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Sugar Uptake in Lily Pollen[1, 2]

A PROTON SYMPORT

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ABSTRACT

The data presented here are consistent with a proton-sugar co-transport in germinated pollen of *Lilium longiflorum* Thunb. Optimal uptake occurs at pH 5.0. A K_m of 1.7 to 1.8 millimolar is obtained from the initial rate of pH change induced by sucrose uptake as well as from uptake of [U-^{14}C]-sucrose. The energy of activation is −11 kilocalories mole^{-1}. The effect of several inhibitors and sugar competitors on [U-^{14}C]sucrose and D-[U-^{14}C] glucose uptake is given. The possibility of hydrolysis of sucrose prior to its transport into the pollen tube has been considered and reasons for choosing a sucrose-type uptake are presented. The possible *in vivo* significance of this co-transport process during pollen germination is discussed. Germinated pollen has features to recommend it as an experimental system of choice for studies of sugar uptake.

Proton co-transport of sugar as first described in *Chlorella* (15) is now known to occur in higher plants and is probably a process found in all species. The literature has been reviewed (2). In recent studies, proton co-transport has been implicated in pea-stem segments (4, 5), maize coleoptiles and roots (4), intact duckweed (18, 20), tomato internode segments and discs (21, 22), sugar-beet leaves (9), and isolated protoplasts and vacuoles from pea-leaf mesophyll cells (10, 11). Use of highly differentiated tissue, such as *Ricinus* cotyledons (12, 13), creates certain difficulties in comparing the relationship between proton motive force and sugar uptake. Here, we show that germinated lily pollen, a relatively simple experimental system, also exhibits sugar uptake by a proton symport.

MATERIALS AND METHODS

Pollen Germination. Pollen was harvested during July, 1978, from field-grown *Lilium longiflorum* Thunb. cv. Nellie White and stored at −22 C (3). Pollen was germinated at 28 C in a medium containing 0.29 M pentaerythritol, 1.27 mM $Ca(NO_3)_2$, 0.99 mM KNO_3, and 0.167 mM H_3BO_3 (7). Germinated pollen was resuspended in a test medium containing 0.29 mM pentaerythritol and 2.54 mM $Ca(NO_3)_2$ for pH and uptake studies. Pollenkitt, a gummy residue representing about 12% of the fresh weight of pollen, was removed from the pollen suspension with a glass rod prior to each measurement. Values of fresh weight reported here include pollenkitt. Following germination, pollen was filtered with gentle suction on a 0.8-μm Millipore or Sartorium membrane, washed with test medium, and gently resuspended in test medium. Large quantities of germinated pollen were allowed to settle for 5 min in a tall glass cylinder in order to decant about 0.5 volume prior to filtering. In some studies, resuspended pollen was held for periods up to 2 h at 20 C without impairment of cytoplasmic streaming and with little additional tube elongation (see "Results" and Table II).

Measurements. In pH studies, germinated pollen (usually 50 mg) was resuspended in 3.5 ml test medium at 28 C in a thermostated titration vessel (Metrohm, Herisau, Switzerland). A fine stream of air bubbles kept the pollen in suspension. pH changes were measured with a combination glass electrode (Radiometer GK2321-C) and were recorded with a 1-mv Varian A-5 recorder. The initial rate of proton uptake was calculated from the difference in slope of the pH curve before and after addition of sugar to the medium.

In uptake studies involving labeled sucrose, 1 ml pollen suspension was added to 1 ml 100 mM Mes/Ca buffer at pH 5.0 [Mes buffer was adjusted to the indicated pH with $Ca(OH)_2$] and held for 2 min to attain 28 C. One-half ml sugar analog, inhibitor, or test medium was added and, 0.5 min later, 0.5 ml 6 mM [U-^{14}C]sucrose was added. The final concentration of sucrose was 1 mM. Addition of label was taken as zero time. After incubation, uptake was terminated by filtering 1-ml aliquots on 0.8-μm membranes and washing the pollen with 3 ml test medium. The washed pollen and its membrane support were transferred to 5 ml "tritosol" (8) and counted in a Packard model 3320 liquid scintillation spectrometer at a ^{14}C efficiency of 70%.

