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Sucrose Release into the Endosperm Cavity of Wheat Grains Apparently Occurs by Facilitated Diffusion across the Nucellar Cell Membranes[1]

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Nutrients required for the growth of the embryo and endosperm of developing wheat (*Triticum aestivum* L.) grains are released into the endosperm cavity from the maternal tissues across the nucellar cell plasma membranes. We followed the uptake and efflux of sugars into and out of the nucellus by slicing grains longitudinally through the endosperm cavity to expose the nucellar surface to experimental solutions. Sucrose uptake and efflux are passive processes. Neither was sensitive to metabolic inhibitors, pH, or potassium concentration. *p*-Chloromercuribenzene sulfonate, however, strongly inhibited both uptake and efflux, although not equally. Except for *p*-chloromercuribenzene sensitivity, these characteristics of efflux and the insensitivity of Suc movement to turgor pressure are similar to those of sucrose release from maize pedicels, but they contrast with legume seed coats. Although the evidence is incomplete, movement appears to be carrier mediated rather than channel mediated. In vitro rates of sucrose efflux were similar to or somewhat less than in vivo rates, suggesting that transport across the nucellar cell membranes could be a factor in the control of assimilate import into the grain.

Because symplastic connections are absent between the embryonic and maternal tissues of a developing seed, nutrients for embryo growth must take an apoplastic pathway to move between the two. The presence of this step, necessarily involving the release of solutes from the maternal symplast, has been used to considerable advantage in studies of phloem unloading and post-phloem transport in developing seeds (see reviews by Thorne, 1985; Patrick, 1990; Wolswinkel, 1992). The site of solute release in *Vicia* seeds (Offler and Patrick, 1993) and in wheat (*Triticum aestivum* L.) grains (Wang and Fisher, 1994b) is a layer of transfer cells in the maternal tissues facing the embryo or endosperm, respectively. Because plasma membranes are typically very effective barriers to solute movement, the transmembrane release of nutrients from these cells (the nucellus, in the case of wheat grains) deserves careful attention as a possible control site for the rate of assimilate import by the seed.

Various treatments have been used to probe the characteristics of solute movement through the maternal seed tissues. Solute release into the apoplast is typically reduced by metabolic inhibitors (although not always [Porter et al., 1987]), including low temperature (see review by Thorne, 1985). In this sense, the process may be characterized as "metabolically dependent," but this does little to clarify the mechanism involved. As noted by Thorne (1985), and appropriately reemphasized by Oparka (1990), movement from the phloem into the apoplast is a multistep process along a symplastic pathway. Aside from possible direct or indirect effects on transmembrane movement, metabolic dependence might arise simply as an indirect effect of metabolism on phloem transport, unloading from the SE/CC complex, on some stage of subsequent symplastic movement or on subcellular compartmentation. There is, in fact, no evident requirement for energized transport at any step, since imported solutes are moving down substantial gradients in concentration and, in some steps at least, along a pressure gradient as well.

More direct lines of evidence concerning the role of transmembrane efflux come from studies using detached seed coats, which eliminate the contribution of phloem transport, and from treatments more likely to affect membrane transport directly. Most work (summarized by Fieuw and Patrick, 1993) has been done with legumes, in which, although results are not entirely consistent, transmembrane efflux appears to be an energy-linked, carrier-mediated process. Thus, it is inhibited by PCMBS, protonophores, metabolic inhibitors, pH extremes, and low temperatures and is stimulated by high K^+ concentration.

In recent work with wheat grains, we found that solute release from the maternal tissues into the endosperm cavity was inhibited by CN and by CCCP (Wang and Fisher, 1994a). However, because inhibition of import was accompanied by a reduction of the maternal Suc pool, phloem transport or SE/CC unloading, and not membrane transport, appeared to be the likely site of inhibition. Also, we found that perfusion of the endosperm cavity with Suc solutions led to an increase in the Suc pool within the maternal tissues without greatly hindering the movement

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Abbreviations: CCCP, carbonyl cyanide *m*-chlorophenyl hydrazone; DNP, 2,4-dinitrophenol; PCMBS, *p*-chloromercuribenzene sulfonate; Q_{10}, rate at (T + 10)°/rate at T°; R_h, hydrodynamic radius; RUR, relative unloading ratio; SE/CC, sieve element/companion cell; SRR, Suc release rate.

of imported Suc into the endosperm cavity. Thus, the nucellar cell plasma membrane appeared to be capable of accommodating bidirectional Suc transport.

