Planta (Berl.) 130, 339–340 (1976)

Planta

Short Communication

Hydrolysis and Resynthesis of Sucrose and Related Sugars in the Developing Stem Tissues of *Eucalyptus regnans* Trees

S.H. Tham and C.M. Stewart

Division of Chemical Technology, C.S.I.R.O., South Melbourne, Victoria 3205, Australia

Summary. When cambial tissues are removed from tree stems of *Eucalyptus regnans* F. Muell. and incubated *in vitro* with [^{14}C]glucose, [^{14}C]galactose and [^{14}C]fructose in the presence of sufficient PVP (polyvinylpyrrolidone) radioactive sucrose, members of the raffinose family and related sugars are synthesized. These results suggest that PVP inactivates substances which, in *in vitro* experiments, inhibit the action of enzymes which are essential to the resynthesis of nutrient sugars (sucrose, raffinose and stachyose). In living trees, such enzyme inhibition is less likely to occur.

In trees of *Eucalyptus regnans*, excess photosynthates (sucrose, raffinose and stachyose) travel down the stem in the assimilate stream of the inner phloem, and are utilized by the developing (cambial and differentiating) cells after enzymic hydrolysis to monosaccharides. The enzyme system within these cells appears to be responsible for maintaining a dynamically balanced mixture of mono-, di- and oligosaccharides (mainly glucose, fructose, sucrose, *myo*inositol, galactinol, raffinose, stachyose and a little galactose).

The state of balance seems to depend chiefly on seasonal and diurnal sugar supply and demand. The balance, within the developing tissues, probably results from the hydrolysis and resynthesis of sucrose, raffinose and stachyose with *myo*inositol acting as a galactosyl acceptor during the hydrolysis cycle, and galactinol acting as a galactosyl donor during the resynthesis cycle (Stewart *et al.*, 1973; Tham and Stewart, 1974). In these reports, it was shown that [^{14}C]sucrose, when incubated with developing tissues containing PVP (final concentration, 0.4%), is hydrolyzed to fructose and glucose in approximately equal amounts. The experiments, whilst also demonstrating that radioactive raffinose and stachyose are probably resynthesized from [^{14}C]monosaccharides (especially from [^{14}C]-galactose), provided no evidence for the resynthesis of sucrose. The lack of sucrose resynthesis was attributed to partial inactivation of the developing-cell enzyme system by substances such as polyphenols released from vacuoles during or after sampling (see also Baldry *et al.*, 1970). Evidence is now presented to show that resynthesis of sucrose does occur in isolated cambial and differentiating tissues and, no doubt, within these tissues *in situ*.

Cambial and adjacent soft tissues containing 10% (w/v) final concentration of PVP were incubated with individual radioactive sugars. Aliquots taken at various time intervals were inactivated by addition of ethanol, and the isolated sugar mixture was separated by paper chromatography. Radioactivity traces were obtained by running the papers through a radiochromatogram scanner. Detailed experimental procedures have been described by Stewart *et al.* (1973). Samples of tissues were collected during late autumn, winter and late spring.

The results obtained are indicated in Table 1.

Table 1. Sucrose resynthesis during different seasons

Hr	Autumn		Winter		Spring	
	Sucrose from		Sucrose from		Sucrose from	
	fructose	glucose	fructose	glucose	fructose	glucose
1	nd	nd	nd	nd	++	–
2	+	+	nd	nd	nd	nd
3	nd	nd	+	+	+++	+
5	+	+	+	+	nd	nd
7	+	+	nd	nd	++++	+
24	nd	nd	+	+	+++++	+

nd, not determined; –, no [^{14}C]sucrose; +, small amount of [^{14}C]sucrose; +++++, very large amount of [^{14}C]sucrose

Late autumn and winter are the dormant seasons of the year in evergreen trees like *E. regnans*; during this period, the majority (ca. 80%) of the total soluble sugars are present as sucrose and the raffinose family of oligosaccharides; furthermore, the hexoses (glucose and fructose) and sucrose approach equilibrium

conditions (ca. 49% sucrose) during this period; thus, the non-reducing more stable sugars seem to represent soluble readily available storage carbohydrates during the dormant period; also, during this period, the reducing hexoses are probably maintained in equilibrium with sucrose whilst they are being utilized for respiratory and other resting cell reactions, the sucrose supply being augmented by enzymic hydrolysis of raffinose-family oligosaccharides and/or starch (see also Stewart *et al.*, 1973).

