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EFFECTS OF ENZYME CONCENTRATION ON OLIGOFRUCTAN SYNTHESIS FROM SUCROSE

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Abstract At low concentrations of partially purified fructosyltransferase, only trisaccharide products were detected. At higher enzyme concentrations, larger oligofructans were detected. Depending upon enzyme concentration, the results indicated either sucrose : sucrose fructosyltransferase activity exclusively, or a more complex fructan polymerizing system. Consequences for the interpretation of fructosyltransferase data are discussed.

INTRODUCTION

The established model for fructan biosynthesis [1–3] was based upon studies of inulin synthesis in roots of *Helianthus tuberosus*. It involves two fructosyltransfereases: SST*, which forms a trisaccharide intermediate from sucrose, and FFT*, a sucrose-inhibited enzyme which elongates fructan from trisaccharide and larger fructans. This model and its terminology have been widely adopted for studies of fructan synthesis in a range of plant species [4]. An important prediction of this model is that the enzymic *de novo* synthesis of fructan of degree of polymerization > 3 (DP > 3) from sucrose by a mixture of the two fructosyltransferases is impossible, due to sucrose inhibition of FFT [1, 4]. Hence, the majority of data in support of the model are concerned only with the synthesis of trisaccharide. Demonstrations of higher oligomer synthesis are rare [4]. By contrast, we have placed our primary emphasis upon achieving species-specific *de novo* synthesis of representative large fructan (DP > 3) from sucrose. We have reported such specific syntheses by enzymes from a number of grasses and have shown that these reactions are not inhibited by sucrose [4–8]. These studies and a recent report of fructan synthesis by a preparation from *Asparagus* roots [9] have indicated a requirement for a high enzyme concentration in the synthesis of fructans of DP > 3. The present study further explores the effect of enzyme concentration on the products of *in vitro* oligofructan synthesis.

Abbreviations DP: degree of polymerization; SST: sucrose sucrose fructosyltransferase (EC 2.4.1.99); FFT: fructan:fructan fructosyltransferase (EC 2.4.1.100); FSA: fructan synthetic activity; PFT: pectolyase fructosyltransferase; SFT: sucrose fructosyltransferase.

RESULTS AND DISCUSSION

The effect of enzyme concentration on oligofructan synthesis

Pectolyase synthesized fructan from sucrose with the concomitant stoichiometric release of glucose as the sole reducing product [10]. Rates of oligofructan synthesis increased with increasing pectolyase concentration over the range studied (Fig. 1a). The trisaccharide product was detected in all reactions and increased with enzyme concentration (Fig. 1b). The tetrasaccharide product was not detected at enzyme concentrations below 90 $\mu g\,cm^{-3}$, but increased with enzyme concentrations above this threshold (Fig. 1b).

Increasing FSA concentration resulted in increased rates of total fructan synthesis in the range investigated (Fig. 2a). Trisaccharide was detected in all reactions, though higher oligofructans were not detected below 0.3 g fr. wt equivalent cm^{-3} (Fig. 2b). Detection of fructans of increasingly higher DP occurred with increases in enzyme concentrations above this threshold.

Consequences of the effect of enzyme concentration for the interpretation of enzymological studies of fructan biosynthesis

The increase in reaction rate with increased enzyme concentration was unremarkable and to be expected. Where the results are particularly significant is in the variation in the nature of the fructan products detected. With both preparations at low enzyme concentration, the results give the impression of trisaccharide formation exclusively. Experiments performed with comparable enzyme concentrations and with comparable product sensitivity have previously been interpreted as evidence for

