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Subcellular Localization of Dhurrin β-Glucosidase and Hydroxynitrile Lyase in the Mesophyll Cells of *Sorghum* Leaf Blades[1]

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ABSTRACT

Studies with purified mesophyll and epidermal protoplasts and bundle sheath strands have shown that the cyanogenic glucoside dhurrin (*p*-hydroxy-(*S*)-mandelonitrile-β-D-glucoside) is localized in the epidermis of sorghum leaves whereas the enzymes involved in its degradation (dhurrin β-glucosidase and hydroxynitrile lyase) are localized in the mesophyll tissue (Kojima M, JE Poulton, SS Thayer, EE Conn 1979 Plant Physiol 63: 1022–1028). The subcellular localization of these enzymes has now been examined using linear 30 to 55% (w/w) sucrose gradients by fractionation of mesophyll protoplast components. The hydroxynitrile lyase is found in the supernatant fractions suggesting a cytoplasmic (soluble cytoplasm, microsomal or vacuolar location). The dhurrin β-glucosidase (dhurrinase) is particulate and mostly chloroplast-associated. The dhurrinase activity peak has a shoulder of activity more dense than that of the intact chloroplasts. This shoulder does not coincide with markers of any other cell fraction.

In studies of chloroplasts isolated from ruptured mesophyll protoplasts by differential, low-speed centrifugation, the dhurrinase partitions in the same manner as the chloroplast marker triose phosphate dehydrogenase. Chloroplast localization of the β-glucosidase has also been shown in histochemical studies using 6-bromo-2-naphthyl-β-D-glucoside substrate coupled with fast Blue B.

There are two enzymes in young *Sorghum bicolor* seedlings which catalyze the degradation of the cyanogenic glucoside, dhurrin (*p*-hydroxy-(*S*)-mandelonitrile-β-D-glucoside), to HCN, *p*-hydroxybenzaldehyde, and glucose when the tissue is disrupted or crushed. It has been shown recently by Kojima *et al.* (8) using purified mesophyll and epidermal protoplast preparations and bundle sheath strands that these two enzymes, dhurrin β-glucosidase (dhurrinase) and hydroxynitrile lyase (oxynitrilase), are almost exclusively localized in the mesophyll tissue of light-grown *Sorghum* leaf blades and that dhurrin is localized entirely in the epidermal tissue.

In this study, we have used sucrose density gradients, isolated chloroplasts, and light microscopic enzyme histochemistry of mesophyll protoplasts from light-grown sorghum leaf blades to determine the subcellular localization of dhurrinase and oxynitrilase.

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MATERIALS AND METHODS

CHEMICALS

Dhurrin was isolated from 4-day-old etiolated *Sorghum* shoots and purified (15). *p*-Hydroxy-(*R,S*)-mandelonitrile was synthesized according to the method of Ladenburg *et al.* (9). Cellulysin, B grade, was purchased from Calbiochem. Oxynitrilase was purified from *Sorghum* as described by Bové and Conn (5). The 10- and 20-μm nylon nets were purchased from Tetko Inc., Elmsford, NY, and the 44-μm mesh nylon net was obtained from Cistron, Lebanon, PA. Cytochrome *c*, type III (horse heart), was obtained from Sigma. Glucosylamine was prepared by the method of Isbell and Frush (7).

PLANT MATERIALS

Six-day-old light-grown seedlings of *S. bicolor* (Linn.) Moench, var. Redland × Greenleaf were obtained as described by Kojima *et al.* (8). Etiolated plants were obtained by soaking and planting seeds as above and growing them in total darkness for 7 days at 30 C. Only expanded leaf blades were used in the preparation of protoplasts and chloroplasts.

