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Intracellular Localization of Peptide Hydrolases in Wheat (*Triticum aestivum* L.) Leaves[1]

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ABSTRACT

Protoplasts from 8- to 9-day-old wheat (*Triticum aestivum* L.) leaves were used to isolate organelles which were examined for their contents of peptide hydrolase enzymes and, in the case of vacuoles, other acid hydrolases. High yields of intact chloroplasts were obtained using both equilibrium density gradient centrifugation and velocity sedimentation centrifugation on sucrose-sorbitol gradients. Aminopeptidase activity was found to be distributed, in approximately equal proportions, between the chloroplasts and cytoplasm. Leucyltyrosine dipeptidase was mainly found in the cytoplasm, although about 27% was associated with the chloroplasts. Vacuoles shown to be free from Cellulysin contamination contained all of the protoplast carboxypeptidase and hemoglobin-degrading activities. The acid hydrolases, phosphodiesterase, acid phosphatase, α-mannosidase, and β-*N*-acetylglucosamidase were found in the vacuole to varying degrees, but no β-glucosidase was localized in the vacuole.

Studies of protein degradation in plant tissues have demonstrated the existence of a diverse group of peptide hydrolase enzymes. Protein degradation has been envisaged as a process initiated by endopeptidase action and continued through the action of exopeptidases, including aminopeptidases, carboxypeptidase, and di- and tri-peptidases to eventually release amino acids for storage and/or transport (24). Characterization of these enzymes (5, 15, 22, 29) has shown that, despite their common role in protein breakdown, they have few similarities with respect to the conditions required for optimal *in vitro* activity. These differences, particularly differences in optimum assay pH, have led to suggestions of enzyme compartmentalization as a means of regulating protein degradation, since the plant cell contains a number of localized pH zones by virtue of its various organelles.

Although acid proteinases are known to be localized in leaf vacuoles (3, 11), no other peptide hydrolases have been reported there. Carboxypeptidase activity has been located in protein bodies from the cotyledons of germinating mung beans (7, 27) and in vacuoles prepared from the endosperm tissue of 4-d-old castor bean seedlings (18) but has not been reported in vacuoles isolated from leaf tissue. In a previous publication (30), we examined the patterns of activity of a number of enzymes during senescence of the wheat plant. The present study was undertaken to examine the spatial relationship between these enzymes, which include acid proteinase, amino- and carboxypeptidases, and an alkaline dipeptidase.

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MATERIALS AND METHODS

Plant Material. Wheat seeds (*Triticum aestivum* cv. Egret) were imbibed for 24 h prior to being transferred to 34- × 29- × 6-cm trays containing a compost soil mixture. Plants were grown in a glasshouse under natural daylength and light-intensity conditions. Plants were watered daily without additional nutrients.

Protoplast Preparation. Primary leaves from 8- to 9-d-old wheat seedlings were used to isolate protoplasts. The lower epidermis was removed, and the leaves were placed, with stripped surface downward, into 13.8-cm diameter Petri dishes with 10 ml of a 0.4 M sorbitol solution containing 1 mM K_2HPO_4 (pH 7.0, adjusted with concentrated HCl), 5 mM $MgCl_2$, and 1.5% (w/v) Cellulysin (Calbiochem). Sufficient wheat leaves were stripped to cover completely the surface of six Petri dishes. The dishes were placed, in the dark, in a gently shaking water bath for 16 h at 20°C. All subsequent procedures were performed at 4°C.

