Communication

UDP-Glucose-Dependent Sucrose Translocation in Tonoplast Vesicles from Stalk Tissue of Sugarcane

Andrew Maretzki and Margaret Thom*
Hawaiian Sugar Planters' Association, Aiea, Hawaii 96701

ABSTRACT

Tonoplast vesicles isolated from stalk parenchyma tissue of sugarcane plants transport sucrose via a uridine diphosphate glucose (UDPGlc)-dependent group translocator. No sucrose transport via an ATP-dependent system could be detected. The products of UDPGlc uptake in the vesicles were sucrose and sucrose phosphate which, upon hydrolysis with alkaline phosphatase and invertase, showed that both hexose moieties are derived from UDPGlc.

The ability of tonoplast vesicles to take up externally added UDPGlc and to convert this nucleotide sugar to internally accumulated sucrose by a process of vectorial group translocation has been reported for vesicles prepared from sugarcane cell cultures (2) and tomato fruit (4). Intact vacuoles isolated from sugarcane cell suspensions (6) and beet root (5) also carry out this vectorial bioconversion. The information now available supports the initial hypothesis (6) that a series of tonoplast-bound enzymes, or enzymes closely associated with the tonoplast, account for compartmentalization of sucrose in excess of metabolic requirements. Vacuoles from the parenchyma cells of the stalk tissue are the primary location for assimilated sucrose in sugarcane.

The group translocator was originally identified and characterized using a cell culture which originated from explants of young sugarcane stalks (2). However, the presence of the group translocator for sucrose transport has, until now, not been verified in stalk tissue. The present report demonstrates that UDPGlc is taken up by tonoplast vesicles isolated from fresh sugarcane stalks and that the group translocation mechanism presumably operates in intact plants.

MATERIALS AND METHODS

Vigorously growing primary stalks from nine month old plants of clone H73-6110 were randomly selected and cut in the field. Protoplasts and vacuoles were prepared from immature internodes as previously described (8) except that the medium used for protoplast isolation was 25 mM Tris-Mes containing 0.6 mM mannitol, 2 mM DTE, and 1% BSA, adjusted to pH 5.6. The vacuole wash medium was the same as above except that no BSA was used and the pH was adjusted to 6.9. Tonoplast vesicles were prepared by breaking washed vacuoles in a glass homogenizer using a large volume of 25 mM Tris-Mes containing 0.25 mM mannitol and 2 mM DTE adjusted to pH 6.9. The membrane suspension was layered over a cushion of 10% (w/v) dextran T70 and centrifuged at 100,000 g for 60 min. The membrane fraction at the interface was used for experiments.

To determine the labeling pattern of sucrose/sucrose-P after incubation of tonoplast vesicles with UDPGlc, the vesicles were incubated with 200 μM UDPGlc [glc-14C] for 10 min and extracted with 70% ethanol. The ethanol was removed under N2 and the aqueous extract was successively treated with alkaline phosphatase and invertase. The extract was chromatographed on Whatman No. 1 paper and developed 2× in 1-butanol:acetic acid:H2O 4:1:5 (upper phase). Chromatograms were cut into 1-cm sections and the radioactivity was determined.

Other uptake measurements and determination of products of uptake were performed as previously described (2). Protein was determined using Coomassie blue (1).

RESULTS

The method of protoplast and vacuole isolation from sugarcane stalk tissue required modifications from that used for sugarcane cell suspensions (6). Protoplasts from the two types of preparations appeared to differ in their densities sufficiently so that stalk protoplasts could not be pelleted by low speed centrifugation and a flotation procedure was necessary. The addition of 1% BSA to the protoplast isolation media was found important for maintaining integrity of the protoplast. Use of BSA in the isolation medium required an additional wash of isolated vacuoles so that residual BSA would not interfere with the tonoplast protein assay. In a prior publication describing the characteristics of a tonoplast-bound ATPase (8), a similar isolation procedure for tonoplast vesicle preparation was used. At that time it was demonstrated that cytoplasmic enzyme contaminated the vacuole preparation to the extent of about 15%. We have assumed that, in the present modified procedure, the extent of contamination is no greater and is probably less than this.