The purpose of the present experiments was to test further the extent to which energized membrane transport might be involved in solute movement across nucellar cell plasma membranes, whether H^+ and/or K^+ co-transport might be involved, and the extent to which sugar movement might be reversible. To clarify some of the effects of earlier mannitol and sorbitol perfusion treatments (Wang and Fisher, 1994a), we also addressed the possibility that these sugars might be absorbed by the maternal tissues from the endosperm cavity. Our results indicate that solute efflux from the maternal tissues of wheat grains differs in important respects from the corresponding step in legume seeds.

MATERIALS AND METHODS

Plant Material

Wheat (*Triticum aestivum* L. cv SUN 9E) plants were grown in a growth chamber on a 16-h photoperiod at a PPFD of 450 μmol m^{-2} s^{-1} at a day/night regime of 22/16°C. Plants were irrigated with water at 16-h intervals and with a complete nutrient solution once a week. Grain filling was complete about 30 d postanthesis. Ears were tagged at anthesis and used for experiments 17 to 22 d later.

Perfusion Experiments

The endosperm cavities of attached grains were perfused with various solutions to determine their effect on assimilate import into the grain. With the exception of a faster perfusion rate (12 μL h^{-1} instead of 6 μL h^{-1}), perfusion conditions and the measurements made to estimate import rates were as described previously (Wang and Fisher, 1994a). In brief, endosperm cavities of four grains on each ear were perfused with a test solution and, to measure the rate of assimilate import, the flag leaf was pulse labeled with $^{14}CO_2$. Effluent samples from the endosperm cavity were collected over 20-min intervals during the next 5 h. The perfused grains and four intact control grains were removed from the ear, and endosperm cavity sap was collected from the control grains. The "crease tissues" (i.e. a sample consisting of the vascular strand, chalaza, nucellus, and surrounding pericarp; for anatomy, see Wang and Fisher, 1994b; Fisher and Wang, 1995) were dissected from each grain by making parallel longitudinal slices along the pericarp on either side of the crease tissues. Suc, Glc, and Fru were assayed enzymatically (Jones et al., 1977). Radioactivity was assayed by liquid scintillation counting.

Two measures of assimilate import were used (Wang and Fisher, 1994a). The RUR is the ratio of the ^{14}C import by perfused grains compared to nonperfused grains. The second measure, an estimate of the SRR from the nucellus, was calculated by assaying Suc in the effluent and adding to it the Suc absorbed by the endosperm, as estimated from the proportion of ^{14}C in the effluent versus the endosperm.

Solutions used to vary perfusate K^+ contained 1 mM $CaCl_2$, 1 mM $MgCl_2$, 2 mM KH_2PO_4 and, except the lowest K^+ treatment, 15 mM Mes-KOH buffer, at pH 6.5. K^+ was varied by adding KCl. Mannitol was added to adjust the osmotic concentration to 300 mOsm.

Solutions used to vary pH were similar to the above, except KCl was omitted and the buffer (15 mM) was varied. The buffers used to adjust the starting pHs were Glu (pH 4.0), citric acid (pH 4.5–5.0), Mes (pH 6.3), Hepes (pH 7.5–8.0), (*N*-tris[hydroxymethyl]methyl-3-aminopropane sulfonic acid (pH 9.0–9.5), and (3-[cyclohexylamino]-1-propane sulfonic acid) (pH 11.0). pH was adjusted with KOH or HCl as needed. In perfusion experiments, effluent pH was measured in 2-μL samples with a small combination electrode. Because the effluent pH of perfusing solutions was shifted toward the normal cavity pH of 6.4 (see "Results"), the starting pH of the perfusate was adjusted about a half pH unit beyond the pK_a of the buffer to use its full buffering capacity. This procedure was unnecessary for the much larger solution volumes used when sliced grains were incubated (see further).

Sugar Movement Into and Out of the Crease Tissues of Detached Grains

Because the use of endosperm cavity perfusion limits the number of grains that can be used to follow sugar movement into or out of the crease tissues via the nucellus, an alternative approach was devised that relied on slicing the grain longitudinally through the endosperm cavity to expose the nucellus to experimental solutions. Only the lowermost pair ("a" and "b") of grains of the central 10 to 12 spikelets of an ear were used. The exact experimental protocol depended on the length of the incubation and on whether uptake or efflux was to be followed. In most uptake experiments, the possibility that the inner pericarp might provide an alternative site of sugar uptake was minimized by peeling off the outer pericarp and incubating the grains for 15 min in 5 mM PCMBS, followed by three 15-min washes with standard perfusion medium. The osmotic potential of these and other solutions in these experiments was adjusted to about −0.7 MPa, close to that of the endosperm cavity sap. However, PEG 6000 was used to avoid mannitol or sorbitol, which we found are taken up by the crease tissues. After the third post-PCMBS wash, the grains were sliced longitudinally parallel to the nucellar surface (see fig. 1B of Fisher and Wang, 1995), exposing about 75% of the length of the nucellus. The remainder of the nucellus, at the embryo end where the cavity usually bends, was exposed by pulling off the overlying aleurone with forceps. The opened grains were incubated for another 3 to 4 h in perfusion medium to deplete the crease tissues of their Suc (Fisher and Wang, 1993), after which they were transferred to the uptake medium at room temperature (solution about 20°C). For good aeration, all incubations were performed on an oscillating shaker in solutions 5 mm or less in depth. After the desired uptake period, free space sugar was removed by a 10-min wash at 0°C in standard perfusion medium without PEG. The crease tissues were dissected from the grain half, crushed, and extracted for 10 min in 1 mL of water at 95°C, and assayed for sugars or ^{14}C as appropriate.