Protoplasts for vacuole isolation, density gradient centrifugation, and velocity sedimentation centrifugation were prepared using a modification of the procedure of Wallsgrove *et al.* (28). The protoplasts were harvested by gently swirling the incubation medium and decanting. Use of cheesecloth to separate the protoplasts from undigested material was found to cause some protoplast breakage. The undigested leaf material was rinsed twice with 0.4 M sorbitol solution containing 1 mM K_2HPO_4 (pH 7.0) and 5 mM $MgCl_2$. The rinsings were combined with the decanted medium and centrifuged at 200*g* for 5 min. The protoplast pellet was resuspended in 0.5 M sucrose solution containing 50 mM Hepes buffer adjusted to pH 7.5 with concentrated NaOH, 5 mM $MgCl_2$, 1 mM DTT, and 0.5% (w/v) soluble PVP. The resuspended material was made up to 24 ml with the resuspension medium and divided equally between four glass centrifuge tubes (100 × 16 mm). The protoplast suspension was overlaid by 2 ml of 0.3 M sucrose and 0.2 M sorbitol, followed by 2 ml of 0.5 M sorbitol. Both solutions were made up in 50 mM Hepes (pH 7.5) buffer containing 5 mM $MgCl_2$, 1 mM DTT, and 0.1% (w/v) soluble PVP (mol wt, 44,000). The tubes were centrifuged for 30 min at 200*g*, and the protoplasts were harvested from the 0.3 M sucrose + 0.2 M sorbitol-0.5 M sorbitol interface using a Pasteur pipette. The protoplasts were then washed by diluting to 25 ml with 0.5 M sorbitol solution containing 50 mM Hepes (pH 7.5), 5 mM $MgCl_2$, 1 mM DTT, and 0.1% (w/v) soluble PVP and centrifuged for 5 min at 200*g*.

Both DTT and soluble PVP were included for the preparation of protoplasts for subsequent vacuole isolation, in order to minimize vacuole clumping. In the equilibrium density gradient centrifugation experiments, PVP was included to minimize chloroplast clumping. DTT was not included in the velocity sedimentation separation, inasmuch as it was found to interfere with the mitochondrial marker, Cyt *c* oxidase, in the cytoplasmic fractions (originating from mitochondrial breakage).

