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

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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 fluorine-18 [^{18}F] 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 ^{18}F 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'-[^{18}F]fluoro-1'-deoxysucrose and 6-[^{18}F]fluoro-6-deoxysucrose ([^{18}F]FDS) analogs. Each [^{18}F]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-) [^{18}F]FDS derivatives were efficiently and equally translocated, similarly to carbon-14 [^{14}C]-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. [^{18}F]FDS imaging afforded several advantages compared to [^{14}C]-sucrose detection. We calculated that 1'-[^{18}F]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 [^{18}F]FDS analogs are valuable tools to probe sucrose-SUT interactions and to monitor sucrose transport in plants.

Keywords: fluorine-18, fluorodeoxysucrose, maize, phloem, sucrose, transport, ZmSUT1

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; Slewinski and Braun, 2010a). In sink tissues, sucrose is hydrolyzed by invertase 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 Slewinski, 2009). In apoplastic phloem loading species, sucrose is thought to be effluxed to the apoplast (cell wall) and actively transported into the companion cell-sieve element complex (Evert et al., 1978; Baker et al., 2012; Braun, 2012; Chen et al., 2012; Eom et al., 2015). Physiological, genetic, and biochemical evidence showed that the maize SUCROSE 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; Slewinski et al., 2010).

Numerous studies have investigated the substrate-binding specificities and transport mechanism of sucrose transporter (SUT) proteins (Hitz et al., 1985; Card et al., 1986; Hitz 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; Sun et al., 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; Derrer 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 [^{14}C]-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 fluorodeoxysucrose (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, ^{14}C -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', 6'-trideoxy-galactosucrose) 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; Derrer 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 ^{14}C -labeled [methyl- ^{14}C](4'-methoxyphenyl) α -D-glucopyranoside 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- ^{14}C]2-deoxy (4'-methoxyphenyl) α -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 (Turgeon, 2010; Knoblauch et al., 2016). Further, despite the importance of the phloem transport process, anatomical constraints have imposed challenges to its investigation. In particular, phloem tissue is physically inaccessible because it is embedded within the veins, which are in turn located in the middle layers of leaves and other organs (Turgeon and Wolf, 2009). Therefore, a method to visualize phloem transport without disturbing the structure of the phloem in intact plants could provide a great advantage in understanding the mechanism of sucrose transport and the regulation of carbohydrate partitioning (Grignon et al., 1989; Slewinski et al., 2012; Bihmidine et al., 2015; Knoblauch et al., 2015).

Long half-life radioisotopes, such as ^{14}C (half-life = 5730 years), have been applied to the study of sugar transport through the phloem. However, they require destructive sampling to visualize the radiation through autoradiography or to quantify its abundance via liquid scintillation counting (Webb and Gorham, 1964; Geiger et al., 1969; Hofstra and Nelson, 1969; Fisher, 1970; Margolis et al., 1991). To overcome some of the limitations associated with long half-life isotopes, short-lived, positron-emitting isotopes (e.g., carbon-11 [^{11}C], fluorine-18 [^{18}F]) have been used in non-invasive imaging studies 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. In addition, the combination of positron emission tomography (PET) and a positron-emitting isotope permits the real-time dynamics of sugar transport to be visualized (Fiorani and Schurr, 2013; Lee et al., 2013; Partelová et al., 2014; Fraas and Lüthen, 2015; Hubeau and Steppe, 2015; Karve et al., 2015). Previously, [^{11}C]-labeling has been used to study phloem transport and the effects of different stresses on translocation (Moorby et al., 1963; Troughton et al., 1977; Thorpe et al., 1998; Minchin and Thorpe, 2003; Matsuhashi et al., 2006; Thorpe et al., 2007; Suwa et al., 2008; Kawachi et al., 2011; Karve et al., 2015). ^{11}C is often used in the form of [^{11}C] CO_2 , a substrate of photosynthesis, which is directly incorporated into photoassimilates, such as sucrose. However, ^{11}C has a short half-life of 20.3 min, potentially limiting its utility; thus, ^{11}C is only suitable for short time-frame experiments. Over the past few years, a growing number of studies have used ^{18}F -labeled sugars (^{18}F 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). Initial studies of phloem transport in plants used 2-[^{18}F]fluoro-2-deoxy-D-glucose ([^{18}F]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). Hence, this tracer may not adequately reflect in vivo sugar transport (but see (Fatangare and Svatoš, 2016)). Therefore, using a ^{18}F -labeled sucrose molecule may overcome these potential disadvantages of [^{11}C] CO_2 or [^{18}F]FDG.

