut end of petioles and stems, invertase, sucrose synthase[64] and oligosaccharides synthesizing-enzymes which are released from the disrupted tissues might hydrolyze sucrose to hexoses or might synthesize oligosaccharides, respectively, in the buffer solution.

To explain the translocation of raffinose sugars, Turgeon[94] suggested the symplastic phloem loading of these sugars against the concentration gradient. By this model(the polymerization trap mechanism), galactinol and sucrose diffuse from the mesophyll to the intermediary cells where raffinose is. The molecular size of the raffinose prevents symplastic back-flow to the mesophyll, but allows transfer to the SE via broader plasmodesmata. Recently this hypothesis of Turgeon was confirmed by immunolabeling technique[48]. Considering molecular weights and chemical structures of oligosacchrides, which are different from sucrose, oligosaccarides seem not to translocate via the sucrose carrier. However, that does not mean that there is no possibility of the existence of a raffinose carrier in the plasma membrane.

The carrier specificity for sucrose translocation is based on transport studies that showed little competition by a ten fold excess of glucose, fructose, raffinose, maltose, mannose, melibiose or lactose[7,9]. Similarly, carrier mediated flux is consistent with the observation that PCMBS which can covalently modify proteins is a potent inhibitor of pH-dependent sucrose transport[9,65,102]. Among 14 compounds(sugars, amino acids, hormones), sucrose uptake was the most sensitive to PCMBS. This may suggest that labeled PCMBS could preferentially mark this carrier, but there is also a possibility that the inhibitor may bind to other proteins in nonactive sites, hence creating a background of labeled molecules.

Studies on carrier-specific analogs gave us a light to solve the universal question of apoplastic loading of sugars. Tightly binding and nonpermeable sucrose analogs could be used to block the putative loading site carrier. If the radioactive analogs are used as a marker for sucrose carrier proteins, the isolation of the sucrose carrier will be easy and the isolated carrier can be used in characterization and reconstitution experiments.

The result of exogenously supplied L-glucose or 3-O-MeG[17] showed that these sugar analogs were poorly phloem loaded in leaf discs, compared to sucrose. Results on the study of the surface properties of the sucrose and of the specifically derivatized sucrose analogs[45,62] show that sucrose recognition occurs from the interaction of a relatively hydrophobic portion of the sucrose molecules and a hydrophobic region of the carrier protein binding site.

Proton-sucrose transport activity is expected to be mediated by a single polypeptide rather than a heteromeric protein complex. This expectation is based on a knowledge of other cation-substrate

symports and on recent success with solubilization and reconstitution. Several cation-driven symports from a variety of prokaryotes and eukaryotes have been purified and cloned : including, the proton-lactose symport, the cation-melibiose symport, several cation-glucose symports, the proton-xylose symport, the proton-arabinose symport, the sodium-proline symport, and several yeast amino acid porters[3,47,69]. Each cation-coupled transport system listed above is mediated by a single polypeptide. Recent solubilization and reconstitution of the sucrose symport into proteoliposomes is consistent with a single polypeptide[60].

Carrier-driven symport might reflect the possibility of finding the oligosacchride carrier. Study on oligosacchride analogs will help to understand the substrate and carrier.

Recent advances on X-ray crystallography, nuclear magnetic resonance spectroscopy and computer-based image processing may give us more information on the carrier and carrier specificity since some aspects of a substrate like the geometric arrangement of atoms-its charge and size, and influence its interaction with sucrose-binding proteins.

Kinetics and biochemical characteristics of the sucrose carrier

Kinetics of ^{14}C sucrose loading into sugar beet leaf discs revealed the presence of a two component system[71]. At low exogenous sucrose concentrations, a saturable component, which exhibited Michaelis-Menten characteristics, was the main mode of transport and the saturable component was identified with proton-sucrose cotransport. At high concentration(>50mM), phloem loading was dominated by a linear component which appeared to operate as a first order kinetic transport process.

Sucrose uptake kinetics show multi-phasic systems : saturable[99], linear non-saturable or saturable + linear system[14,87]. Physiological assessment of the multi-phasic isotherms are difficult since it might be due to the superimposition of non-membrane events on a carrier-mediated system.

Evidence for the concentration dependence of sucrose uptake and phloem loading is abundant. However, comparisons and generalization of the results are impossible since experimental designs are different.