Equilibrium Density Gradient Centrifugation. After washing,

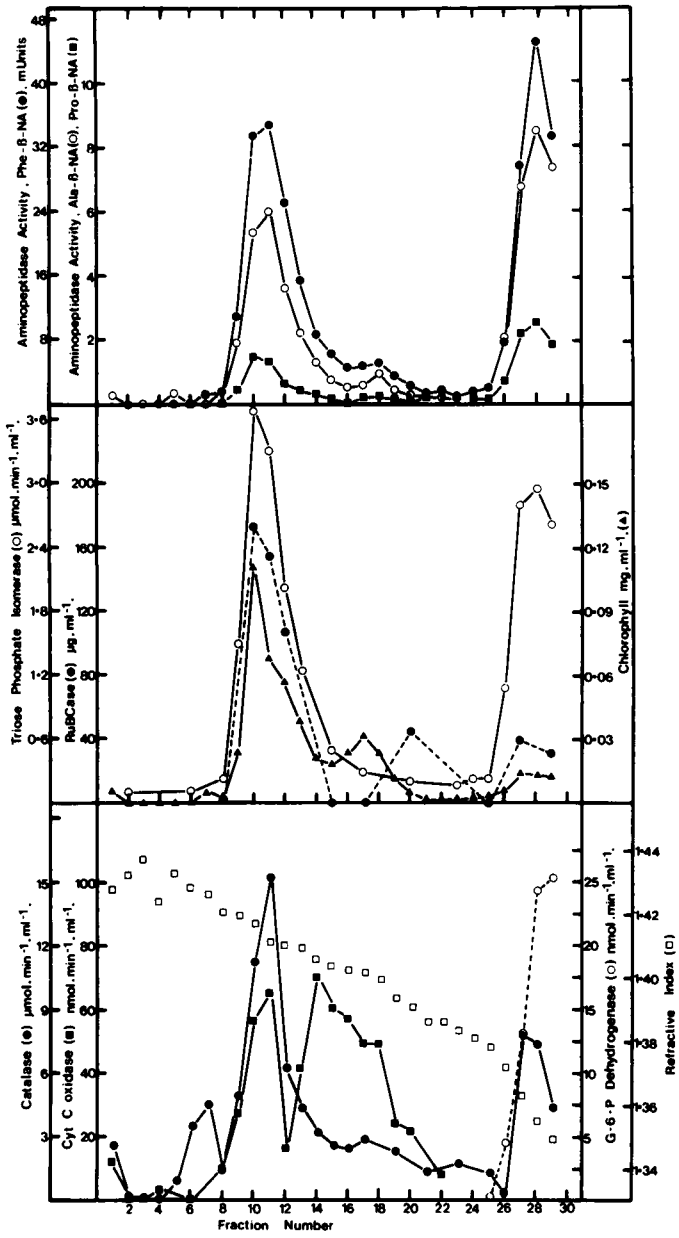

FIG. 1. Distribution of aminopeptidase activity and organelle-marker enzymes following equilibrium density gradient centrifugation of wheat leaf protoplasts. Ruptured protoplasts (4 ml) were loaded onto a sucrose gradient, as described in the text, centrifuged at 90,000*g* for 3 h, and fractionated into 3.3-ml fractions. Top, Aminopeptidase activity against Ala-β-NA (○); Phe-β-NA (●); and Pro-β-NA (■). Middle, Triose-P isomerase (○); RuBPCase protein (●); and chlorophyll (▲). Bottom, catalase (●); Cyt *c* oxidase (■); glucose-6-P dehydrogenase (○); and refractive index (□).

protoplasts were resuspended in 9 ml of 0.3 M sorbitol containing 0.1 M K_2HPO_4 (pH 7.5), 10 mM DTT, 50 mM Hepes, and 5 mM $MgCl_2$. Protoplasts were broken by first shaking gently in a 28°C water bath for 5 min, cooled to 4°C, and passed once through a 20-μm nylon mesh (2-cm diameter orifice). The ruptured protoplasts were mixed with 3 ml BSA and dissolved in the sorbitol-Hepes resuspension medium to give a final concentration of 0.5% (w/v). BSA was included to minimize aggregation of chloroplasts. Two identical sucrose density gradients were prepared by mixing stock solutions of 30%, 42%, and 60% sucrose; dissolved in 5 mM MeS-KOH buffer (pH 7.5) to the desired concentrations; and then layered by pipette into 34-ml cellulose nitrate tubes (2.5-cm diameter × 7.5-cm long). The final composition of the sucrose gradient from bottom to top was: 60% sucrose (5 ml); 56.5% (2 ml); 51.5% (4 ml); 50% (2 ml); 47.5% (2 ml); 45% (4 ml); 42.5% (2 ml); 40% (2 ml); 37.5% (2 ml); 35% (2 ml); 32.5% (1 ml); and 30% sucrose (1 ml). Ruptured protoplasts (4 ml per gradient) were carefully layered on top, and the gradients were spun in a Beckman L2-65B ultracentrifuge with a swinging bucket SW 25 rotor for 3 h at 90,400*g* (maximum). The gradients were fractionated, commencing at the base of the centrifuge tubes, using a peristaltic pump. The two gradients were fractionated separately, and the corresponding fractions (each 1.15 ml) were then combined.

Velocity Sedimentation Centrifugation. Protoplasts used in velocity sedimentation experiments were prepared as described for the equilibrium density gradient centrifugation. The sucrose gradients were also prepared in a similar manner. However, the composition was altered as follows: 60% sucrose (5 ml), linear gradient from 60% to 42% (6 ml); 42% sucrose (5 ml), linear gradient from 42% to 30% (10 ml); and 30% sucrose (3 ml). Samples of ruptured protoplasts (4 ml) were layered onto each of two identical gradients and spun in a type SW 25 swinging bucket rotor for 5 min at 2,300*g* (maximum) and then for 10 min at 14,500*g* (maximum). Fractions from the two gradients were combined to give 2.4-ml fraction.

Vacuole Isolation. The washed protoplasts were resuspended in 12 ml of 0.08 M sorbitol, 0.1 M K_2HPO_4 (pH 8.0), 1 mM DTT, 0.1% (w/v) soluble PVP, and 0.5% (w/v) BSA solution and gently shaken for 5 min at 28°C. After cooling to 4°C, the protoplasts were gently broken by slow passage through a 60-μm mesh (2-cm diameter orifice). Sucrose was added to a concentration of 0.5 M, and 0.5 ml of 0.02% (w/v) neutral red solution was then added to locate the vacuoles.