PREPARATION OF PROTOPLASTS

Approximately 3 g leaf blades were abraded with 150 grit carborundum and digested in 30 ml 1.5% w/v Cellulysin in 0.5 M mannitol in 25 mM K-phosphate-citrate (pH 5.5), essentially as previously described (8). Leaves from 7-day-old etiolated plants were excised and abraded in dim light and digestion was carried out in foil-wrapped Petri dishes. The protoplasts released after 3 h digestion were harvested by passing the digestion mixture through a 44-μm mesh nylon net and centrifuging the filtrate for 2 min at 400*g* in a bench-top centrifuge.

LYSIS OF PROTOPLASTS FOR SUCROSE DENSITY GRADIENTS

The pellets consisting of mesophyll and epidermal protoplasts were each washed three times with 10 ml 0.5 M mannitol in 25 mM K-phosphate-citrate (pH 5.5) and once with 5 ml breaking medium composed of 20 mM Tricine-NaOH (pH 7.5) containing 0.5% BSA, 1% PVP-40, and 0.5 M mannitol. Washed protoplasts were resuspended in 1 to 2 ml breaking medium and forced through a 20-μm mesh nylon net attached to the end of a syringe (14). The filtrate then was passed through a 10-μm mesh nylon net attached to a syringe until all protoplasts were broken (one or two times).

SUCROSE GRADIENT FRACTIONATION

An aliquot of this filtrate was layered onto a 30 to 55% (w/w) linear sucrose gradient containing 20 mM Tricine-NaOH (pH 7.5),

0.5% (w/v) BSA, and 1% (w/v) PVP-40. The gradients were centrifuged in a SW 27 swinging bucket rotor in a Sorvall OTD-50 ultracentrifuge for 20 min at a maximum speed of 25,000 rpm. An ISCO model 185 density gradient fractionator was used to fractionate the gradients. Fractions of 1.35 ml were collected.

ENZYME ASSAYS

Dhurrin β-Glucosidase. The production of *p*-hydroxybenzaldehyde from dhurrin was measured in the presence of extract and exogenous oxynitrilase (11). The assay was started by adding enzyme sample to a quartz cuvette containing 516 nmol dhurrin, 55 milliunits purified oxynitrilase, and 25 μmol citrate-K-phosphate (pH 6.2) in a total volume of 1.1 ml. Incubation was carried out at 30 C and the increase in *A* at 285 nm was followed.

Oxynitrilase. This activity was assayed according to Kojima *et al.* (8).

Catalase. This enzyme was measured by following the decrease in *A* at 240 nm in 1 ml 18 mM H_2O_2 in 50 mM K-phosphate (pH 7.0) at room temperature (10).

Cyt *c* Oxidase. Cyt *c* was reduced using sodium ascorbate according to Wharton (20) and was incubated for 1 min with an equal volume of 0.1% digitonin. To this, 7.5 μmol K-phosphate (pH 7.0) was added. The reaction was initiated by the addition of 5 μmol reduced Cyt *c* in a total volume of 1 ml. The decrease in *A* at 554 nm was followed at 30 C.

NADPH-dependent TPDH.[2] This enzyme was assayed according to Heber *et al.* (6) by following the decrease in *A* at 340 nm.

PEP Carboxylase. This enzyme was assayed according to Ting (19).

Chl was determined by the technique of Arnon (1).

ELECTRON MICROSCOPY OF GRADIENT FRACTIONS

Gradient fractions were diluted to 0.6 M sucrose gradually using 0.4 M sucrose containing 1% glutaraldehyde. The samples then were pelleted in a Beckman microfuge, resuspended in 3.5% glutaraldehyde, and maintained at 0 C for 1 h. They were postfixed in 2% OsO_4, dehydrated in ethanol, and embedded in Spurr's embedment (18).

