Biochemical and molecular characterization of sucrose and amino acid carriers in *Ricinus communis*

Lorraine E. Williams¹, Julie A. Bick, Anil Neelam, Kim N. Weston and J.L. Hall

Department of Biology, School of Biological Sciences, University of Southampton, Bassett Crescent East, Southampton SO9 3TU, UK

Received 14 August 1995; Accepted 29 November 1995

Abstract

The use of energized plasma membrane vesicle preparations from cotyledons and roots of *Ricinus communis* seedlings is described, and evidence is presented for the existence of plasma membrane H⁺/sucrose and H⁺/amino acid symporters. Using fractions isolated from roots, there is evidence for at least two carriers which can transport neutral amino acids and one which can also transport basic amino acids. A method for the solubilization and reconstitution of glutamine transport activity is described. Preliminary results on the molecular characterization of two putative amino acid carriers and a putative sucrose transporter from *Ricinus* are presented.

Key words: Carrier, glutamine, plasma membrane, *Ricinus communis*, sucrose.

Introduction

The membrane transport of solutes such as sugars and amino acids is an important process in the partitioning of assimilates within the plant. Studies using intact tissues, suspension-cultured cells and protoplasts have suggested that sucrose and amino acid uptake across the plasma membrane of higher plants is mediated by specific carrier proteins and driven, in many cases, by the proton electrochemical gradient existing across this membrane (Reinhold and Kaplan, 1984). However, there are a number of complications arising when using intact cells and tissues for studying transport processes; these include diffusional barriers, cell wall binding, metabolism, compartmentalization, interference by solute transport processes at other membranes, and indirect effects on the driving force for uptake. Significant advances in the characterization of the transport properties of sugar and amino acid carriers have been made by studying these solute transport processes in isolated membrane vesicles (Bush, 1993). Much of our work has concentrated on the transport of sucrose and amino acids in the *Ricinus* seedling since this shows high uptake rates for these solutes.

The germinating *Ricinus* seedling receives nutrients such as sucrose and amino acids from the endosperm which acts as a storage facility until the seed germinates. During germination, contents of the endosperm are mobilized and nutrients are released into the apoplasm and absorbed by the cotyledons. These are then transferred via the phloem to other tissues for use in growth and development. The solute composition of the sieve tube sap from the *Ricinus* seedling, when the endosperm is attached, is similar to that from adult *Ricinus* plants (Komor et al., 1991). The major solutes are sucrose (270 mol m⁻³), amino acids (160 mol m⁻³) and K⁺ (25 mol m⁻³). The neutral amino acids are present at the highest concentrations within the phloem sap, with glutamine being the dominant amino acid at 50 mol m⁻³. Amino acids are also found in the xylem sap of *Ricinus* seedlings at a concentration of 25 mol m⁻³, with glutamine occurring at the highest concentration of 12 mol m⁻³ (Schobert and Komor, 1990).

This paper summarizes the data which have been obtained from studies of sucrose and amino acid transport in source and sink tissues of *Ricinus* using isolated membrane vesicles. In addition, preliminary results characterizing *Ricinus* carriers at the molecular level are presented.

Transport studies in *Ricinus* using isolated membrane vesicles

The technique of aqueous two-phase partitioning, which separates microsomal membranes according to their charge and hydrophobicity (Larsson, 1983), has been

¹ To whom correspondence should be addressed. Fax: +44 1703 594269. E-mail: L.E.Williams@soton.ac.uk

© Oxford University Press 1996
used to produce highly purified plasma membrane vesicles from both the cotyledon and root of Ricinus seedlings (Williams et al., 1990a, b, 1992). In addition to eliminating some of the problems encountered with more intact systems, a particular advantage of using vesicles is that the composition of the intra- and extravesicular solution can be manipulated. This experimental system is particularly useful in studying proton-coupled transporters since pH and electrical gradients across the membrane can be altered independently and, therefore, important information can be obtained concerning the relative contribution of each to the transport process.

