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GLUCOFRUCTOSAN BIOSYNTHESIS IN *FUSARIUM OXYSPORUM*: REGULATION AND SUBSTRATE SPECIFICITY OF FRUCTOSYL TRANSFERASE AND INVERTASE

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Key Word Index—*Fusarium oxysporum*; Ascomycetes; glucofructosan; fructosan; fructosyl transferase; invertase; biosynthesis; regulation.

Abstract—Mycelium of *Fusarium oxysporum* grown on a glucose-containing medium lacked fructosyl transferase and invertase activities. Synthesis of fructosyl transferase and invertase were induced when sucrose was added to glucose-metabolizing cells. The specific activities of fructosyl transferase and invertase from mycelium of *F. oxysporum* grown on a sucrose-containing medium were significantly higher compared to mycelium obtained from fructose- and fructose–glucose-containing media. Invertase extracted from mycelium was purified up to 173-fold by ethanol precipitation and DEAE-cellulose CC. It hydrolysed sucrose (K_m 1.31 M at pH 5.0) into glucose and fructose and raffinose into fructose and melibiose. No extracellular fructosyl transferase and invertase was detected on day six of growth. Fructosyl transferase transferred fructose from sucrose to xylose to form fructosyl xylose and di-fructosyl xylose. These were hydrolysed by invertase, thus establishing the β-linkage between fructose–fructose and fructose–xylose. Fructosyl transferase was unable to transfer fructose from sucrose to galactose, D-mannose, arabinose, D-ribose, L-sorbose, L-rhamnose, cellobiose, maltose, melezitose, 2-deoxyglucose and 6-deoxyglucose.

INTRODUCTION

Fructosans are stored as reserve carbohydrates in some higher plants and enzymes related to their metabolism have been studied by many workers [1–20]. Fungi, i.e. lower plants, form only low MW glucofructosans when grown on a sucrose-containing medium [21–27]. It has recently been postulated that invertase (EC 3. 2. 1. 26) and fructosyl transferase (EC 2. 4. 1. 9) act on sucrose primarily to supply glucose needed for the growth of *Fusarium oxysporum* and that synthesis of glucofructosans may be only a side reaction [21]. Only when the sucrose supply in the medium is inadequate are glucofructosans utilized as an energy source [21]. Glucofructosans, therefore, constitute a transitory reserve of carbohydrate between the initial and final growth of fungi. The regulation of fructosyl transferase and invertase is the subject of the present paper.

RESULTS AND DISCUSSION

Effect of different carbon sources on glucofructosan synthesis, fructosyl transferase and invertase

The carbon sources tried for the growth of *F. oxysporum* in liquid medium were ethanol, glucose, fructose and sucrose at a concentration of 3% and glucose plus fructose each at a concentration of 1.5%. Glucofructosans were detected only in the medium containing sucrose. *Phytophthora parasitica*, when grown in the presence of carbohydrates other than sucrose, i.e. glucose, fructose, galactose, and raffinose, or with a combination of glucose, fructose and galactose, did not synthesize low MW fructosans: these were produced only in sucrose medium [26]. However, when leaf discs of chicory, dandelion and lettuce were incubated in glucose-, fructose- or sucrose-containing media, fructo-oligosaccharides from DP (degree of polymerization) 3 to *ca* 21 were synthesized *de novo* and sucrose appeared to be the best exogenous sugar source [28]. This is not surprising, since higher plants have the capacity to change glucose and fructose into sucrose which can be utilized for glucofructosan biosynthesis, whereas lower plants like *Fusarium* and *Claviceps* [25] lack such a capability.

Mycelium grown on either glucose or ethanol was devoid of fructosyl transferase and invertase activities. The specific activity of fructosyl transferase from 6-day-old mycelium grown on 3% fructose was 70, whereas it was 29 from mycelium grown on glucose plus fructose. However, the specific activity of mycelium grown on 3% sucrose ranged from 200 to 500 (Table 1). The ability of *F. oxysporum* to synthesize fructosyl transferase and invertase in a medium containing no sucrose but only fructose, albeit at a lower rate, indicates that the fructose unit alone may suffice as a carbon source for the initial synthesis of these enzymes. Subsequent synthesis, however, depends upon the presence of sucrose in the medium. In media containing different carbon

Table 1. Effect of different carbon sources in the growth medium on fructosyl transferase and invertase activities

Carbon source*	Time after inoculation (days)	Specific activity of enzymes in crude extract (units/mg protein)		Presence of glucofructosans in the medium
		Fructosyl transferase	Invertase	
Fructose	6	70	52	—
Glucose fructose (1.5% each)	6	29	17	—
Sucrose	3	228	110	+
Sucrose	6	504	176	+
Sucrose	16	—	410	—
Glucose	up to 12	—	—	—
Ethanol	up to 12	—	—	—

*3% at time of inoculation.
+, present; —, undetectable.

