Plant Physiol. (1981) 67, 163–169
0032-0889/81/67/0163/07/$00.50/0

Co-transport of Potassium and Sugars across the Plasmalemma of Mesophyll Protoplasts[1]

Received for publication June 24, 1980 and in revised form August 28, 1980

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ABSTRACT

Sugars (sucrose + hexoses) produced photosynthetically by isolated mesophyll protoplasts of wheat and tobacco were effluxed across the plasma membrane (3 to 10 micromoles hexose equivalents per milligram chlorophyll per hour). The efflux was sensitive to uncouplers and oligomycin which indicated a requirement for energy. A proton gradient was probably not coupled directly to the transport because changing the proton gradient across the plasma membrane by varying the pH of the medium or by adding sodium acetate had no significant effect on the rate of sugar release.

A release of K^+ was associated with sugar efflux from the protoplasts. The molar ratio of K^+ to sugar varied between 1.5 and 2.5, depending on the species. Exogenous KCl, RbCl, and LiCl (50 millimolar each), but not NaCl or CsCl, significantly inhibited sugar efflux. Conditions that reduced sugar efflux (exogenous KCl, LiCl, mersalyl, or oligomycin) also reduced K^+ release and caused a time-dependent reduction in photosynthetic sucrose formation and increased amino acid and starch formation. Results obtained support the postulate that a K^+ symport is involved in the transport of sugar across the energized plasmalemma of photosynthetically active mesophyll cells.

In most higher plants, sucrose is a major end product of photosynthesis and is also the principal form in which photosynthetically derived carbohydrates are translocated within the plant. When translocation of assimilates from the leaf is reduced, photosynthetic sucrose formation is reduced, whereas starch formation is increased (38). Studies such as these indicate an influence of end-product accumulation (*e.g.*, sucrose) on the partitioning of carbon between starch and sucrose in the leaf cell.

Sucrose is also a principal end product of photosynthesis by isolated leaf cells and protoplasts (5, 18, 35). Because of continued interest in the use of isolated cells and protoplasts for various photosynthetic studies, it seemed desirable to establish the site of sucrose accumulation during photosynthesis *in vitro* by isolated leaf preparations. It has been demonstrated convincingly that sucrose is synthesized in the cytoplasm of the mesophyll cell (33). Photosynthetic formation of sucrose by mesophyll protoplasts from various species is linear with time (18), which suggests either that internal sucrose accumulation has no effect on carbon partitioning or that sucrose does not accumulate in the cytoplasm. Previous studies (19, 23, 33) showed that less that 10% of the $^{14}CO_2$ fixed over 60 min by isolated leaf preparations is released to the medium, which has been taken to suggest that the products of photosynthesis are retained within the protoplast. Recently, we (19) reported an efflux of sucrose and hexoses, measured enzymically, from isolated mesophyll protoplasts at a rate similar to that of photosynthetic sugar formation *in vitro*. Release of radioactivity to the medium did not quantitatively reflect the sugar released because of dilution with internal pools. The results provided the first evidence for a significant transport of photosynthetic end products across the mesophyll plasma membrane.

Characterization of the mechanism involved in transport of sugars across the mesophyll plasma membrane was the subject of the study reported herein. Specific objectives of the study presented here were to determine whether sugar efflux required an energized membrane and, if so, whether it involved co-transport (symport) of H^+ or alkali cations.

MATERIALS AND METHODS

Protoplast Isolation. Protoplasts were isolated from fully expanded leaves of greenhouse-grown 10- to 14-day-old wheat (*Triticum aestivum* L.) and 4- to 6-week-old tobacco (*Nicotiana tabacum* L.) plants. Wheat protoplasts were isolated and purified as previously described (6). The purified protoplasts were washed three times in 0.5 M sorbitol and 1 mM $CaCl_2$ after flotation on sucrose. Tobacco leaf segments (0.5 mm) were incubated in a medium containing 0.5 M sorbitol, 1 mM $CaCl_2$, 2% (w/v) Cellulysin[2] (Calbiochem), 0.4% (w/v) Macerozyme (All Japan Biochemicals), 0.5% (w/v) BSA, and 0.5% (w/v) PVP-40 at pH 5.5 Protoplasts were released after a 1-h incubation at 30 C into a solution containing 0.5 M sorbitol and 1 mM $CaCl_2$. Tobacco protoplasts were purified using the two-phase system of Kanai and Edwards (24).

Sugar Efflux and Assays. Unless noted otherwise, protoplasts (30–40 μg Chl) were incubated at 25 C in a 0.4-ml reaction mixture containing 0.5 M sorbitol, 0.2 mM $CaCl_2$, 50 mM Hepes-NaOH (pH 8.0), and 7.5 mM $NaHCO_3$. Saturating illumination of about 60 $nE/cm^2 \cdot s$ was provided by 75-w incandescent light bulbs.