Suc efflux from the nucellus was followed by removing the grain from the plant, exposing the nucellus as described above, and placing the grain halves in standard perfusion medium containing PEG. After the incubation period, the crease tissues were dissected out and extracted, and the sugars remaining in the crease tissues were assayed. The initial crease sugar content was determined by cutting open the grain and immediately washing in perfusion medium for 10 min at 0°C to remove free space sugars before extraction.

In one set of experiments, raffinose and stachyose were assayed to determine their uptake by the crease tissues. This was approached by first assaying Suc (Jones et al., 1977), after which 1 mg of invertase was added to each sample to accelerate the hydrolysis of raffinose and stachyose, the amounts of which were estimated from the response to this large amount of invertase.

Radiotracers were obtained from New England Nuclear. Enzymes for sugar assays were obtained from Boehringer Mannheim. Other chemicals were from Sigma.

Respiration Measurements

Rates of O_2 uptake by the crease tissues were measured with an O_2 electrode (Rank Brothers, Cambridge, UK). Crease tissue samples were dissected from 10 to 15 grains and incubated at 25°C in 1 mL of standard perfusion medium in the electrode chamber. Because measurements were made at intervals over several hours, samples were transferred between measurements to perfusion medium in beakers on a rotary shaker.

RESULTS

Perfusion of Attached Grains

The effect of endosperm cavity pH on assimilate import, as indicated by the RUR, showed an optimum at about pH 7.0 (Fig. 1), close to the normal pH of the endosperm cavity (6.37 ± 0.10, n = 12 plants, 16–23 d postanthesis). Especially in the more alkaline range of perfusate solutions, effluent pH was shifted markedly toward the normal cavity pH. Import declined substantially at both pH extremes. Inhibition was reversible, at least on the alkaline side. There was no evident effect of pH on the SRR except at pH 9.5 (Table I), at which the SRR was about 60% higher than expected for intact grains (220 nmol h^{-1} $grain^{-1}$; Fisher, 1990). However, when making comparisons of import, we regard the RUR as a more reliable indicator, because the SRR can be biased upward by leakage of Suc from the endosperm (Wang and Fisher, 1994a). Quite likely, this explains the higher SRR at pH 9.5.

Inhibition of RUR at pH extremes was not accompanied by an increase in the size of the crease Suc pool or in the proportion of the ^{14}C retained in crease (Table I). If anything, the Suc pool showed a slight decrease, suggesting that the decreased RUR resulted from an inhibition of phloem transport or sieve tube unloading.

The RUR was not strongly affected by variation in perfusate K^+ concentration (Fig. 2), nor were the SRR or the proportion of ^{14}C remaining in the crease tissues (Table I). The crease Suc pool size may have declined slightly during perfusion with higher K^+ concentrations (40, 60, and 80 mM; Table I).

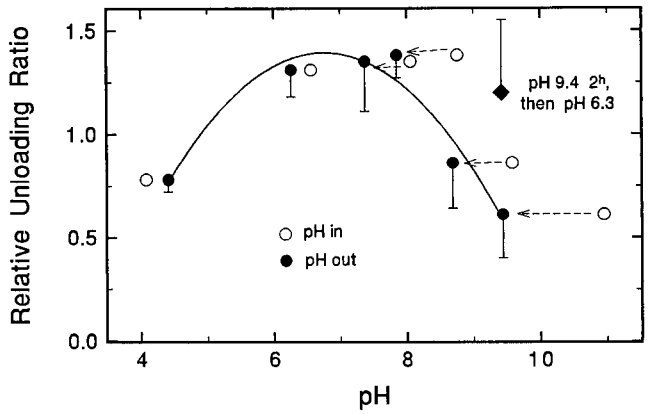

Figure 1. Effect on the RUR of perfusing the endosperm cavity of attached grains with solutions of various pHs. Arrows indicate the change in pH between the perfusing solutions (○) and the effluent solutions (●). The data are fitted to a second-order polynomial. The inhibition at alkaline pH was reversible (◆), since no inhibition occurred when 2 h of perfusion at pH 11.0 (effluent pH 9.4) was followed by 5 h of perfusion at pH 6.5.