**Energization of vesicles**

In plant cells, it is thought that the plasma membrane H⁺-ATPase creates an electrochemical potential gradient of protons which drives the uptake of sugars and amino acids via proton-coupled symports. In vesicles, artificial pH and electrical gradients can be created across the plasma membrane to provide the driving force for uptake. The techniques for creating these gradients in Ricinus membrane vesicles have been described previously (Williams et al., 1990b, 1992) and are summarized in Table 1. To create an internally alkaline pH gradient (ΔpH), the vesicles are loaded with a buffered medium at pH 7.5 and uptake is then carried out in a medium buffered at pH 5.5. To clamp the membrane potential (Δψ) at zero, potassium is present in both the loading and uptake buffers and the potassium ionophore, valinomycin, is included in the medium. To create a negative internal membrane potential, vesicles are loaded with a potassium-containing buffer and diluted into a sodium-containing buffer with valinomycin present. The establishment of these artificial gradients across the membrane has been confirmed using radiolabelled acetate and tetraphenylphosphonium (Williams et al., 1992; Weston et al., 1994). Acetate acts as a lipophilic weak acid and accumulates in alkaline interior vesicles while tetraphenylphosphonium acts as a lipophilic cation, moving into vesicles with a negative internal membrane potential.

**Sucrose and glutamine transport in Ricinus cotyledon plasma membrane vesicles**

The uptake of sucrose and glutamine was determined in cotyledon plasma membrane vesicles following the generation of artificial pH and electrical gradients (Fig. 1, Williams et al., 1990b). In the absence of gradients, little uptake of either sucrose or glutamine occurred. Imposition of a ΔpH resulted in a rapid uptake of both solutes and this uptake was inhibited by the protonophore CCCP. Uptake of sucrose and glutamine was stimulated further when a negative internal Δψ was imposed in addition to the ΔpH. Saturation kinetics were observed for ΔpH + Δψ-driven sucrose and glutamine uptake indicating carrier-mediated transport (Fig. 2; Williams et al., 1990b, 1992). The results obtained provide strong evidence for the existence of plasma membrane proton/sucrose and proton/amino acid symporters in cotyledons of Ricinus (Williams et al., 1990b, 1992).

Proton-sucrose cotransport has also been demonstrated in membrane vesicles isolated from source leaves of sugar beet and spinach (Bush, 1989; Lemoine and Delrot, 1989; Slone et al., 1991) while evidence for several proton-amino acid cotransporters has been obtained using vesicles isolated from source leaves of sugar beet (Li and Bush, 1990, 1991).

**Amino acid transport in Ricinus roots plasma membrane vesicles**

Although there have been a number of studies using vesicles to investigate sugar and amino acid transport in source tissues (for a review see Bush, 1993), less attention has been given to sink tissues. Therefore, a detailed characterization of sugar and amino acid transport was carried out using Ricinus root plasma membrane vesicles (Williams et al., 1992; Weston et al., 1994, 1995). In the presence of energized conditions (ΔpH + Δψ), little uptake was observed for sucrose, glucose and fructose, but high rates of glutamine transport were seen (Williams et al., 1992). Further studies indicated that both components of the proton-motive force are responsible for glutamine transport (Fig. 3; Weston et al., 1994). There is evidence for a direct effect of pH on the transport properties of the carrier, independent of the pH gradient, since Δψ-driven transport is greater at acid pH (Fig. 3, Weston et al., 1994).