At various times (usually 0, 10, 20, and 30 min), 0.1-ml aliquots were withdrawn and centrifuged at 200*g* for 15 s to pellet the protoplasts. Eighty μl of the clear supernatant was added to 100 μl 0.04 N KOH and stored on ice prior to assay. Samples (80 μl) for K^+ measurements were added to 100 μl 0.04 N HCl and stored frozen, pending analysis by atomic absorption spectroscopy. Supernatants were analyzed for sugars by the method of Jones *et al.*

[1] Cooperative investigations of the North Carolina Agricultural Research Service and Agricultural Research, Science and Education Administration, United States Department of Agriculture, Raleigh, NC. This is Paper No. 6244 of the Journal Series of the North Carolina Agricultural Research Service, Raleigh, NC.

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(22). For sucrose determinations, samples (160 μl) were added to 1.0 ml of a mixture containing 100 mM Hepes-NaOH (pH 7.0), 0.4 mM NADP, 1 mM ATP, 5 mM $MgCl_2$, 0.5 mM DTT, 16 units/ml invertase, 0.5 units/ml hexokinase, 2 units/ml phosphoglucoisomerase, and 6 units/ml glucose-6-P dehydrogenase. *A* at 340 nm was measured after 30 min at 25 C. For hexose (glucose + fructose) assays, samples were treated similarly except that invertase was omitted, the mixture was buffered at pH 8.0, and the length of incubation was reduced to 10 min. The amount of sugars present in the supernatant of the zero-time sample was subtracted from determinations in subsequent samples. All data are expressed on an hexose-equivalent basis and are either typical results or the average of several different experiments.

$^{14}CO_2$ Fixation and Product Distribution. Protoplasts (50 to 75 μg Chl/ml) were incubated at 25 C with or without illumination (60 nE/cm^2·s) in a reaction mixture containing 0.50 M sorbitol, 7.5 mM $NaH^{14}CO_3$ (2 to 5 μCi/μmol), 50 mM Hepes-NaOH (pH 8.0), and 0.2 mM $CaCl_2$. Other conditions were as specified below in the text. At various times, aliquots of the reaction mixtures were terminated in acid to measure total $^{14}CO_2$ fixed.

For separation of labeled products into various fractions, 0.1-ml aliquots of the total reaction mixture were mixed with an equal volume of 1 N acetic acid. The insoluble fraction, obtained by centrifugation at 10,000*g* for 5 min, was washed twice with H_2O and saved for ^{14}C-starch analysis. The combined supernatants were separated into neutral, basic, and acidic fractions by ion-exchange chromatography (1). Samples were passed sequentially through 1-ml columns of Dowex 50W-X8 (H^+) and Dowex 1-X8 (acetate). The neutral fraction was that radioactivity that washed through both columns with 3 ml H_2O. Paper-strip chromatography of the neutral fraction in butanol-acetic acid-H_2O (3:1:1) indicated that greater than 90% of the label was in sucrose, which concurs with earlier findings (5). The basic fraction was eluted from the Dowex 1-X8 (acetate) column with 8 ml 5 N acetic acid and the acidic fraction was eluted with 8 ml 2 N HCl. The washed pellets (insoluble radioactivity) were resuspended in 100 μl 50 mM citrate (pH 4.5) containing 500 units/ml amyloglucosidase (Sigma). After incubation at 40 C for 90 min, the samples were centrifuged at 10,000*g* for 4 min. Radioactivity in the supernatant was taken to indicate [^{14}C]glucose derived from labeled "starch."

RESULTS

Efflux of Sugars and K^+ from Protoplasts. During photosynthesis by isolated wheat and tobacco mesophyll protoplasts, an efflux of sugars (sucrose and hexoses) and K^+ to the medium was observed. With wheat protoplasts, the release of both K^+ and total sugars (sucrose + hexoses) was nearly linear between 10 and 30 min and the K^+-to-sugar ratio was 2.6 (Fig. 1). In other experiments, the K^+-to-sugar ratio with wheat protoplasts averaged 2.3. As previously reported (19), sucrose was the predominant sugar released (on a hexose equivalent basis), and the relative amount of hexoses (glucose and fructose) in the medium increased with time. After 30 min photosynthesis, about 1.1 and 0.8 μmol hexose eq/mg Chl were released to the medium in the form of sucrose and hexoses, respectively. Assuming a reaction volume of 0.4 ml containing about 40 μg Chl, the concentration of sucrose and hexoses in the medium would be about 55 and 80 μM, respectively. Because the sugar concentrations in the bathing solution were very low, it can be assumed that the sugar efflux measurements represent unidirectional fluxes. Results similar to those presented in Figure 1 were obtained with tobacco and barley protoplasts, except that the K^+-to-sugar ratio averaged about 1.5 and 2.6, respectively.

Typical results showing the effect of various alkali cations on the kinetics of release of total sugars are presented in Figure 2. The release of sugars (Fig. 2) to the medium was stimulated slightly by 50 mM CsCl and NaCl and was inhibited significantly

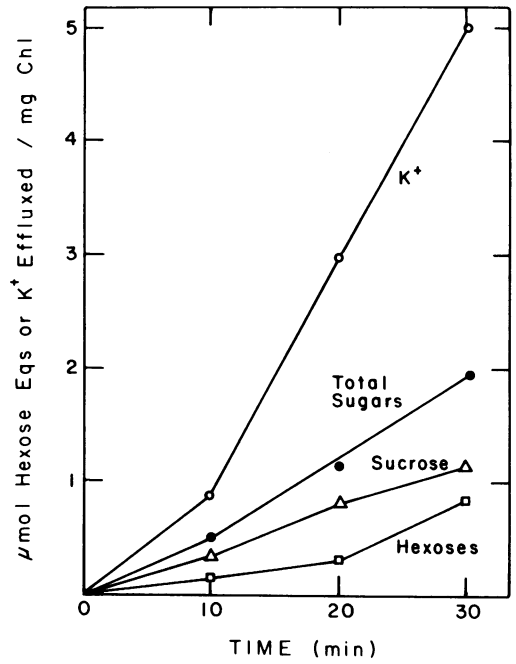

FIG. 1. Kinetics of K^+ and sugar release from wheat protoplasts.