To characterize the nature of labeled products of [U-^{14}C]sucrose uptake after transport into the pollen tube, a sample composed of 560 mg pollen was germinated for 1.5 h and, by established procedure, resuspended in 10 ml test medium. To this was added 5 ml 100 mM Mes/Ca buffer (pH 5.05) and 5 ml 6 mM [U-^{14}C] sucrose (5 μCi). After incubating the suspension for 2.5 min at 28 C, the pollen was filtered, washed with 30 ml test medium, resuspended in 9 ml 90% ethanol at 80 C, and homogenized. Residues were recovered by centrifugation for 15 min at 8,000*g*, re-extracted for 3 min at 80 C in 10 ml 90% ethanol, sonicated with two 30-s bursts in an ultrasonic bath, and centrifuged. Labeled sugars thus extracted were analyzed by TLC and high-performance liquid chromatography. Separation by TLC was done in methyl ethyl ketone/glacial acetic acid/2-propanol/H_2O (2:1:1:2, v/v) on cellulose. Radioactive regions were first scanned and then quantitatively determined by removing the cellulose

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powder in bands and counting the bands individually in liquid scintillation vials. Samples to be separated by high-performance liquid chromatography were first partially purified by absorption from water on a 1- × 5-cm column of acid-washed Norit A charcoal. Sugars were eluted in 10% ethanol, diluted with a mixture of sucrose, glucose, and fructose as carrier, and separated on a μBonapak carbohydrate column (Waters Assoc., Milford, MA) with acetonitrile-H_2O (9:1, v/v) at a flow rate of 1 ml min^{-1}. Sugars were detected by the change in refractive index, identified by comparison with authentic standards, and analyzed by ^{14}C content.

RESULTS AND DISCUSSION

pH Studies. As seen in Figure 1, addition of sucrose to a suspension of germinated pollen in test medium at 28 C initiated a rapid rise in the pH of the medium. The pH of the unbuffered medium at the time that sucrose was added varied from 5.2 to 6.0, depending upon the experiment. An increase, up to 0.04 pH unit min^{-1}, followed addition of sucrose. Other sugars and sugar analogs, including D-glucose, D-xylose, maltose, 3-*O*-methyl-D-glucose, and 2-deoxy-D-glucose also produced this pH rise, but the response, in all cases, was less than that obtained with sucrose. Trehalose, L-glucose, phloridzin, and *myo*-inositol failed to induce a pH shift. In subsequent studies involving measurement of change in pH, the test sugar was sucrose.

The initial rate of change in pH was dependent on the time selected for measurement following onset of germination. This

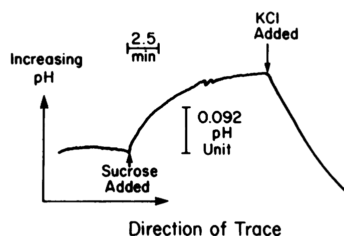

FIG. 1. Recorder trace showing change in pH after adding 19 mg sucrose in 0.5 ml test medium to a suspension of 50 mg pollen in 3 ml test medium at 28 C to yield a final sucrose concentration of 15.9 mM. Pollen had germinated for 4.25 h prior to this determination. The effect of a subsequent addition of 2.5 mg KCl in 0.5 ml test medium (final concentration, 8.4 mM) is also shown.

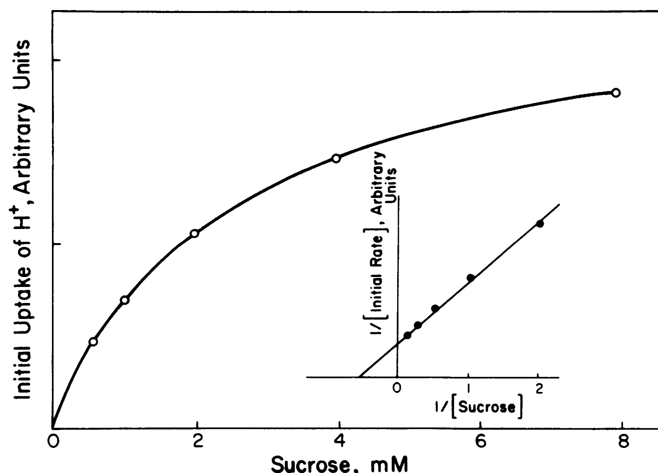

FIG. 2. Plot of initial slope of proton uptake as a function of sucrose concentration. Batches of 50 mg pollen were germinated for 3.5 h prior to pH measurements at each sucrose concentration. Inset, double-reciprocal plot of proton uptake as a function of sucrose concentration.