Preperfusion of the endosperm cavity for 15 min with 5 or 10 mM PCMBS strongly inhibited [^{14}C]Suc import into the grain (Fig. 3). This was accompanied by a substantial increase in the crease Suc pool and especially in the proportion of ^{14}C retained in the crease tissues. The PCMBS treatment had no effect when it was followed by a 15-min perfusion with DTT (Fig. 3, open symbols). The decreased RUR the increase in the crease Suc pool and in ^{14}C are the effects expected of a PCMBS-induced blockage of Suc movement across the nucellar cell membranes.

Uptake of Sugars by the Nucellus

Several approaches were taken to verify that most of the increase in crease sugar content occurred via uptake by the nucellus rather than the inner pericarp, which was also exposed to the solutions. When starved grains with their outer pericarp removed were incubated for 2 h in 250 mM Suc, the crease Suc content increased by about 31 ± 20 nmol (n = 8 grains). This is considerably less than the 160- to 180-nmol increase observed when the nucellus was exposed directly to 250 mM solutions (Fig. 4 and other experiments). If the grains were treated for 15 min with 5 mM PCMBS after their outer pericarp was removed, the increase in crease Suc content was barely detectable (15 ± 9 nmol).

In contrast, when the possibility of uptake via the pericarp was entirely eliminated by perfusing the endosperm cavities of detached, starved grains with 250 mM Suc medium, the crease Suc pool increased by 115 ± 6 nmol (n = 4 grains). This increase is somewhat less than for half grains incubated in 250 mM solutions, but much of the difference can be ascribed to dilution of the perfusate to 210 mM during passage through the endosperm cavity. These observations, combined with the fact that free space

Table I. *SRR, crease Suc pool size, and percentage of ^{14}C remaining in the crease tissues of attached perfused grains 5 h after pulse labeling the flag leaf with $^{14}CO_2$*

Treatment	No. of Grains	Crease ^{14}C	Crease Suc	SRR
		% total	*% controls*	*nmol h^{-1} $grain^{-1}$*
Intact grains[a]	108	17 ± 3	100	220
pH treatments				
RUR > 1.0	11	16 ± 2	96 ± 16	194 ± 22
RUR < 1.0	11	14 ± 4	81 ± 9	214 ± 29
pH 9.5	4	14 ± 4	90 ± 13	357 ± 41
K^+ treatments				
1–20 mM	14	16 ± 6	94 ± 9	236 ± 25
40–80 mM	16	16 ± 2	83 ± 5	268 ± 33

[a] Data from Wang and Fisher (1994a).

solutes cannot readily penetrate beyond the nucellus, because of an apoplastic barrier in the chalaza (Wang and Fisher, 1994b), specifically implicate the nucellar cell membranes as the principal uptake site for Suc in our uptake experiments.

The time course for net Suc uptake into the crease tissues from solutions of two Suc concentrations is shown in Figure 4. Net uptake was relatively rapid at first but almost ceased after 3 to 4 h. Since the water content of a crease sample after incubation was about 1.31 ± 0.15 μL (n = 10), the average Suc concentration of the crease tissues at steady state approximated that of the uptake solution, indicating that Suc was not actively accumulated. In another experiment in which crease Suc content was determined after 4 h in various Suc concentrations, the amount was a linear function of the bathing Suc concentration (r^2 = 0.98). The regression extrapolated to 16 nmol of Suc per crease at 0 mM Suc concentration, suggesting that a small proportion of the Suc pool might not be available for transport (the symplastic crease pool in undisturbed grains is about 250 nmol).

When the rate of Suc uptake was followed as a function of concentration, the uptake rate continued to increase over the entire range of concentrations tried (to 300 mM; Fig. 5).