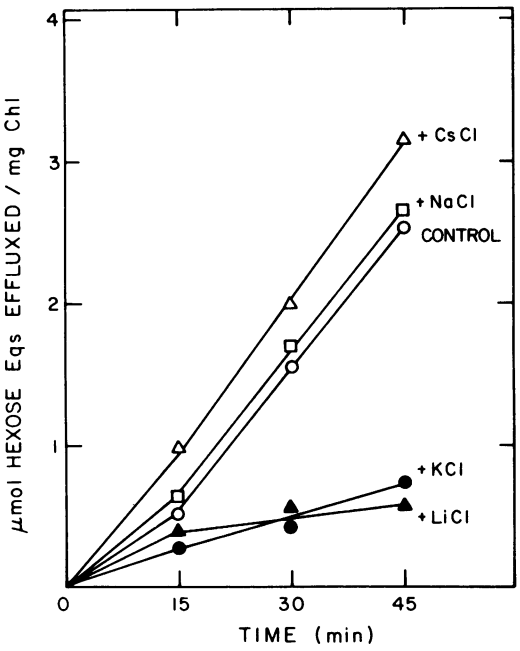

FIG. 2. Effect of alkali cations on efflux of sugars from wheat protoplasts. Reaction mixtures containing the indicated salts (50 mM) had decreased sorbitol concentrations (0.40 M) to maintain constant osmotic potential (0.5 M total). Photosynthetic rates were not affected by the salts.

by exogenous KCl and LiCl. Inhibition similar to that of KCl was caused by RbCl but, for clarity, is not presented. Photosynthetic rates were not significantly affected by any of the salts and, thus, the observed inhibition was not caused by reduced photosynthetic sugar formation.

The concentration dependence of the effects of exogenous NaCl and KCl on the rate of sugar release from wheat protoplasts is shown in Figure 3. The concentration of sorbitol used as the osmoticum was reduced proportionally with salt to maintain a constant medium osmolarity of 0.5 M. NaCl had no effect on sugar

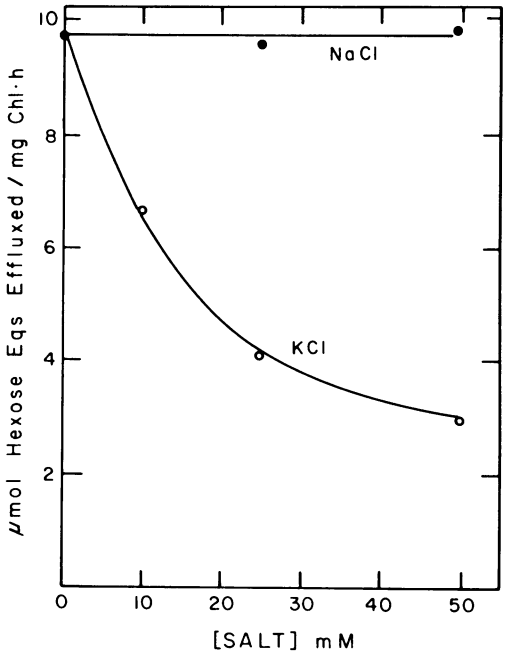

FIG. 3. Effects of exogenous NaCl or KCl on the rate of sugar efflux from tobacco protoplasts. The concentration of sorbitol in the reaction mixtures was varied to maintain a constant medium osmolarity of 0.5 M. Photosynthetic rates were not affected by the salts.

Table I. *Effect of Divalent Cations and Chelation on Sugar Efflux from Tobacco Protoplasts in Light*

Addition[a]	Concn.	Total Sugars Effluxed at		Rate[b]
		10 min	20 min	
	mM	μmol hexose eq/mg Chl		
None		0.43	1.43	6.0
$CaCl_2$	2	0.50	1.50	6.0
	5	0.43	1.70	7.2
$MgCl_2$	2	0.50	1.42	5.5
EDTA	2	0.90	2.0	6.6

[a] Reaction mixtures contained 0.2 mM $CaCl_2$.

[b] Rate of sugar efflux between 10 and 20 min is expressed as μmol hexose eq/mg Chl·h.

efflux over the concentration range tested. In contrast, KCl significantly inhibited it and produced maximum inhibition of about 70% at a concentration of 50 mM.

The rate of sugar efflux from tobacco protoplasts was not affected significantly by mM concentrations of the divalent cations Ca^{2+} and Mg^{2+} or by the chelator EDTA (Table I). However, total sugar released after 10 and 20 min photosynthesis was slightly greater in the presence of 5 mM EDTA.