Equal volumes of the broken protoplast suspension were overlaid by 2 ml each of the following solutions: 0.4 M sucrose + 0.1 M sorbitol; 0.2 sucrose + 0.3 sorbitol; and 0.5 M sorbitol; each made up in 50 mM Hepes (pH 7.0), 0.5% (w/v) BSA, 0.075 M K_2HPO_4, 1 mM DTT, and 0.1% (w/v) soluble PVP. Following centrifugation for 20 min at 200*g*, two major vacuole bands were present. The band at the 0.2 M sucrose + 0.3 M sorbitol and the 0.5 M sorbitol interface contained a mixture of vacuoles and protoplasts, while a further band at the top of the 0.5 M sorbitol layer was virtually free from protoplast contamination (less than 3%). Glucose-6-P dehydrogenase assays (10) indicated that the vacuoles were not contaminated by cytoplasm. The vacuole band was removed by Pasteur pipette and used for subsequent enzyme localization studies. The vacuoles and protoplasts were counted with a hemocytometer.

Control Preparations. Crude extract was prepared from wheat leaves incubated overnight in 0.4 M sorbitol solution (pH 7.0) containing 1 mM KH_2PO_4 and 5 mM $MgCl_2$. The leaves were ground in a chilled mortar with a pestle in 25 mM sodium phosphate buffer (pH 7.0) containing 1 mM DTT, 0.1% (w/v) soluble PVP, and 0.05% (v/v) Triton-X-100. Protoplast and vacuole preparations were also made up to 0.05% (v/v) with Triton-X-100 and homogenized in a Fortuna glass homogenizer. All preparations were then centrifuged at 25,000*g* for 10 min.

After samples were taken for protein determination, BSA was added to the protoplast and crude extract preparations to a concentration of 0.5% (w/v).

Enzyme Assays. Aminopeptidase and leucyltyrosine dipeptidase activities were determined as described previously (30). Hemoglobin-degrading activity was measured by incubating 0.2 ml of extract with 0.2 ml of hemoglobin (6 mg/ml) and 0.2 ml of 0.2 M sodium acetate buffer (pH 4.5). The reaction was stopped after 120 min at 50°C by adding 0.5 ml of 12% (w/v) TCA. Activity was determined by measuring the release of TCA-soluble amino nitrogen, using the modified ninhydrin method of Yemm and

Cocking (31). Carboxypeptidase activity was assayed as described previously (30), except that the assay pH was 5.0. The reaction was stopped after 120 min at 35°C by adding 0.5 ml of 12% (w/v) TCA. TCA-soluble amino nitrogen was measured as described above. Acid phosphatase, β-glucosidase, phosphodiesterase, β-*N*-acetylglucosamidase, and α-mannosidase were measured by following the release of PNP[2] from their respective substrates, PNP-phosphate, PNP-β-D-glucoside, bis-PNP-phosphate, PNP-*N*-acetyl-β-D-glucosaminide, and PNP-β-D-mannoside. Activities were measured according to the procedure of Murray and Collier (17). Soluble protein was measured by the method of Lowry *et al.* (12), following precipitation with 12% (w/v) TCA. BSA was used to prepare a standard curve.

Specific enzymic markers were assayed to locate the various cell organelles. Mitochondria were located by Cyt *c* oxidase (25), peroxisomes by catalase (1), cytoplasm by glucose-6-P dehydrogenase (10), and chloroplasts by RuBPCase protein (21). Additional chloroplast markers used were Chl (2) and triose-P isomerase (6), which is also located in the cytoplasm. For the organelle isolation experiments, sucrose gradients are expressed in terms of refractive indices.

