Research article

In vivo transport of three radioactive [18F]-fluorinated deoxysucrose analogs by the maize sucrose transporter ZmSUT1

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ARTICLE INFO

Article history:
Received 12 December 2016
Received in revised form 5 March 2017
Accepted 6 March 2017
Available online 8 March 2017

Keywords:
Fluorine-18
Fluorodeoxysucrose
Maize
Phloem
Sucrose
Transport
ZmSUT1

ABSTRACT

Sucrose transporter (SUT) proteins translocate sucrose across cell membranes; however, mechanistic aspects of sucrose binding by SUTs are not well resolved. Specific hydroxyl groups in sucrose participate in hydrogen bonding with SUT proteins. We previously reported that substituting a radioactive fluoride-[18F] at the C-6' position within the fructosyl moiety of sucrose did not affect sucrose transport by the maize (Zea mays) ZmSUT1 protein. To determine how [18F] substitution of hydroxyl groups at two other positions within sucrose, the C-1' in the fructosyl moiety or the C-6 in the glucosyl moiety, impact sucrose transport, we synthesized 1'-[18F]fluoro-1'-deoxysucrose and 6-[18F]fluoro-6-deoxysucrose ([18F]FDS) analogs. Each [18F]FDS derivative was independently introduced into wild-type or sut1 mutant plants, which are defective in sucrose phloem loading. All three (1'-, 6'-, and 6-) [18F]FDS derivatives were efficiently and equally translocated, similarly to carbon-14 [14C]-labeled sucrose. Hence, individually replacing the hydroxyl groups at these positions within sucrose does not interfere with substrate recognition, binding, or membrane transport processes, and hydroxyl groups at these three positions are not essential for hydrogen bonding between sucrose and ZmSUT1. [18F]FDS imaging afforded several advantages compared to [14C]-sucrose detection. We calculated that 1'-[18F]FDS was transported at approximately a rate of 0.90 ± 0.15 m.h⁻¹ in wild-type leaves, and at 0.68 ± 0.25 m.h⁻¹ in sut1 mutant leaves. Collectively, our data indicated that [18F]FDS analogs are valuable tools to probe sucrose-SUT interactions and to monitor sucrose transport in plants.

1. Introduction

Nearly 80 percent of the carbon assimilated during the day in source leaves is exported to the sink (importing) tissues, such as roots, developing shoots, or reproductive tissues (Clauss et al., 1964; Hartt and Kortschak, 1967; Kalt-Torres et al., 1987). For the majority of crop plants, sucrose, a disaccharide combination of the monosaccharides glucose and fructose, is the primary form of carbohydrate utilized for long-distance transport via the phloem (Zimmermann and Ziegler, 1975; Ohshima et al., 1990; Slewninski and Braun, 2010a). In sink tissues, sucrose is hydrolyzed by inverte into glucose and fructose, or degraded by sucrose synthase into UDP-glucose and fructose, which are substrates of multiple pathways involved in metabolic, signaling, structural, or storage functions (Koch, 2004; Bihmidine et al., 2013; Braun et al., 2014; Ruan, 2014). Therefore, understanding sucrose transport is key for developing novel strategies for crop improvement (Ayre, 2011; Yadav et al., 2015; Bihmidine et al., 2016).

In maize (Zea mays) leaves, sucrose moves symplasmically through plasmodesmata from the mesophyll cell into the bundle sheath cell, and then on into the phloem parenchyma cell (Evert et al., 1978; Braun and Slewninski, 2009). In apoplastic phloem loading species, sucrose is thought to be effluxed to the apoplas (cell wall) and actively transported into the companion cell-sieve element complex (Evert et al., 1978; Baker et al., 2012; Braun, 2010a).
Physiological, genetic, and biochemical evidence showed that the maize SUCCROSE TRANSPORTER1 (ZmSUT1), a sucrose/H⁺ symporter, is responsible for transporting sucrose into companion cells (Aoki et al., 1999; Carpaneto et al., 2005; Slewinski et al., 2009; Baker et al., 2016). Loss-of-function mutations of ZmSUT1 (termed sut1) result in reduced sucrose export from leaves, hyperaccumulation of carbohydrates in leaves, and reduced biomass partitioning (Slewinski et al., 2009, 2010).