With wheat protoplasts, the addition of FCCP[3], the K^+ ionophore valinomycin, and oligomycin, after 10 min photosynthesis, inhibited the release of both sugars (Fig. 4A) and K^+ (Fig. 4B). In the control, the K^+-to-sugar ratio averaged 2.2 over the period tested. A similar ratio was maintained in the presence of oligomycin, although total release was inhibited about 80% or more (Fig. 4; Table II). Sugar efflux from tobacco protoplasts was also sensitive to oligomycin, which caused more than 75% inhibition at a concentration of 5 μg/ml (Table II). Occasionally, inhibition by oligomycin increased with time, so that the observed sugar release

[3] Abbreviations: FCCP, carbonyl cyanide *p*-trifluoromethyoxyphenylhydrazone; NaOAc: sodium acetate.

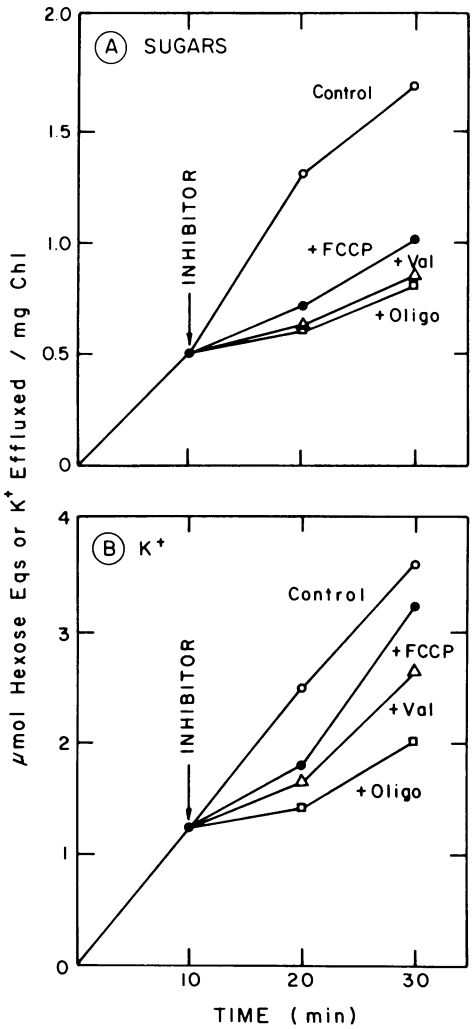

FIG. 4. Effect of 1 μM FCCP, 0.1 μM valinomycin (Val), and 5 μg/ml oligomycin (Oligo) on sugar efflux (A) and K^+ efflux (B) from wheat protoplasts. Inhibitors were added at 10 min (arrow). None of the treatments inhibited photosynthesis more than 25%.

Table II. *Effect of Sulfhydryl Reagents and Oligomycin on Rate of Sugar Efflux from Wheat and Tobacco Mesophyll Protoplasts*

Treatment	Species	
	Wheat	Tobacco
	μmol hexose eq/mg Chl·30 min	
Control	2.0 (100)[a]	3.0 (100)
p-Chloromercuriphenylsulfonic acid, 0.1 mM	1.95 (97)	3.1 (103)
Mersalyl		
0.09 mM	1.42 (71)	2.4 (80)
0.18 mM	1.15 (58)	2.0 (67)
0.36 mM	0.85 (42)	1.6 (53)
Oligomycin, 5 μg/ml	0.20 (10)	0.7 (23)

[a] Per cent of control rate is indicated parenthetically.

was curvilinear. Valinomycin and FCCP reduced sugar efflux by a relatively constant amount, whereas the inhibition of K^+ efflux was transient (Fig. 4). Twenty min after addition of either FCCP or valinomycin, the ratio of K^+ to sugar effluxed was increased to 3.2. Presumably, other mechanisms exist for K^+ release that are not coupled to sugar transport and may tend to occur in the

presence of inhibitors. Sugar and K^+ efflux from tobacco protoplasts were inhibited also by valinomycin and FCCP (data not shown).

The effects of FCCP concentration on photosynthesis and the efflux of sugars from wheat protoplasts are shown in Figure 5. FCCP was most inhibitory to photosynthesis by isolated protoplasts when added in the dark prior to illumination, as compared to addition after 10 min of photosynthesis. A similar effect of time of addition of dinitrophenol on inhibition of photosynthesis also was observed (data not shown). Concentrations of FCCP in excess of 0.5 μM were required to inhibit photosynthesis significantly when the uncoupler was added after 10 min illumination (Fig. 5). In contrast, the efflux of sugars from protoplasts was inhibited significantly by FCCP concentrations (added after 10 min photosynthesis) as low as 0.25 μM and about 50% inhibition was obtained at 0.5 μM FCCP (Fig. 5). Consequently, the effect of FCCP on sugar efflux was clearly not related to inhibition of photosynthesis.

Sugar efflux from both wheat and tobacco protoplasts was sensitive to mersalyl and insensitive to *p*-chloromercuriphenylsulfonic acid (Table II). Fifty per cent inhibition by mersalyl appeared to require concentrations of about 0.3 and 0.4 mM with wheat and tobacco protoplasts, respectively. At the concentrations tested, the sulfhydryl reagents did not affect photosynthetic rates.