In a previous study, we synthesized a new radioactive tracer 6'-[^{18}F]fluoro-6'-deoxysucrose (6'-[^{18}F]FDS) to monitor sucrose transport in maize leaves. We showed that 6'-[^{18}F]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 [^{18}F]-fluorinated deoxysucrose ([^{18}F]FDS) analogs (1'-[^{18}F]fluoro-1'-deoxysucrose (1'-[^{18}F]FDS) and 6-[^{18}F]fluoro-6-deoxysucrose (6-[^{18}F]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 moiety, respectively. We found that these two [^{18}F]FDS were efficiently transported through the leaf blades. Furthermore, and somewhat surprisingly, all three [^{18}F]FDS analogs were

transported similarly to each other and to [^{14}C]-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'-[^{18}F]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 $\mu\text{mol.m}^{-2}.\text{s}^{-1}$ 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 (Dyma Green, Hummert International, MO, USA).

All plants used in experiments were 3 to 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, they were moved into the growth chamber at least 48 hours before an experiment. The plants were maintained in this growth chamber before and during the transport assay, under a mixture of incandescent and fluorescent lights (400 $\mu\text{mol.m}^{-2}.\text{s}^{-1}$ at the canopy height), with a 14-h-day (32°C)/10-h-night (25°C), and 75% relative humidity.

2.2 Genetic materials, genotyping, and phenotyping

The *zmsut1-m1* (abbreviated *sut1*) mutant allele has been described (Slewinski et al., 2009). The *sut1* mutants were backcrossed five generations into the inbred B73, and heterozygous F5 plants were self-pollinated to create segregating F6 families, which were used for the experiments. Plants carrying the *sut1* allele were identified by PCR genotyping using insertion-deletion polymorphism primer set IDP8570, forward 5'-CGCTAAGCTCGTCCTTCTCC-3' and reverse 5'-GGTTTGTGATCTTTGTGTCACC-3'. PCR conditions were 95°C for 30s, 60°C for 45s, and 72°C for 1 min for 40 cycles. PCR products were separated on a 2% agarose gel and stained with SYBR safe DNA gel stain (ThermoFisher Scientific, MA, USA). We used homozygous mutant and wild-type plants for radioactive tracers transport assays.

All mutant and wild-type plants used for labeling experiments were also confirmed by phenotyping. The *sut1* mutant leaves displayed a chlorotic leaf phenotype. In addition, the hydathodes of *sut1* mutant leaves exuded high amounts of sucrose in the guttation fluid, which dried and formed sucrose droplets on the margin of the leaves (Slewinski et al., 2010). Wild-type or other characterized carbohydrate hyperaccumulating maize mutant leaves do not exhibit this phenotype (Baker and Braun, 2007; Baker and Braun, 2008; Ma et al., 2008; Slewinski and Braun, 2010b).

2.3 Radiosynthesis of [^{18}F]FDS analogs

[^{18}F]FDS analogs were individually fluorinated with ^{18}F at the C-1' or C-6' positions within the fructosyl moiety, or the C-6 position within the glucosyl moiety. The structures of three analogs are shown in Figure 1.

^{18}F was produced by the nuclear reaction $^{18}\text{O}(\text{p},\text{n})^{18}\text{F}$ using a GE PET Trace cyclotron as described (Rotsch et al., 2015). The precursors (6'-O-Trifluoromethanesulfonyl-2,3,4,6,1',3',4'-hepta-O-benzoylsucrose for 6'-[^{18}F]FDS; 1'-O-Trifluoromethanesulfonyl-2,3,4,6,3',4',6'-hepta-O-benzoylsucrose for 1'-[^{18}F]FDS; and 6-O-Trifluoromethanesulfonyl-2,3,4,1',3',4',6'-hepta-O-benzoylsucrose for 6-[^{18}F]FDS) were prepared as described (Gaddam and Harmata, 2013; Ying et al., 2013; Gao et al., 2014).

