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Update on Biochemistry

Fructan: More Than a Reserve Carbohydrate?[1]

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Most plants store starch or Suc as reserve carbohydrates, but about 15% of all flowering plant species store fructans, which are linear and branched polymers of Fru. Among the plants that store fructans are many of significant economic importance, such as cereals (e.g. barley, wheat, and oat), vegetables (e.g. chicory, onion, and lettuce), ornamentals (e.g. dahlia and tulip), and forage grasses (e.g. *Lolium* and *Festuca*) (Hendry and Wallace, 1993). Fructans isolated from these plants have a variety of applications. Small fructans have a sweet taste, whereas longer fructan chains form emulsions with a fat-like texture and a neutral taste. The human digestive tract does not contain enzymes able to degrade fructans; therefore, there is strong interest from the food industry to use them as low-calorie food ingredients. In plants, fructans may have functions other than carbon storage; they have been implicated in protecting plants against water deficit caused by drought or low temperatures (Hendry and Wallace, 1993; Pilon-Smits et al., 1995).

The substrate for fructan synthesis is Suc, and like Suc, fructans are stored in the vacuole. Although Suc is synthesized in the cytoplasm, fructans are produced in the vacuole by the action of specific enzymes (fructosyltransferases) that transfer Fru from Suc to the growing fructan chain. Fructan synthesis is modulated by light, which changes the availability of Suc in the cell (Fig. 1). The biosynthetic enzymes are evolutionarily related to invertases, enzymes that hydrolyze Suc.

The biochemistry of fructan synthesis has been determined, and the first genes encoding these biosynthetic enzymes have recently been cloned, opening new biotechnological opportunities for the use of fructans. Until now the major obstacles have been the limited availability of long-chain fructans and the heterogeneity of harvested fructans. It will now be possible to genetically engineer plants to produce large quantities of fructans of defined structure and size. Furthermore, fructan accumulation in plants that normally do not produce them may contribute to protection from water stress in these plants.

A number of research groups have studied fructan accumulation in plants in an attempt to understand fructan synthesis and the physiological role of fructan accumulation in plants and to improve the commercial availability of fructans. In this *Update* we give an overview of these attempts and discuss their impact on our insight into fructan production in plants. First, a few words on fructan synthesis in bacteria, which is simpler than plant fructan biosynthesis because only a single biosynthetic enzyme is involved.

BACTERIAL FRUCTAN BIOSYNTHESIS

Fructan-producing bacteria can be found in a wide range of taxa, including plant pathogens and the bacteria present in oral and gut floras of animals and humans. Examples of bacterial genera in which fructan-producing strains can be found are *Bacillus*, *Streptococcus*, *Pseudomonas*, *Erwinia*, and *Actinomyces* (Hendry and Wallace, 1993). In general, bacteria produce fructan molecules consisting mainly of β(2-6)-linked fructosyl residues, occasionally containing β(2-1)-linked branches (Dedonder, 1966). Such fructans are called levans and can reach a DP of more than 100,000 Fru units.

Bacterial levan is produced extracellularly by a single enzyme, levansucrase, which produces levan directly from Suc. In addition to fructosyltransferase activity, several of these bacterial levansucrases can transfer fructosyl units to water (invertase activity) and to other sugars such as Glu, Fru, and raffinose (Cote and Ahlgren, 1993) as a side reaction. Although most of the bacteria produce levans, a few strains of *Streptococcus mutans* known for their involvement in dental caries produce fructan consisting mostly of β(2-1)-linked fructosyl units (for review, see Uchiyama, 1993).

For the degradation of levan, bacteria produce specific enzymes called levanases, which are divided into endo- and exo-levanases. Exo-levanases hydrolyze only levan and the product is usually levanbiose, meaning that a terminal di-fructosyl unit is removed. Endo-levanases hydrolyze levan and levan oligomers consisting of more than three fructosyl units. These levan molecules are randomly

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Abbreviations: 1-FFT, fructan:fructan 1-fructosyltransferase; 1-SST, Suc:Suc 1-fructosyltransferase; 6G-FFT, fructan:fructan 6G-fructosyltransferase; 6-SFT, Suc:fructan 6-fructosyltransferase; DP, degree of polymerization.