Effect of KCl and Oligomycin on Photosynthetic Products. It was of interest to determine whether inhibitors of sugar efflux affected the products formed during $^{14}CO_2$ assimilation. The effect of oligomycin and KCl on the kinetics of ^{14}C incorporation into starch and the neutral fraction (mainly sucrose) with tobacco protoplasts is presented in Figure 6. In the control, labeling of neutrals and starch was relatively linear during the period tested. In the presence of oligomycin or KCl, a progressive reduction in labeling of the neutral fraction was observed after about 20 min

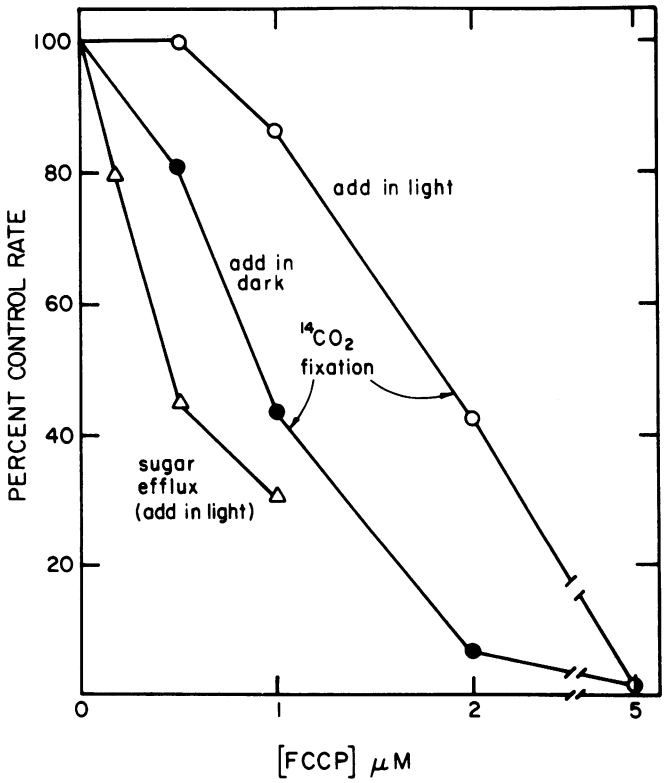

FIG. 5. Effect of FCCP concentration on photosynthetic rate and efflux of sugars from wheat protoplasts. The FCCP was added in the dark (●) or after 10 min of illumination (△, ○); the control rates were 80 μmol CO_2 fixed/mg Chl·h and 4.8 μmol hexose eqs effluxed/mg Chl·h, respectively.

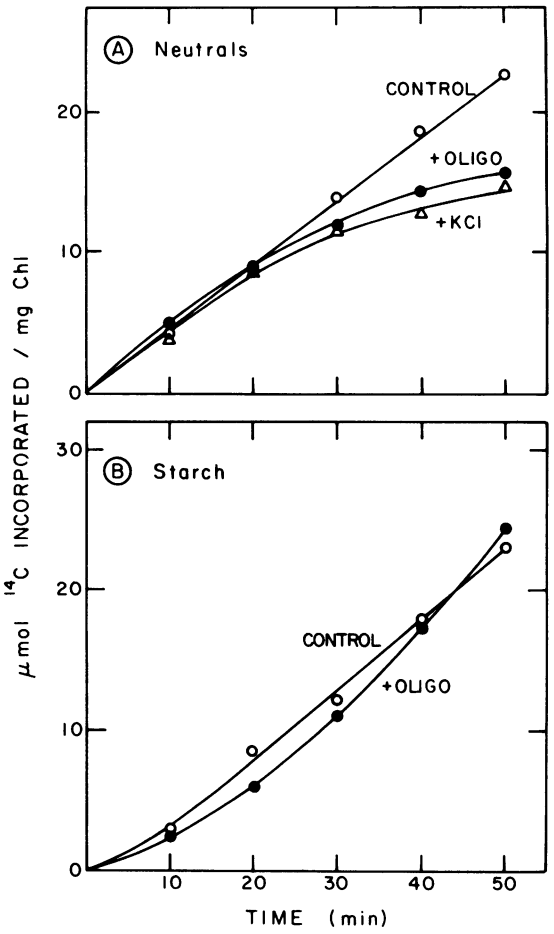

FIG. 6. Effect of 50 mM KCl and 5 μg/ml oligomycin (oligo) on incorporation of $^{14}CO_2$ into neutral fraction (A) and starch (B) by tobacco protoplasts. The rate of total $^{14}CO_2$ assimilation was about 90 μmol $^{14}CO_2$ fixed/mg Chl·h in the control and treatments.

