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Assimilate Unloading from Maize (*Zea mays* L.) Pedicel Tissues[1]

II. EFFECTS OF CHEMICAL AGENTS ON SUGAR, AMINO ACID, AND ^{14}C-ASSIMILATE UNLOADING

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ABSTRACT

Sugar, amino acid, and ^{14}C-assimilate release from attached maize (*Zea mays* L.) pedicels was studied following treatment with several chemical inhibitors. In the absence of these agents, sugar release was nearly linear over a 7-hour period. At least 13 amino acids were released with glutamine comprising over 30% of the total. Release was not affected by potassium concentration, 10-minute pretreatments with *p*-chloromercuribenzene sulfonic acid (PCMBS) or dithiothreitol, and low concentrations of $CaCl_2$. Three hours or more exposure to PCMBS, dinitrophenol, *N*-ethylmaleimide, or 2,4,6-trinitrobenzene sulfonic acid strongly inhibited ^{14}C-assimilate, sugar, and amino acid release from the pedicel. These treatments also reduced ^{14}C-assimilate movement into the kernel bases. It is, therefore, likely that reduced unloading, caused by these relatively long-term exposures to chemical inhibitors, was related to reduced translocation of assimilates into treated kernels. Whether this effect is due to disruption of kernel metabolism and sieve element function or reduced assimilate unloading and subsequent accumulation of unlabeled assimilates within the pedicel tissues cannot be determined at this time.

Assimilate unloading processes are difficult to understand because unloading mechanisms apparently differ among crop species and among the various sink tissues within a plant (21). In growth sinks, such as root tips and expanding leaves, there is evidence that unloading is via the symplast (5, 19). Within stem tissues, unloading occurs into the apoplast from which assimilates may be taken up for storage or reloaded into the phloem (7, 11). In reproductive sinks, such as legume seeds, unloading occurs from the maternal seed coat tissue into the apoplast which surrounds the developing embryo (21). Assimilates must then be taken up into the embryo. Since no vascular or plasmodesmatal connections exist between the maternal tissues and the developing endosperm cells in maize, a similar assimilate pathway to that of legumes must be followed in maize kernels (3, 20). We conclude from available evidence (3, 12, 15, 16, 20) that sugars move passively from the pedicel symplast of maize as sucrose and are then hydrolyzed to monosaccharides within the apoplast.

Wolswinkel and Ammerlaan (24) noted that sink tissues may utilize energy during unloading from the sieve elements, uptake into storage cells, or for biosynthetic and respiratory transformations. In this regard, the use of metabolic inhibitors and other chemical agents can enhance our understanding of transport processes within sink tissues. Assimilate unloading from legume seed coats and the maize pedicel has been characterized recently using a procedure known as the 'empty seed' technique (13–16, 21, 22, 24–28). Using this technique, the embryo and endosperm, if present, are surgically removed and replaced with a suitable medium for trapping unloaded assimilates (21, 22). Assimilate unloading from seed-coat tissues of French bean (13), soybean (22), pea (28), and broad bean (24) is believed to be an energy-dependent process and is markedly inhibited by sulfhydryl reagents. Sugar and amino acid unloading from legume seedcoats is also sensitive to metabolic inhibitors (13, 22, 24, 28); however, the active site of these inhibitors is difficult to establish because chemical inhibitors induce many secondary effects (4). In contrast to results obtained for legumes, Porter *et al.* (15) demonstrated that sugar efflux from the maternal tissues of attached maize kernels is not inhibited by short-term exposure to PCMBS[3] or other metabolic inhibitors.

The studies reported herein were conducted to further characterize assimilate unloading from the maternal tissue of the maize kernel. Specifically, we studied the sensitivity of the unloading process to various chemical agents and established the usefulness of the present technique for studying amino acid unloading from the pedicel tissues.

MATERIALS AND METHODS

Plant Material. Plants of the dent maize (*Zea mays* L.) DeKalb hybrid 'XL55' were grown in a greenhouse at University Park, PA. Plant culture and experimental conditions for these greenhouse studies have been described (15). Plants which were 21 or 22 d postpollination were utilized for all experiments. Kernels from these plants were in the linear phase of dry matter accumulation and had attained less than 50% of their final dry weight. Kernel water content ranged from 55 to 65% by weight. The experiments reported herein were initiated between 9:00 and 11:00 AM local time.

Agar Efflux Studies. Kernels on intact maize ears were pre-

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[3] Abbreviations: PCMBS, *p*-chloromercuribenzene sulfonic acid; DNP, 2,4-dinitrophenol; CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; MOPS, morpholinopropane sulfonic acid; NEM, *N*-ethylmaleimide; TNBS, 2,4,6-trinitrobenzene sulfonic acid; KIDA, potassium iminodiacetic acid; THF, Tetrahydrofuran.

pared from assimilate transport and unloading studies as described previously (15). Briefly, the distal halves of kernels were removed with a scalpel and the endosperm tissue was subsequently removed with a sharpened spatula such that each kernel base formed a cup which remained attached to the ear. These pedicel cups consist of pericarp, vascular, and pedicel parenchyma tissues so that assimilates from the source regions of the plant continue to enter the pedicel normally during the course of an experiment. Each pedicel cup was briefly rinsed with water and the excess water was removed with a micropipette. The pedicel cups were then filled for 10-min with 70 μl of pretreatment solution. All pretreatment solutions were made up in 20 mM MOPS (pH 7), 0.1 mM $CaCl_2$, except that in some experiments the $CaCl_2$ concentration was varied to study the effects of $CaCl_2$ on unloading. Following pretreatment, the excess solution was withdrawn with a micropipette and replaced with 70 μl of 1% purified agar (Oxoid No. 3; Consolidated Laboratories, Inc., Chicago Heights, IL) made up in similar buffer, which also contained 0.1 mM PCMBS in experiments studying sugar unloading rather than ^{14}C-assimilate unloading. This low concentration of PCMBS was added to the agar to inhibit invertase activity in the agar traps. In several experiments, chemical inhibitors were applied to the cup for a 10-min pretreatment period, while in other experiments the long-term effect of exposure to these inhibitors was studied by also including the inhibitors within the agar collection media. All PCMBS used in these experiments was purified in a column packed with Chelex 100 ion exchange resin (Bio-Rad Laboratories). Assimilates were allowed to accumulate in the agar solute traps for varying time periods and then the agar traps were removed and stored as previously described (15). When experiments did not involve the use of $^{14}CO_2$, sugars were extracted from the agar solute traps and quantified using the methods described by Porter *et al.* (15).