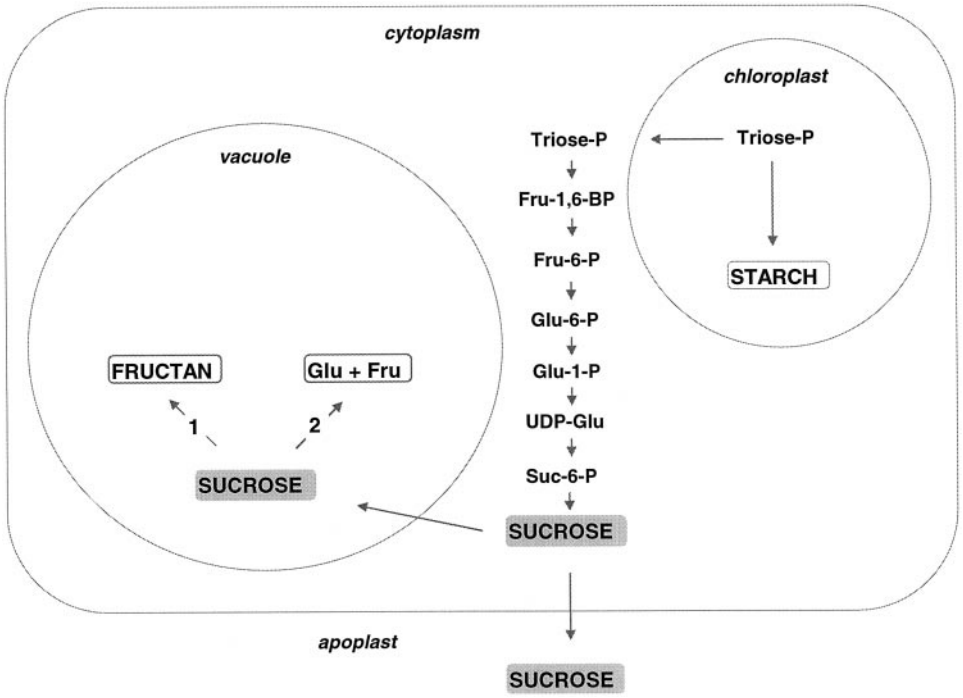

Figure 1. Schematic representation of carbohydrate metabolism in a plant cell. High photosynthetic activity is associated with high rates of carbon export from the chloroplast to the cytoplasm, resulting in an increase of intermediates for Suc synthesis. The synthesized Suc is either distributed to the vacuole (storage) or to the apoplast (export). In the vacuole, Suc can be converted into fructan by fructosyltransferases (1) or hydrolyzed into Glu and Fru by invertase (2).

split into short-chain levans. Nonspecific β-fructosidases are produced to split off the terminal Fru residue (Uchiyama, 1993).

STRUCTURAL DIVERSITY OF FRUCTANS

In contrast to the seemingly uniform structure of bacterial fructans, plant fructans show much more structural diversity (Pollock and Cairns, 1991). The fructosyl chain length in plants varies greatly and is much shorter than that of bacterial fructan. In general, DPs of 30 to 50 fructosyl residues are found, but occasionally DPs can exceed 200. Furthermore, plant fructans have a greater variety in the linkage of the fructosyl residues. In higher plants five major classes of structurally different fructans can be distinguished: inulin, levan, mixed levan, inulin neoseries, and levan neoseries.

Inulin consists of linear (2-1)-linked β-D-fructosyl units (G1-2F1-2Fn) and is usually found in plant species belonging to the order Asterales, such as chicory and Jerusalem artichoke (Bonnett et al., 1994; Koops and Jonker, 1996). The shortest inulin molecule is the trisaccharide 1-kestose, also called isokestose (Fig. 2A).

Levan consists of linear (2-6)-linked β-D-fructosyl units (G1-2F6-2Fn) and is found in some grasses (e.g. *Dactylis glomerata*; Bonnett et al., 1997). Mixed levan is composed of both (2-1)- and (2-6)-linked β-D-fructosyl units. This type of fructan is found in most plant species belonging to the Poales, such as wheat and barley (Carpita et al., 1989; Bonnett et al., 1997). An example of this type of fructan is the molecule bifurcose (Fig. 2B).

The inulin neoseries are linear (2-1)-linked β-D-fructosyl units linked to both C1 and C6 of the Glu moiety of the Suc molecule. This results in a fructan polymer with a Fru chain (mF2-1F2-6G1-2F1-2Fn) on both ends of the Glu molecule. These fructans are found in plants belonging to the Liliaceae (e.g. onion and asparagus; Shiomi, 1989). The smallest inulin neoseries molecule is neokestose (Fig. 2C).

The levan neoseries are polymers of predominantly β(2-6)-linked fructosyl residues on either end of the Glu moiety of the Suc molecule. These fructans are found in a few plant species belonging to the Poales (e.g. oat; Livingstone et al., 1993).

Although most fructan molecules consist of fructosyl residues linked to Suc, fructan molecules have also been isolated from species of the Asteraceae that contain only β(2-1)-linked Fru molecules (Ernst et al., 1996).

PLANT FRUCTOSYLTRANSFERASES

In plants, fructan is synthesized from Suc by the action of two or more different fructosyltransferases. According to the classical model of Edelman and Jefford (1968), two enzymes are involved in the synthesis of the most simple form of fructan, inulin. The first enzyme, 1-SST, initiates de novo fructan synthesis by catalyzing the transfer of a fructosyl residue from Suc to another Suc molecule, resulting in the formation of the trisaccharide, 1-kestose (G1-2F1-2F;