Isolated conducting tissue of *Cyclamen persicum* Mill, Grimm et al.[42] showed similar sucrose uptake to sugar beet leaf discs : i.e., the total uptake of sucrose yielded saturation kinetics of a Michaelis-Menten type with an apparent Km = 5.2mM and Vmax = 4.2μmol g $FW^{-1}h^{-1}$. In the high concentration range 20-400mM the total uptake was linear.

Traditional kinetics studies used isolated mesophyll cells, isolated phloem tissues and leaf discs. However, these experimental methods have disadvantages. Use of isolated mesophyll cells presents problems. First, the techniques used to isolate the mesophyll cells or protoplasts inevitably alter the cells in unknown ways[19,95]. Second, release in the mesophyll is only pertinent to the retention of assimilates in the symplastic section of the pathway to the phloem[19]. These problems provide arguments against kinetic results.

Leaf disc experiments do not provide the exact kinetics, site and mechanism of release of sucrose from the mesophyll symplasts-mesophyll cells, mesophyll sheath, phloem parenchyma- and subsequent uptake by the SE-CC complex. Leakage from discs probably includes : loss from the mesophyll, and leakage from the cut veins[2,48]. In addition, the leakage solute is contaminated with the compounds in the apoplast at the start of the washing. Misleading information from leaf discs also comes from an unknown contribution of mesophyll cells, as mesophyll cells seem to pos-

sess more high-affinity low capacity system[99].

Based on the idea that active sucrose transport across the plasmalemma is consistent with cotransport of protons and sucrose, plasmalemma vesicle study has been performed to understand the kinetics. In plasmalemma vesicles isolated from the leaves of sugar beet[9,58] and *Ricinus*[102], proton-coupled sucrose transport showed an apparent Km of 1. This value is different from 25 mol m^{-3} from intact *Ricinus* leaves which indicates the presence of a saturable carrier mediated component and a linear component[52]. The discrepancy in the Km values from intact cotyledons and plasmalemma vesicles may result from problems encountered measuring transport in intact tissues : complications from diffusional barriers and difficulties in ascertaining exactly at which cells and membranes uptake is occurring[102].

The kinetic parameters associated with a saturable system vary widely. The high-affinity system is always sensitive to PCMBS, however, the lower-affinity system is not[15,71] or only partially sensitive to this inhibitor. This suggests that the lower-affinity system is not purely diffusional.

These multi-phasic system might be described by one of these possibilities or a combination :

(a) carrier located in different cells have different functions.

(b) different carriers located in different cells, and

(c) different carriers located in different membranes of one cellular type.

In general, many data have shown that the presence of two uptake components is a general characteristics of many different plant cells and that the high-affinity system is more concentrated in the conducting complex than the other cells[71].

Properties of the proton-sucrose transport

Sucrose uptake from the apoplast with the proton-sucrose cotransporter is a common feature of apoplastic sucrose loading. If it is a common property shared by the cells of the conducting complex and the parenchyma cells, why is this process more efficient in the conducting cells than in the parenchyma cells? This could be due to a greater activity of the ATPase in the conducting complex, which would allow a faster rate of functioning of the sucrose carrier than in parenchyma cells, or the functioning of a higher density of sucrose carrier.

Identification of proton-sucrose cotransport showed two different experimental approaches. Hitz et al.[45] systematically examined the structural determinants required for sucrose transport into protoplasts isolated from developing soybean cotyledon. Based on their observations, a photolyzable sucrose derivative was synthesized and used to specifically label a 62kD sucrose-binding protein in the plasma membrane of these protoplasts. In addition to binding the sucrose analogs, this protein appears in the membrane at the same time as sucrose transport activity[83] and immunocytochemical localization of this protein in leaf tissue places it in the phloem[103].

Gallet et al.[31] labeled the sucrose transport system with the protein modifier, NEM. In the experiment, a 42kD protein from sugar beet leaf tissue was identified based on differential incorporation of ^{14}C-NEM in the presence or absence of 250mM sucrose. The differential labeling was observed only when transported sugars(sucrose, α-D-glucoside) were present in the incubation medium, but not in the presence of the non-transported analogues. Lemoine et al.[58] supported that conclusion using immunological method. Polyclonal antibodies against the 42kD protein decreased sucrose transport by approximately 55%. However, there are several ambiguities associated with the data implicating this polypeptide since the extremely high concentration of