^{14}C Tracer Experiments. Plants were prepared as described above, so that agar-filled kernel bases were in place about 1 h prior to labeling. A pulse of $^{14}CO_2$ was applied to the ear leaf, and often simultaneously to the leaf above the ear, using the technique described by Porter *et al.* (15). Unless stated otherwise, agar traps and kernel bases were sampled 180 min after labeling. Kernel bases were oxidized and prepared for liquid scintillation counting as previously described (15). The agar solute traps were dropped into plastic minivials containing 5 ml of Scinti Verse I (Fisher Scientific, Pittsburgh, PA) cocktail. These vials were allowed to stand at room temperature for approximately 48 h and were shaken every 2 to 3 h during the day. Prior to counting, 1.8 ml H_2O was added to produce a uniform gel phase. Counting was conducted in the gel phase on a Beckman LS-8000 liquid scintillation counter.

Sugar and Amino Acid Determinations. Sugars were extracted from the agar solute traps and the reducing sugars and sucrose present were quantified as previously described (15). Amino acids were similarly extracted and total α-amino acid content was measured using the modified ninhydrin technique of Rosen (18) with glutamine as the standard.

Amino acid separation was conducted on several samples using the microcomputer controlled HPLC system described by Gustine and McCulloch (8). Amino acids were derivatized with *o*-phthalaldehyde (Fluoropa, Pierce Chemical Co., Rockford, IL) and separated on a reversed phase system as described previously (8) except that final conditions of 75% THF were maintained for 10 min to allow elution of lysine. Detection was by measurement of fluorescence at 425 nm (excitation at 339 nm). Peak identities were established using internal authentic amino acid standards. Glutamine content of the samples was determined by peak integration (8) with glutamine as the standard.

RESULTS AND DISCUSSION

Response to PCMBS. Thorne and Rainbird (22) demonstrated that the slowly penetrating sulfhydryl group inhibitor, PCMBS, inhibited ^{14}C-assimilate unloading from soybean seed coats by 75%, while no inhibition of translocation into the seed coat tissue was observed. Their procedure utilized the empty-seed technique with a 10-min, 2.5 mM PCMBS pretreatment followed by a 10-min wash and assimilate collection in agar traps for 2 h. Using very similar treatment techniques, but measuring sugar unloading rather than ^{14}C-assimilate unloading, Porter *et al.* (15) observed no inhibition of unloading from maize pedicel tissues. Similarly, more recent studies on soybean seed coats indicated that treatment for up to 20 min with 5 mM PCMBS did not affect steady state rates of sucrose import or export (6). Since sugar collection in maize could be influenced somewhat by sugars already present in the pedicel free space, a ^{14}C-tracer experiment was conducted to confirm the observations presented in our earlier paper (15). A 10-min PCMBS pretreatment clearly did not influence the amount of ^{14}C-assimilate transported into the kernel base or unloaded from the maize pedicel into the agar (Table I). Pedicel tissues unloaded ^{14}C-assimilates regardless of whether the tissue was washed with buffer or buffer containing 25 mM DTT (Table I), a disulfide reducing agent. These data support our earlier results (15) and provide further evidence that assimilate unloading from maize pedicel tissues is not mediated by an exposed sulfhydryl-containing carrier protein. Gifford and Thorne (6) recently observed that DTT can have an inhibitory or a stimulatory effect on sucrose efflux from soybean seed coats, depending on tissue conditions.

Long-Term Exposure to PCMBS. Inclusion of PCMBS in the agar during the 3-h chase period significantly decreased ^{14}C-assimilate content within the agar trap and kernel base (Table I). This apparent inhibition of translocation resulting from 'long-term' exposure to PCMBS may have been caused by slow penetration of PCMBS into the maize pedicel cells and subsequent disruption of metabolism and phloem transport. Alternatively, inhibition of sugar unloading from the pedicel cells could have increased accumulation of unlabeled assimilates within the pedicel prior to arrival of the ^{14}C-pulse. Such an accumulation of unlabeled assimilates would probably decrease sink strength and total ^{14}C-assimilate transport into the treated kernels. In support of the former suggestion, long exposure times to PCMBS also have been reported to inhibit both ^{14}C-import and unloading within pea seed coats (28). Using excised maize pedicels and bathing these pedicels in buffer solutions containing 0 to 5 mM PCMBS, Orr (12) observed no inhibition of sugar efflux from the pedicels as PCMBS rates were increased. Unfortunately, exposure to PCMBS in these studies was for only 45 min. Longer exposures to PCMBS using excised pedicels are needed to determine the mechanism by which long-term PCMBS exposure affects ^{14}C-assimilate transport and unloading in intact systems. A similar more detailed approach using excised broad bean and pea seed coats previously loaded with ^{14}C-assimilates was used by Wolswinkel *et al.* (24, 28). Detailed studies such as these but using maize pedicels are needed to separate the above alternative mechanisms for inhibition of transport caused by long-term exposure to PCMBS. Consistent with data reported by Porter *et al.* (15), the information presented herein indicates that the mechanism for assimilate unloading in maize kernels is different from that of soybean. A 3-h exposure of maize pedicels to PCMBS (Table I) did not decrease ^{14}C-assimilate unloading to the same extent as was reported for 10-min treatments with soybean seed coats (22). In a more recent report (6), continuous exposure to 0.5 mM PCMBS or frequent applications of 5 mM PCMBS were required to inhibit steady state sucrose efflux from soybean seed coats. Long exposure times to PCMBS also have been reported to inhibit both ^{14}C-import and unloading within