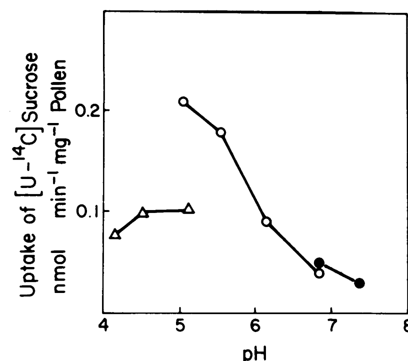

FIG. 3. pH profile of ^{14}C uptake after [U-^{14}C]sucrose addition to germinated pollen that had germinated for 1.5 h. Points in the right-hand curve were obtained with 100 mM Mes buffer (○) or Hepes buffer (●) after adjusting to desired pH with $Ca(OH)_2$. Points in the left-hand curve were obtained with 100 mM acetate buffer.

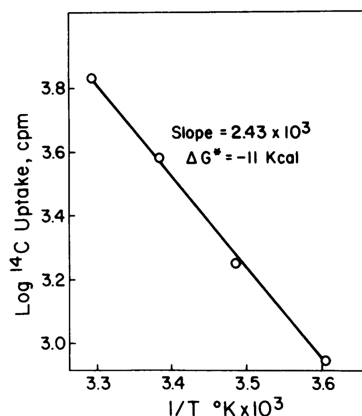

FIG. 4. Plot of temperature dependence of ^{14}C uptake by germinated pollen after addition of [U-^{14}C]sucrose to pollen suspension in pentaerythritol medium at pH 5.05 in 100 mM Mes/Ca buffer. Pollen was germinated for 1.5 h.

rate rose during the first hour of germination, then gradually declined. During this period, endogenous sucrose dropped to 72 mg g^{-1} pollen, less than one-half of its initial value, with the greatest decline occurring in the first hour following germination. A similar decline in the endogenous reserves of germinating pollen from *Camillia japonica* has been reported (17). Addition of KCl in a final concentration of 8.4 mM led to the release of protons into the medium conceivably due to a proton/K^+ antiport similar to that described in *Ricinus* cotyledons or due to passive movement of K^+ into the cells, resulting in depolarization of the potential in the plasma membrane.

The initial uptake of protons, measured as a function of sucrose concentration, produced a hyperbolic curve (Fig. 2) and the reciprocal plot (Fig. 2, inset) gave a K_m of 1.74 mM. At a concentration of 5 μM, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone, a proton ionophore which destroys the proton gradient, inhibited by 80% the pH change initiated by addition of sucrose. This is expected if this sugar or its hydrolysis products utilizes proton motive force to enter the pollen tube. *p*-Hydroxymercuribenzoate, a thiol-directed reagent, almost completely inhibited proton uptake at 100 μM, probably by inactivation of the carrier system. Iodoacetamide, another thiol-directed reagent, was without effect at the same concentration. Phloridzin at 43 mM inhibited the pH effect by 46%.

