LOCALIZATION OF ALLANTOINASE IN GLYOXYSOMES OF GERMINATING CASTOR BEANS

by

Allen J. St. Angelo and Robert L. Ory
Southern Regional Research Laboratory[1/]
New Orleans, Louisiana 70119

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Intracellular localization of allantoinase was studied in germinating castor bean endosperm. Glyoxysomes and mitochondria were separated by density gradient centrifugation. Using isocitrate lyase and fumarase as the respective marker enzymes for the separated organelles, allantoinase was found in glyoxysomes but was absent in mitochondria.

In a previous report allantoinase (allantoin amidohydrolase, EC 3.5.2.5) was detected in extracts of castor bean endosperm only after 3-5 days germination (9). The enzyme appeared to be membrane or particle-bound and was solubilized with deoxycholate.

A comparison of the procedure employed in separating the allantoinase-active fraction to those used in preparing mitochondria and glyoxysomes (2, 3) showed several similarities in the conditions for their respective centrifugations, suggesting that the allantoinase might be associated with one or both of these particles. Glyoxysomes have a greater density than mitochondria and therefore can be separated from them by density gradient centrifugation. Also, glyoxysomes contain certain glyoxylate cycle enzymes but not fumarase, NADH oxidase, succinate dehydrogenase, or the cytochromes which are found in mitochondria (2-5).

While no previous evidence for the presence of ureide-metabolizing enzymes in either of these two particles had been reported, the report of Ory, et al. (9) suggested that one or both of them might also contain the allantoinase. The purpose of this work was to isolate intact mitochondria

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1/ One of the laboratories of the Southern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.

and glyoxysomes by essentially the same procedure as Breidenbach and Beevers (2) and to determine if allantoinase was associated with either of the particles. Identities of the two fractions were confirmed by simultaneous assay for fumarase and isocitrate lyase, the marker enzymes designated for mitochondria and glyoxysomes, respectively (2). In this communication, we present evidence to show that allantoinase is absent in mitochondria, but present in glyoxysomes.

MATERIALS AND METHODS

Castor beans, Ricinus communis L., Baker 296 variety, were germinated at 30° in the dark in moist vermiculite after prior dusting with Spergon. After 6 days, washed endosperm tissue was homogenized in 1.5 volumes of the grinding medium described by Breidenbach and Beevers (2), but in an Omnimix for 10 seconds at a rheostat setting of 50 rather than by hand chopping and mortar and pestle. The homogenate was then squeezed through cheesecloth and centrifuged at 500 x g for 5 minutes. All operations were conducted at 4°. The resulting supernatant solution (S-500) was centrifuged at 11,000 x g for 20 minutes and the pellet (P-11,000) carefully resuspended in 10 ml of the grinding medium in a loose-fitting Potter-Elvejhem homogenizer.

Ten ml of this resuspended fraction (P-11,000) was layered over a discontinuous Ficoll gradient consisting of 16 ml 51% Ficoll, 16 ml 36% Ficoll, and 20 ml 20% Ficoll; then centrifuged four hours at 23,000 rpm in a Beckman Model L-2 Ultracentrifuge equipped with a SW 25.2 rotor. Figure 1 shows the three major fractions: B, which lies between densities of 1.08 (g/ml) and 1.14; C, between 1.14 and 1.21; and D, which contains particles heavier than 1.21. Fraction A contains those materials lighter than 1.08 density. All fractions were separated by syringe, appropriately diluted with the grinding medium minus sucrose, and promptly assayed for the various enzymes.

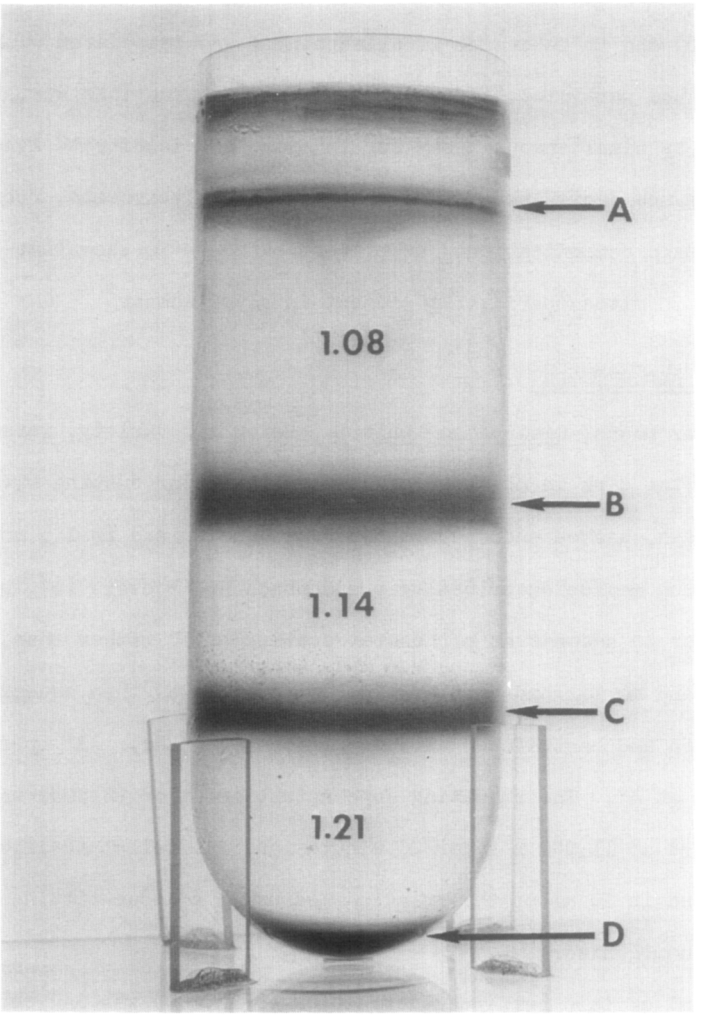

Figure 1. Photograph of components of P-11,000 fraction separated over Ficoll gradient. Densities of 1.08, 1.14, and 1.21 represent 20%, 36%, and 51% Ficoll solutions, respectively. Fraction A, Residual lipid; B, Mitochondria, C, Proplastids, designation used by Breidenbach and Beevers (2); and D, Glyoxysomes.