Numerous studies have investigated the substrate-binding specificities and transport mechanism of sucrose transporter (SUT) proteins (Hitz et al., 1985, 1986; Card et al., 1986; Madore and Lucas, 1987; Hitz, 1988; Hecht et al., 1992; Griffin et al., 1993; Chandran et al., 2003; Carpaneto et al., 2005; Sivitz et al., 2005; Reinders et al., 2006; Carpaneto et al., 2010; Sun et al., 2010, 2012; Sun and Ward, 2012). Additionally, studies using biophysical approaches have demonstrated that a proton and then a sucrose molecule binds ZmSUT1, after which the transporter undergoes a conformational change, resulting in proton and sucrose symport across the plasma membrane (Carpaneto et al., 2010; Geiger, 2011; Derrey et al., 2013). Based on 3D modeling of the structure of the rice (Oryza sativa) OsSUT1 orthologous protein, site-directed mutagenesis, and electrophysiological experiments (Sun et al., 2012) demonstrated the importance of five charged amino acids in two of the transmembrane domains of OsSUT1 for sucrose transport. In addition, Sun and Ward (2012) found that the hydroxyl groups at the C-3 and C-4 positions within the glucosyl moiety of sucrose were crucial for sucrose transport by OsSUT1.

Some of the most extensive biochemical characterizations of substrate-binding specificity for sucrose uptake transport were performed in the 1980s using soybean (Glycine max) cotyledons. Hitz et al. (1985, 1986) synthesized a number of different fluorinated- or phenyl glucopyranoside-sucrose derivatives and tested their ability to compete with [14C]-sucrose uptake into protoplasts derived from developing soybean cotyledons. Individually replacing the hydroxyl groups with fluorine [F] at the C-1', C-4', or C-6' positions within the fructosyl moiety of sucrose increased the hydrophobicity of these fluoroedeoxysucrose (FDS) derivatives, and, for the C-1' - and C-6'-FDS analogs, but not the C-4'-FDS derivative, increased their binding affinity by SUTs (Card et al., 1986). In addition, F substitution eliminated the potential for hydrogen bonding from the hydroxyl groups at these positions (Dunitz and Taylor, 1997). Interestingly, 18F-labeled 1'-FDS was transported in soybean and maize tissues (Hitz et al., 1985; Schmalstig and Hitz, 1987), but to our knowledge, 6-FDS has not been previously tested as a transport substrate by SUTs. Hitz et al. (1986) also showed that deoxy-derivatives lacking hydroxyl groups at the C-3, C-4, and C-6 positions in the glucosyl moiety of sucrose were involved in substrate recognition and apparently donate their hydrogen atoms to the protein for hydrogen bonding. More recent kinetic analyses on Xenopus laevis oocytes expressing different SUT proteins found that sucralose (4, 1', 6'-trichloro-4', 1'-trideoxygalactosucrose) inhibits sucrose transport by ZmSUT1, sugarcane (Saccharum spp.) ShSUT1, and OsSUT1, demonstrating the importance of these positions for transport (Reinders et al., 2006; Sun et al., 2010; Derrey et al., 2013). Sucralose is a derivative of sucrose that contains a chlorine at the C-1' and C-6' positions of the fructosyl moiety and the C-4 position of the galactosyl moiety. Additionally, Griffin et al. (1993) showed that 14C-labeled [methyl-14C]-4'-methoxyphenyl] z-D-glucopiranoside was taken up into celery (Apium graveolens) phloem tissues, and that the unlabeled compound competitively inhibited sucrose uptake. However, by removing the hydroxyl group at the C-2 position they found that [methyl-14C]-2-deoxy (4'-methoxyphenyl) z-D-glucopyranoside was a non-competitive inhibitor of sucrose uptake. Collectively, these reports showed that the hydroxyl groups at the C-2, C-3, C-4, and C-6 carbon positions of the glucosyl moiety of sucrose are involved in binding to SUTs, likely through hydrogen bonding (Hitz, 1988). Furthermore, the ability of several phenylglycoside-deoxy-sucrose derivatives to compete with sucrose and to serve as transport substrates suggested that it is the hydrophobicity of the fructosyl moiety of sucrose, not the capacity for hydrogen bonding via the hydroxyl groups at the substituted positions, which contributes to binding by soybean SUTs (Card et al., 1986; Hitz et al., 1986).