The non-enzymatic and automated radiosynthesis of the three [^{18}F]FDS analogs was performed as described (Rotsch et al., 2015) using a dual reactor synthesis Modular-Lab system (Eckert & Zielger, 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 [^{18}F]FDS analog was resuspended in water.

2.4 [^{18}F]FDS and [^{14}C]-sucrose transport assays

[^{18}F]FDS analogs and universally labeled [^{14}C]-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 [^{18}F]FDS and 556.52 Bq [^{14}C]-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 ^{18}F . The next day, the same labeled leaf was exposed to a phosphor plate for 5 days to image the ^{14}C . For the 1'-[^{18}F]FDS transport experiment, n=11 wild-type plants and n= 12 *sut1* mutant plants were assayed. For the 6'-[^{18}F]FDS transport experiment, n=9 wild-type plants and n=9 *sut1* mutant plants were assayed. For the 6-[^{18}F]FDS transport experiment, n=18 wild-type plants and n=20 *sut1* mutant plants were assayed. The phosphor plate was scanned with a GE Typhon 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 [^{18}F]FDS transport

To calculate the rate of sucrose transport, 1'-[^{18}F]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 1'-[^{18}F]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 [^{18}F]FDS analog and [^{14}C]-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 [^{18}F]FDS analog was normalized by the intensity of [^{14}C]-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'-[^{18}F]FDS and 6-[^{18}F]FDS were efficiently translocated in maize leaves

To test if 1'-[^{18}F]FDS and 6-[^{18}F]FDS would be transported in vivo, these two analogs and 6'-[^{18}F]FDS were independently introduced into leaves of wild-type plants and *sut1* mutant plants that lacked *ZmSut1* function (Figures 2 and 3). We also introduced the universally labeled [^{14}C]-sucrose tracer along with each [^{18}F]FDS analog as a positive control to test whether these analogs would function as substrates of ZmSUT1 for phloem transport. One hour after [^{18}F]FDS analogs were applied to the tip of the leaf, the ^{18}F radioactivity signal was detected at the base of wild-type leaves but not *sut1* mutant leaves (Fig. 3A-C). Transport of the three [^{18}F]FDS analogs was visibly reduced in *sut1* mutant leaves compared to wild-type leaves. The transport patterns of these three [^{18}F]FDS analogs were similar to the [^{14}C]-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 [^{18}F]FDS analogs better defined the veins than [^{14}C]-sucrose (Fig. 3).

3.2 All three [^{18}F]FDS analogs show comparable transport

We compared the [^{18}F]FDS/[^{14}C]-sucrose normalized transport values of the three [^{18}F]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}F]FDS analogs and their distance transported from the application site. The effect of the different [^{18}F]FDS analogs on transport was not significant ($p = 0.661$) (Table 1). Therefore, these data suggest that all three [^{18}F]FDS analogs were similarly transported through the phloem.

Table 1. Statistical analysis of transport of three different [^{18}F]FDS analogs. A mixed-model ANOVA was used to examine the effects of analog and distance from application site to the [^{18}F]FDS/[^{14}C]-sucrose intensity.

Factors	wild type		<i>sut1</i> mutant	
	F-value	p-value	F-value	p-value
Analog	0.49	0.611	1.06	0.3493
Distance from application site	43.19	<.0001	7.82	<.0001
Analog x Distance from application site	2.05	0.0105	0.85	0.6274

3.3 The rate of [^{18}F]FDS transport in maize source leaf phloem

Since our results suggested that the three [^{18}F]FDS analogs were transported similarly through the phloem, we selected 1'-[^{18}F]FDS to study the rate of [^{18}F]FDS transport through the maize source leaf phloem. Activity from 1'-[^{18}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 Figure 6A is not statistically different from the background. We also observed that toward the tip of the leaves, the ^{18}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 Figure 6A, B), we calculated the minimum rate of 1'-[^{18}F]FDS transport as $v = dx/dt = 0.90 \pm 0.15 \text{ m.h}^{-1}$ in the wild-type leaf blade, and $0.65 \pm 0.25 \text{ m.h}^{-1}$ in the *sut1* mutant leaf blade, where v is the rate

