The Metabolism of Fructose Polymers in Plants

TRANSFRUCTOSYLATION IN TUBERS OF *HELIANTHUS TUBEROSUS* L.

By J. EDELMAN* AND A. G. DICKERSON†

Department of Plant Physiology, Imperial College of Science and Technology, London, S.W. 7

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1. A transfructosylase was separated from Jerusalem artichoke-tuber extracts. The partially purified enzyme was practically free from fructosans and its specific activity was increased more than sevenfold. 2. The enzyme was highly specific for terminal β -(2 $\rightarrow$ 1')-linked fructofuranosyl residues. 3. The most active donor found was 1^F-fructosylsucrose, which had about five times the reactivity of the next higher homologues (degree of polymerization 4–6); (1-fructosyl)₂fructose was intermediate in reactivity; sucrose was inactive. 4. Sucrose, and polymers of high degree of polymerization (>20), were the most efficient acceptors for transferred fructosyl residues of the compounds tested. 5. A variety of transferase activities appeared to be catalysed by the enzyme, namely, dismutation ('self-transfer') giving rise to a series of polymers ranging in degree of polymerization both above and below that of the original substrate, transfer to polymer giving rise to limited quantities of higher polymers, and transfer to sucrose; in the last case 1^F-fructosylsucrose/sucrose transfer (resulting in exchange labelling of sucrosyl groups) was used to investigate the mechanism of the reaction more fully. 6. Since fructose transfer can be demonstrated in the living plant tissue, the inter-relationships of the various activities and their significance in fructosan metabolism are discussed.

In a previous paper (Edelman & Jefford, 1964) some properties of β -fructofuranosylases were reported, with regard to their involvement in mobilization of the fructosans of the Jerusalem artichoke tuber during sprouting. Characteristics of the compounds in the tuber have been described (Bacon & Edelman, 1950; Edelman & Jefford, 1964).

Several transfructosylase activities in this tissue have already been reported. They are summarized in equations (a)–(e), where Glc~Fru represents a sucrose residue, and the other D-fructofuranosyl units are linked β -(2 $\rightarrow$ 1').

The relationships among these reactions were obscure, all the more so as some of them were investigated in ignorance of the existence of others, and enzyme preparations were likely to have contained fructosans of high degree of polymerization and hydrolases, all of which, in the light of later knowledge, were likely to take part in relevant reactions.

This paper is concerned with some detailed characteristics of transfructosylase in artichoke-tuber extracts with a view to clarification of the

complexities of the reactions; the preparations used were free from contaminating fructosans and their hydrolases. A preliminary report of some of this work has been presented (Edelman & Dickerson, 1963).

METHODS

Jerusalem artichokes were obtained from Chelsea Physic Garden and the Botanical Supply Unit, University of London.

Estimations. Total fructose (free and combined) was measured colorimetrically by the method of Cole (see Bacon & Bell, 1948), and reducing sugar by the method of Somogyi (1952) and Nelson (1944). Soluble protein was measured colorimetrically with Folin's reagents (see Lowry, Rosebrough, Farr & Randall, 1951), and spectrophotometrically by the nomograph method based on extinction coefficients of Warburg & Christian (1942).

Preparation of substrate-free enzyme. A column of DEAE-cellulose (Whatman DE50, powder form: 10g.; 2.5cm. $\times$ 18.0cm.) was washed with 10 bed-volumes of N-NaOH and then equilibrated with 5mm-phosphate buffer, pH 6.35, at 5° until the pH of the effluent was 6.25. Artichoke juice (250ml.) prepared in the manner described below and containing 10ml. of 0.1M-cysteine and 0.1M-sodium diethyldithiocarbamate in phosphate buffer, pH 7.0, was cooled to 5° and the precipitated inulin removed by centrifugation at 8000–10000g for 15 min. The supernatant liquid (100ml.) was then applied to the column and washed through with

*Present address: Department of Biology, Queen Elizabeth College, London, W. 8.

†Present address: Department of Biochemistry, Imperial College of Science and Technology, London, S.W. 7.