After being loaded into the phloem in leaves, sucrose is translocated to sink tissues through the transport phloem. In the phloem, sucrose movement is modeled by the Münch pressure flow theory. In the leaf, the high concentration of sucrose in the collection phloem (0.9–1.4 M for maize) (Ohshima et al., 1990; Weiner et al., 1991) versus the low concentration in the vein apoplasm (estimated to be in the low mM range for maize) (Heyser et al., 1978; Lohaus et al., 2001) creates an osmotic gradient that draws water from the xylem into the phloem. In the sink tissues, sucrose exits the release phloem, and water from the phloem is recycled back to the xylem, thereby generating a hydrostatic pressure differential that drives sucrose from the source toward the sink tissues (van Bel, 2003). Thus, solute transport inside the phloem is highly sensitive to the changes in phloem pressure. Over the past few years, a growing number of studies have used 18F-labeled sugars (18F has a half-life of 110 min) to trace sugar transport in plants (Hattori et al., 2008; Ferrieri et al., 2012; Partelová et al., 2014; Hubeau and Steppe, 2015; Rotsch et al., 2015). It is also important to note that 11C is only suitable for short time-frame experiments. In addition, the combination of positron emission tomography (PET) and a positron-emitting isotope permits the real-time dynamics of sugar transport. Two of their key advantages are higher energy decay products and short half-lives, which make non-invasive, quantitative, and repeatable measurements possible.
studies of phloem transport in plants used 2-[18F]fluoro-2-deoxy-D-glucose ([18F]FDG), which was originally developed for medical research (Ido et al., 1978; Massoud and Gambhir, 2003; Phelps, 2004). However, hexoses are not thought to be substrates for long-distance phloem transport (Liu et al., 2012). Therefore, using a 18F-labeled sucrose molecule may overcome these potential disadvantages of [14C]C2O2 or [18F]FDG.

In a previous study, we synthesized a new radioactive tracer 6’-[18F]fluoro-6-deoxy-sucrose ([6’-18F]FDS) to monitor sucrose transport in maize leaves. We showed that 6’-[18F]FDS could be taken up into veins, and was transported out of the leaf in a ZmSut1-dependent manner (Rotsch et al., 2015). Based on these previous findings, we extended our studies to understand some of the chemical features of sucrose that are involved in substrate recognition, binding, and long-distance transport via the ZmSUT1 protein. We radiosynthesized two additional [18F]-fluorinated deoxyosucrose ([18F]FDS) analogs (1’-[18F]fluoro-1’-deoxyosucrose (1’-[18F]FDS) and 6-[18F]fluoro-6-deoxyosucrose (6-[18F]FDS)), and introduced them into maize leaves to investigate the roles of the hydroxyl groups at the C-1’ and C-6 positions within the fructosyl or glucosyl moieties, respectively. We found that these two [18F]FDS were efficiently transported through the leaf blades. Furthermore, and somewhat surprisingly, all three [18F]FDS analogs were transported similarly to each other and to [14C]-sucrose, suggesting that these specific fluorinated positions have no effect on transport by ZmSUT1. In addition, we were able to utilize the transport data to calculate the rate of 1’-[18F]FDS movement in a maize source leaf.

2. Methods

2.1. Plant materials and growth conditions

Maize plants were grown in the Sears Greenhouse at the University of Missouri. Plants were grown with supplemental lighting provided by high pressure sodium lamps (1000 μmol.m-2.s-1 at the canopy height) under a 16-h-day (30 °C)/8-h-night (25 °C) cycle. Dawn was at 5AM and dusk was at 9PM. Plants were grown in 11 × 12 cm pots, in peat moss-based potting soil (Pro-Mix BX, Premier Tech, Canada), watered daily, and fertilized once with an iron chelate (Sprint 330) and N-P-K 12-12-12 fertilizer (Dyana Green, Hummert International, MO, USA).

All plants used in experiments were 3–4 weeks old. To perform the radioactive labeling experiments, plants were transferred to a growth chamber at the MU Research Reactor to be physically close to the cyclotron. To acclimate the plants to the new environment, transport assays were performed 4 h after the lights turned on, corresponding to when the sucrose concentration in the leaves is high and stable, and the assimilate export rate is highest (Kalt-Torres et al., 1987).