Table I. *Effect of 10-Minute Pretreatment with PCMBS on ^{14}C-Assimilate Accumulation in Maize Pedicels and Pedicel Cups during a 180-min Chase Period following Exposure of the Ear Leaf to $^{14}CO_2$*

Following a brief water wash, the appropriate solutions were added to pedicel cups. After 10 min, the residual solution was removed and buffer ± DTT was added to the cup as a wash. Again after 10 min, the solution was removed and replaced with 70 μl of 1% agar containing 20 mM MOPS (pH 7), 0.1 mM $CaCl_2$. No buffer wash was employed where the agar traps contained 5 mM PCMBS during the 3-h chase period (long exposure). The ear leaf of a plant was exposed to 100 μCi $^{14}CO_2$ approximately 1 h after treatments were imposed. Values are means of five replications. Greenhouse temperatures ranged from 19 to 24°C during the experiment.

PCMBS Pretreatment in 20 mM MOPS	Buffer Wash Treatment	^{14}C Assimilate Content 180 min after $^{14}CO_2$ Exposure			
		In agar		In kernel base	
mM	*±25 mM DTT*	*dpm/kernel*	*% of control*	*dpm/kernel*	*% of control*
		Agar without PCMBS			
0	−	29900	100	25900	100
	+	31500	105	27000	104
2.5	−	28500	95	28800	112
	+	26300	88	23700	92
5.0	−	28600	96	26700	103
	+	29300	98	22400	87
		Agar with 5 mM PCMBS			
5.0		14700	49	17400	67
Waller-Duncan LSD K = 100		6700		6800	

pea seed coats (28).

Response to Metabolic Inhibitors. Compounds such as KCN, NaF, DNP, CCCP, and NaN_3 markedly inhibit transport of labeled assimilates into legume seeds and may also inhibit assimilate unloading (6, 13, 22, 24, 28). Thorne and Rainbird (22) treated empty soybean seed coats with agar collection traps containing 1 mM DNP, 5 mM NaF, or 5 mM $NaAsO_2$ and noted that essentially no ^{14}C-assimilate moved into the treated seed coats within 2 h. NaN_3 effectively inhibited both ^3H- and ^{14}C-transport into and unloading from the seedcoats of *Vicia faba* while CCCP affected only ^3H- and ^{14}C-assimilate unloading (24). Conversely, experiments reported by Porter *et al.* (15) indicated that sugar unloading from maize pedicel tissues was not inhibited by NaF, CCCP, $HgCl_2$, NaN_3, or DNP. Several possible explanations exist for the observed differences in response to rapidly penetrating inhibitors in our previous study (15) when compared to the work of others (22, 24). Perhaps measurement of sugar unloading in our studies rather than ^{14}C-assimilate unloading obscured unloading inhibition by these chemicals. This seems unlikely since Wolswinkel and Ammerlaan (24) and Wolswinkel *et al.* (28) observed similar inhibition patterns regardless of whether the experiments measured labeled assimilates or total sugars. Another possibility is that the exposure time of maize pedicel tissues to the inhibitors was 10 min in the experiments reported by Porter *et al.* (15), while in some experiments reported by Wolswinkel and Ammerlaan (24), Thorne and Rainbird (22), and Wolswinkel *et al.* (28), exposure was for 2 to 24 h. While the 10-min pretreatments were apparently adequate for introducing substances, such as PCMBS, which presumably had a direct effect on exposed carrier proteins, these short-term treatments may not have allowed penetration of the chemicals into tissues whose roles are not directly linked to unloading (*i.e.* sieve elements embedded in the tissue). A third possible explanation for the observed lack of inhibition by these agents is that the maize pedicel tissues are completely insensitive to the chemicals tested.

Several experiments were conducted to test these alternative hypotheses and to further characterize the unloading process within the maize pedicel. Pedicel cups were treated for 10 min with various chemical agents and then sugar unloading into agar traps containing the same inhibitors was measured over a 7-h period (Fig. 1). Sugar unloading from buffer-treated controls continued at a constant rate for the final 6 h of the collection period. Sugar accumulation in agar was significantly decreased by the penetrating sulfhydryl reagent, NEM, and by the penetrating respiratory uncoupler, DNP, within 1 h of treatment. These results are consistent with results of similar experiments using NaF, $NaAsO_2$, and DNP on soybean seed coats (22) and using CCCP, NaN_3, PCMBS, and low temperatures on broad bean seed coats (24). Long-term exposure to 5 mM PCMBS resulted in a 50% reduction in sugar collecting within the agar traps (Fig. 1). This inhibition is consistent with data for ^{14}C-