(Dedonder, 1951a,b)

(Edelman & Bacon, 1951a,b)

(Edelman & Recaldin, 1961)

(Recaldin, 1961)

(Dickerson, 1965)

the same buffer at 50–60 ml./hr. until the effluent contained <3.0 μg . of total fructose/ml. The column was then eluted after the method of Bock & Ling (1954) with a linear gradient of ionic strength (increasing from 0.02 to 0.6) with NaCl and phosphate buffer (Na_2HPO_4 and KH_2PO_4), pH 6.1. The eluate was collected at approx. 1 ml./min. in 40 ml. fractions. The fractions were assayed for transferase activity (μmoles of fructose transferred/ml. of incubated system/hr./ μg . of protein) by incubating dialysed samples containing equal amounts of protein (0.5 μg .) with 25 mm-[U^{14}C]-1 $^{\text{F}}$ -fructosylsucrose in 0.02 M-phosphate buffer, pH 6.35, for 1 hr. at 25°. The products of the incubation were separated by chromatography and their radioactive content was determined. Fractions containing the activity which emerged from the column between the ionic strengths 0.29 and 0.45 were bulked and subjected to ultrafiltration in $\frac{1}{4}$ in. diam. Visking tubing and washing with 5 mm-phosphate buffer, pH 6.1, at 5°. The residual solution was freeze-dried to yield approx. 100 mg. of pale-brown powder, which was stored at -20°. When dissolved in water this preparation gave pH 6.1; it contained no detectable fructose and had a specific enzyme activity 7.5 times that of the original protein in the juice. It is henceforth called 'buffered transferase'. No hydrolase activity was detected in this preparation (see also the Results section) because: (1) its activity in tuber juice is much lower than that of the transferase; (2) it was presumably at least partially separated on the column; (3) conditions of pH were unsuitable (Edelman & Jefford, 1964).

Enzymic procedure. Incubations were carried out at 25°, pH 6.1. The recommendations of the International Union of Biochemistry were followed for general procedure. Small volumes of incubation mixtures (<50 μl .) were contained in 20 mm. $\times$ 3 mm. (internal diam.) glass tubes; larger volumes (50–150 μl .) were placed in specially designed tubes (with a constricted neck to reduce evaporation) with an internal volume 150 μl . Pipetting of small volumes (1–20 μl .) was in E-Mil Gold-Line auto zero high-precision pipettes or Drummond Microcaps (Drummond Scientific Co., Philadelphia, Pa., U.S.A.). Enzyme activity was terminated by the use of 10 mm-HgCl₂; 20–30 μl . of this solution was applied to the chromatogram before the sample.

Preparation of enzyme-free substrates. Tuber juice contains a series of fructosans ranging from sucrose to inulin (Edelman & Jefford, 1964). One litre of juice was expressed from the tissues of debudded and washed tubers macerated in a Waring Blendor, and the mixed oligo- and poly-saccharides were precipitated at 0° with ethanol (final concn.

95%, v/v); approx. 30 g. of mixed fructosans was obtained after separation by centrifugation at 0° for 15 min. at 10000g.

Partial separation of the oligosaccharides from the polysaccharides was effected both by differential precipitation in 70–75% ethanol, whence most of the oligosaccharide fraction (D.P. ≤ 6) remained in solution, and by the use of column chromatography. For the latter, the method of Jones, Wall & Pittet (1960) was followed with slight modifications: Dowex 50 W (200–400 mesh; 2% divinylbenzene) in the Li⁺ form was carefully packed in a 200 cm. $\times$ 1.7 cm. column and the polymer mixture containing about 0.5 g. of total fructose in 5.0 ml. was applied and the column eluted with water at about 15–20 ml./hr.; individual oligosaccharides were not completely separated but 10 ml. fractions were collected in which 50–70% of total fructose was in one compound within the lower D.P. range (3–10); the compounds left the column in descending order of molecular weight. The crude fractions were concentrated under an infrared lamp in a current of air, applied to Whatman 3MM paper and the individual fructosans separated by descending chromatography at 25° in propan-1-ol-ethyl acetate-water [for 48 hr. in 7:1:2 (by vol.) for monosaccharides, up to 5 days in 6:1:3 (by vol.) for oligosaccharides to about D.P. 14, and for up to 3 weeks in 11:2:7 (by vol.) for higher polymers and 'inulin' (>D.P. 25)]. Identification of the compounds on chromatograms was carried out with sprays used by Edelman & Jefford (1964), after which the polymers were eluted from the paper with water. Isolated polymers were further purified by rechromatography.