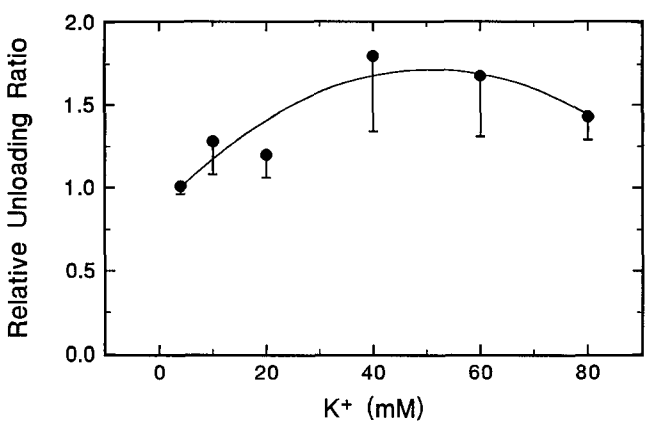

Figure 2. Effect on the RUR of perfusing the endosperm cavity of atttached grains with solutions of various K^+ concentrations. The data are fitted to a second-order polynomial.

However, the relationship was curvilinear, suggesting that uptake might saturate at higher concentrations.

The possibility that uptake might be sensitive to turgor was investigated by comparing [^{14}C]Suc uptake from 10 mM solutions into the crease tissues of grains that had been starved for 4 h to deplete their solute content or into crease tissues that had been preloaded for 3 h with 250 mM Suc. None of the samples, taken at 10-min intervals over 50 min, differed by more than 10% (data not shown). The time course of Suc efflux into perfusion media with or without added PEG, a difference in water potential of about 0.6 MPa, was also similar for the two treatments (data not shown).

The specificity of sugar absorption was investigated by following the uptake of ^3H-labeled mannitol or sorbitol from 125 mM solutions and of unlabeled raffinose or stachyose from 200 mM solutions. Appreciable amounts of mannitol and sorbitol were absorbed, with virtually identical time courses for the two, and net uptake ceased when the tissue concentration approached that of the medium (data not shown). However, the initial rate of uptake was

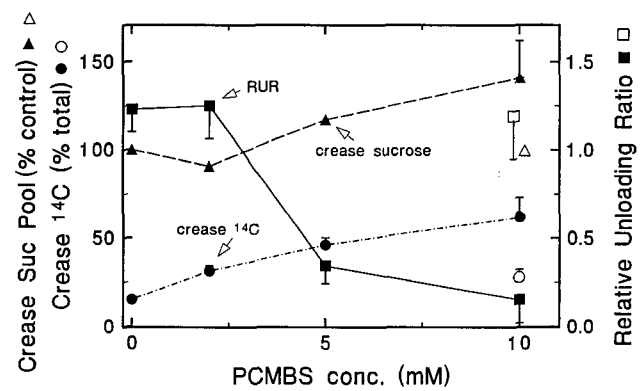

Figure 3. Effect of preperfusing the endosperm cavity of attached grains for 15 min with various concentrations of PCMBS before pulse labeling with $^{14}CO_2$ to determine the RUR. The crease Suc pool size and the proportion of ^{14}C remaining in the crease after the 5-h transport period were also determined. Open symbols indicate the effect of following a 10 mM PCMBS perfusion with a 15-min perfusion with 5 mM DTT. For clarity, the upper symbols are slightly displaced along the abscissa.

only about 50 nmol h^{-1}, or about half that from a 125 mM Suc solution (Fig. 5). Very little raffinose or stachyose was absorbed. Although the assay of these sugars was less precise than for Suc, their uptake rate was clearly no more than 5 nmol h^{-1}, or only about 5% of that expected from a 200 mM Suc solution (Fig. 5).

Effect of Inhibitors, pH, K^+, and Temperature on Suc Uptake and Efflux

Even at the highest concentrations used, neither the respiratory inhibitors DNP or CN nor the protonophore CCCP had a significant effect on Suc movement across the nucellar cell membrane (Table II). PCMBS, however, was very effective in blocking Suc efflux from the nucellus, with no detectable loss of Suc occurring in 30 min into PCMBS solutions of 2, 5, or 10 mM (data not shown). PCMBS was not so effective in blocking Suc uptake into the crease tissues. When uptake was followed by incubating for 30 min in 20 and 200 mM [^{14}C]Suc solutions with or without 5 mM PCMBS, uptake from the PCMBS solutions was 40 to 45% of that from the PCMBS-free media (data not shown).

In contrast to the inhibition at pH extremes of Suc import into the wheat grain (Fig. 2), pH had no evident effect on Suc uptake into or efflux from the nucellus (Fig. 6). Varying the K^+ concentration was similarly without effect (Fig. 7).

The effect of temperature on both uptake and efflux was followed simultaneously by incubating recently detached grains in 50 mM [^{14}C]Suc over the range of 0 to 30°C. Crease ^{14}C was assayed to measure uptake, and efflux was determined as the difference in crease Suc content before and after incubation. Arrhenius plots of the data are presented in Figure 8. Both processes had similar activation energies of about 36 kJ mol^{-1}, or a Q_{10} of about 1.7.