CHLOROPLAST ISOLATION FROM RUPTURED PROTOPLASTS BY DIFFERENTIAL CENTRIFUGATION

Protoplasts were prepared as described above and washed four times with digestion buffer. They then were resuspended in 0.33 M sorbitol containing 0.2 mM $MgCl_2$ and 20 mM Mes-Tris (pH 6.5) and ruptured as described above. This suspension was centrifuged at 3000*g* for 30 s with hand-braking. The supernatant was immediately decanted (Table II, first supernatant), and the pellet was washed with 0.33 M sorbitol adjusted to pH 7.5 using 0.1 M Tris and centrifuged at 1500*g* for 60 s with hand-braking. The supernatant was decanted immediately (Table II, second supernatant) and the pellet was resuspended in 0.33 M sorbitol (pH 7.5) medium. In this manner, chloroplasts were isolated in less than 10 min after rupture of the protoplasts.

Chloroplast intactness in the pellet fraction was determined by assaying the activity of TPDH with and without 0.33 M sorbitol. The difference between the "no sorbitol" and "plus sorbitol" rates was divided by the "no sorbitol" rate to give the fraction of intact chloroplasts in the pellet.

ENZYME HISTOCHEMISTRY

Washed protoplasts prepared as above were fixed for 30 min in 10% neutral formalin in 50 mM citrate-phosphate (pH 5.5) containing 0.5 M mannitol. The fixed protoplasts were rinsed three times in 50 mM citrate-phosphate (pH 5.5) and incubated for 30 min in one of the following: (*a*) a solution of substrate prepared by initially dissolving 2 mg 6-bromo-2-naphthyl-β-D-glucoside in 0.4 ml dimethyl formamide and subsequently diluting it to 10 ml with warm 50 mM citrate-phosphate (pH 5.5); (*b*) 0.4 ml dimethyl formamide made to 10 ml with buffer (no substrate control); (*c*) a solution of substrate containing either 10 mM δ-gluconolactone or 10 mM glucosylamine (inhibitors of β-glucosidase activity). Protoplasts then were washed three times in buffer [50 mM citrate-phosphate (pH 5.5)] and incubated in a filtered solution of 0.2% fast blue B salt in 90 mM K-phosphate (pH 8.0) containing 7% ethanol (postcoupling method). In the case of simultaneous coupling, 2 mg/ml fast blue B salt was added to solutions *a*, *b*, or *c* and filtered before use. Protoplasts were washed and observed using a Zeiss Universal photomicroscope.

RESULTS

Protoplasts were used as our source of subcellular fractions because we found it difficult to obtain intact organelles from young light-grown *Sorghum* leaves by mechanical disruption (blender grinding, electric knife, mortar and pestle, or Polytron homogenizer). As used in this study, the protoplast method also eliminated the presence of bundle sheath cells and organelles which do not contain dhurrinase or oxynitrilase (8) but do contain Chl and other markers which could obscure our results. The protoplast preparations used as a source of organelles, however, did contain epidermal protoplasts. Inasmuch as gradients obtained using highly purified mesophyll protoplasts (less than 2% epidermal cells) showed the same pattern of Chl and dhurrinase (and because their presence does not alter the banding pattern), the mesophyll protoplasts were not further purified as in Kojima *et al.* (8).

Gradient Results. A summary of a number of experiments (10–15) showing the peak density of enzymes and markers and the percentage of their activities recovered from the gradients is found in Table I.

Figure 1A shows the normal banding pattern of dhurrinase and Chl in linear 30 to 55% (w/w) sucrose gradients (shown in a gradient prepared from the older leaf blade). In this gradient system, intact plastids have a peak density of 1.199 (see Table I) as shown by the co-migration of TPDH activity (Fig. 1C) and the denser Chl peak (Fig. 1, A and B). In light-grown tissue (Fig. 1, A and B), the dhurrinase activity also has a peak density of 1.199, which corresponds to the intact chloroplast peak. In all experi-

Table I. *Peak Density of Enzymes and Markers and Percentage of Starting Protoplast Activity Recovered from Linear 30 to 55% (w/w) Sucrose Gradients of Ruptured Mesophyll Protoplasts*

The protoplasts were prepared from 6-day-old light-grown *Sorghum* leaf blades.