O- β -D-Fructofuranosyl-(2 $\rightarrow$ 1')-D-fructose (1-fructosyl-fructose) and **O- β -D-fructofuranosyl-(2 $\rightarrow$ 1')-O- β -D-fructofuranosyl-(2 $\rightarrow$ 1')-D-fructose** [(1-fructosyl)₂fructose] were obtained by the partial hydrolysis of inulin with 0.1 N-oxalic acid at 60° for 30 min. and were separated by paper chromatography in propan-1-ol-ethyl acetate-water (7:1:2, by vol.) for 40 hr.

Preparation of radioactive substrates. A detached leaf of *Canna indica* was exposed in sunlight to $^{14}\text{CO}_2$ prepared by the action of 50% (v/v) lactic acid on Ba $^{14}\text{CO}_3$ (125 μC /mg.) obtained from The Radiochemical Centre, Amersham, Bucks.; [U^{14}C]sucrose (100 μC /mg.) isolated from the leaf was incubated with a Taka-diestase preparation (1%, w/v; Parke Davis and Co. Ltd., Hounslow, Middlesex) for 24 hr. at 30°. The [U^{14}C]-O- β -D-fructofuranosyl-(2 $\rightarrow$ 1')-O- β -D-fructofuranosyl-(2 $\rightarrow$ 1')- α -D-glucopyranoside (1 $^{\text{F}}$ -fructo-

* Abbreviation: D.P., degree of polymerization.

ylsucrose) isolated from the digest by chromatography as above is known to be contaminated with *O*- β -D-fructofuranosyl-(2 $\rightarrow$ 6')-*O*- α -D-glucopyranosyl-(1 $\rightarrow$ 2)- β -D-fructofuranoside (6^G-fructosylsucrose), which runs together with the 1^F compound on the chromatogram (Bacon, 1960 *a, b*). Purer radioactive substrates were therefore prepared as follows: 0.40 mg. (100 μ C) of repurified [¹⁴C]sucrose was incubated with 0.52 mg. of carrier sucrose and 1.27 mg. of repurified 1^F-fructosylsucrose in 50 μ l. of solution (i.e. both substrates at 0.05 M), containing 0.50 mg. of the buffered transferase, for 15 min. at 25°. The products of the incubation were isolated by chromatography as above. The 1^F-fructosylsucrose/sucrose-transferase activity equilibrated the radioactivity and gave rise to about 45 μ C of 1^F-fructosylsucrose labelled only in the sucrose residue; a small amount (about 6% of the total radioactivity) of the next higher homologue [(1^F-fructosyl)₂sucrose] was also formed. Approx. 300 μ C. (10–12 μ C) of 1^F-fructosylsucrose thus isolated was incubated alone in 10 μ l. of water (0.06 M) with 0.26 mg. of the buffered transferase for 2 hr. at 25°. From this incubation it was possible to isolate, in addition to 1^F-fructosylsucrose, the higher homologues (1^F-fructo-

yl)₂sucrose, (1^F-fructosyl)₃sucrose and (1^F-fructosyl)₄sucrose formed by transfructosylation and all labelled in the sucrose residue alone. After repurification by chromatography the final yields were approx. 1 mg. containing 40 μ C, 0.26 mg. containing 8.5 μ C, 0.06 mg. containing 1.5 μ C and 0.04 mg. containing 0.75 μ C for the polymers of D.P. 3, 4, 5 and 6 respectively.