It is important to emphasize that although the studies with isolated membrane vesicles have concentrated mainly on glutamine transport (because of the high levels found in the phloem and xylem sap of Ricinus seedlings), and have referred to a glutamine/proton cotransporter, it can not be assumed that this is the main substrate of the

**Table 1. Creation of artificial gradients across the plasma membrane in Ricinus root vesicles**

<table>
<thead>
<tr>
<th>Gradient</th>
<th>Intra-vesicular solution</th>
<th>Extra-vesicular solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔpH + Δψ (at pH 7.5)</td>
<td>pH 7.5, K⁺</td>
<td>pH 7.5, K⁺</td>
</tr>
<tr>
<td>ΔpH + Δψ (at pH 5.5)</td>
<td>pH 5.5, K⁺</td>
<td>pH 5.5, K⁺</td>
</tr>
<tr>
<td>ΔpH (Δψ = 0)</td>
<td>pH 7.5, K⁺</td>
<td>pH 5.5, K⁺, valinomycin</td>
</tr>
<tr>
<td>Δψ (at pH 5.5)</td>
<td>pH 5.5, K⁺</td>
<td>pH 5.5, Na⁺, valinomycin</td>
</tr>
<tr>
<td>ΔpH + Δψ (at pH 7.5)</td>
<td>pH 7.5, K⁺</td>
<td>pH 7.5, Na⁺, valinomycin</td>
</tr>
</tbody>
</table>
Sucrose and amino acid carriers in Ricinus

Fig. 1. Sucrose (a) and glutamine (b) uptake into Ricinus cotyledon plasma membrane vesicles. Transport assays were carried out in the presence of \( \Delta \text{pH} + \Delta \psi \) (internal negative) (●), \( \Delta \text{pH} \) (■), or \( \Delta \text{pH} 10 \text{ mmol m}^{-3} \) CCCP (▲). For non-energized transport, pH 7.5-loaded vesicles were diluted into pH 7.5 uptake medium (○) or pH 5.5-loaded vesicles into pH 5.5 uptake medium (□). The final sucrose and glutamine concentrations were 107 and 113 mmol m\(^{-3}\), respectively.

Fig. 2. Kinetics of sucrose (a) and glutamine (b) uptake into plasma membrane vesicles of Ricinus cotyledons. Uptake in non-energized conditions (▲) was subtracted from uptake occurring in the presence of \( \Delta \text{pH} + \Delta \psi \) (●) for each concentration to give the \( \Delta \text{pH} + \Delta \psi \)-driven uptake (■). \( K_m \) values obtained from Hanes-Wolf plots of this data were 0.87 mol m\(^{-3}\) for sucrose and 0.35 mol m\(^{-3}\) for glutamine.

Studies using plasma membrane vesicles (Weston et al., 1995) have indicated that in Ricinus roots, there are at least two neutral amino acid transporters; one which transports (or binds) all the neutral amino acids with a lower affinity for asparagine, and another which discriminates against isoleucine and valine. There is evidence that there may be an additional carrier for the basic amino acids, arginine and lysine, which also transports or binds some of the neutral amino acids. There may also be a separate carrier transporting acidic amino acids although there is some evidence that glutamic acid may be transported via the glutamine transporter at low pH (Weston et al., 1995). The data from vesicle studies indicate that the transport of glutamine, isoleucine, glutamic acid, and aspartic acid is driven by both components of the proton-motive force, suggesting that they are taken up by proton-coupled symporters (Weston et al., 1995). In contrast, the transport of the basic amino acids, lysine and arginine, appears to be driven only by a negative internal membrane potential, suggesting that the carrier may be a voltage-driven uniporter (Weston et al., 1995). Further studies are required to determine whether some of the inter-amino acid competition that has been evident from the experiments conducted in vesicles can be resolved by pursuing experiments in intact plant tissues.
Fig. 3. Time-dependent transport of glutamine (100 mmol m$^{-3}$) into Ricinus root plasma membrane vesicles. (a) The effect of $\Delta\phi + \Delta\psi$. Glutamine uptake in the presence of $\Delta\phi + \Delta\psi$ (•) and no gradients at pH 7.5 (■). (b) The effect of $\Delta\phi$. Glutamine uptake in the presence of $\Delta\psi$ (membrane potential not clamped) (▲); $\Delta \phi$ (membrane potential clamped at zero) (■); and no gradients (■). (c) The effect of $\Delta\psi$. Glutamine uptake in the absence of gradients (open symbols) and in the presence of $\Delta\psi$ (closed symbols) determined at pH 5.5 (■, □) and pH 7.5 (●, ○).