of transport, dx is the distance of the front of 1'-[^{18}F]FDS transport from the tip after 15 min, and dt is the duration of transport. The quantitative data in Figure 7 presents the transport of 1'-[^{18}F]FDS in *sut1* mutant and wild-type leaves at three time points. The total amount of 1'-[^{18}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, C). However, in the *sut1* mutant leaves, most of the 1'-[^{18}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}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}C -labeled 1'-FDS was taken up into soybean cotyledons and maize roots (Hitz et al., 1985), and that ^{14}C -labeled 1'-FDS was transported into maize endosperm (Schmalstig and Hitz, 1987). In our previous study, we found that 6'-[^{18}F]FDS was efficiently transported in maize leaves (Rotsch et al., 2015). However, to our knowledge, in vivo transport studies in plants using 1'-[^{18}F]FDS or 6'-[^{18}F]FDS have not been previously reported, although the radiosynthesis of 1'-[^{18}F]FDS has been accomplished (Gifford et al., 2012). In this work, we found that 1'-[^{18}F]FDS and 6'-[^{18}F]FDS were readily taken up and transported in maize leaves. Moreover, [^{14}C]-sucrose and the three tested ^{18}F 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 [^{18}F]FDS analogs and [^{14}C]-sucrose utilize the same transport process for phloem entry (Lin et al., 1984; Schmitt et al., 1984; Hitz et al., 1985; Card et al., 1986; Hitz 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 [^{18}F]FDS molecules are bound and transported by ZmSUT1. In addition, we observed that both 1'-[^{18}F]FDS and 6'-[^{18}F]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; Carpaneto et al., 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- ^{18}F FDS would be bound by ZmSUT1 at lower affinity and would not compete efficiently with sucrose. However, our results showed that relative to ^{14}C -sucrose, 6- ^{18}F FDS, 6'- ^{18}F FDS, and 1'- ^{18}F FDS were all similarly transported in maize leaves. Therefore, replacement of hydroxyl groups at these three positions with ^{18}F 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; Carpaneto et al., 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; Aldape et al., 2003; Aoki 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- ^{18}F FDS transport data.

Although ^{18}F FDS had a similar transport pattern to ^{14}C -sucrose in maize leaves, the signal detected from ^{18}F FDS analogs was always consistently stronger than that from ^{14}C -sucrose, and it more clearly defined that transport occurred in the veins. An explanation lies in the much higher energy of the positron from ^{18}F decay vs the beta particle from ^{14}C . As a result, the counting/imaging system is much more sensitive to ^{18}F than ^{14}C . Therefore, ^{18}F 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 was a poor substrate for invertase activity (Hitz et al., 1985), suggesting that substantially less ^{18}F FDS would enter cellular metabolism through hydrolysis 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 ± 0.15 m.h⁻¹ in the wild-type leaf and 0.65 ± 0.25 m.h⁻¹ in the *sut1* mutant leaf. Quantitative analysis also showed that the amount of 1'- ^{18}F 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'- ^{18}F FDS remained in the tip of the leaf after 60 min, suggesting that far less sucrose was exported from the *sut1* mutant leaf. According to the Münch model for phloem transport, the