Detection and estimation of radioactivity. Radioactive areas on paper chromatograms were located with a thin end-window Geiger-Müller counter (Monitor Radiation Alpha Beta, McMichael Radio Ltd.; and a portable Airmec type 255) or by exposure to Kodirex No-screen X-ray film. Quantitative estimation of ¹⁴C was achieved either by chromatogram scanning (Isotope Developments Ltd. scanner 2029 with scaler 1700), or by elution of the compounds followed by thin end-window counting on duralumin disks, or by direct estimation on the radioactive areas cut from the paper and put through an automatic sample changer (I.D.L. 2014 fitted with 2 in. Geiger-Müller tube 2B2; average of both sides taken). Estimates based on these three methods agreed closely.

RESULTS

Self-transfer. This term is used when a compound acts both as fructosyl donor and acceptor. Self-transfer of the trisaccharide 1^F-fructosylsucrose is shown, on a molar basis, in Fig. 1(*a*); all fructose was accounted for at all stages in the reaction by

Fig. 1. Action of the transferase upon 1^F-fructosylsucrose. The incubation mixture contained 0.20 M-1^F-fructosylsucrose and 2.5 mg. of buffered transferase/ml. Results were calculated from analyses of total fructose content (*a*) as μ moles/ml. and (*b*) as mg./ml. ○, Sucrose; ●, D.P. 3; △, D.P. 4.

Fig. 2. Rate of transfer of fructose from individual oligosaccharides in the presence of transferase. Each incubation mixture contained 0.03 M-substrate labelled with ¹⁴C in the sucrosyl group and 2.6 mg. of buffered transferase/ml. Results were calculated from assays of radioactivity. ●, D.P. 3; △, D.P. 4; ▲, D.P. 5; □, D.P. 6.

Fig. 3. Disproportionation of individual oligosaccharides by transferase. Mixtures contained the appropriate substrate (0.05M) as given at the bottom of the Figure and 3.0mg. of buffered transferase/ml. The three samples shown here from left to right for each substrate were removed for paper chromatography after 0, 1 and 5 hr. incubation respectively. Chromatograms were sprayed with benzidine-trichloroacetic acid (Edelman & Bacon, 1951a,b). Direction of development is shown by the arrow. All numerals refer to D.P. values.

Fig. 4. Comparison of rates of self-transfer with 1^F-fructosylsucrose or (1-fructosyl)₂fructose in the presence of transferase. Each incubation mixture contained 0.24M-1^F-fructosylsucrose (●) or 0.28M-(1-fructosyl)₂fructose (■), and 2.5 mg. of buffered transferase/ml. Results were calculated from analyses of total fructose content.

the oligosaccharides present in the reaction mixture: the major components are shown, but some of higher D.P. were present in small amounts which together had a fructose content that was 15% of the total present in the reaction mixture. Fig. 1(b) shows that at equilibrium the major reaction products contain equal weights of fructose, a phenomenon which can be observed among the oligo- and poly-saccharides found in the juice of dormant tubers. Similar activity was shown with the tetra-, penta- and hexa-saccharides. The rate of reaction with these substances is shown in Fig. 2, which demonstrates the greater rate of transfer with the trisaccharide as substrate. Incubation of a variety of oligosaccharides individually leads to the situation demonstrated in Fig. 3. The absence of free fructose in these experiments shows that the buffered transferase is free from hydrolytic fructosylases. Investigation of the specificity of the enzyme showed that terminal β -(2→1')-linked fructofuranosyl groups were transferred, as the enzyme was active against all the natural oligosaccharides tried (D.P. 3-9), as well as against (1-fructosyl)₂fructose (Fig. 4). The enzyme was inactive against 1-fructosylfructose, sucrose, maltose, melibiose, melezitose, raffinose, 6^G-fructosylsucrose and 6^F-fructosylsucrose. The effect of varying substrate concentration is shown in Fig. 5. Because of the nature of the reaction both donor

Fig. 5. Lineweaver-Burk plots for self-transfer. Each incubation mixture contained substrate labelled with ^{14}C in the sucrose residue; those with 1^{F} -fructosylsucrose (●) contained 2.0 mg. of buffered enzyme/ml. and were incubated for 5 min.; those with $(1^{\text{F}}\text{-fructosyl})_2\text{sucrose}$ (Δ) contained 3.65 mg. of enzyme/ml. and were incubated for 15 min. Results were calculated from assays of radioactivity.