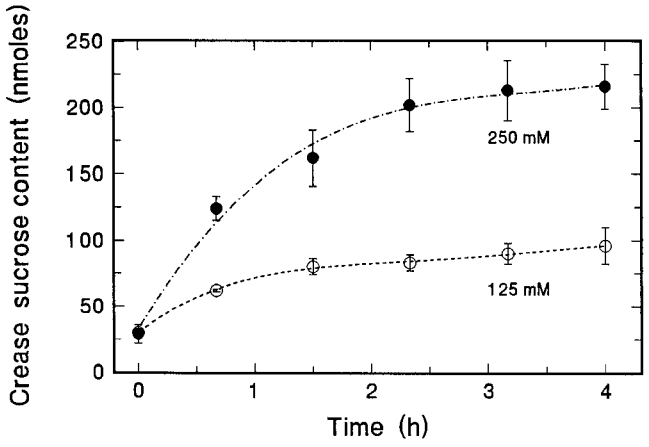

Figure 4. Time course for Suc uptake into the crease tissues from 250 and 125 mM Suc solutions. After the grains were detached, their outer pericarp was peeled off, and they were incubated for 15 min in 5 mM PCMBS. After three 15-min washes, the grains were sliced lengthwise to expose the nucellar surface and incubated for 3 h in Suc-free medium to deplete their crease tissues of Suc. They were then transferred to the Suc solutions. Before analysis of the crease Suc content, free space Suc was removed by a 10-min wash at 0°C. In the 250 mM Suc experiment, the sample size was six grains (combined results of two experiments); for the 125 mM experiment, the sample size was three grains.

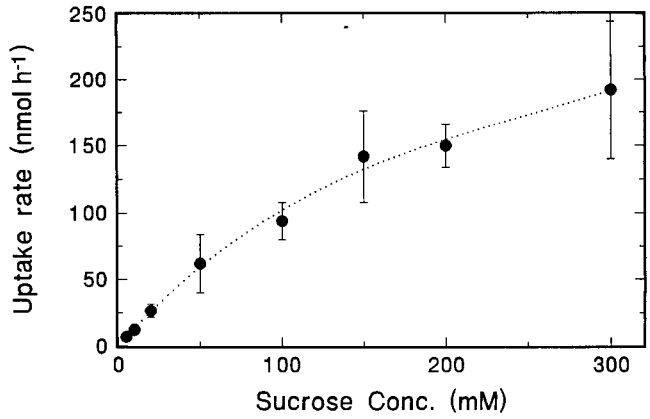

Figure 5. Effect of Suc concentration on uptake rate. Combined results of two experiments (six grains per point). Experimental treatments were as in Figure 4, except solutions contained [^{14}C]Suc; uptake was calculated from the crease ^{14}C content after 30 min in the Suc solution and a 10-min wash at 0°C.

Respiration Rates

The O_2 uptake rate of the crease tissues, measured within 0.5 h after dissecting the samples from the grain, was 16 ± 2 μmol O_2 g^{-1} (fresh weight) h^{-1} (n = 10). This rate often declined by 15 to 20% during the 1st h, after which the rate varied by less than 10% for at least 5 h. Response to the addition of KCN or DNP was fairly prompt, with most of the change in the O_2 uptake rate taking place within 10 min. At 0.2 mM, CN inhibited O_2 uptake by 40% or more, whereas 0.2 mM DNP stimulated uptake by about 45%. When crease tissues were incubated for 15 min in 10 mM PCMBS, followed by washing with perfusion medium, there was no effect on respiration for at least the following 2 h. Thus, these experiments confirm that CN and DNP were penetrating the tissues in metabolically significant amounts, whereas PCMBS was evidently excluded.

DISCUSSION

Our observations clearly indicate that the movement of Suc across the nucellar cell membranes of wheat grains is the result of passive transport and is not energetically dependent, either directly or indirectly. Transmembrane movement was reversible, with net uptake ceasing when the tissue Suc concentration approached that of the external medium. Neither uptake nor efflux was affected by inhibitors of energy metabolism or by treatments (CCCP, H^+, K^+) expected to affect energized membrane transport. Although assimilate import into the grain was pH dependent, Suc movement across the nucellar cell membrane was not. Thus, the site of pH dependence for import appears to be movement along the phloem or unloading from the SE/CC complex. The only suggestion that active transport might occur comes from the fact that import into the grain can continue when the Suc concentration in the endosperm cavity is higher than that in the sieve tubes (approximately

Table II. *Effect of various inhibitors on Suc uptake by and efflux from the crease tissues*

Rates were determined by assaying the Suc content of crease tissues before and after the uptake period (1 h in 100 mM Suc) or the efflux period (1 h in Suc-free medium). For uptake experiments, the half-grains were starved for 3 to 4 h, preincubated for 20 min in the inhibitor solution without Suc, and then transferred to the same medium containing Suc. In efflux experiments, grains were removed from the ear, sliced longitudinally to expose the nucellus, and placed immediately into Suc-free medium. Combined data from two experiments (a total of seven grains for each inhibitor concentration).