In late spring (well into the growing season), the rate of [^{14}C]sucrose synthesis from [^{14}C]glucose is similar to that observed during the dormant season. However, the rate of [^{14}C]sucrose formation from [^{14}C]fructose is very much greater than that observed from [^{14}C]glucose in late spring and from [^{14}C]fructose during the resting season. These results suggest that the concentration of sucrose synthesizing enzymes may be low, that certain enzymes are somewhat inactivated by inhibitors or that *the sucrose-hexose equilibrium is maintained* during the dormant season. Again, fructose is a better source than glucose for sucrose resynthesis during the growing season (Table 1); hence, it is probable that PVP is not an effective anti-inhibitor for glucose isomerase.

Incubation of [^{14}C]sucrose with developing tissues gave peaks corresponding to almost equal amounts of labelled glucose and fructose on a radiochromatogram trace, thus confirming the ability of these tissues to hydrolyse sucrose. The rate of sucrose hydrolysis was quite high during both the growing and dormant seasons. This observation indicates that sucrose hydrolases are not inactivated by the enzyme inhibitors present in the *in vitro* samples of developing tissues.

The evidence that [^{14}C]fructose is a good substrate for sucrose synthesis in springtime suggests that developing tissues at this time are rich in UDP-glucose or other NDP-glucoses, and that the main reaction occurring is:

$$\text{UDP-glucose} + [^{14}\text{C}]\text{fructose} \rightleftharpoons [^{14}\text{C}]\text{sucrose} + \text{UDP}.$$

The enzyme is most likely to be sucrose synthetase rather than sucrose phosphate synthetase, because the latter enzyme appears to be specific for UDP-glucose and fructose-6-phosphate. Sucrose synthetase has been shown to exist in chloroplasts of sugar-cane leaves (Davies, 1974). However, Delmer and Albersheim (1970), after working with *Phaseolus aureus*, suggested that the same enzyme occurred only in non-photosynthetic tissues. Wardrop and Cronshaw (1962) have observed chloroplast-like organelles in the young tissues adjacent to the cambium in eucalypt tree stems. Thus the identity of the sucrose-synthesizing enzyme in developing stem tissues remains open to question, but it is probably sucrose synthetase.

[^{14}C]Galactose, and to a lesser extent the other radioactive sugars, gave rise to radioactive *myo*inositol, galactinol and oligosaccharides during the dormant season, but these sugars were formed only in small or in trace amounts during late spring. These results, considered in conjunction with observations reported previously (*loc. cit.*) for the developing stem tissues of *E. regnans*, indicate that resynthesis of raffinose-family oligosaccharides and hydrolysis of these sugars are predominant processes just prior to and during the dormant and during the growing periods, respectively. Sucrose appears to represent the most vital sugar nutrient present in developing tissues because its ready hydrolysis and its ready resynthesis from fructose take place throughout the year and during the growing season, respectively. This conclusion may be confounded somewhat by the observation that enzymes involved in the synthesis of sucrose and related sugars are more susceptible to inhibition than those involved in the hydrolysis of these sugars. The evidence for cyclic processes of hydrolysis and resynthesis of nutrient sugars (with the probable participation of *myo*inositol and galactinol in these processes) suggests that sugar reactions, similar to those which occur in photosynthetic tissues, take place in the dividing and differentiating tissues within stems of *E. regnans* trees (see also Kandler, 1967).

The authors wish to thank Dr. D.E. Bland and Mr. J. Skicho, of the C.S.I.R.O. Division of Chemical Technology, for doing the radiochromatogram scans.

References

Baldry, C.W., Bucke, C., Coombs, J.: Effects of some phenoloxidase inhibitors on chloroplasts and carboxylating enzymes of sugar cane and spinach. Planta (Berl.) **94**, 124–133 (1970)

Davies, D.R.: Some aspects of sucrose metabolism. In: Plant carbohydrate biochemistry, pp. 61–81, Ed.: Pridham, J.B. London-New York: Academic Press 1974

Delmer, D.P., Albersheim, P.: The biogenesis of sucrose and nucleoside diphosphate glucoses in *Phaseolus aureus*. Plant Physiol. **45**, 782–786 (1970)

Kandler, O.: Biosynthesis of poly- and oligosaccharides during photosynthesis in green plants. In: Harvesting the sun, pp. 131–152, Eds.: San Pietro, A., Greer, F.A., Army, T.J., New York-London: Academic Press 1967

Stewart, C.M., Melvin, J.F., Ditchburne, N., Tham, S.H., Zerdoner, E.: The effect of season of growth on the chemical composition of cambial saps of *Eucalyptus regnans* trees. Oecologia (Berl.) **12**, 349–372 (1973)

Tham, S.H., Stewart, C.M.: Diurnal variations of soluble sugars in *Eucalyptus regnans*. Phytochem. **13**, 1023–1026 (1974)

Wardrop, A.B., Cronshaw, J.: Formation of phenolic substances in the ray parenchyma of angiosperms. Nature (Lond.) **193**, 90–92 (1962)

Received February 5; accepted March 1 1976