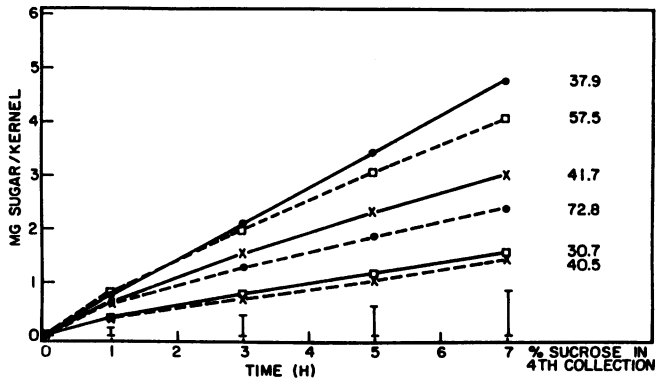

FIG. 1. Time course of sugar unloading from maize pedicel cups following 10-min exposure to PCMBS and long-term exposure to PCMBS and several other chemical agents. Pedicel cups were treated for 10-min with buffer (●——●) or 5 mM PCMBS (□- - -□) in buffer (20 mM MOPS [pH 7], 0.1 mM $CaCl_2$) and then sugar was collected in 70 μl 1% buffered agar (same buffer plus 0.1 mM PCMBS). Agar was replaced at 1, 3, 5, and 7 h after pretreatment. Long-term treatments with 5 mM TNBS (×——×), PCMBS (●- - -●), DNP (□——□), or NEM (×- - -×) consisted of similar treatments plus 5 mM inhibitor was included in the agar for the entire 7-h sampling period. Values represent means of four replications with three kernels per replication. Vertical bars indicate Waller-Duncan LSD K=100 for total unloaded sugars collected up to each sample time. Percentage sucrose is based on moles of total sugar. Greenhouse temperature ranged from 21 to 22°C during the experiment.

assimilate unloading presented in Table I. TNBS, a relatively nonpenetrating amino-reactive inhibitor resulted in 40% reduction in sugar collection following long-term exposure (Fig. 1). When PCMBS was applied for only a 10-min pretreatment period, sugar unloading was not significantly inhibited compared to buffer-treated controls. This lack of inhibition by 10-min pretreatment with PCMBS is consistent with our earlier reports (15) and with recent data for sucrose unloading in soybean seed coats (6), but is very different from data for ^{14}C-assimilate unloading in soybean (22).

Unloading *versus* Translocation. Several ^{14}C-pulse label experiments were conducted to determine if the effects of long-term NEM, PCMBS, TNBS, and DNP treatments were entirely on unloading or if translocation into the treated kernel base was also inhibited. This determination was made by comparing the ^{14}C-content of treated kernel bases and agar traps to those by buffer-treated controls. Three-h treatments with PCMBS, NEM, and TNBS substantially decreased the ^{14}C-assimilate content of treated kernel bases and also reduced ^{14}C-assimilate collection in the agar traps (Table II). In one experiment, 5 mM DNP was also included as a 3-h treatment. This treatment resulted in unloading rates which were only 11% those of buffer controls and ^{14}C-assimilate levels within the kernel base which were only 26% those of controls (data not shown). These data are in agreement with data presented for assimilate transport into soybean (6, 22) and other legume seeds (24, 28). Inhibition of ^{14}C-assimilate transport into the maize kernels in our studies may have been due to inhibition of unloading and subsequent internal assimilate accumulation or may have resulted from indirect effects of long-term exposure to the chemical agents used in these studies. We cannot directly distinguish between these two alternatives; however, Wolswinkel *et al.* (24, 28) used a combination of experiments such as ours and experiments using excised legume seedcoats to make this distinction. Clear separation of the above alternatives in the maize kernel system will require similar extensive studies using detached maize pedicels. Orr (12) studied sugar efflux from excised maize pedicels which were bathed in a buffer solution containing PCMBS. No inhibition of sugar efflux was observed over a 45-min exposure period. From our data and that of Orr (12), we suggest that the former alternative is unlikely and that indirect effects on translocation caused by long-term exposure to chemical agents probably reduced ^{14}C content of treated kernels in our studies. Longer exposure times of PCMBS and other inhibitors to excised maize pedicels will be necessary to conclusively evaluate this contention. Thorne (21) and Geiger and Sovonick (4) previously concluded that long exposure times to metabolic inhibitors at high concentrations can cause secondary effects on transport resulting from altered metabolism and disruption of membranes. Patrick (13) demonstrated that a 10-min pretreatment with NEM or CCCP reduced respiration in bean seed coats by about 50%, while PCMBS had no effect on respiration. Phloem transport can also be disrupted by metabolic inhibitors (4, 23).

The slight inhibition of ^{14}C unloading and transport by the 10-min PCMBS treatment reported in Table II was probably due to omission of the 10-min buffer wash following pretreatment in those experiments. When this wash was utilized, no inhibition by the 10-min pretreatment was observed (Table I). The results are, thus, consistent with the conclusion that sugar unloading from maize pedicel tissues is not directly inhibited by PCMBS.

Type of Sugar Unloaded. The only inhibitor which significantly increased the percentage of total sugar accumulating in the agar traps as sucrose was PCMBS (Fig. 1). Sucrose comprised 58% of the total sugars collected during the period from 5 to 7 h following the 10-min pretreatment. This observation, along with similar data from three previous collections in this experiment (data not shown), is in agreement with values near 60% obtained following similar treatments in past studies (15). Percentage sucrose collected during the period from 5 to 7 h after pretreatment increased to 73% following long-term exposure to PCMBS (Fig. 1). It appears likely that the 7-h exposure to PCMBS allowed enough penetration of PCMBS into the pedicel symplast to inhibit sucrose inversion prior to unloading. Alternatively, the longer treatment may have allowed PCMBS to access free space regions which were not accessed by the 10-min pretreatment. The marked inhibition of ^{14}C-assimilate transport into the kernels treated for long periods (Table I) and the lack of rate dependence observed using 10-min pretreatments (15) provides evidence in favor of the former explanation. However, data in Table III discussed below favor the latter alternative. Thus, it is likely that both PCMBS penetration into the symplast and increased inhibition of free space invertase occurred. It is noteworthy that even a 5- to 7-h exposure to PCMBS did not completely inhibit sucrose inversion (Fig. 1), and thus, it is possible that some sugar is unloaded from the pedicel symplast as monosaccharide.