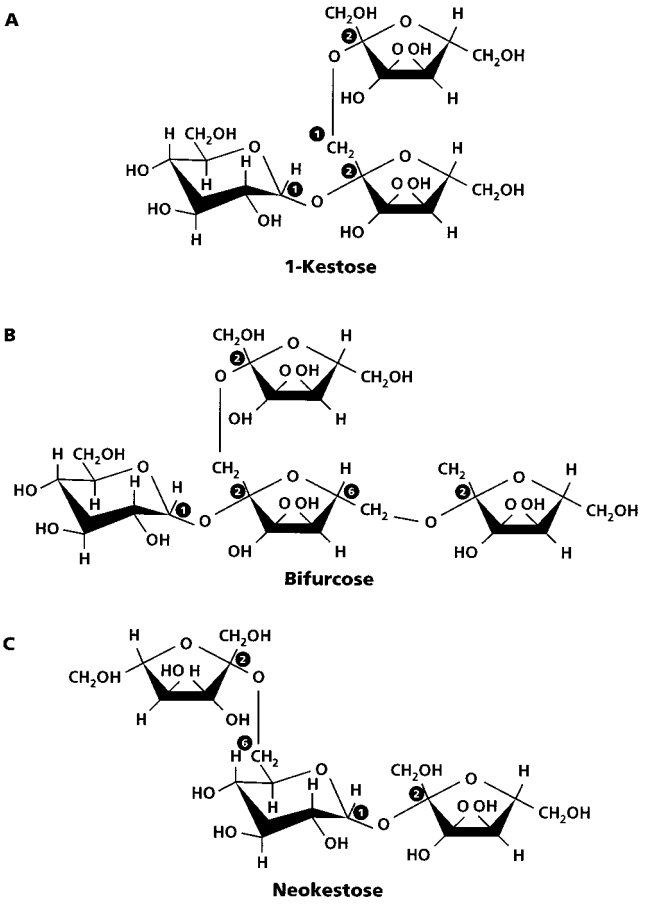

Figure 2. Some examples of structurally different fructan molecules found in plants. A, The trisaccharide 1-kestose consists of a (2-1)-linked β-D-fructosyl unit to Suc and is the shortest inulin molecule. B, The tetrasaccharide bifurcose is an example of a mixed-type levan and consists of a (2-1)- and a (2-6)-linked β-D-fructosyl unit to Suc. C, Neokestose is the smallest inulin neoseries molecule, and in this molecule a β-D-fructosyl unit is linked to the C6 of the Glu moiety of Suc. The numbers encircled in black represent the numbers of the carbon atoms in the sugar molecules. For a detailed description, see text.

Fig. 3). The second enzyme, 1-FFT, transfers fructosyl residues from a fructan molecule with a DP of ≥3 to another fructan molecule or to Suc (Fig. 3). The action of 1-SST and 1-FFT results in the formation of a mixture of fructan molecules with different chain lengths. Although this model for fructan synthesis was proposed by Edelman and Jefford in 1968, it took nearly 30 years before it was shown to be correct. In 1996, several research groups published results of the purification to homogeneity of the enzymes 1-SST and 1-FFT and showed that incubation of these purified fructosyltransferases with Suc resulted in the formation of inulin with a polymer length of up to 20 fructosyl residues (Koops and Jonker, 1996; Lüscher et al., 1996; Van den Ende and Van Laere, 1996). Both 1-SST and 1-FFT are unusual enzymes in that they do not show simple Michaelis-Menten kinetics; their activity depends on both the substrate and the enzyme concentration and is essentially nonsaturable (Koops and Jonker, 1996).

In addition to the enzymes, the cDNAs encoding 1-SST and 1-FFT have also been recently isolated from several plant species. 1-SST has been cloned from Jerusalem artichoke (Van der Meer et al., 1998), chicory (de Halleux and Van Cutsem, 1997), artichoke (Hellwege et al., 1997), and onion (Vijn et al., 1998), and 1-FFT has been cloned from Jerusalem artichoke (Van der Meer et al., 1998) and artichoke (Hellwege et al., 1998). Transformation of 1-SST cDNAs to crops such as sugarbeet and potato has shown that this enzyme is capable of inducing synthesis of 1-kestose and nystose (G1-2F1-2F1-2F) (Hellwege et al., 1997; Sévenier et al., 1998). Remarkably, transgenic sugarbeet plants were obtained that converted 90% of the taproot vacuolar Suc into 1-kestose and nystose.

A long-standing question has been how fructan chain length is determined. For example, in Jerusalem artichoke inulin has an average DP of 8 to 10, whereas in blossom discs of artichoke, inulin has an average DP of 65 (Praznik and Beck, 1985). Transformation of tobacco protoplasts with cDNAs encoding the 1-FFTs from these plants and in vitro incubation of protein extracts from the transformed protoplasts with low-DP (3–5) inulin as the substrate showed that the 1-FFT of artichoke produces fructan molecules with a higher DP (up to 20) than the 1-FFT of Jerusalem artichoke (up to 12) under the same conditions. This suggests that the size of the fructosyl polymers produced by a plant depends mainly on the enzymatic activity of their 1-FFTs, although a role for exohydrolases in defining the final size of the fructosyl chain in the original host plant cannot be excluded (Hellwege et al., 1998). Therefore, cloning of the cDNAs encoding 1-FFT from artichoke and Jerusalem artichoke and their expression in a heterologous system revealed more about the intrinsic properties of these enzymes.