Marker or Enzyme	Peak Density	Amount Recovered from Gradients
	g/ml	%
Chl		
Broken plastids	1.17	95
Intact plastids	1.199	
TPDH	1.199	70–75
Cyt *c* oxidase	1.17	70[a]
Catalase	<1.14	40
PEP carboxylase	<1.13	40–43
Hydroxynitrile lyase	<1.14	85
Dhurrin β-glucosidase	1.199	88–92

[a] In gradients containing 10 mM δ-gluconolactone.

[2] Abbreviations: TPDH, triose phosphate dehydrogenase; PEP, P-enolpyruvate.

ments, however, there is a shoulder or second peak of dhurrinase activity (Fig. 1, A, B, and E) which is more dense (1.21) than the intact chloroplast markers, Chl and TPDH. This shoulder, or second peak, of dhurrinase activity represents 20 to 40% of the total dhurrinase peak. In most gradient experiments performed, the percentage of the recovered TPDH and dhurrinase activity and Chl put on the gradient collected in the 1.199 to 1.21 density peak is the same (69.2% dhurrinase and 64.7% Chl in Fig. 1A and 68.8% TPDH in Fig. 1C).

The percentage of intact chloroplasts as determined by per cent of Chl and/or TPDH in the 1.199 peak varied from 30 to 70% in sucrose gradients. There was a marked decrease in the percentage of intact plastids in experiments in which gradients were centrifuged longer than 20 min (*i.e.* 1, 3, or 10 h). Although it is customary to centrifuge gradients 1 to 3 h, there was no effect on the peak density of the intact chloroplasts or the dhurrinase activity by shortening the ultracentrifugation period to 20 min; therefore, this time was used in all experiments shown.

In this sucrose gradient system, all of the recovered activity of PEP carboxylase and oxynitrilase is found in the top of the gradient (Fig. 1, C and D; Table I). PEP carboxylase has frequently been shown to be a soluble cytoplasmic enzyme in plant tissues (13). Because oxynitrilase and PEP carboxylase have the same elution pattern, it is likely that oxynitrilase is also a cytoplasmic enzyme. On the basis of the gradient work alone, we cannot exclude a vacuolar or microsomal localization.

Mitochondria, as indicated by Cyt *c* oxidase activity, have a peak density of 1.17 (Fig. 1C) and co-migrate with the broken chloroplasts. The absence of any Cyt *c* oxidase activity in the top fraction of the gradient is probably the result of inhibition by the HCN produced by the action of dhurrinase and oxynitrilase even when inhibitors of dhurrinase (10 mM gluconolactone or glucosylamine) were included in the protoplast breaking medium and gradient solutions.

Microbodies, as indicated by catalase activity, never migrated to a density greater than 1.14 (Fig. 1D; Table I) under these conditions. Miflin and Beevers (12) report a mean density of 1.10 to 1.13 for pea and spinach leaf microbodies in sucrose gradients.

Endoplasmic reticulum (microsomal) markers were not included here as Saunders *et al.* (17) established a mean density of 1.10 for *Sorghum* microsomes. The microsomes should not have entered the 30 to 55% (w/w) gradient under these conditions. As can be seen from these gradient profiles, there is no detectable contamination of the intact chloroplast or dhurrinase activity peak by mitochondria, microbodies, or cytoplasm.

Kojima *et al.* (8) observed that the first (older) leaf blade had 1.5 times the specific activity of dhurrinase activity seen in the sucrose gradients. We therefore prepared protoplasts separately from the basal half of the second (younger) leaf-blade and the tip half of the first (older) leaf-blade and layered the ruptured protoplasts onto separate gradients (Fig. 1, A and B). There was no significant difference in these two samples in per cent intactness, peak density of Chl and dhurrinase activity, or their elution profiles. There is also no difference between either of these profiles and that obtained when the entire leaf blades of both leaves are used. We concluded that the shoulder of dhurrinase activity in the dhurrinase peak was not the result of a developmental difference in the two leaf-blades.