Transport assays were performed on fully expanded leaves 5 or 6. The leaf was cut 2.54 cm from the tip. Two ml of 1 mM unlabeled sucrose, spiked with 742.02 Bq [18F]FDS and 556.52 Bq [14C]-sucrose, was applied by dipping the leaf tip into the labeling solution (Gaddam and Harmata, 2013; Ying et al., 2013; Gao et al., 2014).

The non-enzymatic and automated radiosynthesis of the three [18F]FDS analogs was performed as described (Rotsch et al., 2015) using a dual reactor synthesis Modular-Lab system (Eckert & Zieglers, Germany). The radiosynthesis process took approximately 150 min and included three steps. The first step was labeling the precursor, the second step was hydrolysis of the product of the first step, and the third step was purification by HPLC separation. The mobile phase was evaporated from the separated product, and the [18F]FDS analog was resuspended in water.

2.4. [18F]FDS and [14C]-sucrose transport assays

[18F]FDS analogs and universally labeled [14C]-sucrose (American Radiolabeled Chemicals, St. Louis, MO, USA) transport assays were performed as described (Rotsch et al., 2015). The experiments were performed 4 h after the lights turned on, corresponding to when the sucrose concentration in the leaves is high and stable, and the assimilate export rate is highest (Kalt-Torres et al., 1987).

Transport assays were performed on fully expanded leaves 5 or 6. The leaf was cut 2.54 cm from the tip. Two ml of 1 mM unlabeled sucrose, spiked with 742.02 Bq [18F]FDS and 556.52 Bq [14C]-sucrose, was applied by dipping the leaf tip into the labeling solution for 3 min (Fig. 2A). After 3 min, the leaf tip was removed from the solution, and was cleaned twice by wiping with dry Kimwipes and then with wet ones, after which the leaf tip remained in air. The plant was allowed to translocate the labeled sucrose for up to 60 min in the growth chamber (Fig. 2B). After which, a ~26 cm leaf segment, measured from the cut tip, was excised from the plant, taped to paper, and exposed to a phosphor plate (Fig. 2C). The plate was exposed for 60 min to image the [18F]. The next day, the same labeled leaf was exposed to a phosphor plate for 5 days to image the [14C]. The transport experiment, n = 8 wild-type plants and n = 11 sut1 mutant plants were assayed. For the 6’-[18F]FDS transport experiment, n = 9 wild-type plants and n = 9 sut1 mutant plants were assayed. For the 6-[18F]FDS transport experiment, n = 18 wild-type plants and n = 20 sut1 mutant plants
were assayed. The phosphor plate was scanned with a GE Typhoon FLA 9000 scanner, the laser was set up at 635 nm, and pixel size was 100 μm. Image data were quantified using GE ImageQuant TL Toolbox program 7.0.

2.5. Rate of [18F]FDS transport

To calculate the rate of sucrose transport, [18F]FDS was applied to wild-type (n = 8–11) and sut1 mutant (n = 8–11) leaves at each time point as described above. The transport time analyzed was varied from 15, 30, or 60 min. Transport rates were calculated as the distance from the cut leaf tip to the position of the radiation front of [18F]FDS divided by the transport time. The radiation front was identified where the pixel intensity was 50 arbitrary units higher than the pixel intensity of the background.

2.6. Statistical analyses

To study the transport of each [18F]FDS analog and [14C]-sucrose, the pixel intensity at each measured distance (2.54 cm) from the application site was converted to a percentage of the total intensity within the whole leaf. To limit the error potentially caused by contaminating labeled sucrose on the leaf surface at the application site, the first 2.54 cm distance from the leaf tip was excluded from the analyses. Means and standard errors were calculated using Microsoft Excel. The difference in radioactive tracer transport between sut1 mutant and wild-type leaves was determined using the Student's t-test embedded in the Microsoft Excel program, at the 0.05 probability level.

To compare the transport of different sucrose analogs, the intensity of each [18F]FDS analog was normalized by the intensity of [14C]-sucrose in the same experiment at each distance from the application site. The normalized values were used for statistical analyses. A mixed-model ANOVA was performed to analyze the difference between different analogs using Statistical Analysis System (SAS Institute, NC, USA).