In barley the fructan molecule bifurcose is produced. This molecule is composed of both (2-1)- and (2-6)-linked β-D-fructosyl units linked to Suc (Fig. 2B). Duchateau et al.

1-SST:	G1-2F	+ G1-2F	→ G1-2F1-2F	+ G
	sucrose	+ sucrose	→ 1-kestose	+ glucose
1-FFT:	G1-2F(1-2F)m	+ G1-2F(1-2F)n	→ G1-2F(1-2F)m-1	+ G1-2F(1-2F)n+1
	Elongation of fructan chain, m > 0 and n ≥ 0			
6-SFT:	G1-2F	+ G1-2F1-2F	→ G1-2F1(6-2F)-2F	+ G
	sucrose	+ 1-kestose	→ bifurcose	+ glucose
	G1-2F	+ G1-2F	→ G1-2F6-2F	+ G
	sucrose	+ sucrose	→ 6-kestose	+ glucose
6G-FFT:	G1-2F	+ G1-2F1-2F	→ F2-6G1-2F	+ G1-2F
	sucrose	+ 1-kestose	→ neokestose	+ sucrose
FEH:	G1-2F(1-2F)n		→ G1-2F(1-2F)n-1	+ F
	fructan, n > 0		→ fructan	+ fructose

Figure 3. Enzymatic activities of different plant fructosyltransferases involved in fructan synthesis and of fructan exohydrolase, an enzyme involved in fructan degradation. FEH, Fructan exohydrolase.

(1995) succeeded in purifying to homogeneity 6-SFT, the enzyme that catalyzes the production of the tetrasaccharide bifurcose (G1-2F1[6-2F]-2F) from Suc and 1-kestose. If only Suc is available as a substrate for 6-SFT, it acts mainly as an invertase, resulting in the hydrolysis of Suc to Glc and Fru. Moreover, 6-SFT can also catalyze the formation of the trisaccharide 6-kestose (G1-2F6-2; Fig. 3). The barley cDNA encoding 6-SFT has been cloned and its catalytic activities have been verified by transient expression assays in tobacco protoplasts (Sprenger et al., 1995).

Liliaceous species such as onion and asparagus produce fructan of the inulin neoseries in addition to the normal inulin. These species harbor an additional fructosyltransferase, 6G-FFT, which catalyzes the formation of the trisaccharide neokestose (F2-6G1-2F; Fig. 2C) by the transfer of a Fru residue from 1-kestose to the C6 of the Glu moiety of Suc (Fig. 3; Shiomi, 1989; Wiemken et al., 1995). Extension of the Fru chains on either end of the Glu molecule is catalyzed by the action of 1-FFT. A cDNA encoding such a 6G-FFT has been cloned from onion, and its functionality has been proven (Vijn et al., 1997).

Next to fructan-synthesizing enzymes, fructan-degrading exohydrolytic enzymes have been purified from several fructan-accumulating plants. An exohydrolase with a β-(2-6)-linkage-specific fructan-β-fructosidase activity has been purified from the grass *Lolium perenne*, and a β-(2-1)-linkage-specific exohydrolase has been purified from Jerusalem artichoke (Marx et al., 1997a, 1997b). These enzymes degrade the fructan polymers by removing the terminal Fru residue, resulting in the release of free Fru (Fig. 3; Henson and Livingston, 1996; Marx et al., 1997a, 1997b). To our knowledge, no endo-inulinases have yet been purified from plants, but they are present in fungi. Also, no reports have yet appeared regarding the cloning of cDNAs encoding plant exohydrolases, but this is likely to happen in the near future.

MODEL FOR PLANT FRUCTAN BIOSYNTHESIS

Based on the activities observed for the above-mentioned fructosyltransferases, a model can be proposed for biosynthesis of the structurally different fructan molecules found in plants (Fig. 4). Starting from Suc, 1-SST produces 1-kestose, which can be elongated by 1-FFT, resulting in the formation of inulin. From Suc and 1-kestose, 6G-FFT produces neokestose, which can be elongated by 1-FFT or 6-SFT, resulting in the production of the inulin or levan neoseries, respectively. From Suc and 1-kestose, 6-SFT produces bifurcose, which can also be elongated by either 1-FFT or 6-SFT, resulting in branched, mixed-type levans. When only Suc is available as a substrate, 6-SFT produces 6-kestose, which can also be elongated by 6-SFT to produce levans. Another possibility for the production of levans, which was proposed by Wiemken et al. (1995), involves the removal of the β(2-1)-linked fructosyl residue from bifurcose by either 1-FFT or exohydrolase (Fig. 4).

Looking at the proposed model (Fig. 4), we must keep in mind that fructan synthesis in plants might be much more complex. All of the different fructosyltransferases tested so far are able to produce several fructan molecules depending on the available substrate or on incubation conditions. For example, in addition to 1-kestose, 1-SST is able to produce tetra- and even pentasaccharides from Suc (Koops and Jonker, 1996; Lüscher et al., 1996; Van den Ende and Van Laere, 1996), and in addition to neokestose, 6G-FFT produces the inulin tetrasaccharide nystose (Vijn et al., 1997). This suggests that as-yet-unidentified physiological conditions of the plant influence the substrate affinities of the different fructosyltransferases.