When compared to the 10-min pretreatments, long-term exposure to PCMBS increased the percentage of sugars collected as sucrose, but reduced the amount of sugar unloaded (Fig. 1). An experiment utilizing a 30-min PCMBS pretreatment period was conducted to determine if the percentage of sugars collected as sucrose could be increased without concurrent inhibition of sugar unloading. A 30-min pretreatment with either buffer or 5 mM PCMBS resulted in increased unloading during the first 3-h

Table II. *Inhibition of ^{14}C-Assimilate Transport and Unloading in Maize Pedicel Cups following Long-Term Exposure to Several Chemical Agents*

Following a brief water wash, the appropriate pretreatment solutions were added. After 10 min, the residual solution was removed and the pedicel cups were filled with 70 μl of 1% agar 20 mM MOPS (pH 7), 0.1 mM $CaCl_2$. In some treatments (+) the agar also contained 5 mM inhibitor for the entire 3-h collection period. Values are means for combined data from two experiments with five replications per experiment. Greenhouse temperatures ranged from 20 to 23 and 27 to 29°C for the two experiments.

Pretreatment in 20 mM MOPS	Inhibitor Included in Agar	^{14}C Accumulated in 180 min after $^{14}CO_2$ Exposure			
		In agar		In kernel base	
5 mM	+/−	*dpm/kernel*	*% of control*	*dpm/kernel*	*% of control*
Buffer control	−	34700	100	32300	100
PCMBS	−	26800	77	28100	87
PCMBS	+	12100	35	15800	49
NEM	+	500	1	2000	6
TNBS	+	18900	54	21000	65
Waller-Duncan LSD K = 100		11300		11100	

Table III. *Effect of PCMBS Pretreatment and Pretreatment Duration on Sugar Unloading and Accumulation in Agar Traps*

Treatments were imposed as described in Table I except that pretreatment solutions consisted of buffer or 5 mM PCMBS in buffer for 10 or 30 min. Agar used in this experiment contained 0.1 mM PCMBS. Following the first 3-h collection period a repeat of the original pretreatment was imposed and fresh agar was applied to the cups. Percentage sucrose is based on moles of total sugar. Values are means of four replications. Contrasts were significant at P = 0.05 (*) and P = 0.01 (**), respectively. Greenhouse temperatures ranged from 25 to 27°C during the experiment.

Pretreatment		First 3-h Collection		Intermediate Pedicel Cup Treatment	Second 3-h Collection	
Time	Solution	Total sugar	Sucrose		Total sugar	Sucrose
min		*mg/kernel*	*%*		*mg/kernel*	*%*
10	Buffer	0.73	25.4	10 min, buffer	0.46	21.8
10	PCMBS, 5 mM	0.70	54.6	10 min, 5 mM PCMBS	0.38	65.4
30	Buffer	0.96	27.5	30 min, buffer	0.59	23.7
30	PCMBS, 5 mM	1.10	73.0	30 min, 5 mM PCMBS	0.50	76.4
Orthogonal contrasts						
Comparison of 10- *versus* 30-min pretreatment		*	**		NS	NS
Comparison of Buffer *versus* PCMBS pretreatment		NS	**		NS	**
Interaction: Pretreatment time × (buffer *versus* PCMBS)		NS	*		NS	NS

collection period when compared to a 10-min pretreatment (Table III). We have no satisfactory explanation for this phenomenon; however, increased unloading rates were not observed following the second 30-min pretreatment period. When the 30-min PCMBS pretreatment was utilized, 73% of the sugar collected was sucrose (Table III). About 55% of the sugar collected following the 10-min PCMBS pretreatment was sucrose. A second 10-min PCMBS pretreatment period superimposed on the original 10-min pretreated kernels resulted in an increase in percentage sucrose collected from about 55 to 65%. No corresponding increase in percentage of sugars collected as sucrose was observed when a second 30-min pretreatment period was superimposed on the original 30-min treated kernels. This indicates that the 30-min PCMBS pretreatment inactivated essentially all free space invertase, while unreacted invertase was still available following the original 10-min PCMBS pretreatment. It is noteworthy that the 10-min PCMBS pretreatment resulted in a percentage sucrose collection similar to past experiments with similar treatments (15, Fig. 1), while sucrose percentages for the 30-min PCMBS pretreatment were similar to those obtained following long-term 5- to 7-h exposure to PCMBS (Fig. 1). The similarity of percentage sucrose values for the 5- to 7-h treatment and 30-min pretreatment suggests that a 30-min pretreatment adequately inhibits essentially all invertase within the pedicel free space.

Effects of Ca^{2+} and K^+ on Unloading. Assimilate unloading from soybean seed coats has been stimulated, in some cases, by exogenous K^+ and by fusicoccin (6, 22). Thorne (21) noted that sugar unloading could be mediated by existing K^+ and H^+ gradients within the seed coat, but that sufficient evidence is lacking to conclusively support such an inference. Porter *et al.* (15) previously demonstrated that K^+ had no effect on unloading from maize pedicel tissues. Two experiments were conducted during the present study to confirm this observation. No significant changes in sugar unloading were observed over a 6-h exposure period when maize pedicel cups were treated with 0 to 100 mM KIDA (Table IV). Similarly, 100 mM KCl had no effect on sugar unloading. During the first 3-h collection period, the percentage of sugars collected as sucrose was slightly higher in the presence of 100 mM KIDA. This may have been an indirect effect due to sucrose leakage from sieve elements caused by KIDA-induced chelation of Ca^{2+}. A direct K^+ effect is unlikely since 100 mM KCl did not induce the same effect (Table IV). These data support the conclusion of Porter *et al.* (15) that sugar release from maize pedicel tissues is not affected by K^+. Similarly, Wolswinkel and Ammerlaan (24) concluded that K^+ does not mediate assimilate unloading from seed coats of *Vicia faba*.