RESULTS

Equilibrium Density Gradient and Velocity Sedimentation Centrifugation. Isolation of organelles by equilibrium density gradient centrifugation resulted in the partial resolution of peroxisomes, chloroplasts, and mitochondria (Fig. 1). A good yield of intact chloroplasts was obtained (peak fractions (10–11), with only a trace of broken chloroplasts (fractions 17–18). Although considerable quantities of mitochondria and peroxisomes were occluded within the intact chloroplast band, chloroplast-free zones of peroxisomes (fractions 5–8) and mitochondria (fractions 13–16) were also located in the gradient. The cytoplasm, identified by glucose-6-P dehydrogenase (fractions 26–29), was contained in the supernatant on top of the sucrose gradient. The supernatant also contains the contents of broken organelles, including the vacuoles, which are too large to pass intact through the 20-μm mesh used to break the protoplasts. Significant catalase activity was found in the supernatant as a result of peroxisome breakage. The mitochondrial marker, Cyt *c* oxidase could not be measured in the supernatant because of interference from DTT that had been present in the protoplast resuspension medium.

The distribution of aminopeptidase activity (Fig. 1), measured against three substrates, closely resembles that of triose-P isomerase, a chloroplast and cytoplasmic marker. No aminopeptidase activity was associated with the chloroplast-free peroxisome or mitochondrial bands. Clearly, some of the aminopeptidase activity associated with the supernatant was released from broken chloroplasts. However, the extent of chloroplast breakage was insufficient to account entirely for the aminopeptidase activity found in the supernatant. Leucyltyrosine dipeptidase activity was found in the supernatant, although some activity (19%) was associated with the chloroplast. Allowing for chloroplast breakage (estimated by Chl), leucyltyrosine activity in the chloroplast was calculated as 27% of the total activity. Hemoglobin-degrading and carboxypeptidase activities were entirely confined to the supernatant (data not shown).

Despite the inclusion of BSA (conventionally used to prevent the nonspecific adherence of membranes) and other additives such as DTT and PVP (to prevent possible interactions between sulfhydryl groups or phenolics, respectively), we were unable to prevent chloroplasts from aggregating. Although we separated high yields