Dhurrinase from 4-day-old etiolated *Sorghum* shoots has been partially purified and characterized (S. Eklund, MS. thesis in preparation). We wanted to determine if the subcellular localization of the dhurrinase was the same in etiolated leaf blades as it was in light-grown tissue. The distribution of dhurrinase and the TPDH marker in sucrose gradients of ruptured protoplasts prepared from etiolated leaf-blades is shown in Figure 1E. The TPDH plastid marker in etiolated *Sorghum* tissue has a peak density of 1.22. Etioplasts have been found to be more dense (mean density,

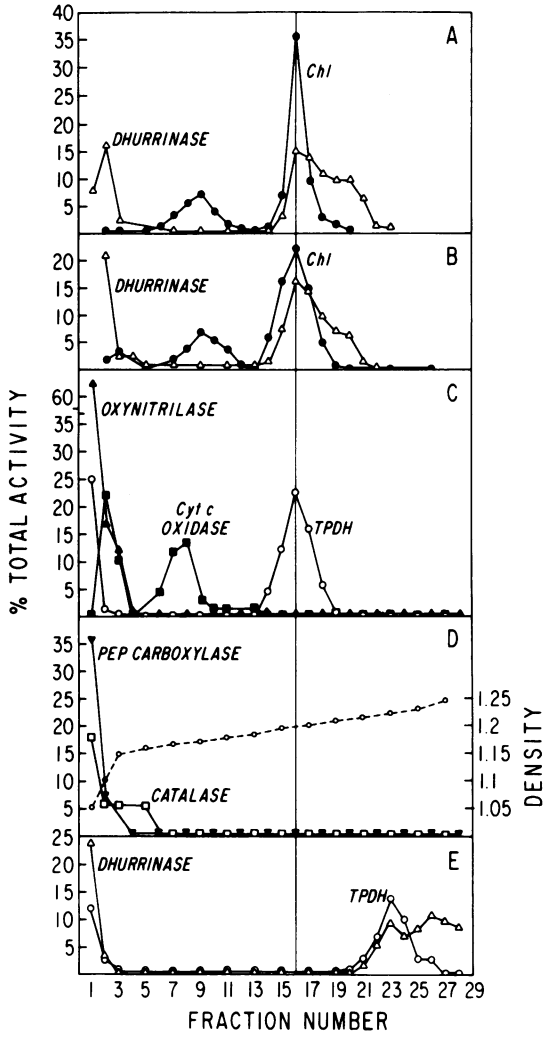

FIG. 1. Localization of various enzyme activities and markers in 1.35-ml fractions of 40-ml linear 30 to 55% (w/w) sucrose density gradients of ruptured mesophyll protoplasts. The percentage of the total activity of each (given below in parentheses) put on the gradient is shown. One unit is equal to 1 μmol product/min. (See Table I for the percentage of activity recovered from the gradient for each enzyme.) A, Chl (1.98 mg) and dhurrinase (11.2 units) from protoplasts prepared from the tip half of the older leaf blade; B, Chl (0.81 mg) and dhurrinase (2.73 units) from protoplasts prepared from the basal half of the younger leaf blade; C, TPDH (5.75 units), Cyt *c* oxidase (0.329 units), and oxynitrilase (2.43 units) from protoplasts prepared from entire leaf blades; D, PEP carboxylase (2.42 units), catalase (9.63 units), and density of sucrose from protoplasts prepared from entire leaf blades; E, dhurrinase (4.24 units) and TPDH (2.63 units) from protoplasts prepared from etiolated leaf blades. (●——●), Chl; (△——△), dhurrinase; (○——○), TPDH; (■——■), Cyt *c* oxidase; (▲——▲), oxynitrilase; (▼——▼), PEP carboxylase; (□——□), catalase; and (○---○) sucrose density.

1.22–1.25) than chloroplasts (mean density, 1.18–1.22) in sucrose gradients (13). *Sorghum* mesophyll etioplasts are more dense (1.22) than chloroplasts (1.199), as shown by the TPDH marker. The dhurrinase activity from ruptured etiolated mesophyll protoplasts appears as a double peak (peak densities of 1.22 and 1.23). The fact that there is a corresponding increase in the peak density of the TPDH and dhurrinase activities suggests strongly that the dhurrinase is plastid-associated in etiolated as well as in green tissue.