sucrose used to protect the sucrose transport system during labeling is known to significantly alter the surface chemistry of biological membranes[13]. A global change in the surface properties of the plasmalemma could lead to unspecific labeling patterns and would be consistent with the high number of proteins that were differentially labeled. Another complication concerning the NEM data emerges from the kinetics of PCMBS interaction with the symport that show PCMBS-dependent inactivation is not substrate protectable. This result suggest PCMBS binding is not linked to the active sites and, therefore, it cannot be expected that differential incorporation of label at the sensitive sulfhydryl group based on substrate protection. Li et al.[60] reported the first successful solubilization and reconstitution of the proton-sucrose symport. The western blot of total plasma membrane protein showed that their antiserum crossreacts with many proteins ranging in size from about 35 to 50kD. Additionally, SDS- denatured proteins derived from the gel filtration peak ranged in size from 30 to >100kD. These observations obscure the significance of the 42kD protein in the reconstituted system.

The non-penetrating reagent, PCMBS has been extensively used to identify membrane-embedded carrier proteins which are involved in the transport of amino acids, sugars, and ions in a variety of organisms[65]. The rationale is that if the sucrose derived from photosynthesis is actively transported across a membrane into the phloem from the free space(cell walls), then a reagent that inhibits uptake of exogenously supplied sucrose into the phloem would inhibit sucrose entry into the phloem loading. Results show that PCMBS markedly inhibits sucrose uptake into leaf tissue and the site of inhibition is limited to the plasma membrane, possibly by reacting with sulfhydryl groups of a carrier proteins. In contrast, the membrane-penetrating chemical modified, NEM, which is also specific for -SH groups, completely inhibits photosynthesis and respiration.

The characterization and isolation of the sucrose carrier in a functional form opens up a wide range of new studies, including the molecular functioning of the sucrose carrier in reconstituted system and its distribution amongst different membranes, cells, organs, and plants.

Evidence for a proton-sucrose cotransport has accumulated, however little progress has been made in identifying the cotransport protein or in characterizing the system with suitable biochemical methods. In order to understand the proton-sucrose cotransport, researchers tried to have get purified membrane vesicles which represent a powerful experimental system. With intact cells membrane transport activity could be examined, however there were complicated problem such as the metabolism and intracellular compartmentation that limit the experimental interpretation.

However, the experimental difficulties have showed progress on the sucrose transport system. One problem of intact cells is that it is difficult to identify the bioenergetics of a transport system because of complex interactions among the primary pumps, ion channels and other unrelated transporters. Unlike the intact cells, isolated plasma membrane vesicles are a simple system which allow to focus on specific transport processes[75,93]. The other difficulty is the purification of plasma membranes from green leaf tissues. This problem seems to be solved, at present, by an efficient purification method for plasma membrane from leaf tissue[56]. With this method, highly purified plasma membrane vesicles that are free of the contaminating chloroplast membranes and proteins can be obtained. These vesicles are known to configurate 'right-side out', the native form.

Use of sugar analogs, sucrose transport inhibi-

tors such as PCMBS and NEM, and purified plasmalemma vesicles would help to understand the kinetics and characteristics of sucrose carrier. Purification of plasmalemma seems to solve one of the difficult problems on sucrose carrier study. Though there are still questions on the sucrose carrier system from plasmalemma vesicles whether the system represents that from phloem tissues, molecular biological study will help to reconstruct the sucrose carrier.

Reactivation of sucrose carrier in plasmalemma and sucrose carrier inducer

During transition from sink to source, photosynthetically active leaves gain positive carbon balance, and in post-transitional leaves of some plants such as sugar beet, sucrose destined to be exported out of leaves enters apoplast prior to loading into the minor veins[41,80]. Based on the assumption of apoplastic loading and the fact that termination of assimilate import coincides with a positive carbon balance in leaves, Giaquinta[41] proposed that apoplastic sucrose might induce the development of a putative phloem-located sucrose carrier, leading to vein loading and export out of leaves.

In microbial system and lower plants[27], induction of sucrose carrier by sucrose has been reported. However, in the induction experiment, Pitcher et al.[81] found that sucrose was not a direct and specific inducer of its putative carrier, and it is suggested that sugars may provide the energy for vein loading.

Light has been proposed for sucrose carrier induction[80,81]. Pitcher and Daie[80] found that sink to source transition did not appear to be light-regulated, while the ability to accumulate sucrose in the veins for export out of leaves was light dependent.

Ding et al.[25] suggested that the development of air spaces during the final stage of cell expansion is responsible for the disruption of plasmodesmatal connections which results in the import of sucrose. However, Pitcher and Daie[80] found that the intracellular air space were not developed beyond that of a normal sink leaf.