Inhibitor	Concentrations Used	Control Rate (no inhibitor)	Rate at the Highest Inhibitor Concentration
		nmol h^{-1}	
Uptake			
DNP	0.02, 0.2, 2 mM	55 ± 12	47 ± 12
CN	0.02, 0.2, 2 mM	55 ± 12	40 ± 14
CCCP	1, 10, 100 μM	62 ± 15	54 ± 12
Efflux			
DNP	0.02, 0.2, 2 mM	185 ± 40	166 ± 50
CN	0.02, 0.2, 2 mM	185 ± 40	173 ± 37
CCCP	1, 10, 100 μM	122 ± 22	137 ± 32

450–600 mM; Wang and Fisher, 1994a). As we noted, however, it is more likely that such movement occurs by pressure-driven symplastic flow out of the SE/CC complex rather than by active membrane transport.

Although the evidence is incomplete, it appears most likely that transmembrane movement involves facilitated transport via a carrier rather than movement through a channel (i.e. a continuous aqueous pathway through the membrane [Stein, 1986]). Nucellar cell membranes are almost impermeable to carboxyfluorescein and Lucifer Yellow (R_hs = 0.61 and 0.68 nm, respectively; Wang and Fisher, 1994b). However, these molecules are not only somewhat larger than Suc (R_h = 0.47 nm) but anionic as well and might be excluded on either basis from a channel capable of accommodating Suc.

Our present results indicate that the membranes are also fairly impermeable to the trisaccharide raffinose (R_h = 0.57 nm; Schultz and Solomon, 1961) and to the tetrasaccharide stachyose. The smaller polyols, mannitol (R_h = 0.36 nm; Schultz and Solomon, 1961) and sorbitol, are absorbed by the nucellus but at only half the rate for Suc. Thus, although there may be some relationship between size and transmembrane movement, there are major irregularities, and the cutoff appears to be much more abrupt than would be expected as the size-exclusion limit of a channel was approached (Nikaido and Rosenberg, 1981; Stein, 1986). More importantly, the Q_{10} for movement in both directions (1.7) is substantially higher than would be expected for movement through a water-filled pore. For the latter mechanism, Nikaido and Rosenberg (1981) measured a Q_{10} of 1.25, close to that for free diffusion in bulk water. Also, the apparent insensitivity of transport to turgor pressure argues against the presence of large, water-filled channels in the plasma membrane.

The Suc flux across the nucellar cell membranes (about 2 × 10^{-8} mol m^{-2} s^{-1}; Wang et al., 1994) is well within the range of rates observed for facilitated diffusion. The red blood cell membrane, for example, can accommodate about 100-fold this flux from a 5 mM solution (Jung, 1975).

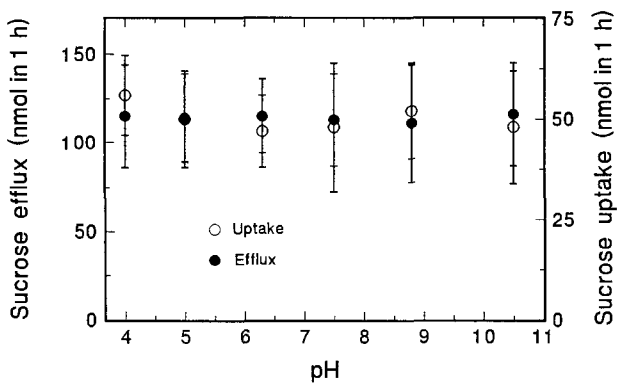

Figure 6. Effect of pH on Suc uptake into and efflux from the crease tissues. Experimental treatments for uptake experiments were as in Figure 4. Uptake was for 1 h from a 100 mM Suc solution. For efflux experiments, grains were detached, sliced longitudinally to expose the nucellus, and placed immediately in Suc-free medium for 1 h. Initial crease Suc content was 247 ± 20 nmol. Uptake and efflux were calculated from the difference in crease Suc content before and after the 1-h incubation period. Each plot is the combined data from two experiments (eight grains per point).