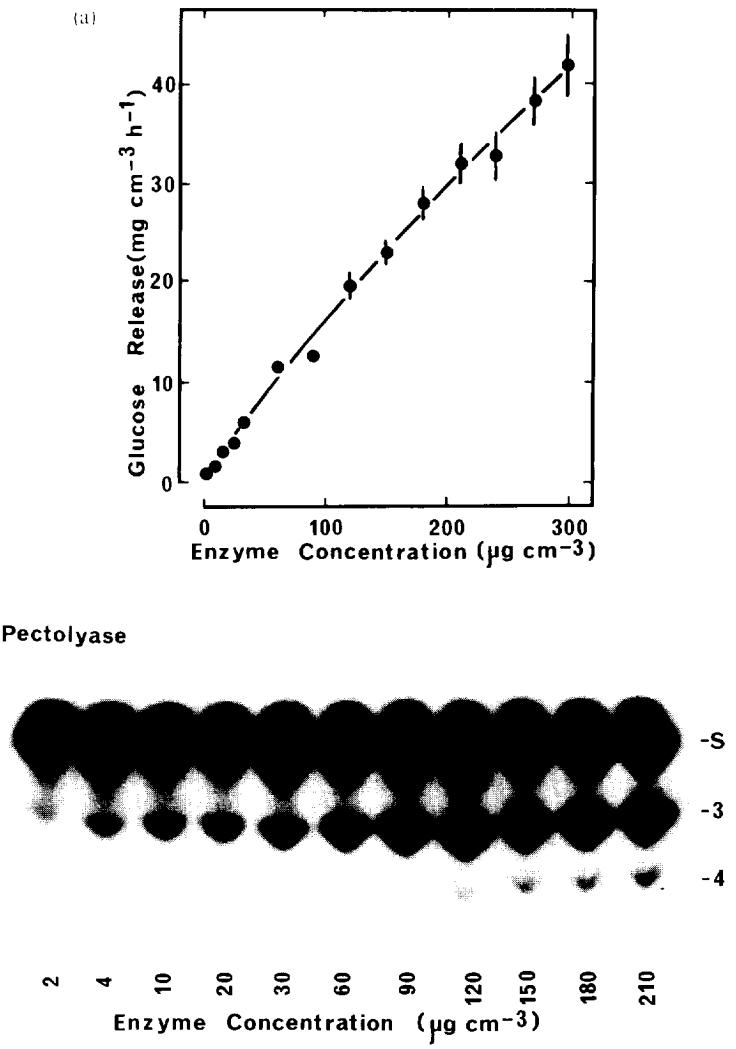

Fig. 1. Fructan synthesis from 1000 mol m^{-3} sucrose at pH 5.5 and 30°, catalysed by various concentrations of Pectolyase Y-23. (a) Variation in reaction rates determined as glucose released in a 1.0 hr reaction. Data points are means of triplicate assays ± standard error. Where no error bar is shown, the error was smaller than the symbol. (b) Qualitative analysis of reaction products by TLC. Each lane contained total sugars in a 1.0 mm^3 sample of a 1.0 hr digestion of sucrose at each Pectolyase concentration. Markers S, I and Nys refer to the mobilities of sucrose, isokestose and nystose, respectively.

trisaccharide synthesis exclusively. Considered in isolation, such data give the impression of an SST reaction, and would be interpreted as evidence for SST activity in the context of the model of Edelman and Jefford [1].

By comparison, identical enzyme preparations, under identical conditions except for the higher enzyme concentrations, permitted the detection of fructan products of DP $\geqslant$ 3. In terms of the established model, results obtained with the high enzyme concentrations could be interpreted as evidence for the presence of both SST and FFT, but would imply that sucrose did not inhibit polymerization. Equally, the results could be explained by the activity of a single SFT, which requires the attainment of a threshold concentration of trisaccharide intermediate before the synthesis of higher oligofructans commences [4].

The status of SST as a distinct enzyme of fructan synthesis

The results show that for the commonly used short reaction periods (*ca* 2–3 hr), high enzyme concentration is crucial for the *in vitro* expression of detectable higher fructan synthesis. Reactions at low enzyme concentration give an incomplete picture of the range of activities possible. This has two important implications: (i) specific designation of SST activity to reactions performed at low enzyme concentration may be erroneous; and (ii) trials of enzyme concentrations sufficient to allow higher oligofructan synthesis are necessary before designation of specific function(s). In practice, 3–5 g fr. wt equivalent cm^{-3} is required for enzymes from grasses [5–8]. Most previous studies of fructosyltransferases used low enzyme concentration and short reaction periods. [4, 11–13]. The