At present, air space, sucrose, and light have been proposed as sucrose carrier inducers. However, there is no direct evidence that one of the proposed inducers induce sucrose carrier. As Pitcher and Daie[80] suggested, cessation of import may be independent of the development of export capacity. In order to understand the induction mechanism, further study on sink to source transition is needed.

Control mechanism of sucrose carrier

Since photoassimilates produce in the mesophyll and selectively and actively load into SE-CCC, control of sucrose export from leaves can be explained at the level of :

(a) production and partitioning of photosynthate ;
(b) intra- and inter-cellular compartmentation of assimilates ;
(c) localized entry of sucrose into the apoplast near the loading sites ;
(d) the active loading mechanism on the SE-CCC.

Considering that translocation in a plant is a well-integrated and complicated process, no single site can be treated independently from the entire tissue. In this report, however, I will focus the control mechanism at the loading sites.

High sucrose concentrations within the phloem have the potential of inhibiting the further accumulation of sucrose[81]. The high internal concentrations of sucrose are presumed to prevent the dissociation of the hypothetical carrier substrate complex on the inside surface of the plasma membrane. Giaquinta[40] found that a high internal sucrose concentration inhibited the uptake of [^{14}C] sucrose in sugar beet source leaves. Inter-

nal solute concentrations have been proposed to exert allosteric control of transport carriers.

Based on transport kinetics, it was suggested that the binding of K^+ to allosteric sites on the carrier complex induces a conformational change which reduces the affinity of the carrier toward additional K^+. Uptake of K^+ and K^+-stimulated, Mg^{2+}-dependent plasmalemma ATPase activity displayed kinetics which were consistent with a carrier. Lindberg[63] confirmed the fact that sucrose allosterically affects *in vitro* ATPase activity in the presence of K^+ or Na^+ on a plasmalemma ATPase in sugar beet roots. Sucrose in the presence of the substrate MgATP and Na^+ was suggested to activate ATPase activity.

Turgor has been reported to affect the phloem loading. Increase in sink demand would decrease phloem turgor and it results in compensatory changes in sucrose and K^+ loading. Experiments with isolated leaf discs of *Ricinus*[89], of *Beta*[36], of *Phaseolus*[15], and with isolated veins of *Pisum*[25] showed that a decrease in turgor promoted phloem loading. Kinetic studies with *Phaseolus* leaf discs demonstrated that turgor affects only the saturable component of sucrose uptake, whereas the linear component was unchanged. The proton-pump seems a good target sensor and the transducer of osmotic conditions[82]. And this sensitivity is confirmed by electrophysiological measurements[61].

Membrane potential may affect the phloem loading possibility via ATPase. Turgor changes have also been linked to membrane conformational changes and in membrane potential changes[37].

As the plasma membrane H^+-ATPase plays a central role in the phloem loading of assimilate, the plant growth factors which can affect H^+-ATPase are presumed to control phloem loading of assimilate[76]. However, very little is known about the mechanisms that regulate plasma membrane H^+-ATPase activity in the intact cells. The identification of an auto-inhibitory domain in the C-terminal of the plant plasma membrane H^+-ATPase[77] implies that there are several possible ways of self-control mechanism for ATPase such as the binding of effector molecules, phosphorylation, partial proteolysis, or removal of the inhibitory domain at the gene level. In addition, proton pumping across the plasma membrane could be regulated by changes in the transcriptional activity of H^+-ATPase genes or by differential expression of pump isoforms varying in their C-terminal domain.

Plant growth regulating hormones such as IAA have been implicated in the regulation of phloem-mediated assimilate translocation[74]. Sturgis and Rubery[92] have reported that, in the leaf discs of *Phaseolus vulgaris*, sucrose uptake was promoted by IAA in the presence of 2.5 mol m^{-3} KCl. Aloni et al.[1] reported that application of GA_3 to mature leaves of broad bean promoted export of assimilates. Hormonal effects were also found with isolated vascular bundle of celery[16]. Estruch et al.[25] showed that hormonal action could be focused on the H^+-ATPase which is located at the plasma membrane. The hormonal response of the vein system was dependent on the osmotic concentration of the solution which indicated an interaction between hormonal effects and cell turgor.

Regulation of phloem loading can be made by changes of sucrose concentration, turgor, membrane potential, H^+-ATPase activity, and plant hormones. These factors, separately or combined, affect the common parameter in the phloem loading : the transmembrane sucrose gradient, and the energization status of the membrane.

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