Although the general model for fructan synthesis suggests that Suc is the only substrate for de novo fructan synthesis, fructan molecules consisting only of Fru are found in plants (Ernst et al., 1996; Van den Ende et al.,

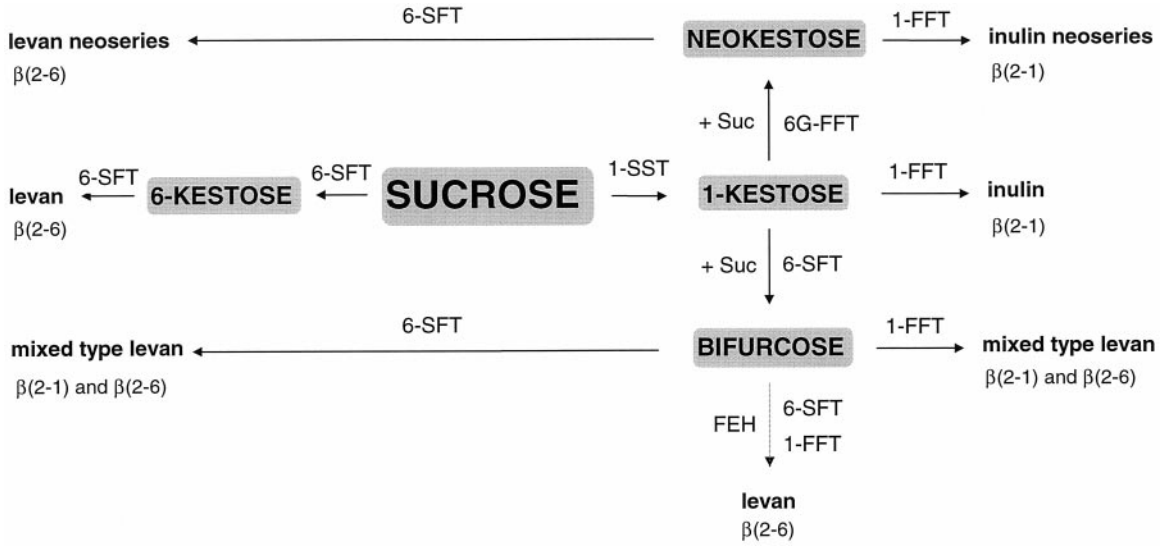

Figure 4. Model of fructan biosynthesis in plants. Starting from Suc, structurally different fructan molecules can be produced by the concerted action of different fructosyltransferases. For a detailed description, see text. The dotted arrow shows an alternative route for the production of levan, as suggested by Wiemken et al. (1995). FEH, Fructan exohydrolase.

1996). These reducing fructofuranosyl-only fructan molecules lack the terminal Glu residue. Although such fructan molecules can be produced by the action of endo-inulinases or, possibly, α-glucosidases that remove the Glu moiety, it has recently been shown that 1-FFT is able to produce fructofuranosyl-only oligosaccharides from Fru and inulin (Van den Ende et al., 1996).

DID FRUCTOSYLTRANSFERASES EVOLVE FROM INVERTASES?

The cloning of cDNAs encoding fructosyltransferase enables us to study the properties of these enzymes in detail. It also allows us to take a closer look at their molecular relations and evolutionary origin. Biochemical analyses of fructosyltransferases have already shown that some of these enzymes also have Suc-hydrolytic (invertase) activity (e.g. barley 6-SFT; Sprenger et al., 1995). Furthermore, it has been observed that at high Suc concentrations most invertases have 1-SST activity (Obenland et al., 1993; Vijn et al., 1998). Not surprisingly, comparison of the amino acid sequences of fructosyltransferases and plant invertases reveals a high degree of identity. For example, at the amino acid level the onion acid invertase shows 61% identity to the onion 1-SST and 63% identity to the onion 6G-FFT (Vijn et al., 1998). The highest homology is found between the fructosyltransferases and the acid invertases, but identity with cell wall invertases is also substantial (approximately 39% similarity) (Fig. 5A).

Recent studies of yeast invertase have revealed the catalytic mechanism for Suc hydrolysis (Reddy and Maley, 1996). The amino acids involved in Suc hydrolysis are the Asp (D) in the Suc-binding box NDPNG (Fig. 5A, region A) and the Glu (E) in box G (Fig. 5A, region G). These amino acids are also conserved among the plant invertases and fructosyltransferases, suggesting an analogous mechanism for Suc hydrolysis (Fig. 5A). However, which amino acids are involved in fructosyltransferase activity is not yet known. For bacterial levansucrases it has been shown that a single point mutation can convert the enzymes into invertases (Chambert and Petit-Glatron, 1991). The availability of the genes encoding plant invertases and fructosyltransferases now allows us to address the question of which domains of the enzymes carry the specificity for hydrolysis and Fru transfer.