Table III. *Effect of KCl and Oligomycin on Distribution of Labeled Products after 50 min $^{14}CO_2$ Assimilation by Tobacco Protoplasts*

Treatment	Soluble Fraction			Starch
	Acidic	Basic	Neutral	
	% total ^{14}C			*% total ^{14}C*
Control	13.0	19.0	32.1	35.9
+ 50 mM KCl	15.4	23.1	23.0	37.6
+ 5 μg/ml oligomycin	14.0	23.6	24.3	37.9

photosynthesis (Fig. 6A) and, concurrently, an increase was observed in the rate of ^{14}C-starch formation (Fig. 6B). The effect of KCl on starch labeling was similar to that produced by oligomycin but, for the sake of clarity, is not shown. Both KCl and oligomycin initially caused a slight decrease in starch labeling relative to the control. However, oligomycin and KCl increased the rate of ^{14}C-starch formation, measured between 40 and 50 min, 48 and 30%, respectively. After 50 min photosynthesis, the absolute amount of radioactivity in starch in the treatments exceeded that of the control. The decrease in incorporation of radioactivity into the neutral fraction in the presence of oligomycin or KCl was compensated by increased labeling of the acidic, basic, and insoluble (starch) fractions (Table III). Qualitatively similar results were obtained when sugar release was inhibited with mersalyl and when wheat protoplasts were used (data not shown).

Table IV. *Effect of Incubation pH on Rate of Sugar Efflux from Wheat and Tobacco Mesophyll Protoplasts*

Rates were calculated on the basis of sugars appearing in the medium between 15 and 45 min after illumination.

pH	Species	
	Wheat	Tobacco
	μmol hexose eq/mg Chl·30 min	
8.0	1.9	3.5
7.5	1.9	3.6
7.0	1.8	3.3
6.5	1.9	—[a]

[a] —, efflux was not determined because photosynthetic rates were significantly inhibited at this pH.

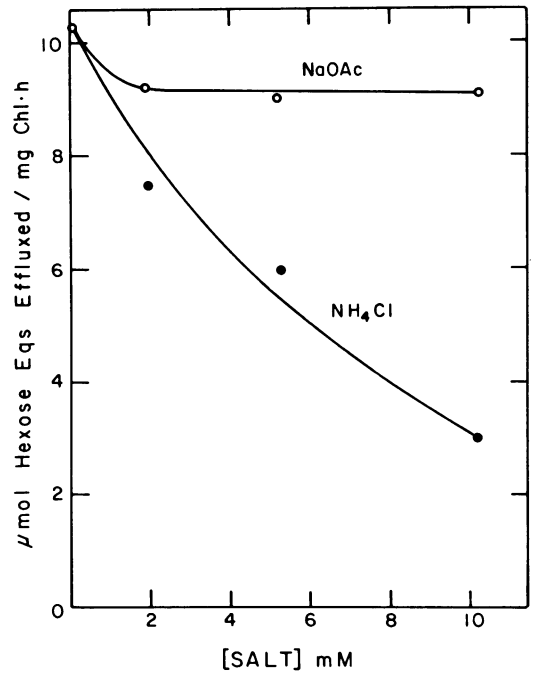

FIG. 7. Effects of NaOAc and NH_4Cl on the rate of sugar efflux from tobacco protoplasts.

Involvement of Proton Gradient. Decreasing the external pH from 8.0 to 6.5 did not significantly affect the rate of sugar efflux from wheat or tobacco protoplasts (Table IV) when photosynthetic rates were unaffected. Changing the external pH with a nonpermeant buffer would be expected to perturb the existing proton gradient across the plasmalemma (12). The relative constancy of sugar efflux suggested that transport was not coupled directly to movement of protons or hydroxyl ions.

The effects of the salt of a weak acid (NaOAc) and base (NH_4Cl) on sugar efflux from tobacco protoplasts at constant external pH (pH 8.0) are shown in Figure 7. Relatively low concentrations of NaOAc maximally inhibited sugar efflux by about 12%. In contrast, inhibition of efflux by NH_4Cl was concentration-dependent, with apparently about 6 mM NH_4Cl required for 50% inhibition. Qualitatively similar results were obtained with wheat protoplasts (data not shown).

Permeability Properties of Plasma Membrane. Photosynthesis by isolated wheat protoplasts was remarkably unaffected by exogenous materials that would be expected to affect photosynthesis if they permeated the plasma membrane. Table V shows that exogenous Pi and mannose had no significant effect on photosynthetic rate or the partitioning of fixed carbon between starch and sucrose. Exogenous glucosamine (20 mM) also had no effect (data not shown).

Table V. *Effects of Exogenous Pi and Mannose on $^{14}CO_2$ Fixation and End-product Formation by Wheat Protoplasts*

Protoplasts were preincubated in the dark at 25 C for 10 min with additions as indicated.

Pretreatment of Protoplasts	Additions to Assay Medium	$^{14}CO_2$ Fixation Rate	^{14}C Incorporation	
			Sucrose	Starch
		μmol/mg Chl·h	%	
Control	None	92	48	7.3
+ 15 mM Pi	+ 10 mM Pi	90	46	7.2
+ 20 mM Mannose	+ 10 mM Mannose	82	48	9.0

DISCUSSION

The sugar transport characterized herein involved two distinct steps: first, the internal generation of sugars by photosynthetic carbon assimilation and, second, the efflux of sugars across the plasma membrane. That the sugar efflux observed during photosynthesis by isolated protoplasts is not attributable to general leakiness of the plasma membrane is suggested by the following lines of evidence: (*a*) no release of hexose-P and proteins (19); (*b*) no loss of cytoplasmic Pi as evidenced by linear incorporation of $^{14}CO_2$ into sucrose (18, 19) and no release of internal Pi to the medium (D. W. Edgecomb and S. C. Huber, unpublished); (*c*) Internal sugar pools are constant as opposed to declining with time (19); (*d*) sugar efflux was not inhibited by divalent cations (Table I) which tend to stabilize membranes; and (*e*) sugar efflux was inhibited by a variety of treatments that would not be expected to reduce membrane leakiness (*e.g.* K^+, Rb^+, Li^+, FCCP, valinomycin, and oligomycin).