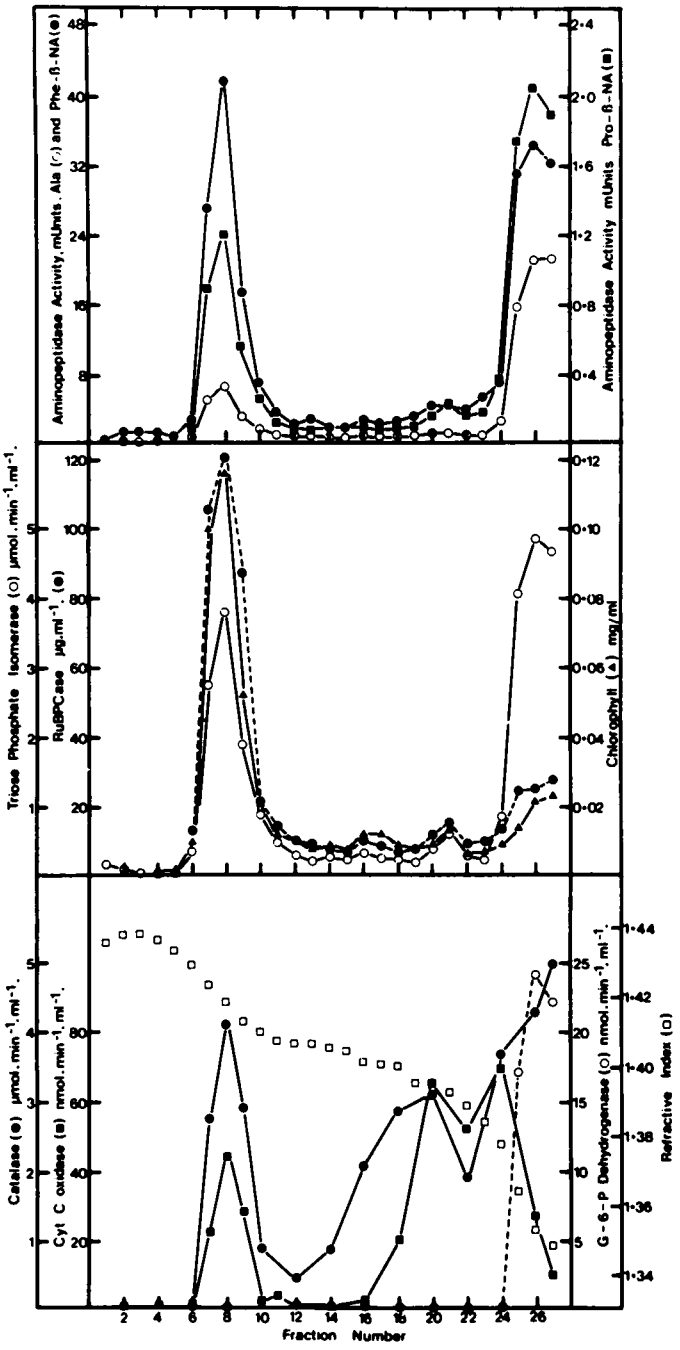

FIG. 2. Distribution of aminopeptidase activity and organelle-marker enzymes following velocity sedimentation centrifugation of wheat leaf protoplasts. Ruptured protoplasts (4 ml) were loaded onto a sucrose gradient, as described in the text, and centrifuged for 5 min at 2,300*g*, followed by 10 min at 14,500*g*. The gradient was fractionated into 2.4 ml fractions. Symbols are the same as those described in Figure 1.

of intact chloroplasts from chloroplast-free mitochondria and peroxisomes, it appears that the mitochondria and peroxisomes (although no cytoplasm) were occluded within the aggregated chloroplasts as they descended through the sucrose gradient. As an alternative approach, broken protoplasts were subjected to velocity sedimentation centrifugation, which results in the rapid sedimentation of chloroplasts. Good yields of intact chloroplasts were obtained (77% of intact chloroplasts), as estimated by Chl, but we were unable to isolate an uncontaminated band of chloroplasts (Fig. 2). Nevertheless, aminopeptidase activity was again

[2] Abbreviations: PNP, *p*-nitrophenyl; RuBPCase, ribulose-1,5-bisphosphate carboxylase. β-NA, β-naphthylamide; pCMB, 4-hydroxymercuribenzoic acid; ZZMSF, phenylmethylsulfonylfluoride.

closely associated with the chloroplasts (fractions 6–10) and the supernatant (fractions 25–27). There was no correspondence of aminopeptidase activity with the mitochondrial or peroxisomal markers, nor were there any cytoplasmic contamination of the chloroplast band. A proportion of the leucyltyrosine dipeptidase activity was again found in the intact chloroplast band (27% after allowing for chloroplast breakage), but the majority of the activity was contained in the supernatant (data not shown). Although aminopeptidase from barley has been shown to hydrolyze leucyltyrosine at neutral pH, it does not show appreciable activity against the dipeptide at pH 10.2, the pH of the leucyltyrosine dipeptidase assay (9). All of the carboxypeptidase and hemoglobin-degrading activities were found in the supernatant.

Vacuole Isolations. Both hemoglobin-degrading and carboxypeptidase activities were completely localized within or bound to the outside of the vacuole (Table I). No aminopeptidase or leucyltyrosine dipeptidase activities were found in the vacuole. Acid phosphatase, phosphodiesterase, α-mannosidase, and β-*N*-acetylglucosamidase, which are acid hydrolases commonly used to demonstrate the lysosomal nature of the vacuole (3, 13), were also found to be associated with the vacuole. β-Glucosidase has been found previously in vacuoles from corn root meristems (14) and the endosperm tissue of castor bean seedlings (18), but no β-glucosidase was found in the vacuoles isolated from wheat leaves (Table I).