The close relationship between invertases and fructosyltransferases at the biochemical and molecular levels is strong evidence for the notion that fructosyltransferases evolved from invertases by relatively few mutational changes. This is especially evident in the close relationship between acid invertases and fructosyltransferases (Fig. 5B). The different fructosyltransferases cluster together with the acid or vacuolar invertases, whereas the cell wall invertases form a separate cluster (Fig. 5B). Several unrelated fructan-accumulating plant families, e.g. the Poales and Asteraceae, acquired fructosyltransferases during evolution. This polyphyletic origin of the fructan-accumulating trait raises questions concerning the nature of the selective forces that have led to the evolution of invertases to fructosyltransferases.

PHYSIOLOGICAL ROLE OF FRUCTAN IN PLANTS

The physiological role of fructans in plants is not fully understood. The presence of fructosyltransferases and fructans in the vacuole has been shown convincingly; fructans are probably synthesized there as well (Wagner et al., 1983; Vijn et al., 1997). However, fructan synthesis in compartments other than vacuoles, such as prevacuolar compartments, cannot yet be ruled out. In addition to its role as a major reserve carbohydrate, fructan synthesis might control Suc concentration in the vacuole. Vacuolar fructan synthesis lowers the Suc concentration in the cell and prevents sugar-induced feedback inhibition of photosynthesis (for review, see Pollock, 1986). Continuous illumination or feeding Suc to excised leaves of fructan-accumulating plants induces fructan synthesis, suggesting a correlation between high Suc levels and the induction of fructan synthesis (Wagner et al., 1983; Vijn et al., 1997). In the vacuole, fructan accumulation can reach levels in excess of 70% of dry weight without inhibiting photosynthesis. Moreover, unlike starch, fructans are soluble.

Another reason for plants to use Suc or fructan as major storage carbohydrates is related to climate. The global distribution of fructan-accumulating plants shows that they are especially abundant in temperate climate zones with seasonal drought or frost, but they are almost absent in tropical regions (Hendry and Wallace, 1993). Although starch biosynthesis decreases dramatically when the temperature drops below 10°C, photosynthesis and fructan production are much less sensitive to low temperature (Pollock, 1986). For example, 1-SST of Jerusalem artichoke retains 50% of its activity at 5°C compared with its activity at optimal temperatures from 20°C to 25°C (Koops and Jonker, 1996). For the above-mentioned reasons fructan storage would be advantageous for plants that are photosynthetically active during the winter or early spring. The protection of the photosynthetic apparatus and the mobilization of stored fructan reserves for rapid growth when temperatures rise are strong factors influencing the evolution of the fructan-accumulation trait.

The involvement of fructans in drought and cold tolerance has been suggested repeatedly (for review, see Wiemken et al., 1995). However, studies of environmental stress resistance are complex, and it is difficult to show a direct correlation between stress and fructan accumulation. In one recent attempt, Puebla and colleagues (1997) compared fructan synthesis in two *Bromus* species adapted to different climatic conditions. These two species were subjected to cold stress and water deficit, and it was found that the species adapted to a cold desert climate exhibited constitutive fructan synthesis, whereas the species adapted to a warmer climate produced fructan only under cold stress. In this study, drought did not influence fructan synthesis. Such an increased tolerance to drought was also reported by Pilon-Smits et al. (1995), who introduced a bacterial levansucrase into tobacco plants, a species normally incapable of forming fructan. These fructan-producing transgenic tobacco plants were found to be more resistant to PEG-induced drought stress, as determined by their growth properties and biomass accumulation.

