Sucrose and Invertase, an Uneasy Alliance

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Sucrose is the main "currency" of higher plants. It is the most important product of photosynthesis and is transported from leaves throughout the plant. It can either be stored or broken down for use in the synthesis of all the other components of living cells. Sucrose, however, is not just a passive carrier of carbon between energy-producing and energy-utilising tissues. Sucrose also acts as an active messenger, conveying information on the energy status of individual tissues. By direct induction or repression of gene transcription, sucrose plays a central role in balancing the needs of carbohydrate-producing and consuming cells under a wide range of environmental conditions.

**SUCROSE AND INVERTASE, AN UNEASY ALLIANCE**

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**Figure 3.1.** Scheme showing the involvement of acid invertase in regulation of cellular hexose content. Fast exchanges are shown by thick arrows, and slow exchanges by thin arrows. Fru, fructose; glu, glucose; INV, invertase; TP, triose phosphate; *, symplastic export of sucrose; **, apoplastic export of sucrose; I transporter.
Carbohydrates as signals
Sucrose consists of a molecule of glucose and a molecule of fructose joined through a specific chemical bond. Before sucrose can be used, this bond has to be broken by the action of specific enzymes which are present in all tissues which require sucrose. The most common enzyme of this type is invertase, which hydrolyses sucrose, releasing equal amounts of glucose and fructose (Figure 3.1).

Both glucose and fructose are implicated in the signalling pathways by which sucrose alerts the plant cell to nutritional requirements or constraints. Thus invertase action amplifies the sucrose signal by producing two “messenger” molecules for the price of one. We therefore consider invertase to be a bi-functional enzyme, both catalysing sucrose breakdown and amplifying information on carbon status. This may be sensed by plant cells such that metabolism is modified by altering gene expression.

Invertase activity is high in many tissues which need sucrose. For example, young leaves, which have not developed the ability to fix carbon through photosynthesis, rely on imported sucrose to grow. Invertase activity falls as the leaf matures (Figure 3.2). In many leaves, however, we observe a subsequent rise in activity even though the leaf is fully mature and functioning as a source of sucrose for the rest of the plant. The activity is high enough, when measured in the test tube, to break down in thirty minutes all the sucrose made during an eight-hour day! Clearly this does not happen within the leaf, and we have tried to identify the mechanisms which allow sucrose and invertase to co-exist peacefully in the same organ.

We first investigated the possibility that the enzyme, although present, was not active. There is evidence from studies on the purified enzyme from leaves that fructose partially inhibits invertase even at concentrations as low as 2 millimolar. Thus, if sucrose was broken down and the fructose not used, this would tend to inhibit subsequent hydrolysis. Measurements of fructose concentration in grass leaves are consistent with some inhibition occurring but it seems unlikely that this would entirely prevent sucrose breakdown.

Cellular location
The next factor that we studied was the location of invertase within the leaf, and whether that differed from the location of sucrose. Sucrose is synthesised in the cytoplasm of photosynthetic cells from where it can be exported to the vacuole or out of the cell. Within a given cell, invertase was not uniformly distributed between compartments but was located mainly in the large central vacuole and in the cell-wall. A very small amount of invertase activity has been found in the cytosol of fully-grown leaves, but this would have little effect on the total amount of sucrose that accumulated. Also, when we looked at the whole leaf it was apparent that invertase was not uniformly distributed between different tissues. In many plants much more invertase is present around

Figure 3.2 Changes in soluble invertase activity during growth of the fourth leaf of ryegrass.
the veins than in the rest of the leaf (Figure 3.3). This distribution of invertase may affect sucrose export from the leaf. Sucrose is exported in the phloem (one of the tissues in the veins) but a high invertase activity in this region would be expected to lower the concentration of sucrose and thus to reduce export. Since this distribution occurs in normal, healthy plants which are growing actively, invertase located in the veins must be regulated in some way in order to allow unrestricted export of sucrose.

Implications
The co-existence of invertase and sucrose in leaves is a clear paradox since continuous synthesis and breakdown of sucrose within the same cells or compartments would represent an energy-consuming “futile” cycle. This poses questions concerning the relationship between invertase and its substrate. In the leaves of many plant species, maximal extractable invertase activity is constant during the day, whilst the accumulation of sucrose, hexoses and starch vary markedly with light (Figure 3.4). The fact that invertase activity has no apparent influence over the gross accumulation of these carbohydrates implies that this enzyme has a more subtle function.

The mobility of sugars within leaf cells may provide a clue. Whilst sucrose can move relatively easily in and out of the vacuole, the movement of hexoses is much more restricted. We propose the following hypothesis: sucrose is indeed broken down by

*Figure 3.3 Localisation of invertase protein in barley leaves. Darker staining areas show cross-reaction of an antibody recognising the invertase protein in the barley leaf blade.*
invertase to generate hexoses within the vacuole but these are released only slowly to the cytosol and therefore form a reserve of potentially accessible carbon. Hexoses in the vacuole will also inhibit invertase and slow its further action. At times when export to non-photosynthetic tissues takes most of the sucrose produced by photosynthesis, this reserve would provide an adequate supply of carbon to the cytosol without triggering the sugar-sensitive signalling pathways that affect gene expression. However, if changes in the environment reduced the export of sucrose from leaves, this would result in a sustained period of sucrose accumulation within the vacuoles. This would overcome the inhibition of invertase by fructose, resulting in a massive accumulation of hexose in the vacuole and the subsequent liberation of more signalling molecules (Figure 3.1). The observed reduction in photosynthesis that is associated with sugar accumulation in leaves appears to be mediated by changes in the expression of specific genes and could be the result of a process like the one we propose.

This is obviously not the whole story as far as invertase in leaves is concerned. There are, for example, non-photosynthetic cells in the epidermis which have their own specific invertase in order to provide the hexoses they require for metabolism. Our collaborators in Bangor, led by Deri Tomos, have shown that the vacuoles in these cells contain almost no sucrose. Currently, we are trying to measure sucrose concentrations and invertase activity in vacuoles from photosynthetic cells at different distances from the veins, in order to establish whether or not there is a gradient of metabolism out from the transport tissues.

The findings which we have outlined, based on recent studies from IGER and elsewhere, have indicated an important role for an enzyme long neglected, which now appears to be an essential component of one of the major signalling pathways in higher plants.

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