Uptake of sucrose in the presence of MgATP and uptake of UDPGlc were measured using the same vesicle preparation (Fig. 1). Compared to the uptake of UDPGlc, the amount of sucrose taken up by the preparations over a 13-min period was almost negligible, in spite of the presence of both Mg2+ and ATP in the incubation mixture. This is consistent with results obtained on vacuoles and vesicles isolated from cell culture (6). The uptake rate of UDPGlc by vesicles prepared from stalk parenchyma amounted to 4.7 nmol·min⁻¹·mg⁻¹ protein. Although the vesicles deteriorated with time, uptake measurements made within an hour following completion of the isolation procedure were linear over the 13-min time period.

The stimulatory effect of glucose on the uptake of UDPGlc...
has been previously reported for cell culture tonoplast preparations (7). A similar effect was obtained in tonoplast of fresh parenchyma tissue; i.e. a more than twofold stimulation of UDPGlc uptake was measured when 10 mM glucose was added to the uptake medium simultaneously with the UDPGlc substrate (Fig. 1). The cause of UDPGlc uptake stimulation by a sugar molecule such as sucrose or glucose can only be postulated, in the absence of more definitive evidence at the present time. Conceivably it involves an allosteric effect on a membrane site for UDPGlc bioconversion.

Chromatographic analysis of the labeled products resulting from the incubation of [U-14C glucose] UDPGlc with tonoplast vesicles showed that in the tonoplast vesicles, the radioactivity taken up was converted to sucrose and sucrose-P, while in the incubation medium, glucose and fructose phosphates accounted for more than 50% of the radioactivity (Table 1). These results are similar to those obtained for preparation of sugarcane suspension cultures (2, 6, 7) as well as red beet vacuoles (5).

The labeling pattern of sucrose recovered from the tonoplast vesicle extract after incubation with UDPGlc was investigated. Since there were no hexosephosphates in the tonoplast extract, all glucose and fructose recovered after phosphatase and invertase hydrolysis can be assumed to have originated from sucrose or sucrose-P. The glucose and fructose recovered after hydrolysis with alkaline phosphatase and invertase were labeled approximately to the same extent. From the UDPGlc-derivated sucrose pool in the tonoplast vesicles, 46% of the radioactivity was glucose and 54% was fructose. This indicates that the glucose portion of the UDPGlc molecule provides both hexose units for the formation of sucrose-P and sucrose, as originally proposed (6).

DISCUSSION

The apparent absence of an active APT-dependent sucrose uptake system on the tonoplast of sugarcane stalk parenchyma cells is consistent with a similar lack of evidence for sucrose uptake in suspension-cultured cells (2, 6). These results could be interpreted as indicating that the tonoplast transport systems in Saccharum are particularly sensitive to conditions used during the isolation procedure and that the sites are physically damaged. However, this interpretation is weakened by the fact that the sucrose carrier apparently remained fully functional (6). A second possibility for the absence of sucrose transport is that the carrier is sensitive to a factor, possibly a natural regulator, released when the tissue is perturbed by cutting or isolation. This could invoke an irreversible reaction unaffected by repeated washes such as used in the preparation of tonoplasts in this study. This seems unlikely since the sucrose carrier in Beta vulgaris does not show this inactivation (5). A third explanation that must now be considered seriously is that, unlike B. vulgaris and other plants, Saccharum sp. does not have a functional carrier for sucrose at least on the tonoplast.

With verification that sugarcane stalk parenchyma accumulates sucrose into vacuoles via the group translocator, one could now consider comparing the group translocation characteristics of different Saccharum species. Wild types of sugarcane such as S. spontaneum, which account for increased vigor, and S. officinarum, which accounts for most of the high sucrose content of modern sugarcane hybrids, might exhibit quite different transport characteristics. However, we suspect that this survey could be an exercise in futility since at present no evidence exists that, in vivo, the group translocator for sucrose represents an overall limiting reaction for sugar storage in stalk sink tissue. Rather, the contribution of the sucrose group translocator to the overall economy of the plant's metabolism should become a focus of attention. The difference in the sucrose storage mechanism of Saccharum hybrids and B. vulgaris, i.e. the apparent lack of electrogenic sucrose transport in the case of the former and the clearcut existence of such mechanisms in the case of the latter, indicates that even plants which can accumulate relatively high concentrations of the same disaccharide partition and accumulate their excess carbohydrate pools differently. Precise temporal studies to follow relevant hydrolytic as well as synthetic enzyme activities in the cytoplasm are needed to address this aspect.

LITERATURE CITED


6. Thom M, AMARETZKI 1985 Group translocation as a mechanism for sucrose transfer into vacuoles from sugarcane cells. Proc Natl Acad Sci USA 82: 4697–4701