Labeled Sugar Uptake Studies. [U-^{14}C]Sucrose and D-[U-^{14}C]-glucose were used in these studies. With [U-^{14}C]sucrose, uptake remained linear at least 12 min following its addition to germi-

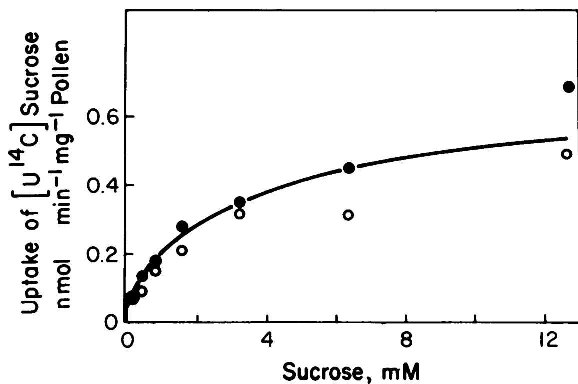

FIG. 5. Plot of ^{14}C uptake as a function of sucrose concentration after addition of [U-^{14}C]sucrose to germinated pollen in pentaerythritol medium at pH 5.05 in 100 mM Mes/Ca buffer. Pollen was germinated for 1.5 h. Data are reported for two experiments involving 4 (●) and 8 min (○).

Table I. *Effect of Various Sugars and Related Compounds on Uptake of [U-^{14}C]Sucrose by Germinated Lily Pollen*

[U-^{14}C]Sucrose uptake was measured in the presence of a 10-fold excess of the test compound using pollen that had germinated for 1.5 h. Pollen was preincubated for 0.5 min prior to addition of label. Incubation was 4 min. Inhibition of uptake is reported as per cent of the control.

Compound Added	Inhibition of [14]C Uptake
	%
None	0
D-Galactose	84
3-*O*-Methyl-D-glucose	80
D-Glucose	73
2-Deoxy-D-glucose	72
Maltose	57
D-Glucurono-6,3-lactone	43
D-Xylose	48
6-Deoxy-D-glucose	46
5-Thio-D-glucose	30
Cellobiose	21
myo-Inositol	14
D-Mannose	5
L-Glucose	0
D-Fructose	0
scyllo-Inositol	0

nating pollen in test medium containing Mes/Ca^{2+} buffer (pH 5.45) at 28 C. In subsequent studies, uptake was limited to 4 min or less to minimize efflux of label and metabolism of the sugar. Uptake of sucrose was maximal at pH 5.0 (Fig. 3). Compared to Mes buffer, acetate inhibited uptake in this pH region. The energy of activation of sucrose from the Arrhenius plot (Fig. 4) was calculated to be −11 kcal mol^{-1} (46 kJ mol^{-1}), which is typical of a facilitated transport rather than passive diffusion.

Uptake of [U-^{14}C]sucrose as a function of substrate concentration gave a hyperbolic curve (Fig. 5) similar to that of proton uptake (Fig. 2). K_m values calculated from inverse plots of proton and sucrose uptake were similar: 1.74 as compared to 1.8 mM. In contrast to this, the K_m for D-[U-^{14}C]glucose uptake at pH 5.5 and 28 C was significantly less, 0.34 mM.

In the presence of carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone, *p*-hydroxymercuribenzoate or phloridzin at concentrations similar to those used in pH-change studies, [U-^{14}C]sucrose uptake was inhibited to the same extent as the pH effect, but [U-^{14}C]glucose uptake was inhibited to a lesser extent. Iodoacetamide failed to inhibit uptake of either sugar. Based on K_m value and response to metabolic inhibitors, results are more consistent with the idea of a proton-sucrose symport.

Table II. *Reproducibility in Measurement of Sucrose Uptake and Inhibition by D-Glucose after Holding Germinated Pollen at 21.5 C*

A single batch of 560 mg lily pollen was germinated for 1.5 h, filtered, washed with test medium, and resuspended in 20 ml test medium at 21.5 C. One-ml aliquots were added to 1 ml 100 mM Mes/Ca buffer (pH 5.5) at 28 C and incubated 1 min. To this suspension was added 0.5 ml test medium or 100 mM D-glucose and, 0.5 min later, 0.5 ml 6 mM [U-^{14}C]sucrose containing 0.5 μCi. The final concentrations of D-glucose and sucrose were 16.7 and 1 mM, respectively. Uptake proceeded for 4 min followed by filtering, washing, and counting of the pollen together with its membrane support.