Allantoinase activity was measured by the method described earlier (9); isocitrate lyase, by the method of Dixon and Kornberg (6); and fumarase, according to Racker (10).

RESULTS AND DISCUSSION

Table 1 shows that approximately 81% of the total allantoinase

Table 1

Allantoinase Activity and Distribution in Fraction from Differential Centrifugation

Fraction	Total Activity (units*)	Distrib. (%)	Total Activity (units*)	Distrib. (%)
Original Extract	0.7690	100		
P-500	0.2288	25		
S-500	0.6750	75	0.6075	100
P-11,000			0.4763	81
S-11,000			0.1155	19
Recovery		117		98

*One unit is defined as the change of one absorptivity unit per min. per ml of enzyme.

activity was found in the 11,000 g precipitate (P-11,000). Breidenbach and Beevers (2) reported that this fraction contains intact mitochondria and glyoxysomes, which could be separated from each other by density gradient centrifugation.

Further fractionation of P-11,000 (Table 2) showed that allantoinase activity was predominantly in Fraction D, i.e., in those particles heavier than 1.21 density. No activity was found in the top two fractions, but 21% was found in Fraction C. Significantly, 73% of the glyoxysomal marker, isocitrate lyase, was also found in Fraction D, and only 15% in Fraction C. The distributions of allantoinase and isocitrate lyase in Fractions D and C parallel each other. The small amounts of enzymatic activities in C could be attributed to ruptured glyoxysomes not sedimented through the 51% Ficoll, density 1.21. Small amounts of isocitrate lyase activity found in the upper zones of the gradient are perhaps due to bursting of some glyoxysomes. Similar distribution results were noted for isocitrate lyase and malate synthetase activities

Table 2

Enzyme Activity and Distribution in Fractions
from Density Gradient Centrifugation

Fraction	ALLANTOINASE		FUMARASE		ISOCITRATE LYASE	
	Total Activity (units*)	Distrib. (%)	Total Activity (units*)	Distrib. (%)	Total Activity (units*)	Distrib. (%)
Homogenate (P-11,000)	0.0392	100	1.60	100	44.7	100
A	0	0	0.05	9	0.67	3
B	0	0	0.47	91	1.94	9
C	0.0150	21	0	0	3.19	15
D	0.0550	79	0	0	15.49	73
Recovery	-	178	-	33	-	48

*One unit is defined as the change of one absorptivity unit per min. per ml of enzyme.

by Gerhardt and Beevers (8). Fumarase, the mitochondrial marker enzyme, was found only in Fractions A and B. While complete recoveries of all three enzymes were not obtained after centrifuging over the Ficoll gradient, these results show that allantoinase is primarily located in glyoxysomes and absent in mitochondria.

Allantoinase catalyzes the hydrolysis of allantoin to allantoic acid, which is then cleaved to glyoxylic acid and urea by the enzyme, allantoicase, as part of the purine catabolic pathway. During the present investigations on ureide-metabolizing enzymes, we first isolated the mitochondria and glyoxysomes using the sucrose gradient described by Breidenbach and Beevers (2). However, the method used to determine allantoinase activity employed phenylhydrazine-HCl and traces of reducing sugars in the sucrose interfered with the assay. We tried various combinations of sucrose and Ficoll, a synthetic copolymer of sucrose and epichlorohydrin, but an all Ficoll gradient proved best and was

finally chosen. We also noted that fumarase seemed to lose activity during the four hour centrifugation at 23,000 rpm, while isocitrate lyase and allantoinase activities remained relatively constant when the intact glyoxysomes were assayed.

When endosperm tissue was macerated with a food chopper and mortar and pestle for 6-7 minutes as done by Breidenbach and Beevers (2), the mitochondria appeared to rupture; most of the fumarase was found in the soluble fraction (S-11,000) and not in the sediment (P-11,000). After numerous trials, grinding the tissue in an Omnimix for 10 seconds at very low speeds was found to yield the best results. Fumarase obtained from this procedure was distributed approximately 63% in the P-11,000 fraction and 37% in the soluble, S-11,000, fraction.

In a recent report by Visentin and Allen (11) allantoinase was shown to be associated with amphibian peroxisomes. These particles resembled peroxisomes rather than mitochondria or lysosomes in their density distribution. They also reported finding catalase, D-amino acid oxidase, and urate oxidase in this fraction. While mammalian peroxisomes and plant glyoxysomes have some common features, Breidenbach et al. (3) concluded that they are different particles with different metabolic functions, since peroxisomes lack the enzymes of the glyoxylate cycle. Our report of allantoinase in castor bean glyoxysomes represents another feature common to both particles.

Gerhardt and Beevers (7) recently reported finding RNA in castor bean glyoxysomes. Since allantoin is one of the products of purine metabolism, and there appears to be at least two enzymes of this pathway associated with glyoxysomes (peroxisomes), namely, allantoinase and urate oxidase (1, 11), perhaps other enzymes of the purine metabolic pathway are present in these organelles.

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