3. Results

3.1. 1-[18F]FDS and 6-[18F]FDS were efficiently translocated in maize leaves

To test if 1-[18F]FDS and 6-[18F]FDS would be transported in vivo, these two analogs and 6-[18F]FDS were independently introduced into leaves of wild-type plants and sut1 mutant plants that lacked ZmSut1 function (Figs. 2 and 3). We also introduced the universally labeled [14C]-sucrose tracer along with each [18F]FDS analog as a positive control to test whether these analogs would function as substrates of ZmSUT1 for phloem transport. One hour after [18F]FDS analogs were applied to the tip of the leaf, the [18F] radioactivity signal was detected at the base of wild-type leaves but not sut1 mutant leaves (Fig. 3A–C). Transport of the three [18F]FDS analogs was visibly reduced in sut1 mutant leaves compared to wild-type leaves. The transport patterns of these three [18F]FDS
analogs were similar to the [14C]-sucrose transport patterns. Quantification of the radiation at different locations within the labeled leaves showed that after the first 10 cm, the difference in transport between sut1 mutant and wild-type leaves was statistically significant at $p \leq 0.05$ (Fig. 4A–F). In addition, we observed that the visible radioactivity of all three [18F]FDS analogs better defined the veins than [14C]-sucrose (Fig. 3).

3.2. All three [18F]FDS analogs show comparable transport

We compared the [18F]FDS/[14C]-sucrose normalized transport values of the three [18F]FDS analogs at each distance from the application site (Fig. 5). The data showed no statistically significant differences in the transport of these analogs at any distance from the application site, in both wild-type or sut1 mutant leaves (Fig. 5).
In addition, we used a mixed-model ANOVA to examine the effects of the different \(^{18}\text{F}\)FDS analogs and their distance transported from the application site. The effect of the different \(^{18}\text{F}\)FDS analogs on transport was not significant \((p = 0.661)\) (Table 1). Therefore, these data suggest that all three \(^{18}\text{F}\)FDS analogs were similarly transported through the phloem.

### 3.3. The rate of \(^{18}\text{F}\)FDS transport in maize source leaf phloem

Since our results suggested that the three \(^{18}\text{F}\)FDS analogs were transported similarly through the phloem, we selected \(^{18}\text{F}\)FDS to study the rate of \(^{18}\text{F}\)FDS transport through the maize source leaf phloem. Activity from \(^{10}\text{F}\)FDS was visible in the phosphor image at the base of the wild-type leaves, but not the sut1 mutant leaves 15 min after application (Fig. 6A and Fig. 7). The faint amount of signal below the red arrow in the image of the sut1 mutant leaf in Fig. 6A is not statistically different from the background. We also observed that toward the tip of the leaves, the \(^{18}\text{F}\) signal was visible in lateral veins, which function in long-distance transport (Fritz et al., 1989). Toward the base of the wild-type leaves, most of the radiation was visible within the midvein, the largest vein in the leaf with the greatest transport capacity (Russell and Evert, 1985). By measuring the location of the radiation front in the leaves (red arrows in Fig. 6A and B), we calculated the minimum rate of \(^{18}\text{F}\)FDS transport as \(\nu = \frac{dx}{dt} = 0.90 \pm 0.15\) m.h\(^{-1}\) in the wild-type leaf blade, and \(0.65 \pm 0.25\) m.h\(^{-1}\) in the sut1 mutant leaf blade, where \(\nu\) is the rate of transport, \(dx\) is the distance of the front of \(^{18}\text{F}\)FDS transport from the tip after 15 min, and \(dt\) is the duration of transport. The quantitative data in Fig. 7 presents the transport of \(^{10}\text{F}\)FDS in sut1 mutant and wild-type leaves at three time points. The total amount of \(^{10}\text{F}\)FDS in the wild-type leaves was greatly reduced after 30 and 60 min, suggesting that the labeled sucrose was exported out of the base of the wild-type leaves (Fig. 6B and C). However, in the sut1 mutant leaves, most of the \(^{10}\text{F}\)FDS remained in the tip of the leaves after 60 min, suggesting that far less sucrose was exported. In addition, we observed the \(^{18}\text{F}\) activity distribution peak in the midrib was near the middle of the wild-type leaves after 15 min, while after 30 min the peak was located at the base of the wild-type leaves (these peaks are marked by white arrows in Fig. 6A and B).