Storage Time of Pollen at 21.5 C	Addition of D-Glucose	[14]C Recovered in Pollen	Inhibition of Uptake
min		*cpm*	*%*
0	−	454	
10	+	94	79
25	−	490	
35	+	90	82
55	−	472	
65	+	84	82
85	−	452	
95	+	78	83

To test the specificity of co-transport, several sugars and related polyhydroxy compounds were examined for an effect on uptake of [U-^{14}C]sucrose (Table I). Each was present in a molar amount that was 10-fold greater than that of sucrose. Sugars causing significant inhibition were D-galactose, glucose (D- but not L-), maltose, and D-xylose. The reproducibility of such measurements is illustrated in Table II. Here, aliquots from a single batch of germinated pollen, held at 21.5 C, were transferred at intervals to a vessel at 28 C for measurement of sucrose uptake and glucose inhibition. Consistent data were obtained for four such comparisons in a period of 95 min. Although D-glucose was a potent inhibitor of [U-^{14}C]sucrose uptake, sucrose inhibited uptake of D-[U-^{14}C]glucose only slightly (≃20%) under similar conditions, suggestive of low specificity for proton-glucose co-transport. That certain stereospecific requirements are present is seen in the lack of inhibition of sucrose uptake by L-glucose, D-fructose, and D-mannose.

To characterize the products of [U-^{14}C]sucrose uptake, germinated pollen was incubated in the labeled sugar for 2.5 min and then fractionated to obtain the distribution of radioisotope. Free sugars contained 26%, phosphorylated sugars contained 39%, and polymeric products contained 35%. In free sugars, the bulk of the ^{14}C appeared in sucrose, glucose, and fructose in a 1.0:0.31:0.19 ratio. Appearance of 74% of the label in phosphorylated sugars and polymeric material revealed a process of rapid utilization of sucrose for biosynthetic needs following uptake, an observation contrasting to that found in *Ricinus* cotyledons (14). This intense metabolic consumption of sucrose occurred despite a relatively high internal concentration of sucrose and suggests the presence of at least two sucrose pools in germinated lily pollen. Since 2-deoxy-D-glucose and 3-*O*-methyl-D-glucose, both nonmetabolizable sugars which competed with sucrose uptake and promoted a pH shift when added to a suspension of germinated pollen, it is then unlikely that the metabolism of sucrose caused the pH effect.

The data presented here are consistent with a proton-sugar co-transport in germinating lily pollen, but the question of sucrose specificity must still be decided. Invertase is present in lily pollen (6) and its potential role in sucrose uptake must be considered (17). Hydrolysis of sucrose by invertase prior to uptake would be detected as an accumulation of reducing sugars in the external medium. Analysis of the original sucrose solution and that of the incubation medium after the 2.5-min experimental period did not

show any significant change in concentration of hexoses. Further, as mentioned earlier, K_m values of proton and sucrose uptake are similar, but both differ from that of glucose. Also, sucrose inhibits [U-^{14}C]glucose uptake only weakly rather than strongly, as should have been the case if sucrose were hydrolyzed. Finally, there is a similarity in the effect of certain metabolic inhibitors on sucrose and proton uptake, but a somewhat different effect of these inhibitors on glucose uptake. Collectively, these considerations lend support to the concept of a co-transport involving sucrose rather than its hydrolysis products.

Arguments to favor a hydrolytic cleavage of sucrose prior to transport are found in the similar pH changes effected by sucrose, glucose, and glucose analogs and in the effect of other sugars on sucrose uptake (Table I).

We propose a proton-sugar co-transport system in germinating lily pollen which may be specific for sucrose or, alternatively, provide a mechanism for uptake of certain monosaccharides. Aside from its use as an excellent experimental system, co-transport of sugar may have a functional role during pollination. *In vivo,* lily pollen germinates in the presence of a pistil exudate that is rich in arabinogalactan (1). As the pollen tube penetrates the exudate-filled stylar canal, it utilizes this exudate as a nutritional source for tube wall formation (16). The proton-sugar cotransport system may be involved in mobilizing exudate-derived carbohydrate, possibly galactose and oligosaccharides, produced by carbohydrases that are secreted by the elongating pollen tube (19).

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