flow of solution through the sieve tubes is driven by a hydrostatic 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 hydrostatic pressure in the *sut1* mutant phloem, and consequently, result in a slower rate of transport. As predicted, the 1'-[¹⁸F]FDS transport data showed that the rate of sucrose transport in the phloem of the *sut1* mutant leaf was slower compared to the wild-type leaf. Interestingly, we observed limited radiotracer transport in *sut1* 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 companion cell-sieve element complex in maize leaves is not entirely symplasmically isolated from surrounding cells (Evert et al., 1978), so it is possible that some sucrose can enter the phloem in the *sut1* mutant 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, Biddulph and Cory (1957) calculated the velocity of [¹⁴C]-sucrose translocated in red kidney bean (*Phaseolus vulgaris*) stems at 0.79 m.h⁻¹. Hofstra and Nelson (1969) estimated the transport rate of [¹⁴C]-sucrose in a mature maize leaf at 1.5 m.h⁻¹, and they suggested that the transport rate changed depending on the temperature. Later, Troughton et al. (1977) used [¹¹C]CO₂ and reported a wide range of transport rates in maize leaves from 0.15 to 6.6 m.h⁻¹, depending on the light conditions. In *Sorghum bicolor*, photosynthetically assimilated [¹¹C]CO₂ fed to a mature leaf was transported at a rate of 1.5 to 2.0 m.h⁻¹; interestingly, the authors also found that the transport rate was different in leaf, stem, and root tissues (Karve et al., 2015). Knoblauch et al. (2016) estimated the translocation rate of photosynthetically assimilated [¹¹C]CO₂ in the morning glory (*Ipomoea purpurea*) phloem at 0.44 m.h⁻¹. Clearly, the transport rate depends on the plant, its age, the experimental growth conditions, etc., but our measurements of 1'-[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 fructosyl moiety and at C-6 within the glucosyl 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'-[¹⁸F]FDS transport in the phloem of maize source leaves at approximately 0.90 ± 0.15 m.h⁻¹ in the wild-type leaf and 0.65 ± 0.25 m.h⁻¹ in the *sut1* mutant leaf. We demonstrated that these [¹⁸F]FDS analogs can be used as radiotracers in plants to visualize sucrose transport. Future research will combine these [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.

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Figure legends

Figure 1. Structures of three [^{18}F]FDS analogs.

- A. 1'-Deoxy-1'-[^{18}F]-Fluorosucrose (1'-[^{18}F]FDS).
- B. 6'-Deoxy-6'-[^{18}F]-Fluorosucrose (6'-[^{18}F]FDS).
- C. 6-Deoxy-6-[^{18}F]-Fluorosucrose (6-[^{18}F]FDS).

Figure 2. [^{18}F]FDS and [^{14}C]-sucrose transport assay.

- A. A solution of [^{18}F]FDS and [^{14}C]-sucrose was applied to the cut tip of the mature leaf 5 or 6 of 3-week-old plants for 3 minutes. Black arrow indicates where the leaf tip was dipped into the solution.
- B. Maize plants were left to transport assimilates for 15, 30, or 60 min in a growth chamber.
- C. The leaf was excised at 25.08 cm from the leaf tip and exposed to phosphor imaging plate. Photograph of maize leaves (left) and corresponding autoradiography (right) showing the distribution of ^{18}F in the leaves. ^{18}F phosphor images were obtained after one hour exposure. Black coloration indicates low radioactivity with increasing radioactivity shown in yellow and red. Bar = 2.54 cm.

Figure 3. Transport of [^{18}F]FDS and [^{14}C]-sucrose in wild-type (WT) and *sut1* mutant leaves after one hour.

- A, D. Autoradiographs of leaves showing 1'-[^{18}F]FDS (A) and [^{14}C]-sucrose (D) transport in the 1'-[^{18}F]FDS translocation experiment.
 - B, E. Autoradiographs of leaves showing 6'-[^{18}F]FDS (B) and [^{14}C]-sucrose (E) transport in the 6'-[^{18}F]FDS translocation experiment.
 - C, F. Autoradiographs of leaves showing 6-[^{18}F]FDS (C) and [^{14}C]-sucrose (F) transport in the 6-[^{18}F]FDS translocation experiment.
- Scale bars = 2.54 cm.

Figure 4. Distribution of translocated [^{18}F]FDS and [^{14}C]-sucrose in wild-type (WT) and *sut1* mutant leaves. Translocation was measured by the signal intensity at each distance (in cm) from the application site, and was expressed as a percentage of intensity at each distance over the total

intensity in the leaf. The graphs represent transport averages and the error bars represent the SE.

* indicates statistically different at $p \leq 0.05$ using Student's *t* test.

A, B. Quantification of 1'-[^{18}F]FDS and [^{14}C]-sucrose transport in the 1'-[^{18}F]FDS transport experiment.

C, D. Quantification of 6'-[^{18}F]FDS and [^{14}C]-sucrose transport in the 6'-[^{18}F]FDS transport experiment.

E, F. Quantification of 6-[^{18}F]FDS and [^{14}C]-sucrose transport in the 6-[^{18}F]FDS transport experiment.