We have also investigated the possibility that an inhibitor of dhurrinase might be present in the intact chloroplast peak, which

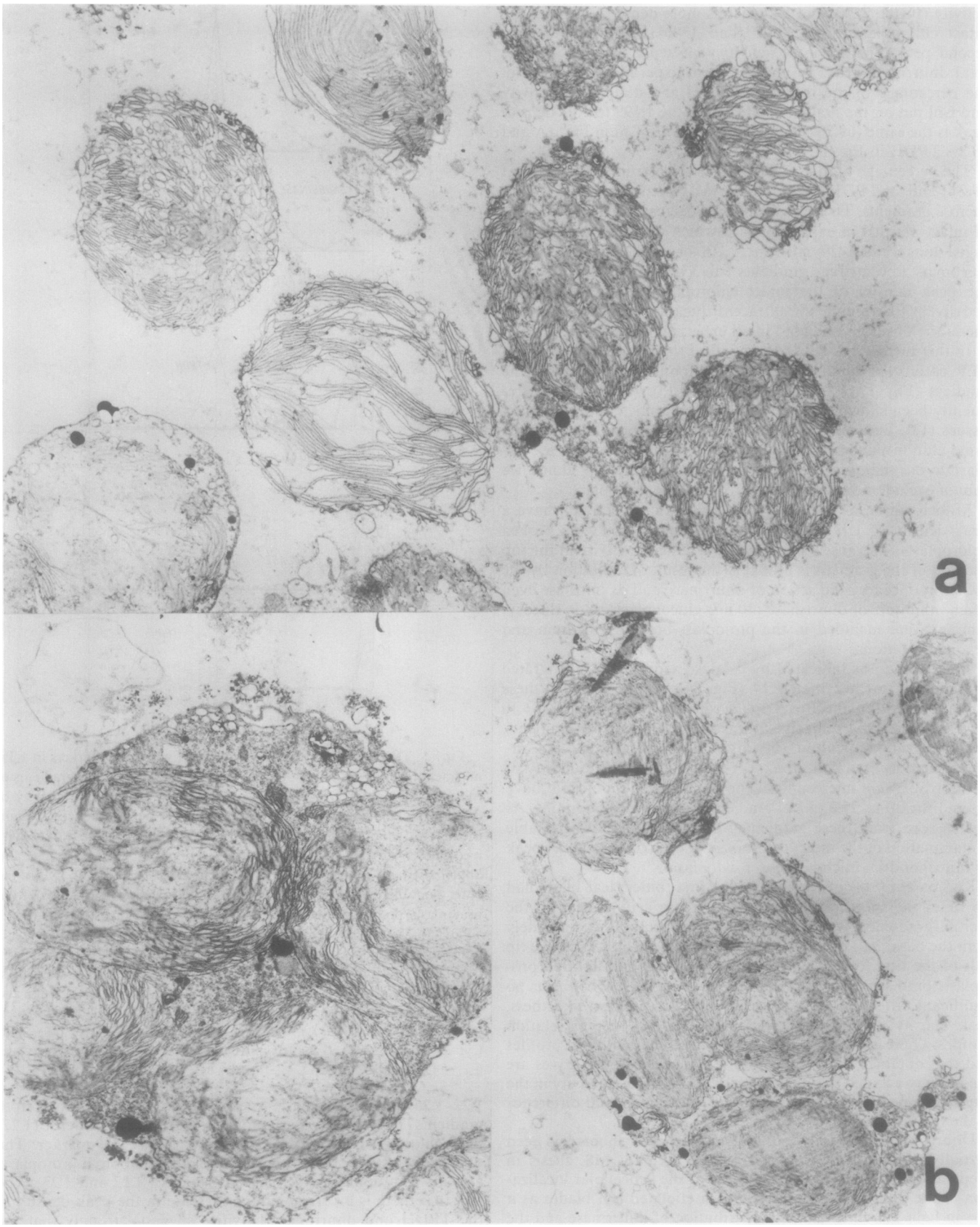

FIG. 2. Electron micrographs of fractions of sucrose gradients of ruptured mesophyll protoplasts. a, fraction 16 (biochemically intact chloroplast peak) showing mesophyll chloroplast (× 6,900); b, fraction 18 (shoulder of dhurrinase activity) showing "subprotoplasts" or clumped chloroplasts which occur in addition to individual mesophyll chloroplasts as shown above (× 8,500).

Table II. *Partitioning of Dhurrinase (DβG), TPDH, and Chl from Ruptured Protoplasts of 6-Day-old Sorghum Leaf Blades in Low-speed Differential Centrifugation*

Results of two experiments are shown. In each experiment, 27% of the TPDH activity shown ("no sorbitol" assay) is in intact chloroplasts (see "Materials and Methods").

Fraction	Crude Activity					
	Experiment 1			Experiment 2		
	DβG	TPDH	Chl	DβG	TPDH	Chl
	%					
First supernatant, 3,000*g*	7.9	12.9	0.4	23.1	37.2	3.4
Second supernatant, 1,500*g*	11.8	6.4	0.5	48.4	25.4	18.8
Pellet, 1,500*g*	48.7	50.8	93.8	21.7	17.6	77.8

would alter the profile of this enzyme in the gradients. However, aliquots of the Chl peak fraction did not inhibit the dhurrinase activity in the shoulder region of the peak or a partially purified dhurrinase preparation from etiolated *Sorghum* (data not shown).

Electron Microscopy of Gradient Fractions. Aliquots of the Chl peak fraction and a fraction in the shoulder region of the dhurrinase activity peak were prepared for electron microscopy. As a result of the dilution of these gradient fractions to permit pelleting, most of the plastids appear broken. The two fractions examined contained mesophyll chloroplasts (Fig. 2, a and b). In the dhurrinase shoulder region, there were also a few clumped mesophyll chloroplasts, or what