sources the level of invertase in the mycelium followed the order: sucrose > fructose > fructose plus glucose (Table 1). Even though the activities of invertase and fructosyl transferase in mycelium grown on either fructose or fructose plus glucose was significantly less than in mycelium grown on sucrose the exact physiological role of these enzymes cannot be pinpointed. It has been reported that an intracellular invertase was induced in cultures of *Clostridium pasteurianum* utilizing sucrose as carbon source. The induction of the enzyme by sucrose was repressed by the addition of fructose, whereas addition of glucose had no such effect [29]. Addition of glucose or fructose to *F. oxysporum* growing on sucrose neither inhibited the growth nor led to a lowering of the specific activity of invertase (Table 2). In this respect, *F. oxysporum* behaved differently to *Aspergillus flavus*, in which synthesis of invertase was repressed by the addition of glucose or fructose to sucrose-metabolizing cells [30]. However, addition of glucose or fructose to sucrose-metabolizing cells of *F. oxysporum* led to a decreased level of fructosyl transferase (Table 2). Invertase and fructosyl transferase could be induced if sucrose was added to the glucose-containing medium (Table 2).

The specific activity of invertase increased significantly on day 16 when no carbohydrate was detected by partition PC (Table 1). A similar situation in *Streptococcus mitis* was explained by assuming a differential release of proteins from cells in the stationary phase or by binding of autolytically released invertase to the cell surface of remaining whole cells [31].

An ultrafiltrate of 6-day-old *F. oxysporum* grown on sucrose was incubated with 3% sucrose plus [^{14}C]-sucrose (*ca* 1 μCi) in 0.5 ml. After incubation at 25° for 6 hr, glucofructosans, fructose and glucose, isolated by PC, had no radioactivity. This clearly established the absence of an extracellular fructosyl transferase and invertase. Low MW glucofructosans were not detected in the mycelium of *F. oxysporum* grown on sucrose, being confined only to the medium. These data indirectly indicated that this enzyme system (fructosyl transferase and invertase) may be present on the outer membrane of the fungus and can act on sucrose present in the medium to form low

Table 2. Effect of addition of different carbohydrates on *F. oxysporum* growing

Nature of medium	Addition of 3% carbohydrate on 4 day of growth	Dry biomass day 8 (g/10 flasks)	Vol. crude extract from 8-day-old mycelium 10(ml/10 flasks)	Protein (mg)	Fructosyl transferase (units)
Glucose	Sucrose	1.69	34	43.2	3264
Sucrose	Glucose	1.47	34	33.6	2108
Sucrose	Fructose	1.20	35	40.2	2940
Sucrose	—	0.94	32	27.1	3584
Glucose	—	1.24	—	—	—

+, present; −, undetectable.

Table 3. Purification of invertase from 7-day-old mycelium of *F. oxysporum*

Purification step	Vol. (ml)	Protein (mg)	Total (units)	Sp. act. (units/mg protein)	Purification (fold)
Crude	91	98.18	9227	94	1.0
Ethanol precipitation	18.5	22.32	12262	549	5.8
DEAE-cellulose chromatography	31.5	0.475	7754	16342	173.6

MW glucofructosans yielding glucose for uptake by the organism. In *Saccharomyces cerevisiae* [32] and *Streptococcus mutans* [33] a major portion of the invertase activity is located outside the cell membrane and is accessible to exogenously added substrates. Maruyama and Onodera [34] have isolated and purified two forms of invertase from *F. oxysporum*, which we believe are similar to our fructosyl transferase and invertase purified from *F. oxysporum* [21]. Invertase activity is localized in the isolated cell-wall fraction of mycelia and microconidia of *F. oxysporum* [35].

Purification of invertase

Invertase from *F. oxysporum* was purified 174-fold by means of ethanol precipitation and DEAE-cellulose CC (Table 3). The enzyme was eluted with 0.5 M sodium chloride–0.1 M sodium acetate buffer, pH 5.0. Its K_m (Lineweaver–Burk) at pH 5.0 with sucrose as substrate is 1.31 M, compared with 0.79 and 1.33 M for the invertases of *Clostridium pasteurlanum* [29] and *Aspergillus flavus* [30] respectively. Fructosyl transferase from *F. oxysporum* was purified as described earlier [21]. Its K_m at the optimum pH (7.0) with sucrose as substrate is 0.32 M, compared with values of 0.28, 0.36, 0.18, 0.15, 0.23 and 0.11 M for the fructosyl transferases from *Chicory intybus* [4], *Agave americana* [6], *Agave vera cruz* [14], *Helianthus tuberosus* [36], *Allium cepa* [19] and *Asparagus officinalis* [18] respectively.