Porter *et al.* (15) demonstrated that 10 mM Ca^{2+} strongly inhibited sugar unloading from maize pedicel tissues. Calcium also inhibited sucrose efflux from leaf discs of *Vicia faba* (1). Removal of Ca^{2+} by chelation with EGTA results in stimulation of ^{14}C-assimilate unloading from soybean seed coats (22) and sugar unloading from maize pedicel tissues (15). A 10-min pretreatment with 1 and 10 mM $CaCl_2$ reduced ^{14}C-assimilate levels in the kernel base and also decreased the collection of ^{14}C-assimilates within the agar solute traps (Fig. 2). Inhibition of unloading into the agar trap was much greater by 10 mM $CaCl_2$ than was inhibition of transport into the kernel base. The observed inhibition of ^{14}C-assimilate transport into kernel base tissue may have been a secondary effect caused by decreased unloading from the pedicel and subsequent accumulation of unlabeled assimilates within the pedicel prior to the arrival of the ^{14}C-assimilates. Alternatively, Ca^{2+} may have inhibited translocation into the kernels via indirect effects on metabolism or sieve element integrity. We favor the former explanation since the inhibition effects of 10 mM $CaCl_2$ on sugar unloading are largely reversed by 10-min pretreatments with EGTA (15) and since the role of Ca^{2+} at unloading sites has been primarily described as that of a divalent, apoplastic cation which binds to the plasmalemma and helps maintain membrane integrity (1, 15, 22, and references therein).

Thorne and Rainbird (22) reported that EGTA greatly increased the inhibitory effect of PCMBS on ^{14}C-transport and unloading within the soybean seedcoat. Although the precise mechanism for the response is unknown, it seems likely that membrane leakiness (15, 22) induced by EGTA, may have

Table IV. *Effect of Varying Potassium Concentrations on Sugar Unloading in Maize Pedicel Cups and Accumulation in Agar Traps*

Procedures were similar to those reported in Table III. Pretreatment solutions and agar contained KIDA or KCl. Following the first 3-h collection period, the pedicel cups were refilled with agar for the second 3-h collection. Percentage sucrose is based on moles of total sugar. Values are means for combined data from two experiments, four replications per experiment. Greenhouse temperature ranges were from 13 to 20 and 14 to 20°C for the two experiments.

Pretreatment in 20 mM MOPS	First 3-h Collection		Second 3-h Collection	
	Total sugar	Sucrose	Total sugar	Sucrose
	mg/kernel	*%*	*mg/kernel*	*%*
Buffer	0.92	27.6	0.66	22.2
KIDA, 5 mM	0.92	28.7	0.66	22.0
KIDA, 20 mM	0.98	29.9	0.77	21.5
KIDA, 50 mM	0.92	29.9	0.73	26.0
KIDA, 100 mM	1.04	32.5	0.84	24.0
KCl, 100 mM	0.94	29.8	0.76	26.6
Waller-Duncan LSD K = 100	NS	4.2	NS	NS

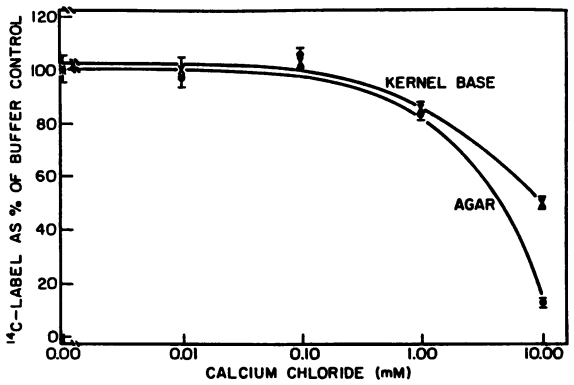

FIG. 2. Effect of pretreatment with $CaCl_2$ on relative ^{14}C-assimilate transport into and unloading from maize pedicel cups. Pedicel cups were treated for 10 min with buffer (20 mM MOPS, pH 7) containing various concentrations of $CaCl_2$, then were filled with 70 μl 1% agar in 20 mM MOPS (pH 7). About 1 h after treatment, the ear leaf of the treated plant was exposed to 100 μCi of $^{14}CO_2$ and samples were collected 240 min after exposure. Unloaded ^{14}C-assimilates and ^{14}C-content of the kernel base are expressed as percentage of the buffer control. Values represent means from four replications with three kernels per sample. Vertical bars represent 1 SE and the average ^{14}C-content of the buffer control was 29,300 dpm in the agar and 21,000 dpm per three kernel bases. Similar results were obtained in a separate sampling 180 min after exposure. Greenhouse temperature ranged from 26 to 27°C during the experiment.

allowed rapid penetration of PCMBS into the symplast from which it is normally excluded. In the present study, an experiment combining PCMBS and EGTA treatments did not result in increased inhibition of unloading by PCMBS (Table V). The treatment responses to $CaCl_2$ and EGTA were mostly similar in magnitude and reversibility to those reported previously in the absence of PCMBS (15). Stimulation of sugar unloading by EGTA in the presence of PCMBS (Table V) was less than that observed previously (15) suggesting that some interaction between EGTA and PCMBS may have occurred. Because the magnitude of such interaction is far less than that reported by Thorne and Rainbird (22), it seems likely that the assimilate transport and unloading system of maize pedicel tissues is simply far less sensitive to PCMBS than that reported for soybean. It should be pointed out that the experiment reported by Thorne and Rainbird (22) utilized ^{14}C-tracers, while sugar efflux was measured during the present study. It is possible that EGTA-induced passive sugar efflux from the pedicel cells may have masked any EGTA/PCMBS interactive effect on sugar transport. Percentage of total sugars collected as sucrose during the second 3-h collection period was lower for Ca^{2+}-PCMBS combinations than for EGTA plus PCMBS or PCMBS alone. While this observation is consistent with data presented for experiments without PCMBS (15), an explanation is not readily apparent. Possibly the effect of Ca^{2+} on membrane permeability is more specific to the sieve element membranes than those of the pedicel parenchyma cells.