may be described as "subprotoplasts" (Fig. 2b). These "clumps" were also evident in light microscopic examination. This clumping phenomenon could possibly explain the increased density of the dhurrinase activity shoulder. The absence of detectable biochemical markers in this zone of the gradient is puzzling however. No organelles other than the mesophyll chloroplasts or the clumped plastids were observed in these fractions.

Chloroplasts Purified from Protoplasts by Differential Centrifugation. Chloroplasts prepared from ruptured protoplasts have equivalent percentages of the TPDH and dhurrinase activities as shown in Table II. Although the two experiments shown differ in the absolute percentage of these two enzymes present in the pellet, it is clear that TPDH and dhurrinase partition in the same way in this procedure. This again supports the theory that the dhurrinase activity is chloroplast-associated.

Light Microscopic Enzyme Histochemistry. The chloroplasts of mesophyll protoplasts stained a dark purple when incubated with 6-bromo-2-naphthyl-β-D-glucoside with either simultaneous or postcoupling with fast blue B dye. There was no staining in the absence of substrate or when either δ-gluconolactone or glucosylamine were added to the substrate solution. Results were the same in fixed and unfixed protoplasts. Protoplasts were used in these studies instead of tissue sections because of the poor penetration of solutions in sections. The only staining observed in ethanol-fixed cryostat-sections of 6-day-old *Sorghum* leaf blades was in the walls of guard cells and bundle sheath cells, presumably due to β-glucosidase activity associated with lignification. It has been shown that dhurrinase partially purified from etiolated *Sorghum* does cleave the histochemical substrate although at a slow rate (S. Eklund and S. S. Thayer, unpublished data).