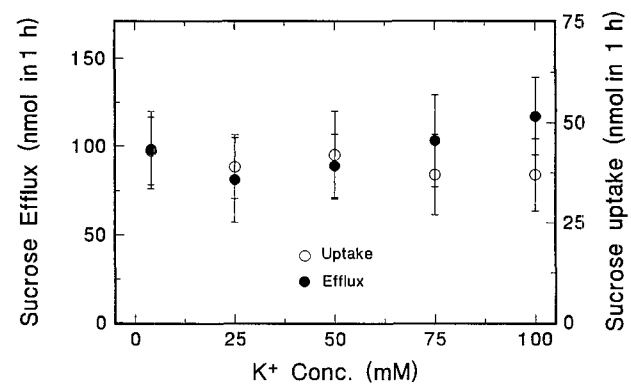

Figure 7. Effect of K^+ concentration on Suc uptake into and efflux from the crease tissues. Experimental treatments were as in Figure 6. Each plot is the combined data from two experiments (eight grains per point).

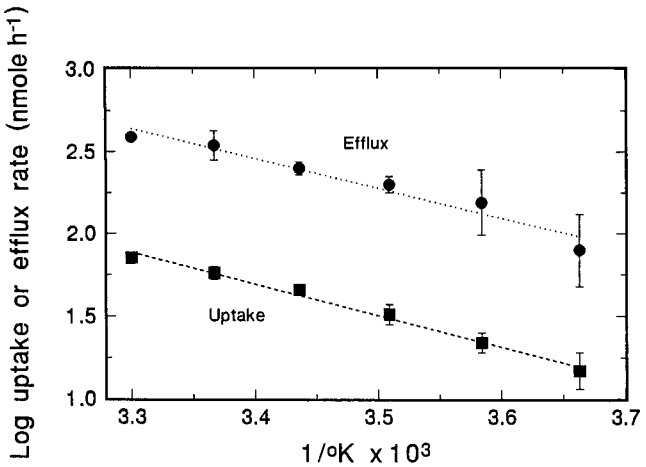

Figure 8. Arrhenius plots of the effect of temperature on Suc uptake into and efflux from the crease tissues. The same grains were used to determine both uptake and efflux. Experimental treatment for uptake was as in Figure 5, except uptake was from a 50 mM [^{14}C]Suc solution. Uptake/efflux times were varied, depending on the temperature: 40 min at 0 and 6°C, 30 min at 12 and 18°C, and 20 min at 24 and 30°C. Efflux was calculated from the difference in crease Suc content before and after the incubation period. Each plot is the combined data from two experiments (six grains per point).

Aside from a somewhat high Q_{10}, however, positive evidence for carrier-mediated transport is sparse. Transport was not strongly specific, nor was there clear evidence that it is saturable, at least for uptake. Under most conditions, the efflux rate was 2 to 3 times the uptake rate. However, this is not necessarily inconsistent with carrier-mediated diffusion (Stein, 1986). What is more of a concern is the virtually complete blockage of efflux by PCMBS concentrations, which still allowed uptake to proceed at 40% of the control rate, suggesting that, despite the similarity in the response of uptake and efflux to most treatments, more than one mechanism of transmembrane movement might be involved. Clearly, confident interpretations of the mechanism(s) involved in Suc efflux across the nucellar cell membranes must await more detailed investigation of the process.

The characteristics of Suc release from the nucellus of wheat grains contrasts in important respects to the analogous process in legume seed coats. As noted earlier in this paper, solute release in legumes appears to be energy linked, as reflected by its sensitivity to metabolic inhibitors, protonophores, and pH extremes, and is stimulated by high K^+ concentrations. Also, legume seed coats have almost no capacity for Suc uptake (Minchin and Thorpe, 1990; Ellis et al., 1992). All of these characteristics differ from the situation in wheat grains. In addition, efflux in legumes is sensitive to external osmotic pressure (Wolswinkel, 1992), whereas in wheat it is not. As in wheat, solute release from corn pedicels is insensitive to osmotica and to metabolic inhibitors (although not to PCMBS; Porter et al., 1987). Whether these characteristics are linked in some way remains to be seen.

The in vitro rates of Suc efflux from the nucellus, even into Suc-free media, never exceeded the Suc import rate of 220 nmol h^{-1}. Instead, the efflux rates ranged from somewhat less to distinctly less than this, which may be attributed in part to the lower temperature of the efflux solutions (about 20°C) compared to the grain temperature under growth conditions (25–28°C). The general similarity of the in vivo and in vitro efflux rates suggests that transport across the nucellar cell membranes could be involved in the control of assimilate import into the grain. Other evidence, however, strongly implicates movement out of the SE/CC complex (Fisher and Wang, 1995) as an important control point.

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