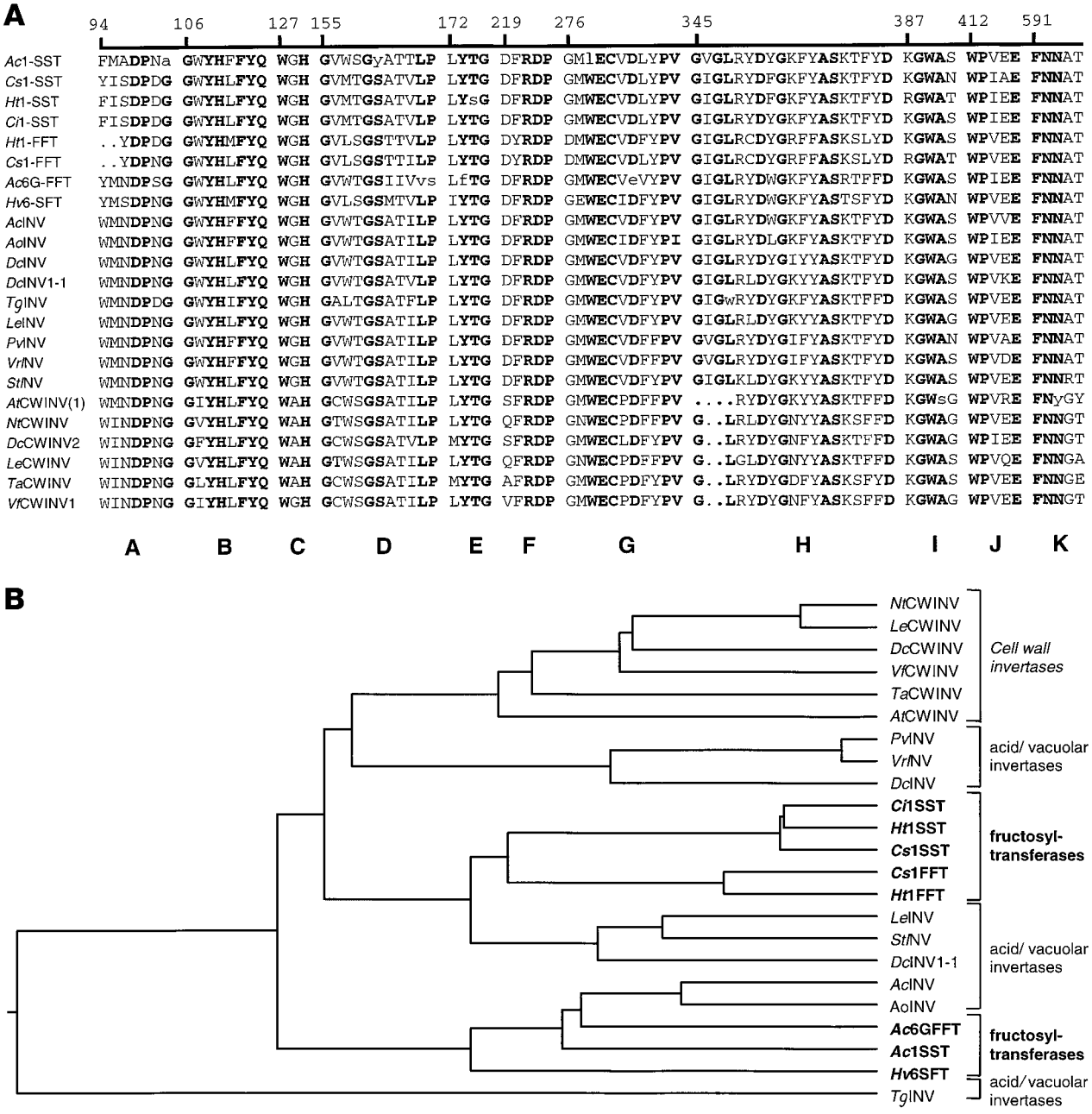

Figure 5. Comparison of amino acid sequences of plant fructosyltransferases with those of acid invertases and cell wall invertases. A, Alignment of well-conserved regions of invertases and fructosyltransferases. Letters in bold type show almost perfectly conserved amino acids. Numbers above the comparisons represent the amino acid sequence of onion (*Allium cepa*) 1-SST (*Ac*1-SST). Region A contains the so-called Suc-binding box NDPNG with the well-conserved Asp. Region G contains the well-conserved Glu, which, together with the Asp, is involved in Suc hydrolysis in invertases. B, Dendrogram showing evolutionary relatedness of sequences. The following sequences were included: 1-SST from onion (accession no. AJ0060660), artichoke (accession no. Y09662), Jerusalem artichoke (accession no. AJ009757), and chicory (accession no. U81520); 1-FFT from Jerusalem artichoke (accession no. AJ009756) and artichoke (accession no. AJ000481); 6G-FFT from onion (accession no. Y07838); 6-SFT from barley (accession no. X83233); acid/vacuolar invertases of onion (accession no. AJ006067), asparagus (accession no. AF002656), carrot (accession no. A67163 [*Dc*INV] and accession no. X75351 [*Dc*INV1-1]), tulip (accession no. X95651), tomato (accession no. D22350), bean (accession no. U92438), mung bean (accession no. D10265), potato (accession no. X70368); and cell wall invertases from Arabidopsis (accession no. X78424), tobacco (accession no. X81834), carrot (accession no. X78424), tomato (accession no. AB004558), wheat (accession no. AJ224681), and fava bean (accession no. Z35162). Abbreviations for the source plants are as follows: *Nt, Nicotiana tabacum*; *Le, Lycopersicon esculentum; Dc, Daucus carota; Vf, Vicia faba; Ta, Triticum aestivum; At, Arabidopsis thaliana; Pv, Phaseolus vulgaris; Vr, Vigna radiata; Ci, Cichorium intybus; Ht, Helianthus tuberosus; Cs, Cynara scolymus; St, Solanum tuberosum; Ac, Allium cepa; Ao, Asparagus officinales; Hv, Hordeum vulgare; Tg, Tulipa gesneriana.*

It should be noted that in these studies a direct correlation between the observed fructan accumulation and drought tolerance was not unequivocally shown. In the latter study (Pilon-Smits et al., 1995) the introduction of a bacterial levansucrase resulted in the production of fructans with a DP of more than 100,000. Such high-DP fructan molecules are normally not found in plants and may induce stress responses. For studies of the physiological role of fructan metabolism in plants it is preferable to use plant fructosyltransferase genes. Such genes are now available and have been transformed into several different plant species that do not normally accumulate fructans, and the performance of these transgenic plants under different environmental stresses can now be studied in detail. Another approach to studying the physiological role of fructan accumulation is by inhibiting its synthesis in plants that normally accumulate fructan. This can be achieved by silencing the endogenous fructosyltransferase genes with the introduction of additional fructosyltransferase genes, either in the antisense or in the sense orientation (cosuppression).