Preston and Kruger (22) have reported the existence of two proteolytic enzymes in extracts of germinated wheat seeds, which are able to degrade both hemoglobin and the carboxypeptidase substrate *N*-carbobenzoxy-L-phenylalanyl-L-alamine. To ensure that the carboxypeptidase and hemoglobin-degrading activities we measured represent distinct enzymes, we looked at the effect of the inhibitors pCMB and PMSF on enzyme activities and the effect of temperature on enzyme stability. At 1 mM PMSF, the carboxypeptidase activity was inhibited 45% compared with a 20% loss of hemoglobin-degrading activity; at 1 mM pCMB, 17% of the carboxypeptidase activity was lost compared with a 42% loss of the hemoglobin-degrading activity. The two activities also showed slight differences in their sensitivity to high temperatures. Treatment at 50°C for 10 min resulted in the loss of 23% of the carboxypeptidase activity, but only 8% of the hemoglobin-degrading activity was lost. The results show that the activities measured do represent distinct enzymes, although this does not exclude the possibility of carboxypeptidase activity against hemoglobin. This would result in an overestimate of endopeptidase activity against hemoglobin, but, since there was no loss of hemoglobin-degrading activity from the protoplasts to the vacuole (Table I), the results still confirm the presence of acid proteinase within the vacuole.

The fact that the Cellulysin mixture contains hemoglobin-degrading activity (Table I) raises the possibility that the activity found in the vacuole is merely the result of Cellulysin contamination. One of the major criteria for uncontaminated protoplasts is that their specific activity must reflect that of the crude leaf extract (3). Since the specific activity of hemoglobin-degrading activity in the protoplasts was lower than that of the crude leaf extract, it appears that intact protoplasts have not adsorbed hemoglobin-degrading activity from the Cellulysin mixture.

Boller and Kende (3), using cultured tobacco cells, found that enzymes present at high levels in Cellulysin were removed during the flotation of intact protoplasts in Ficoll gradient, because contaminating enzymes were preferentially adsorbed to the cell debris in the pellet. In our experiments, although a Ficoll gradient was not used, protoplasts were separated from the cell debris by flotation in a sucrose/sorbitol gradient. Furthermore, following protoplast breakage to release the vacuoles, protoplast membranes pelleted by centrifugation were found to have no hemoglobin-degrading activity.

The Cellulysin mixture was found to be contaminated by β-glucosidase, yet no activity was associated with isolated vacuoles (Table I). Thus, if β-glucosidase can be used as an indicator, Cellulysin enzymes did not seem to be bound indiscriminately to the vacuoles.

DISCUSSION

The vacuolar location of hemoglobin-degrading activity confirms the earlier report of Lin and Wittenbach (11), who found RuBPCase-degrading enzymes in the vacuole of wheat leaves. Carboxypeptidase activity, which, like hemoglobin-degrading activity, has an acid pH optimum, is also associated with the vacuole. Clearly, the vacuole is capable of protein degradation, although

Table I. *Enzyme Distribution during Preparation of Wheat Leaf Vacuoles*

Enzymes were assayed in crude extract, Cellulysin, protoplast, and vacuole homogenates, as described in the text.

Enzyme	Crude Extract	Protoplasts		Vacuoles	Cellulysin	Relative Activity in Vacuole Preparation[c]
	nmol min^{-1} mg^{-1} protein		*nmol min^{-1} 10^6 protoplasts^{-1}*	*nmol min^{-1} 10^6 vacuoles^{-1}*	*nmol min^{-1} mg^{-1} protein*	
Hemoglobin-degrading activity	4.8	0.98	1.27	1.29	15.0	101.6
Carboxypeptidase[a]						
Aminopeptidase[b]	9.5	3.20	4.09	4.10	0	100.2
Ala-β-NA	26.7	22.3	31.8	0	0	0
Phe-β-NA	95.7	65.1	80.3	1.5	0	2
Pro-β-NA	4.0	3.6	4.4	0	0	0
Leucyltyrosine dipeptidase[b]	2.85×10^3	2.01×10^3	6.38×10^3	0.17×10^3	0.15×10^3	2.7
Acid phosphatase[b]	4.3×10^4	2.1×10^4	3.0×10^4	1.6×10^4	0.07×10^4	52.3
β-Glucosidase[b]	8.3×10^3	6.7×10^3	9.5×10^3	0.04×10^3	108×10^3	0.4
Phosphodiesterase[b]	3.5×10^3	2.7×10^3	3.9×10^3	0.40×10^3	0.36×10^3	10.3
β-*N*-Acetylglucosamidase[b]	6.9×10^2	7.3×10^2	10.4×10^2	5.6×10^2	1.3×10^2	53.8
α-Mannosidase[b]	1.90×10^3	0.93×10^3	1.57×10^3	0.73×10^3	0	46.5