Specificity of fructosyl transferase and invertase

Fructosyl transferase is able to transfer a fructose unit from sucrose to [^{14}C]glucose to form fructosyl glucose and di-fructosyl glucose [21] and from fructose to xylose to form fructosyl xylose and di-fructosyl xylose (Fig. 1). Di-fructosyl xylose and fructosyl xylose were hydrolysed to fructose and xylose by invertase (Fluka) thus establishing the β-linkage between xylose–fructose and between fructose–fructose. Similarly, di-fructosyl glucose and tri-fructosyl glucose were completely hydrolysed by invertase. Fructose could not be transferred to galactose, D-mannose, arabinose, D-ribose, L-sorbose, L-rhamnose, cellobiose, maltose, melezitose, 2-deoxyglucose or 6-deoxyglucose. The configurations of C-1 to C-4 of glucose and xylose are identical, and this property may be critical for binding of the acceptor molecule to the active site of the fructosyl transferase. In *C. intybus* [5] fructosyl transferase is unable to transfer fructose from sucrose to glucose and xylose. However, fructosyl transferase from *A. americana* is able to transfer fructose from sucrose to mannose, xylose, ribose, arabinose and glucose [6]. In *A. vera cruz*, fructosyl transferase is able to transfer fructose from sucrose to glucose, fructose and xylose [14].

Invertase was able to hydrolyse sucrose into glucose and fructose and raffinose into fructose and melibiose. It did not hydrolyse maltose.

EXPERIMENTAL

Material. Peroxidase (V.P. Chest Institute, New Delhi), glucose oxidase (BDH), invertase (Fluka, Switzerland), DEAE-cellulose (Pharmacia), 6-deoxyglucose and 2-deoxyglucose (Sigma, U.S.A.) were obtained from commercial sources. The culture of *F. oxysporum* NCIM 1072 was obtained from the National Chemical Laboratory, Poona, India.

either on glucose or sucrose on growth, fructosyl transferase and invertase activities

Invertase (units)	Sp. act. of fructosyl transferase (units/mg protein)	Sp. act. of invertase (units/mg protein)	Fructosyl transferase/g dry biomass	Invertase /g dry biomass	Presence of glucofructosans in the medium
6936	75	160	1931	4104	+
6426	63	191	1434	4371	+
4970	73	124	2450	4141	+
3328	132	122	3812	3540	+
—	—	—	—	—	—

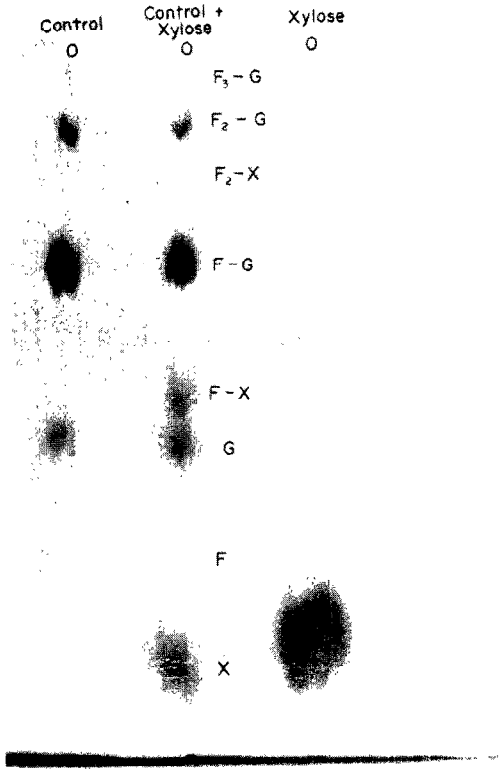

Fig. 1. Paper chromatogram showing the transfer of fructose from sucrose to xylose by fructosyl transferase from *F. oxysporum*. The control incubation contained sucrose and fructosyl transferase. F_3-G, tri-fructosyl glucose; F_2-G di-fructosyl glucose; F-G, fructosyl glucose (sucrose); G, glucose; F, fructose; X, xylose; F-X, fructosyl xylose; and F_2-X, di-fructosyl xylose.

The culture of the organism, enzyme isolation, assay systems for invertase and fructosyl transferase, spray reagents for detection of carbohydrates and other details are as already reported [21]. 1 unit of fructosyl transferase is defined as the quantity of enzyme responsible for the transfer of 1 μmol fructose at pH 5 at 25° in 24 hr. Similarly, 1 unit of invertase is defined as the quantity of enzyme responsible for the release of 1 μmol fructose from sucrose at pH 5 at 25° in 24 hr. *n*-BuOH–HOAc–H_2O (4:1:5) was used for PC [37]. Protein was estimated by the procedure of ref. [38].

Acceptor specificity of fructose from sucrose. To sucrose and the acceptor sugar (0.11 M each) in 1.0 ml NaOAc buffer, pH 5.0, was added 0.1 ml purified fructosyl transferase and the mixture incubated for 24 hr at 25°. The carbohydrates were then detected by PC. When xylose was used as an acceptor molecule, the chromatogram was dried sprayed with 4% invertase (Fluka) preparation in 0.1 M NaOAc buffer, pH 5.0, and kept at 30° for 24 hr. PC was then carried out in the second direction and the sugar spots developed.

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