Further, retention of the semipermeability properties of the plasma membrane is supported by the following: (*a*) no effect of external Pi (up to 20 mM) on photosynthetic rate or the incorporation of $^{14}CO_2$ into starch and sucrose (Table V); (*b*) no effect of external mannose (Table V), which would be expected to reduce cytoplasmic Pi (17) if permeable to the plasma membrane, on photosynthetic products; and (*c*) no uptake of [^{3}H]leucine by pea protoplasts over short (60 min) time periods (20).

These lines of evidence suggest rather strongly that the plasma membrane retains at least some of its semipermeability properties with regard to both the uptake and release of many low molecular weight materials. Here, evidence was obtained that a significant portion of the sugar release occurred from photosynthesizing protoplasts (as opposed to possibly damaged, nonphotosynthetic protoplasts) because inhibition of sugar release with exogenous K^+ or oligomycin caused a shift in products to favor increased starch and decreased sucrose formation (Fig. 6). Inhibitors were also used here to probe the efflux mechanism at concentrations that did not significantly impair photosynthesis so that effects on transport could be separated from those on biosynthesis.

Sensitivity to mersalyl, a nonpermeant sulfhydryl reagent, suggested that the sugar efflux involved membrane proteins with essential sulfhydryl groups accessible to the medium. Inhibition of sugar efflux by oligomycin (Fig. 4; Table II) and the uncoupler FCCP (Fig. 4) suggested that transport required an energized membrane. Oligomycin and uncouplers also have been shown to inhibit rapidly the influx of inorganic ions into protoplasts from tobacco suspension cells (29) and to depolarize the membrane potential of oat leaf protoplasts (34). It is not clear whether the mechanism of oligomycin action involved inhibition of the plasmalemma or mitochondrial ATPase. Balke and Hodges (2) reported that oligomycin inhibited ion absorption in oat roots and

showed that oligomycin preferentially inhibited the mitochondrial ATPase. The results were taken to indicate that oligomycin acted by inhibiting ATP production rather than utilization. The relative sensitivity of the two ATPases in leaf protoplasts and, hence, the mechanism of oligomycin action is not known. The basis for inhibition by valinomycin in the absence of exogenous K^+ is also not understood. It might be expected that valinomycin would cause K^+ release from the protoplasts, which would reduce sugar efflux (see below). Such a release was not observed (Fig. 4B). Valinomycin may affect the intraprotoplast K^+ pools or the cytoplasmic adenylate pools as a result of its uncoupling action on plant mitochondria (15). Further experimentation will be required to determine the basis for inhibition of sugar efflux by valinomycin and oligomycin.

Involvement of a sugar·H^+ symport in the transport across the plasma membrane seems unlikely. Salts of weak acids and bases can perturb proton gradients across membranes at fixed external pH (3, 8, 25). The observed sugar efflux was not significantly affected by decreasing the pH of the medium from 8.0 to 6.5 or by adding NaOAc. Sodium acetate would be expected to decrease internal pH as a result of internal dissociation of HOAc forming $H^+ + OAc^-$. Exogenous NH_4Cl, in contrast, would increase internal pH (3, 25) by equilibration of NH_3 across the plasma membrane and internal hydrolysis to form $NH_4^+ + OH^-$. Sugar efflux should be affected in opposite directions by NaOAc and NH_4Cl if proton movement (symport or antiport) was involved. This was not observed (Fig. 7), which indicated that protons, and the proton gradient, were probably not involved in transport. In systems that are thought to involve a solute·H^+ symport, transport is very sensitive to changes in external pH (7, 12, 28, 31). The concentration-dependent inhibition of sugar efflux caused by NH_4Cl may be related to its action as an uncoupler of photophosphorylation or to depolarization of the plasma membrane by increasing internal pH (25) rather than by altering the pH gradient across the plasmalemma.

Several lines of evidence were obtained, however, for a sugar·K^+ symport: (*a*) an efflux of K^+ from protoplasts was observed that was similar in magnitude to the sugar efflux (Fig. 1); (*b*) exogenous KCl and RbCl, but not NaCl or CsCl, inhibited the sugar efflux (Figs. 2 and 3); and (*c*) the K^+ and sugar effluxes were linked, inasmuch as inhibitors that reduced sugar efflux also blocked the K^+ release (Fig. 4). Taken together, these observations suggested that the effects of exogenous K^+ could not be entirely ascribed to depolarization of the membrane potential (34, 36), although depolarization may be partially responsible. Depolarization and, hence, inhibition might also be expected by Na^+, which was not observed. Inhibition of sugar release by Li^+ may be related to interference with other ion fluxes across the plasma membrane (21). Li^+ was probably not entering the protoplasts at a significant rate because Li^+ is very inhibitory to photosynthesis by isolated chloroplasts (S. C. Huber, unpublished), but protoplast photosynthetic rate was unaffected.