Table 2. Kinetic parameters determined for amino acid transport at the plasma membrane of Ricinus roots

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>$K_m$ (mmol m$^{-3}$)</th>
<th>$V_{max}$ (nmol mg$^{-1}$ protein min$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutamine</td>
<td>356±105</td>
<td>9.37±1.13</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>354±54</td>
<td>5.99±0.36</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>1178±327</td>
<td>29.0±5.13</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>1616±382</td>
<td>58±9.50</td>
</tr>
<tr>
<td>Arginine</td>
<td>155±33</td>
<td>5.06±0.34</td>
</tr>
<tr>
<td>Lysine</td>
<td>285±48</td>
<td>4.07±0.26</td>
</tr>
</tbody>
</table>

observed is due to actual transport or, instead, to competitive binding, and trans-stimulation studies could be used to resolve this question.

Solubilization and reconstitution studies

To determine the specificity of an individual transporter, it is necessary to study it in isolation from other transporters. One approach has been to solubilize the transporter from the plasma membrane and then to purify it prior to reconstitution into an artificial membrane system (Li et al., 1991; Thume and Dietz, 1991). This approach has been used to characterize the Ricinus root glutamine transporter. A method was developed for solubilizing the glutamine transport activity from Ricinus root plasma membrane vesicles and reconstituting the activity into liposomes. CHAPS was used to solubilize the activity from the membrane and a detergent dilution and dialysis method was carried out to remove the detergent to form transport-competent proteoliposomes (Weston et al., 1994). Gel filtration of the solubilized plasma membrane proteins was performed prior to reconstitution and the transport activity for each reconstituted fraction is shown in Fig. 4. The transport activity was found in fractions eluting at 40–60 kDa (as determined by comparison with elution profiles of protein standards). Since this is far removed from the main protein peak, and the $\Delta\phi + \Delta\psi$-driven specific activity of transport was much higher than that observed for the initial reconstituted solubilizate, this suggested that there was considerable purification of the transporter. However, gel electrophoresis and silver staining of the fractions obtained following gel filtration prior to reconstitution, revealed that there was, nevertheless, a large number of proteins in the peak activity fractions. Further purification steps are, therefore, required.

Limitations to the use of vesicles

Although the use of vesicles minimizes many of the problems encountered when using more intact systems, there can be some important limitations in studying transport processes in isolated vesicles. For instance, the purification procedure may damage the membrane and
affect the permeability and orientation of the membrane vesicles. In addition, the transport protein of interest may be inactivated during the isolation and purification of the membrane, or other important regulatory molecules may be lost. There can also be problems with obtaining sufficient material from certain tissues and also maintaining stable gradients in vesicles from some species. In addition, because of the heterogeneous nature of plasma membrane vesicle preparations it is very difficult to determine the tissue and cellular distribution of particular carriers. Another important consideration concerns the resolution of the number of carriers present in vesicle studies. For example, there may be more than one plasma membrane amino acid carrier for a particular amino acid and if the carriers have very similar kinetic properties and substrate specificities then it may be very difficult to distinguish between them.