Fig. 7. Effect of reactant concentration on the 1^{F} -fructosylsucrose/sucrose transfer reaction. Mixtures were incubated for 4 min. and contained a known weight (about 1 mg.) of buffered transferase/ml., and substrates as follows: ●, 1^{F} -fructosyl[^{14}C]sucrose alone; ▽, equimolar 1^{F} -fructosylsucrose and [^{14}C]sucrose; ○, varying 1^{F} -fructosylsucrose and 25 mM-[^{14}C]sucrose; ▼, varying [^{14}C]sucrose and 25 mM- 1^{F} -fructosylsucrose. Results were calculated from assays of radioactivity and for 1.0 mg. of transferase preparation; e.g., where sucrose is acceptor, the appearance of label in the trisaccharide was measured and was found to be equivalent to its loss from the sucrose, and hence fructose transfer was calculated on the basis of equation (c).

Fig. 6. Effect of the transferase upon an equimolar mixture of 1^{F} -fructosylsucrose and [^{14}C]sucrose. The incubation mixture contained each substrate at 25 mM concentration and 5.4 mg. of buffered transferase. Results were calculated from assays of radioactivity; ○, sucrose; ●, 1^{F} -fructosylsucrose.

and acceptor concentrations necessarily varied together, but the apparent K_m values were calculated to be 0.125 M (D.P. 3) and 0.08 M (D.P. 4).

Transfer to sucrose. (1) Trisaccharide as fructosyl donor. Reaction (c) above is demonstrated by the action of the buffered transferase on an equimolar mixture of 1^{F} -fructosylsucrose and [^{14}C]sucrose

(Fig. 6). The pH optimum was found to be a plateau between 6.0 and 7.0 with a sharp fall on either side.

The effect of varying the concentrations of the reactants is shown in Fig. 7: initial specific activities were known, and subsequent relative ^{14}C contents of all reaction products were measured; fructose transfer was calculated on the basis of proportionality of migration of label. Under the conditions of these experiments (i.e. < 10% transfer of fructose residues from the original substrate) we have found by measurement of both ^{14}C migration and fructose content that no detectable self-transfer occurs when sucrose is present. Significant self-transfer was found only after the equilibration reaction had reached completion.

Specificity: a similar reaction was observed with $(1\text{-fructosyl})_2\text{fructose}$ used as donor; this reaction proceeded at one-quarter of the rate of that with 1^{F} -fructosylsucrose as donor. Raffinose behaved like sucrose as an acceptor, and 1^{F} -fructosylraffinose in the same way as the sucrose analogue as a fructosyl donor; in both cases the reaction rate was the same as for 1^{F} -fructosylsucrose/sucrose transfer.

(2) Oligo- and poly-saccharide as donor. The specificity of the buffered transferase towards individual compounds of the natural series is given in

Table 1. *Transfer of fructose residues from oligosaccharides to sucrose*

(A) Mixtures contained 25mm-donor, 25mm-[U-¹⁴C]-sucrose, 2.4mg. of buffered transferase/ml., and were incubated for 10min. Results were calculated from assays of radioactivity. (B) As (A) but mixtures contained 5mm-donor (except where stated otherwise) and 3.2mg. of enzyme/ml., and were incubated for 15min.

D.P. of donor	Fructose transferred (μ moles/hr./ml. of reaction mixture)	
	(A)	(B)
3	46.7	(25mm) 33.8
4	2.34	(5mm) 14.6
5	3.71	
6	3.09	1.92
7	8.4	
8	17.4	7.1
9	16.2	
12		7.6
15		5.8
18		4.4
20		5.5
24-26		5.1
27-35		6.3

Fig. 8. Effect of concentration of inulin primer upon fructose transfer. Mixtures contained inulin (D.P. ≥ 25) (average mol.wt. taken as 5000), 25mm-[U-¹⁴C]-1^F-fructosylsucrose, and 4mg. of buffered transferase/ml., and were incubated for 2hr. Results were calculated from assays of radioactivity. The two curves denote different occasions.

Table 1; all the compounds tested were poorer donors than the trisaccharide, particularly the lower members (D.P. 4-6).