### 4. Discussion

It has previously been shown that \(^{14}\text{C}\)-labeled 1'-FDS was taken up into soybean cotyledons and maize roots (Hitz et al., 1985), and that \(^{14}\text{C}\)-labeled 1'-FDS was transported into maize endosperm (Schmalstig and Hitz, 1987). In our previous study, we found that 6'-\(^{18}\text{F}\)FDS was efficiently transported in maize leaves (Rotsch
et al., 2015). However, to our knowledge, in vivo transport studies in plants using 1-[^18F]FDS or 6-[^18F]FDS have not been previously reported, although the radiosynthesis of 1-[^18F]FDS has been accomplished (Gifford et al., 2012). In this work, we found that 1-[^18F]FDS and 6-[^18F]FDS were readily taken up and transported in maize leaves. Moreover, [14C]-sucrose and the three tested [18F]FDS analogs showed similar transport patterns, with a significant reduction of sucrose transport in *sut1* mutant leaves as compared to wild-type leaves. These and previous results indicated that these [18F]FDS analogs and [14C]-sucrose utilize the same transport process for phloem entry (Lin et al., 1984; Schmitt et al., 1984; Hitz et al., 1985, 1986; Card et al., 1986; Schmalstig and Hitz, 1987; Hitz, 1988; Sun and Ward, 2012). As previously noted, ZmSUT1 is responsible for actively transporting sucrose into companion cells in maize leaves (Slewinski et al., 2009; Baker et al., 2016); therefore, it is very likely that these [18F]FDS molecules are bound and transported by ZmSUT1. In addition, we observed that both 1-[^18F]FDS and 6-[^18F]FDS were exported out of maize leaves, indicating that they are substrates for long-distance phloem transport, and thus, must be transported across the companion cell plasma membrane.

Studies using *Xenopus laevis* oocytes expressing ZmSUT1 suggested a biophysical model for sucrose and proton co-symport (Carpaneto et al., 2005, 2010; Geiger, 2011; Derrer et al., 2013). From biochemical experiments, Hitz et al. (1985, 1986) and Hitz (1988) proposed that the fructosyl moiety of sucrose participates in SUT binding as a hydrophobic surface. Replacement of the hydroxyl groups at the C-1’ and C-6’ positions within the fructosyl moiety by fluorine should increase the hydrophobicity of these analogs. Thus, FDS analogs with substitutions at these positions would be expected to have higher affinity to SUTs compared to sucrose and to compete with sucrose binding. The previous studies also suggested that the hydroxyl groups at the C-2, C-3, C-4, and C-6 positions within the glucosyl moiety are involved in SUT binding by donating their hydrogen atoms to SUT proteins for hydrogen bonding. Furthermore, replacement of the C-6 hydroxyl group with fluorine reduced the binding affinity of soybean cotyledon SUTs for 6-FDS compared to sucrose (Hitz et al., 1986; Hitz, 1988). Hence, we expected that 6-[^18F]FDS would be bound by ZmSUT1 at lower affinity and would not compete efficiently with sucrose. However, our results showed that relative to [14C]-sucrose, 6-[^18F]FDS, 6-[^18F]FDS, and 1-[^18F]FDS were all similarly transported in maize leaves. Therefore, replacement of hydroxyl groups at these three positions with ^18F appears to have no effect on transport by the ZmSUT1 protein. Given the previous observations about the importance of the hydroxyl group at the C-6 position within the glucosyl moiety for substrate binding, this was an unexpected finding.