[a] Protoplast yield was 1.81×10^6/ml, and vacuole yield 2.82×10^6/ml of extract used for enzyme assays and protein determination. Protein concentrations for the crude and protoplast extracts were 1.24 and 1.17 mg/ml, respectively. Cellulysin contained 2.91 mg protein/ml.

[b] Protoplast yield was 1.46×10^6/ml, and vacuole yield 1.83×10^6/ml. Protein concentration for the crude and protoplast extracts were 1.46 and 1.04 mg/ml, respectively. Cellulysin contained 3.36 mg protein/ml.

[c] Assuming one vacuole per protoplast.

in wheat, at least, the major portion of the cellular protein is extravacuolar (11). Ultrastructural studies have shown vacuoles to contain cytoplasmic vesicles (14) which presumably contain cytoplasmic protein. Inside the vacuole, protein hydrolysis is likely to be facilitated by the low pH of the vacuolar sap. Lowering the pH of crude extracts of wheat leaves to pH 5.2 results in the denaturation of more than 80% of the soluble protein (data not shown). If denaturation makes proteins more susceptible to hydrolysis, as occurs in animal systems (4, 19), sequestration of cytoplasmic proteins into the vacuole is likely to acclerate their degradation. In addition, vacuolar enzymes have been shown to be capable of degrading a wide range of proteins (3, 11), either through a few enzymes with broad specificity or, alternatively, by means of a range of enzymes. These observations implicate the vacuole as an organelle capable of the nonspecific degradation of proteins.

While those peptide hydrolases with an acid pH optimum are associated with the vacuole, the neutral/alkaline peptide hydrolases have an extravacuolar location. Wheat-leaf aminopeptidase activity is divided equally between the chloroplast and the cytoplasm, while leucyltyrosine dipeptidase is predominantly in the cytoplasm. The fact that such a large proportion of the aminopeptidase activity is associated with the chloroplast supports the notion that proteins can be degraded in the chloroplast. Despite the lack of evidence for a chloroplast-located endopeptidase, ultrastructural studies of chloroplast senescence have shown major disruption of chloroplast lamellae and stroma before degeneration of the outer double membrane (16, 21). To be effective in protein degradation, however, aminopeptidases appear to require the presence of an endopeptidase. Peoples (20) and Lin and Wittenbach (11) found about 15% of cellular acid proteinase to be associated with wheat-leaf chloroplasts. However, Lin and Wittenbach (11), on the basis of binding studies with ^{125}I-labeled trypsin, considered their results to be an artefact. Ragster and Chrispeels (23), although unable to detect chloroplast endopeptidase by the conventional ninhydrin assay, did show endopeptidase activity by the loss of the large subunit of RuBPCase using SDS-gel electrophoresis. These results confirmed the earlier report of Thomas and Huffaker (26).

The role of leucyltyrosine dipeptidase in protein breakdown is uncertain. In animal cells, involvement of cytoplasmic dipeptidases in the hydrolysis of dipeptides transferred out of the lysosome has been suggested (4). If the vacuole in plant cells is analogous to animal lysosomes as suggested by Matile (14), then plant leucyltyrosine dipeptidase is appropriately located to perform a similar function in plants. The transport of dipeptides across membranes has been demonstrated in plant cells (8), although not across the tonoplast. As yet, however, there has been no firm evidence to support the lysosomal nature of the vacuole.

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