Transfer to polymer. Addition of [U-¹⁴C]-1^F-fructosylsucrose to artichoke juice indicated that

Fig. 9. Course of fructose transfer from 1^F-fructosylsucrose in the presence of inulin. Conditions were similar to those given for Fig. 7, except that concentration of primer was 5mm and that of the buffered transferase was 3.2mg./ml. Results are plotted as the amount of transferred fructose appearing in: Δ , D.P. 4; $\blacktriangle$, D.P. 5; $\square$, D.P. 6; $\bullet$, D.P. > 20 .

radioactive material was transferred preferentially to the polysaccharide, as shown by paper chromatography, namely to material of R_f zero. Graded acid hydrolysis of eluted polysaccharide gave a series of ¹⁴C-labelled oligofructosides showing that the transferred fructose had become an integral part of the polysaccharide molecules. Fig. 8 shows the effect of concentration of priming polymer upon the action of purified enzyme. During this reaction self-transfer of the trisaccharide also occurred, giving rise to higher oligosaccharides; a time-course of the total transfructosylation occurring in a similar experiment is shown in Fig. 9. This Figure demonstrates preferential transfer to material of high D.P. as the D.P. > 20 curve is steep although the acceptor concentration of polymer (about 5mm) was considerably lower than that of the trisaccharide (25mm), which gave rise to the D.P. 4-6 fractions.

Investigation of the acceptor ability of several of the intermediate oligosaccharides gave results shown in Table 2, which demonstrates that from D.P. 6 upwards the accepting ability is fairly constant and is considerably greater than that of the trisaccharide (self-transfer).

Table 2. *Acceptor ability of members of the inulin series*

Mixtures contained 5 mm-primer, 2.5 mm-[U-¹⁴C]-1 F-fructosylsucrose and 2.9 mg. of buffered transferase/ml., and were incubated for 15 min.

Primer... Fructose transferred (μmoles/hr./ ml. of reaction mixture)	... None*	D.P. 6	D.P. 12	D.P. 18	D.P. 21	D.P. 27-n
to form D.P. 4	12.8	9.7	9.6	8.6	8.9	8.7
to form D.P. 5†	3.9	2.2	2.1	1.5	1.9	1.5
to the primer	—	11.5	10.0	7.4	8.4	10.9
		(10.1)‡	(9.5)‡	(8.7)‡	(9.0)‡	(10.7)‡
Total fructose transferred (%)	16.7	23.4	21.7	17.5	19.2	21.1

* Control.
† Radioautography indicated that reactions initiated by self-transfer of D.P. 3 proceeded no further than production of material of D.P. 5.
‡ Values adjusted for equal total transfer (average 20.6).

	HIGH ACTIVITY	LOW ACTIVITY	NO ACTIVITY
Donor			
	Glc ~ Fru 1 ← 2 Fru	Glc ~ Fru 1 ← 2 Fru _{1 to n} ← 2 Fru	Fru 1 ← 2 Fru
	Gal 1 → 6 Glc ~ Fru 1 ← 2 Fru	Fru 1 ← 2 Fru 1 ← 2 Fru	Glc ~ Fru
			Glc ~ Fru 6 ← 2 Fru
			Fru 2 → 6 Glc ~ Fru
Acceptor			
	Glc ~ Fru		
	Glc ~ Fru 1 (← 2 Fru)* _{4 to n}	Glc ~ Fru 1 ← 2 Fru	Glc
	Gal 1 → 6 Glc ~ Fru		Fru

Fig. 10. Donor and acceptor specificity of the artichoke transferase.

* The tetra- and penta-saccharides were not investigated as the products of the 1^F-fructosylsucrose self-transfer reaction are identical with those of direct transfer from 1^F-fructosylsucrose to the two higher oligosaccharides; the highest value of *n* was not established but was at least 23.