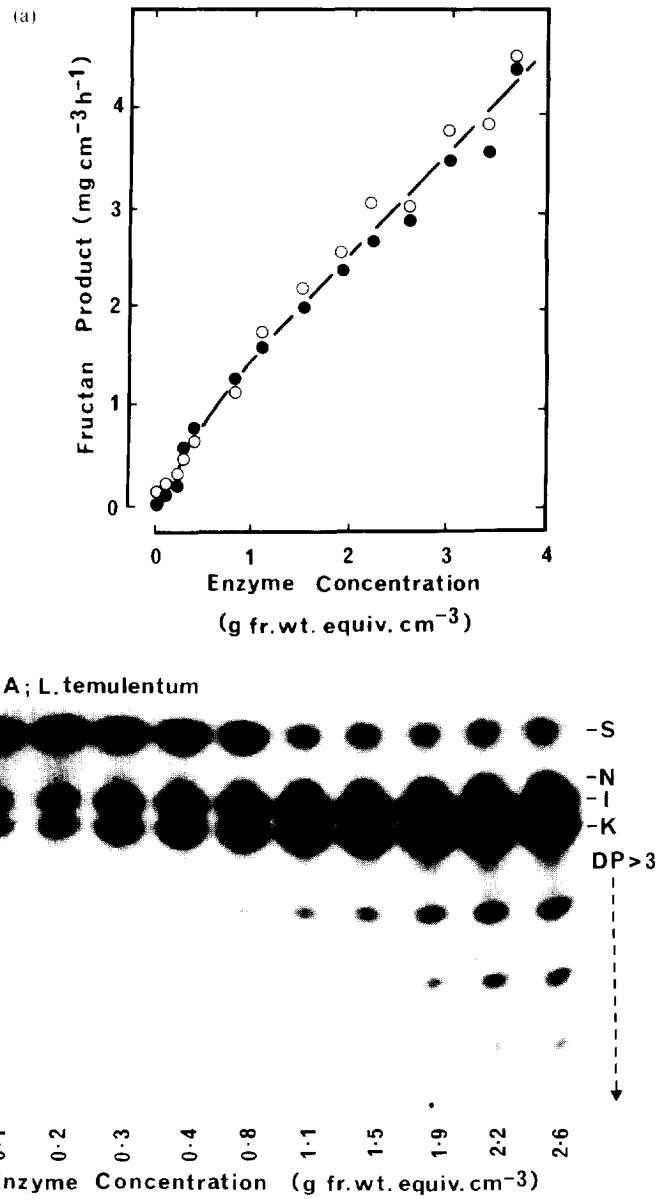

Fig. 2. Fructan synthesis from sucrose catalysed by various concentrations of an enzyme preparation from excised, illuminated leaves of *Lolium temulentum*. (a) Variation in reaction rates estimated from total fructan (DP > 2) formed in a 2 hr reaction at pH 6.0, 1500 mol m^{-3} sucrose and 30°. Data are from two independent determinations. (b) Qualitative analysis of oligofructan products by TLC. Some sucrose substrate and all monosaccharides were removed by HPLC prior to analysis by TLC. Lanes contain products equivalent to 25 mm^3 of a 2 hr reaction. Maximum loading was 140 μg total sugar per lane. S, N, I and K mark the mobilites of standard sucrose and the trisaccharides: neokestose, isokestose and kestose.

formation of trisaccharide was interpreted as evidence for SST and as support for the SST/FFT model. Because of dilution, the full range of possible activities may not have been detected. Specific designation of 'SST' to these activities may be premature.

EXPERIMENTAL

Two different fructan-synthesizing prepns were used, of fungal and of plant origin. PFT [10] concns spanning the range 0.01–3.00 mg cm^{-3} of the dry powder, were prepared in 50 mol m^{-3} NaOAc buffer, pH 5.5. The enzyme solns (0.1 cm^3) were mixed with 0.9 cm^3 of 1100 mol m^{-3} sucrose in the acetate buffer and incubated for 1.0 hr at 30°. Reactions were stopped by heating at 100° for 4 min. Reaction rates were determined as glucose release, as previously described [10]. Fructan products in 1 mm^3 of stopped reaction mixtures were analysed by TLC [13].

FSA from *Lolium temulentum* L. was prepared as described in ref. [7]. FSA was diluted with citrate–phosphate buffer, pH 6.0 (63 mol m^{-3} phosphate) to provide

a concn range spanning 0.2 7.4 g fr. wt equv. cm^{-3}. The enzyme prepns were mixed 1:1 with 3000 mol m^{-3} sucrose, incubated for 2.0 hr at 30° and the reactions were stopped by heating at 100° for 4 min. The neutral sugar fraction of the digests were prepd and analysed by HPLC and TLC as previously described [5, 14]. The sensitivity of the analytical methods and the enzyme reaction conditions reproduced those commonly used in previous studies of enzymic trisaccharide synthesis. The experiments differed in the range of enzyme concns used.

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REFERENCES

1. Edelman, J. and Jefford, T. G. (1968) *New Phytol.* **67**, 517.
2. Luscher, M., Frehner, M. and Nosberger J. (1993) *New Phytol.* **123**, 717.
3. Luscher, M., Frehner, M. and Nosberger J. (1993) *New Phytol.* **123**, 437.
4. Cairns, A. J. (1993) *New Phytol.* **123**, 15.
5. Cairns, A. J. (1992) *New Phytol.* **122**, 253.
6. Cairns, A. J. and Ashton, J. E. (1993) *New Phytol.* **124**, 381.
7. Cairns, A. J. and Ashton, J. E. (1994) *New Phytol.* **126**, 23.
8. Penson, S. P. and Cairns, A. J. (1994) *New Phytol.* **128**, 395.
9. Shiomi, N. (1992) *New Phytol.* **112**, 421.
10. Winters, A. L., Smeekens, S. C. M. and Cairns, A. J. (1992) *New Phytol.* **121**, 525.
11. Simmen, U., Obenland, D., Boller, T. and Wiemken, A. (1993) *Plant Physiol.* **101**, 59.
12. St. John, J. A., Bonnet, G. D. and Simpson, R. J. (1993) *New Phytol.* **123**, 705.
13. Bancal, P. and Gaudillère, J. P. (1993) *New Phytol.* **124**, 375.
14. Cairns, A. J. and Pollock, C. J. (1988) *New Phytol.* **109**, 399.