Inhibition of sugar efflux by KCl, LiCl, or oligomycin altered the distribution of photosynthetic end products as would be expected towards increased starch and decreased sucrose formation (Fig. 6). The situation appears analogous to whole-plant systems which accumulate starch when translocation of photosynthate (sucrose) from the leaf is reduced. It is clear from whole-leaf measurements that the photosynthetic rate continues unchanged (for up to several days) even though translocation is severely reduced (9). Similarly, protoplast photosynthetic rates were unaffected (at least over the periods tested) when sugar efflux and hence product distribution, was altered. It remains to be established how sucrose accumulation in the cytosol affects photosynthetic carbon metabolism.

The large decrease in sugar labeling in the presence of KCl or oligomycin was compensated by increased labeling of starch (Fig. 6B), but also of amino acids and, to a lesser extent, of organic acids (Table III). Isolated protoplasts apparently contain an endogenous nitrate pool which can be utilized for photosynthetic formation of amino acids (32). Formation of amino acids has been shown to occur (in the presence of exogenous NH_4^+) at the expense of sucrose formation (30). On the basis of steady state labeling patterns, Platt *et al.* (30) concluded that ammonia activated pyruvate kinase and P-enolpyruvate carboxylase (and possibly malic enzyme) but inhibited sucrose-P synthetase. The results here suggested that reduced sucrose formation (by end-product inhibition) produced the same qualitative changes as addition of NH_4^+ and may be explained by a mass action effect of increased α-keto acids for ammonia incorporation. Formation of α-keto acids (oxaloacetate, α-ketoglutarate, pyruvate) probably occurs in the cytosol (13), and thus Pi exchange across the chloroplast envelope would still occur even though sucrose formation was reduced. Hence, a smaller effect on starch formation [which reflects the rate of Pi exchange across the envelope (14)] relative to sucrose formation might be expected.

A possible mechanism for sugar transport across the plasma membrane involves a mersalyl-sensitive carrier that catalyzes a sugar·K^+ symport. The transport depends on the transmembrane K^+ gradient and is not energy-linked *per se*. However, based on the sensitivity of sugar efflux to FCCP and oligomycin, it is further postulated that the carrier undergoes an energy-dependent change, perhaps in conformation, that allows transport only when the membrane is in the energized state. Energy-dependent changes in transport have been postulated previously to explain immobilization of sugar transport systems in yeast (27) and *Chlorella* (26) when the membrane is depolarized. It is postulated that the plasmalemma ATPase maintains the energized state of the membrane (25, 36) and, thus, maintains the sugar transport system in a mobile condition. *In vivo*, the ATPase may also be involved in uptake and, hence, recycling of K^+ (29). The precise stoichiometry of K^+ and sugar release is not known. All of the K^+ released was probably not associated with sugar efflux, which makes quantitative comparisons difficult. However, the K^+ release was at least equivalent to the sugar release, which makes the postulated symport mechanism plausible.

Other transport systems in plants have been described which may involve K^+ movement. In *Ricinus* petioles, Malek and Baker (28) showed that high external K^+ stimulated sucrose uptake into the phloem. Recently, Van Bel and Van Erven (37) showed that at high pH ($\geq$5.5), sucrose uptake into internode disks of tomato was accompanied by an influx of K^+, and they proposed a protonated/"potassionated" sucrose carrier. However, there are also reports in the literature which seemingly contradict our findings that external K^+ inhibits the release of sugars from protoplasts. Servaites and Schrader (35) reported that 50 mM K^+ stimulated slightly the release of ^{14}C-labeled metabolites from soybean mesophyll cells. Also, Hawker *et al.* (16), using leaf discs, and Doman and Geiger (4), using abraded attached leaves, reported a K^+ stimulation of sucrose release from the tissue. The reason for the discrepancy between our results and those cited above is not known but the discrepancy may reflect the lack of a cell wall and spatial cellular arrangements that exist in more "intact" systems. However, Doman and Geiger (4) also reported that, with a nontranslocating system of abraded leaf discs, release of sugars labeled by $^{14}CO_2$ assimilation was stimulated by low external KCl (15 mM) but, occasionally, inhibited by high (50 mM) external KCl. The protoplast system may be more analogous to this nontranslocating disc system.

It is thought that sugar is released from the mesophyll cells to the leaf free space *in vivo* only in the immediate vicinity of the phloem (10, 11). Our results with isolated protoplasts suggested that the majority of the mesophyll cells may have the capacity to transport sucrose (19) and that the external medium represents a

large "sink" for photosynthetic end products. Sucrose transport capability may be used only when intercellular connections are absent, as they are *in vitro*. The relation between the mechanism postulated to occur *in vitro* and the sucrose release that occurs in certain cells *in situ* remains to be established. As is the case *in vivo*, transport of sugars across the plasmalemma of isolated protoplasts is dependent on photosynthesis and the rate of transport can influence the partitioning of carbon among end products.

Acknowledgments—The authors thank W. P. Novitzky, D. W. Edgecomb, Jr., and F. S. Farmer for technical assistance and Dr. W. Robarge for help with the atomic absorption measurements.

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