DISCUSSION

With the buffered transferase described here, which was partially purified and substantially free from fructosans and hydrolases acting upon fructosans, the specificity of the transfructosylase for the most relevant sugars is summarized in Fig. 10; these activities could not be ascribed to separate enzymes, hence reactions (b)–(e) are considered to be due to the same enzyme acting under the appropriate conditions. The course of the reactions is conditioned by the nature and concentrations of the donor and acceptor substrates: starting even from a single substance the situation rapidly becomes highly complex as the reaction products themselves act as fructose donors and acceptors which may differ from one another and from the original substrate in their reaction characteristics.

Thus sucrose appears more rapidly the lower the D.P. of the donor oligosaccharide, and, as sucrose is a preferential acceptor, disproportionation is substantially suppressed. For a similar reason the presence of compounds of D.P. greater than about 6 appears to suppress transfer among the lower members.

Since the presence of sucrose in a reaction mixture with the trisaccharide as the sole donor substrate suppresses self-transfer, a competition exists for transferred residues according to the equations (f) and (g).

A comparison of curves (a), (b) and (c) in Fig. 7 shows that the introduction of sucrose, i.e. the substitution of 1^F-fructosylsucrose by sucrose as acceptor, decreases the *K_m* for transfer of fructose residues from 1^F-fructosylsucrose. The effect might be due to the formation of an enzyme–acceptor

complex containing sucrose, which imposes a low K_m on the donating site for trisaccharide, as compared with an enzyme-acceptor complex containing trisaccharide, which imposes a high K_m on the donating site for trisaccharide. Alternatively, the affinity of the donating site for trisaccharide may be relatively high, the rate-limiting step being conditioned by the affinity of the enzyme for acceptor (Stringer & Tsuchiya, 1958), this affinity being higher for sucrose than for trisaccharide.

In the 1^{F} -fructosylsucrose self-transfer reaction (Fig. 7, curve *a*) the concentration of the acceptor necessarily varies with the donor; hence the reaction denoted by curve (*b*) is more comparable with the 1^{F} -fructosylsucrose self-transfer reaction than that of curve (*c*), since the concentrations of both donor and acceptor, namely 1^{F} -fructosylsucrose and sucrose, were varied together and were equimolar throughout; the differences, however, between the slope of curves (*b*) and (*c*) may be considered only marginal, and it would appear from the nearly horizontal slope of curve (*d*) (varying sucrose and constant 1^{F} -fructosylsucrose) that changing the sucrose concentration over the molarity range chosen makes little difference to the overall rate of reaction. Nevertheless, introduction of sucrose (at concentrations between 12.5 and 150 mM) into the reaction mixture does suppress self-transfer and also increases the rate of fructose turnover (except at relatively high 1^{F} -fructosylsucrose concentration), and although over the fairly wide range of concentrations examined here sucrose appears to be equally effective in enhancing the rate of reaction, this does not mean that the reaction is independent of sucrose concentration at lower values.

Significance of transfructosylation in the living tissue. Edelman, Recaldin & Dickerson (1963) have already shown that reaction (*c*) above occurs in the living tissue, both in tubers attached to the plant and in incubated tuber slices. The distribution of fructose among the various compounds in the tuber follows a particular pattern, namely that the total amount present in each of the compounds tends to equality (see Jefford & Edelman, 1961). The transfructosylase described in the present work effects a similar result in cell-free extracts (see Figs. 1 and 10) and in view of the predicted result that labelled sucrose residues would progressively appear in higher oligosaccharides in the living tissue, this enzyme is demonstrably the operative factor.

Whether this enzyme is involved in polymer synthesis *in vivo* still remains in doubt. Certainly, in the presence of added primer, it can form long

chains with the trisaccharide as donor, but the ratio of synthetic polymer to added primer was very low (<1:25, w/w); moreover, translocated sucrose is the primary substrate in the tuber (Edelman & Popov, 1962; Dickerson & Edelman, 1966) and the enzyme does not convert sucrose into trisaccharide. The system of polymer synthesis from sucrose must be more complicated than a straightforward transfructosylation, as extensive conversion of glucosyl into fructosyl residues occurs.

The functional significance of the transfructosylase therefore may lie in controlling the concentrations of sucrose and higher polymers in the tissue [see also the discussion by Edelman & Jefford (1964) of the role of hydrolases in this respect].

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