One possible explanation for these results is that sucrose binding by ZmSUT1 occurs slightly differently than for the SUT protein(s) in soybean cotyledons. Kinetic studies showed that ZmSUT1 belongs to the high-affinity, low-capacity transporter group, which is proposed to function in phloem loading in source tissues (Carpaneto et al., 2005, 2010). However, Aldape et al. (2003) found that the soybean GmSUT1 protein progressively accumulated during cotyledon development, correlating with the increasing sucrose levels in maturing cotyledons, and suggested that GmSUT1 plays an active role in sucrose import into soybean developing cotyledons. Furthermore, kinetic studies showed that GmSUT1 belongs to the low-affinity, high-capacity sucrose transporter group (Aldape et al., 2003). Additionally, based on phylogenetic analyses, SUTs in angiosperms can be classified into five clades or groups (Braun and Slewinski, 2009). Soybean GmSUT1 is a member of the group 2 SUTs, which are unique to eudicot plants, whereas maize ZmSUT1 is a member of the group 1 SUTs, which are only found in monocot plants (Aoki et al., 1999, 2003; Aldape et al., 2003; Braun and Slewinski, 2009; Reinders et al., 2012). Group 1 and group 2 SUT proteins are distantly related and typically share ~40% amino acid similarity, although the five amino acids identified in OsSUT1 that are critical for sucrose transport are conserved in ZmSUT1 and GmSUT1 (Sun et al., 2012). In addition, differences in substrate specificity between group 1 and group 2 SUTs have been reported (Reinders et al., 2012; Knoblauch et al., 2015). Thus, it is possible that the hydrogen group at the C-6 position within the glucosyl moiety is essential for substrate recognition by GmSUT1 but not for ZmSUT1, which could account for our 6-[^18F]FDS transport data.

Although [18F]FDS had a similar transport pattern to [14C]-sucrose in maize leaves, the signal detected from [18F]FDS analogs was always consistently stronger than that from [14C]-sucrose, and it more clearly defined that transport occurred in the veins. An explanation lies in the much higher energy of the positron from ^18F decay vs the beta particle from ^14C. As a result, the counting/imaging system is much more sensitive to ^18F than ^14C. Therefore, [18F]FDS analogs give a stronger radioactive decay signal and require significantly less exposure/counting time, which affords experimental advantages. In addition, enzymatic studies showed that FDS

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**Table 1**

<table>
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<th>Factors</th>
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**Fig. 5.** Comparing transport of different [^{18}F]FDS analogs. Graphs represent averages of [^{18}F]FDS/[^{14}C]-sucrose measurements and error bars represent the SE. A. [^{18}F]FDS/[^{14}C]-sucrose ratio in wild-type leaves. B. [^{18}F]FDS/[^{14}C]-sucrose ratio in *sut1* mutant leaves.
was a poor substrate for invertase activity (Hitz et al., 1985), suggesting that substantially less [$^{18}$F]FDS would enter cellular metabolism compared to [$^{14}$C]-sucrose, which is a substrate of the enzyme. Overall, our results indicated that [$^{18}$F]FDS analogs could be useful tools to elucidate in vivo sucrose transport in intact plants (Leach et al., 2017).

To investigate the rate of transport of [$^{18}$F]FDS in maize leaves, we introduced 1′-[${^{18}$F}]FDS into sut1 mutant and wild-type leaves, and monitored transport after 15, 30, or 60 min. We estimated the minimum rate of 1′-[${^{18}$F}]FDS transport at approximately $0.90 \pm 0.15$ m.h$^{-1}$ in the wild-type leaf and $0.65 \pm 0.25$ m.h$^{-1}$ in the sut1 mutant leaf. Quantitative analysis also showed that the