Amino Acid Unloading. Arruda and DaSilva (2) collected a combination of xylem and phloem sap from the peduncle of the maize ear and determined that this vascular sap contained a complex mixture of at least 18 amino acids. Glutamine comprised 20 to 30% of total amino acids, depending on the age of the tissues. Lyznik *et al.* (10) reported that substantial amino acid pools exist within the pedicel region and that the dominant amino acids within this pool at 15 d postpollination were glutamine (37%), asparagine (16%), alanine (12%), and serine (8%). Since considerable differences in amino acid composition exist among the vascular sap, pedicel, and endosperm, and since available evidence indicates considerable potential for enzymic transformation of amino acids within the pedicel, it appears that the pedicel plays an important role in amino acid metabolism (9, 10). Based on anatomical and microautoradiographic evidence, sugars and nitrogenous compounds must be unloaded from the pedicel symplast and then taken up by the endosperm cells (3). The present agar trap technique could be useful for studying amino acid unloading from the pedicel tissue.

Amino acid unloading was inhibited approximately 50% by 10 mM $CaCl_2$, while removal of Ca^{2+} with EGTA stimulated unloading (Table VI). Presumably these effects were due to changes in permeability of the pedicel cells and sieve elements (15). After the first 3-h collection period, the reversibility of the effects of Ca^{2+} and EGTA was determined by superimposing treatments on the original treated kernels. Following EGTA with a second EGTA treatment resulted in continued stimulation of amino acid unloading, while following EGTA treatment with $CaCl_2$ resulted in the same unloading rate as was observed for pedicels treated twice with $CaCl_2$ (Table VI). Following $CaCl_2$ pretreatment with EGTA resulted in stimulation of amino acid unloading to the control rate. These patterns of inhibition and stimulation of amino acid unloading are identical to those observed for Ca^{2+} and EGTA effects on sugar unloading from the maize pedicel (15), suggesting that similar unloading systems may exist for both sugar and amino acids.

The addition of PCMBS to the 10 mM $CaCl_2$ pretreatment

Table V. *Effect of $CaCl_2$, EGTA, and PCMBS Combinations on Sugar Unloading in Maize Pedicel Cups and Accumulation in Agar Traps*

Procedures were similar to those outlined in Table III. After the first 3-h collection, cups were treated for 10 min with appropriate solutions prior to a second 3-h collection. Percentage sucrose is based on moles of total sugar. Values are means of four replications with 3 kernels per replication. Greenhouse temperatures ranged from 24 to 27°C during the experiment.

Pretreatment in 20 mM MOPS	First 3-h Collection		Intermediate Pedicel Cup Pretreatment	Second 3-h Collection	
	Total sugar	Sucrose		Total sugar	Sucrose
	mg/kernel	%		*mg/kernel*	%
Buffer	1.17	30.4	Buffer	1.05	25.8
PCMBS, 5 mM	1.14	55.3	PCMBS, 5 mM	0.77	67.2
PCMBS, 5 mM + $CaCl_2$, 10 mM	0.76	47.8	PCMBS, 5 mM + $CaCl_2$, 10 mM	0.35	45.3
PCMBS, 5 mM + $CaCl_2$, 10 mM	0.76	45.3	PCMBS, 5 mM + EGTA, 15 mM	1.16	68.1
PCMBS, 5 mM + EGTA, 15 mM	1.40	47.0	PCMBS, 5 mM + EGTA, 15 mM	1.43	61.5
PCMBS, 5 mM + EGTA, 15 mM	1.40	47.2	PCMBS, 5 mM + $CaCl_2$, 10 mM	0.33	40.0
Waller-Duncan LSD K = 100	0.40	11.5		0.17	6.6

Table VI. *Effect of $CaCl_2$ and EGTA on α-Amino Acid Unloading in Maize Pedicel Cups and Accumulation in Agar Traps*

Procedures were similar to those outlined in Table III. After the first 3-h collection, cups were treated for 10 min with appropriate solutions prior to a second 3-h collection. Glutamine was used as the standard for α-amino acid determinations. Values are means of four replications with three kernels per replication. Greenhouse temperature ranged from 21 to 24°C during the experiment.