FRUCTAN UTILITY

Since the mid 1930s, fructans have been used in tests for human kidney function (Shannon and Smith, 1935), and interest in potential medical uses for inulin and inulin derivatives is growing (Fuchs, 1993). Recently, it was found that a fructan-rich diet may have health-promoting effects (Roberfroid, 1993). Fructans are a low-calorie food because they cannot be digested by humans but are instead used efficiently as a carbon source by beneficial bifidobacteria in the colon (Gibson et al., 1995). These bifidobacteria ferment fructans to short-chain fatty acids that have a positive effect on systemic lipid metabolism. Small fructans with DPs of 3 to 6 are sweet tasting and therefore constitute natural low-caloric sweeteners. At this time the most agronomically acceptable crop for fructan production is chicory; however, the function of the fructan isolated from chicory is limited because of the degradation of long fructan chains by fructan exohydrolase upon harvesting. High-DP fructans are now being used in alimentary products where they can replace fat. Emulsions of long-chain fructans in water have organoleptic properties similar to fat. High-DP fructans also hold great promise for a variety of nonfood applications (for review, see Fuchs, 1993). However, the difficulty in obtaining long-chain and complex-branched fructans has thus far limited their application.

FRUCTAN BIOTECHNOLOGY

The availability of cDNA clones encoding fructosyltransferases with different enzymatic activities from several plant species allows the biotechnological exploitation of fructan metabolism. Transformation of fructosyltransferases in agronomically important crops may improve the commercial availability of fructan. The introduction of 1-SST into sugarbeet and potato has shown that large amounts of short-chain fructan molecules are produced (Hellwege, 1998; Sévenier, 1998). The advantage of using crops such as sugarbeet or potato for fructan accumulation is that they lack fructan-hydrolyzing enzymes such as exohydrolase for breaking down the accumulated fructan upon harvesting. Fructan-accumulating sugarbeet is an especially promising crop because of its excellent agronomic performance in temperate zones and its natural Suc storage capacity.

In another approach, novel fructan accumulation was enhanced by the introduction of heterologous fructosyltransferase genes in existing fructan-accumulating crops (Sprenger et al., 1997; Vijn et al., 1997). For example, transformation of onion 6G-FFT into chicory resulted in the production of fructan molecules of the inulin neoseries in addition to normally produced inulin, showing that the type of fructan molecule produced by a plant can be adapted (Vijn et al., 1997).

In the near future it will also be possible to synthesize homogeneous pools of structurally defined fructan molecules in transgenic plants by the introduction of specific sets of fructosyltransferase genes. Pilot experiments in which combinations of protein extracts of tobacco protoplasts transformed with different fructosyltransferases were incubated with Suc produced the expected type of fructan molecules (Vijn et al., 1998).

Other important strides have included the successful expression of an active barley 6-SFT in the yeast *Pichia pastoris* by Hochstrasser and colleagues (1998) and the expression of onion invertase and 1-SST in a mutant *Saccharomyces cerevisiae* strain lacking invertase (I. Vijn and S. Smeekens, unpublished data). The enzymatic activity of the cloned plant fructosyltransferases has so far been studied mainly by transient expression assays in tobacco protoplasts or by stable transformations of plants that normally do not accumulate fructan. These systems are time-consuming and laborious, contain endogenous invertase activities that compete with fructosyltransferases for the substrate, Suc, and also hide possible invertase activity of the introduced fructosyltransferases. The development of an efficient heterologous expression system for plant fructosyltransferases would allow a much more detailed biochemical characterization of these enzymes. In heterologous expression systems it will be possible to study the contribution of specific amino acids to enzymatic activity and to acceptor specificity by site-directed mutagenesis. The influence of posttranslational processing could also be studied.

CONCLUSIONS AND PERSPECTIVES

Since the cloning of the first fructosyltransferase cDNA in 1995 (Sprenger et al., 1995), many others have been isolated. Transformation of these cDNAs into plants that normally do not accumulate fructan and detailed biochemical characterization of the expressed fructosyltransferases have resulted in better insights into fructan biosynthesis in plants. Future studies of fructan synthesis in plants, especially grasses, will probably result in the identification of other fructosyltransferases with additional enzymatic activities, which will lead to a better understanding of the complexity of plant fructan biosynthesis.

Another consequence of the cloning of fructosyltransferases is that their physiological role can now be studied in plants that normally do not accumulate fructans and in plants that inhibit fructan synthesis. Studying the responses of these transgenic plants to environmental stresses such as drought and cold may lead to greater insight into the physiological role of fructans in stress resistance. Furthermore, the transformation of the cloned fructosyltransferases into agronomically important crops shows that such crops have great potential as fructan sources and that it may soon be possible to produce a range of structurally different fructan molecules.

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