Fig. 6. Transport of 1′-[${^{18}$F}]FDS in wild-type and sut1 mutant leaves after 15 min (A), 30 min (B), and 60 min (C). Red arrows indicate the detectable front of the ${^{18}$F} radioactivity. White arrows indicate the peak of ${^{18}$F} distribution. Scale bar = 2.54 cm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Fig. 7. Distribution of 1′-[${^{18}$F}]FDS in wild-type (WT) (A) and sut1 mutant (B) leaves after 15, 30, and 60 min of transport. ${^{18}$F} transport was measured by image intensity (in arbitrary units, au) at different distances (in cm) from the application site. Different symbols (circle, 15 min; triangle, 30 min; x, 60 min) represent the intensity averages for each experiment at each distance from the application site, and the error bars represent the SE.
amount of 1'\textsuperscript{[18F]}FDS in the wild-type leaf was greatly reduced after 30 and 60 min, suggesting that the labeled sucrose was exported out of the wild-type leaf. However, in the mutant leaf, most of the 1'\textsuperscript{[18F]}FDS remained in the tip of the leaf after 60 min, suggesting that far less sucrose was exported from the sug1 mutant leaf. According to the Münch model for phloem transport, the flow of solution through the sieve tubes is driven by a hydrostastic pressure difference between the source phloem and sink phloem. A high pressure is maintained in the collection phloem by an influx of water from the active uptake of sucrose, which is mainly accomplished by ZmSUT1 in maize. As previously mentioned, loss-of-function of ZmSUT1 results in reduced sucrose loading into the companion cells, which is expected to reduce the hydrostastic pressure in the sug1 mutant phloem, and consequently, result in a slower rate of transport. As predicted, the 1'\textsuperscript{[18F]}FDS transport data showed that the rate of sucrose transport in the phloem of the sug1 mutant leaf was slower compared to the wild-type leaf. Interestingly, we observed limited radiotracer transport in sug1 mutant leaves. There are seven Sut genes in the maize genome (Braun et al., 2014). Hence, it is possible that another Sut gene has partial genetic redundancy with Sut1 and is able to catalyze sucrose phloem loading from the apoplasm, although it is unlikely to be the Sut2 gene (Leach et al., 2017). Alternatively, the phloem compartment cell-surface element complex in maize leaves is not entirely symplasmically isolated from surrounding cells (Evett et al., 1978), so it is possible that some sucrose can enter the phloem in the sug1 mutant leaf via plasmodesmata. Further work is necessary to investigate these possibilities.

Several previous studies have measured the rate of sucrose transport in the phloem of different plants. In one of the earliest reports 60 years ago, Bidulph and Cory (1957) calculated the velocity of \textsuperscript{14}C-sucrose translocated in red kidney bean (Phaseolus vulgaris) stems at 0.79 m-h\textsuperscript{-1}. Hofstra and Nelson (1969) estimated the transport rate of \textsuperscript{14}C-sucrose in a mature maize leaf at 1.5 m\textsuperscript{-1}, and they suggested that the transport rate changed depending on the temperature. Later, Troughton et al. (1977) used \textsuperscript{14}C\textsubscript{2}CO\textsubscript{2} and reported a wide range of transport rates in maize leaves from 0.15 to 6.6 m\textsuperscript{-1}, depending on the light conditions. In Sorghum bicolor, photosynthetically assimilated \textsuperscript{14}C\textsubscript{2}CO\textsubscript{2} fed to a mature leaf was transported at a rate of 1.5–2.0 m\textsuperscript{-1}; interestingly, the authors also found that the sucrose rate was different in leaf, stem, and root tissues (Karve et al., 2015). Knoblauch et al. (2016) estimated the translocation rate of photosynthetically assimilated \textsuperscript{14}C\textsubscript{2}CO\textsubscript{2} in the morning glory (Ipomoea purpurea) phloem at 0.44 m\textsuperscript{-1}. Clearly, the transport rate depends on the plant, its age, the experimental growth conditions, etc., but our measurements of 1'\textsuperscript{[18F]}FDS phloem transport speeds in a maize leaf are in good agreement with previous studies.

In summary, this work has revealed that the replacement of hydroxyl groups of sucrose with F at the C-1' and C-6' positions within the fructosylo moiety and at C-6 within the glucosylo moiety does not significantly affect binding and recognition by ZmSUT1. This contrasts with earlier findings on the importance of the C-6 hydroxyl group for binding and transport by soybean cotyledon SUTs. Additionally, we measured the velocity for 1'\textsuperscript{[18F]}FDS transport in the phloem of maize source leaves at approximately 0.90 ± 0.15 m-h\textsuperscript{-1} in the wild-type leaf and 0.65 ± 0.25 m-h\textsuperscript{-1} in the sug1 mutant leaf. We demonstrated that these \textsuperscript{[18F]}FDS analogs can be used as radiotracers in plants to visualize sucrose transport. Future research will combine these \textsuperscript{[18F]}FDS tracers with dynamic radiotracer imaging techniques, especially PET. This will enable investigations of photoassimilate transport and carbohydrate partitioning in living plants in real time under different abiotic or biotic stress conditions.
Phloem-localized, proton-coupled sucrose carrier ZmSUT1 mediates sucrose efflux under the control of the sucrose gradient and the proton motive force. J. Biol. Chem. 286, 21347–21355.