Molecular characterization of *Ricinus* sucrose and amino acid carriers

The use of molecular techniques to isolate the genes encoding transporters and to express the protein heterologously in order to characterize the transport process has proved particularly successful in recent years (Riesmeier *et al.*, 1992; Frommer *et al.*, 1993). Several amino acid transporter genes have now been isolated from *Arabidopsis thaliana* using yeast complementation (Frommer *et al.*, 1993; Hsu *et al.*, 1993; Kwart *et al.*, 1993). In addition, sucrose carrier genes have been isolated from spinach, potato and *Arabidopsis* (Riesmeier *et al.*, 1992, 1993; Sauer and Stolz, 1994). To investigate whether similar or related genes exist in *Ricinus* species has proved particularly successful in recent years (Riesmeier *et al.*, 1993). Several sucrose carrier genes have been isolated from *Ricinus communis* (Table 3) showing extensive sequence homology to the published sequences for sucrose and amino acid carriers (AAP1 and AAP2 from spinach, potato and *Arabidopsis*) showing close homology to published sequences for amino acid and sucrose carriers have been used. Two partial-length cDNA clones (named *Ricinus* amino acid carrier 1, RAAC1 and *Ricinus* amino acid carrier 2, RAAC2) showing close homology to published sequences for amino acid transporters, AAP1 and AAP2 from *Arabidopsis* (Table 3) have been isolated from *Ricinus* roots. In addition, a partial cDNA clone (named *Ricinus* sucrose carrier 1, RSC1) showing extensive sequence homology to the published sequences for sucrose transporters from other species was isolated from *Ricinus* seedlings (Table 4).

Table 3. Percentage identity between the peptide sequences of RAAC1 and RAAC2 and amino acid carriers AAP1 and AAP2

<table>
<thead>
<tr>
<th>% Identity of amino acid sequences</th>
<th>AAP1</th>
<th>AAP2</th>
<th>RAAC2</th>
</tr>
</thead>
<tbody>
<tr>
<td>RAAC1</td>
<td>61</td>
<td>80</td>
<td>63</td>
</tr>
<tr>
<td>RAAC2</td>
<td>75</td>
<td>63</td>
<td></td>
</tr>
</tbody>
</table>

* Based on partially sequenced cDNA clone.

In order to investigate the tissue-specific expression of the RAAC1, RAAC2 and RSC1 clones, Northern analysis was performed, under conditions of high stringency, on total RNA isolated from various *Ricinus* tissues (Bick, Neelam, Hall, and Williams, unpublished data). The RNA was hybridized with 32P-labelled probes, prepared by random priming of the three cloned PCR products. For RSC1, highest expression was observed in the cotyledon (Table 5) with very low expression in the roots and other tissues. For RAAC1 and RAAC2, expression was also highest in the cotyledon, but appreciable levels were also seen in the root. It should be noted that under low stringency conditions, following hybridization with a cDNA probe of the *Arabidopsis* glucose carrier (Sauer *et al.*, 1990), there was a different expression pattern from RAAC1, RAAC2 and RSC1. Highest expression was detected in the root, endosperm and young leaf tissues of *Ricinus*, but there was no detectable signal from the cotyledons (Bick, Hall and Williams, unpublished data).

**Physiological role of amino acid transporters**

The research in this laboratory is particularly concerned with the physiological role of the amino acid transporters in the root. Amino acid carriers may function in the...
uptake of amino acids from the soil and contribute to the nitrogen nutrition of the plant (Schobert and Komor, 1987). Alternatively, they may act in the retrieval of amino acids from the apoplast which have been leaked from the phloem. They may also serve in the recycling of amino acids between the phloem and the xylem. The significance of amino acid uptake from the soil varies with different plant species and their natural habitats. Schobert and Komor (1987) calculated that amino acid uptake may contribute about 15–25% to the nitrogen nutrition of *Ricinus communis*, based on concentrations of amino acids in the soil. These calculations were made with soil that is rich in inorganic nitrogen. However, a study of arctic tundra species growing in soil which is mineral-poor, but is rich in organic nitrogen (in the form of amino acids), determined that amino acid uptake may account for 10–82% of the total plant nitrogen uptake (Kielland, 1994). The role of different amino acid transporters in roots could be investigated by studying their cellular location. This could be achieved by using carrierspecific antibodies in immunocytochemistry and carrier-specific cRNA probes for *in situ* hybridization studies.

**Acknowledgements**

We would like to thank The Royal Society (LEW) and BBSRC (JAB, AN, and KNW) for financial support.

**References**