Figure 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.

Figure 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.

Figure 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.

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Figure 1. Structures of three [^{18}F]FDS analogs.

A. 1'-Deoxy-1' [^{18}F]-Fluorosucrose (1'-[^{18}F]FDS).

B. 6'-Deoxy-6' [^{18}F]-Fluorosucrose (6'-[^{18}F]FDS).

C. 6-Deoxy-6 [^{18}F]-Fluorosucrose (6-[^{18}F]FDS).

Figure 2. [^{18}F]FDS and [^{14}C]-sucrose transport assay.

A. A solution of [^{18}F]FDS and [^{14}C]-sucrose was applied to the cut tip of the mature leaf 5 or 6 of 3-week-old plants for 3 minutes. Black arrow indicates where the leaf tip was dipped into the solution.

B. Maize plants were left to transport assimilates for 15, 30, or 60 min in a growth chamber.

C. The leaf was excised at 25.08 cm from the leaf tip and exposed to phosphor imaging plate. Photograph of maize leaves (left) and corresponding autoradiography (right) showing the distribution of ^{18}F in the leaves. ^{18}F phosphor images were obtained after one hour exposure. Black coloration indicates low radioactivity with increasing radioactivity shown in yellow and red. Bar = 2.54 cm.

Figure 3. Transport of $[^{18}\text{F}]$ FDS and $[^{14}\text{C}]$ -sucrose in wild-type (WT) and *sut1* mutant leaves after one hour.

A, D. Autoradiographs of leaves showing $1'-[^{18}\text{F}]$ FDS (A) and $[^{14}\text{C}]$ -sucrose (D) transport in the $1'-[^{18}\text{F}]$ FDS translocation experiment.

B, E. Autoradiographs of leaves showing $6'-[^{18}\text{F}]$ FDS (B) and $[^{14}\text{C}]$ -sucrose (E) transport in the $6'-[^{18}\text{F}]$ FDS translocation experiment.

C, F. Autoradiographs of leaves showing $6-[^{18}\text{F}]$ FDS (C) and $[^{14}\text{C}]$ -sucrose (F) transport in the $6-[^{18}\text{F}]$ FDS translocation experiment.

Scale bars = 2.54 cm.

Figure 4. Distribution of translocated $[^{18}\text{F}]\text{FDS}$ and $[^{14}\text{C}]\text{-sucrose}$ in wild-type (WT) and *sut1* mutant leaves. Translocation was measured by the signal intensity at each distance (in cm) from the application site, and was expressed as a percentage of intensity at each distance over the total intensity in the leaf. The graphs represent transport averages and the error bars represent the SE. * indicates statistically different at $p \leq 0.05$ using Student's *t* test.

A, B. Quantification of $1'-[^{18}\text{F}]\text{FDS}$ and $[^{14}\text{C}]\text{-sucrose}$ transport in the $1'-[^{18}\text{F}]\text{FDS}$ transport experiment.
 C, D. Quantification of $6'-[^{18}\text{F}]\text{FDS}$ and $[^{14}\text{C}]\text{-sucrose}$ transport in the $6'-[^{18}\text{F}]\text{FDS}$ transport experiment.
 E, F. Quantification of $6-[^{18}\text{F}]\text{FDS}$ and $[^{14}\text{C}]\text{-sucrose}$ transport in the $6-[^{18}\text{F}]\text{FDS}$ transport experiment.

Figure 5. Comparing transport of different $[^{18}\text{F}]\text{FDS}$ analogs. Graphs represent averages of $[^{18}\text{F}]\text{FDS}/[^{14}\text{C}]\text{-sucrose}$ measurements and error bars represent the SE.

A. $[^{18}\text{F}]\text{FDS}/[^{14}\text{C}]\text{-sucrose}$ ratio in wild-type leaves.

B. $[^{18}\text{F}]\text{FDS}/[^{14}\text{C}]\text{-sucrose}$ ratio in *sut1* mutant leaves.

Figure 6. Transport of $1'-[^{18}\text{F}]\text{FDS}$ 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.

Figure 7. Distribution of $1^{\prime}$ - $[^{18}\text{F}]\text{FDS}$ 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.