DISCUSSION

The results of the sucrose density fractionation of mesophyll protoplasts indicate that dhurrinase is particulate and mostly, if not entirely, chloroplast-associated. Some of the dhurrinase activity is found as a dense shoulder on the major dhurrinase activity peak which co-migrates with the intact chloroplasts. We have been unable to identify any particle or subcellular fraction either biochemically or microscopically which is associated with the shoulder of dhurrinase activity in these gradients except for single or clumped mesophyll chloroplasts. The low levels of chloroplast membrane (Chl) or stromal (TPDH) markers and the absence of markers of other subcellular fractions in this region is puzzling. The fact that equal percentages of the dhurrinase and TPDH activity and Chl put on the gradient are associated with the 1.199 density peak (and shoulder) indicates that all three respond similarly during protoplast rupture and ultracentrifugation. Equal percentages of TPDH and dhurrinase activity are also recovered in chloroplasts isolated from ruptured mesophyll protoplasts by low-speed differential centrifugation.

We have shown that the shoulder of dhurrinase activity is not the result of variability of leaf development (*i.e.* not due to proplastids or immature plastids which have a different density and/or specific activity of dhurrinase). No proplastids, bundle sheath chloroplasts, or any other nonmesophyll-type chloroplasts were seen in the electron microscopic examination of the gradient fractions.

It is unlikely that the dhurrinase activity seen associated with intact plastids in sucrose gradients is the result of adsorption of a soluble enzyme onto the chloroplasts. The high concentration of BSA (0.5%) and PVP-40 (1%) in the breaking and gradient solutions should prevent this from occurring. In any case, passive adsorption could not explain the shoulder of dhurrinase activity seen in gradients.

Perhaps the most convincing evidence for the chloroplast localization of the dhurrinase is the corresponding increase in peak density of both the TPDH and dhurrinase activity peaks in sucrose gradients of ruptured etiolated mesophyll protoplasts. If the dhurrinase were associated with some subcellular fraction other than the plastids, there would probably not have been a shift in its peak density in etiolated tissue. Because there was an identical shift in peak density in both the plastid marker peaks TPDH and the dhurrinase peak, the dhurrinase must be associated with the plastids.

The results of the β-glucosidase histochemistry show that the chloroplast localization of β-glucosidase activity in protoplasts is unambiguous within the limits of the method. The major question is of specificity: Is the dye deposition the result of activity of the dhurrin-specific β-glucosidase or other β-glucosidases? In etiolated *Sorghum*, there are three soluble β-glucosidases; two preferentially hydrolyze dhurrin, and one preferentially hydrolyzes the *p*-nitrophenyl glucoside substrate (S. Eklund, MS. thesis in preparation). We cannot be sure as to which of these enzymes is acting on 6-bromo-2-naphthyl-β-D-glucoside, the nonphysiological substrate used in this method. In spite of the question of specificity, it is clear that there is β-glucosidase activity in mesophyll protoplasts and that this activity is associated with the chloroplasts. Other workers using a similar method to localize β-glucosidase activity in plant tissues have found activity primarily associated with cytoplasmic strands and cell walls in corn roots (2–4). Risslar and Millar (16) have studied the localization of *Lotus* linamarase, a β-glucosidase which hydrolyzes another cyanogenic glucoside, linamarin. This enzyme was also localized in cytoplasmic strands and walls in shoots. It seems that β-glucosidases may not be associated with one specific organelle in plants, but in various subcellular fractions in different plants.

Oxynitrilase, the second enzyme involved in the degradation of dhurrin, appears to be a cytoplasmic enzyme. It is interesting that the oxynitrilase and dhurrinase are localized in separate compartments of the mesophyll cells. There appears to be a high degree of compartmentation of the cyanogenic glucoside, dhurrin, and its two degradative enzymes in leaf blades of *Sorghum*. Dhurrin is localized exclusively in the epidermal tissue, whereas dhurrinase

and oxynitrilase are in the mesophyll cells in different subcellular compartments. Disruption and mixing of both the epidermal and adjacent mesophyll cells seem to be necessary for the release of HCN from dhurrin.

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