Pedicel Cup Pretreatment	α-Amino Acid Unloading during the First 3-h Collection Period	Intermediate Treatment	α-Amino Acid Unloading during the Second 3-h Collection Period
	μmol gln equiv/kernel		*μmol gln equiv/kernel*
Water	0.75	Water	0.41
$CaCl_2$, 10 mM	0.34	EGTA, 15 mM	0.40
$CaCl_2$, 10 mM	0.36	$CaCl_2$, 10 mM	0.16
EGTA, 15 mM	1.01	$CaCl_2$, 100 mM	0.23
EGTA, 15 mM	0.94	EGTA, 15 mM	0.67
$CaCl_2$, 10 mM + PCMBS, 2.5 mM	0.30	$CaCl_2$, 10 mM + DTT, 25 mM	0.22
Waller-Duncan LSD K = 100	0.11		0.11

solution did not alter the inhibition of amino acid unloading observed in the presence of Ca^{2+} (Table VI). Likewise, a 10-min pretreatment with 5 mM PCMBS did not inhibit α-amino acid unloading over a 7-h period when compared to buffer-treated controls (Fig. 3). In both cases, amino acid unloading occurred at rates which decreased slightly with time. Seven-h exposure to PCMBS, TNBS, DNP, and NEM resulted in considerable inhibition of α-amino acid unloading (Fig. 3). Inhibition by long-term exposure to PCMBS and TNBS was similar in magnitude to that reported for inhibition of sugar unloading in the same experiment (Fig. 1). Conversely, sugar unloading was reduced 66% by long-term DNP and NEM treatment (Fig. 1), while α-amino acid unloading was reduced by about 40% (Fig. 3). While the magnitude of the effect of these inhibitors on α-amino acid unloading was somewhat different compared to that on sugar unloading, the similarity of inhibition patterns suggests a common unloading mechanism. Observations on amino acid and sugar unloading from pea and broad bean seed coats also indicate similar unloading mechanisms in these species (24, 25, 28).

Buffer-treated control samples from the experiment reported in Table VI produced peaks corresponding to 13 different amino acids (Fig. 4). This probably underestimates the number of amino acids present in the sample, because proline and cysteine would not be derivatized in our system. In addition, three of the peaks eluted with our solvent system probably contain two amino acids each (8). Nevertheless, it is clear that glutamine is the dominant amino acid unloaded from the pedicel. Calculations based on data presented in Figure 4 and other samples from the same experiment indicated that glutamine unloading occurred at a rate of 83 nmol $kernel^{-1}$ h^{-1}. Comparing this value to the total α-amino acid content of these samples (Table VI), indicates that 33% of the α-amino acids were unloaded as glutamine. This percentage of glutamine is similar to that reported for the pedicel region by Lyznik *et al.* (10), but is somewhat lower than estimates

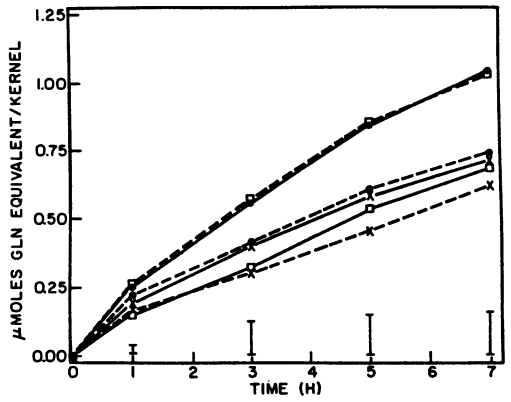

FIG. 3. Time course of α-amino acid unloading from maize pedicel cups following 10-min exposure to PCMBS and long-term exposure to PCMBS and several other chemical agents. Procedures are outlined in Figure 1. Pedicel cups were treated for 10 min with buffer (●——●) or 5 mM PCMBS in buffer (□- - -□) or for the entire 7-h collection period with 5 mM TNBS (×——×), PCMBS (●- - -●), DNP (□——□), or NEM (×- - -×). Values represent means of four replicates with three kernels per replication. Vertical bars indicate Waller-Duncan LSD K = 100 for total unloaded sugars collected up to each sample time. Greenhouse temperature ranged from 21 to 22°C during this experiment.

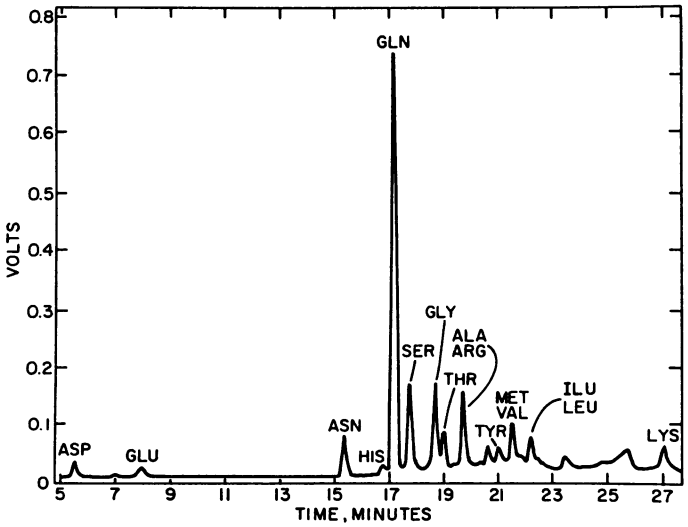

FIG. 4. Separation of amino acids unloaded in maize pedicel cups and collected in agar traps. Collection of amino acids was conducted as described in Table VI. Amino acids were derivatized with *o*-phthalaldehyde, injected onto a C_{18} column, and separated as described in "Materials and Methods." Peak identities were established using internal authentic amino acid standards.

near 50% reported more recently by Lyznik *et al.* (9). Glutamine is also the dominant amino acid constituent unloaded from soybean seed coats (17). Alanine is considered the most important amino acid constituent unloaded from broad bean seed coats, while alanine and glutamine predominate in pea (27).

Conclusion. Assimilate transport into and unloading from the maize pedicel have been further characterized by the studies reported herein. Both processes were readily inhibited by 3 h or more exposure to several chemical agents. It is likely that reduced unloading, caused by these relatively long-term exposures to chemical inhibitors, was related to reduced translocation of assimilates into treated kernels. Whether this effect is due to disruption of kernel metabolism and sieve element function or reduced assimilate unloading and subsequent accumulation of unlabeled assimilates within the pedicel tissues cannot be determined at this time.

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