ISOLATION AND CHARACTERIZATION OF MICROBODIES AND SYMPHYOMICROBODIES WITH DIFFERENT BUOYANT DENSITIES FROM THE FUNGUS BLASTOCLADIELLA EMERSONII

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SUMMARY: Microbodies isolated from sporangia of the aquatic fungus Blastocladiella emersonii have a mean buoyant density of 1.222 g/cm^3 after centrifugation through a linear sucrose gradient, and contain catalase, isocitrate lyase and malate synthase. Microbodies fuse to produce one symphyomicrobody per zoospore at the time of sporogenesis. An increase in density accompanies this process. The symphyomicrobody has a mean buoyant density of 1.292 g/cm^3 while the spore's single mitochondrion has a buoyant density of 1.219 g/cm^3. Statistical data are also provided for both starting levels and purification of symphyomicrobody and mitochondrial enzyme markers.

INTRODUCTION. Since the discovery of the functional nature of mammalian and leaf peroxisomes (1,2) and glyoxysomes (3), there have been many publications on microbodies; they are summarized in a symposium (4), a book (5), and reviews (6,7,8,9). Plant microbodies, extensively characterized morphologically and biochemically (7,9), have been classified as glyoxysomes, peroxisomes and "unspecialized microbodies" (10,11,12). In contrast, descriptions of fungal microbodies have been far more limited (9), identifications being based mainly on fine structural criteria. Analyses of isolated microbodies involving more than one enzyme have been limited to studies with Saccharomyces (13), Neurospora (14) and Coprinus (15).

The zoospores and zoosporangia of the water mold Blastocladiella emersonii (16) contain two kinds of catalase positive organelles, symphyomicrobodies and microbodies (17). They are ontogenetically related in that several microbodies give rise to a single large microbody by symphyogenesis. The purpose of this report is to provide statistically circumscribed data about the enzymatic activities of isolated symphyomicrobodies, and biochemical and physical supporting

evidence for the method of origin of this unusual organelle.

MATERIALS AND METHODS. Synchronous cultures of B. emersonii were grown essentially as described (18) except that synchronous sporogenesis was induced after 18 h at 22°. Zoosporangia were collected by filtration at the time of papilla formation; spores were collected after filtration by centrifugation. Zoosporangia and spore pellets were suspended in homogenizing media consisting of 50 mM Na cacodylate, pH 7.5, containing 0.5 M sucrose, 2.5% Ficoll, 10 mM $MgCl_2$, 10 mM KCl, and 5 mM EDTA. Sporangia were broken by sonication (X3, 30 sec, 60 W, 4°), with one min intervals between each treatment; spores were disrupted by passing through a 27 gauge hypodermic needle until they were broken as observed microscopically. Sporangial homogenates were passed through four layers of 8-ply cellulose gauze, and both the spore and sporangial homogenates were centrifuged at 300 x g for 7 min. The sporangial supernatant was centrifuged at 10,000 x g for 15 min. The pellet was resuspended in homogenizing medium and applied to a continuous sucrose gradient, as was the 300 x g spore supernatant. The sucrose gradient (30-65%, w/w) was formed over a cushion of 65% (w/w) sucrose. All sucrose solutions contained 5 mM EDTA. After centrifugation for 4 h at 24,000 rpm (61,000 x g to 110,000 x g) in a swinging bucket rotar #969, model BD2 International ultracentrifuge, fractions were collected by drops All steps were done at 0-4°. Sucrose concentrations were measured refractometrically; protein was determined colorimetrically (19). Enzyme activities were followed spectrophotometrically at ca 23° using a Gilford model 222 photometer and model 2453 linear potentiometer recorder (Honeywell). The enzymes assayed and methods employed were: succinic dehydrogenase, EC 1.3.99.1 (20); fumarase, EC 4.2.1.2 (21); catalase, EC 1.11.1.6 (22); isocitrate lyase, EC 4.1.3.1 (23); and malate synthase, EC 4.1.3.2 (24). Specific activities are designated as μmoles of substrate used or product formed·min^{-1}·mg $protein^{-1}$.

TABLE I

Specific activities of enzymes associated with organelles, including their initial activities in the zoospore homogenates

	Symphyomicrobody enzymes			Mitochondrial enzymes	
	Isocitrate Lyase	Malate Synthase	Catalase	Succinic Dehydrogenase	Fumarase
S.A., spore homogenate	.0062 ± .0028 (35) (*)	.0091 ± .0057 (18)	1.368 ± .208 (10)	.0741 ± .0396 (18)	.0982 ± .0394 (12)
S.A., isolated organelle	.0642 ± .0358 (15)	.0913 ± .0304 (6)	19.520 ± 4.824 (6)	.1939 ± .0707 (15)	.4015 ± .1189 (6)
Maximum fold purification	25.0	16.2	19.2	5.2	6.0
Average fold purification	10.4	10.0	14.3	2.6	4.1

(*) Mean ± S.D.; number of separate experiments, in brackets.

RESULTS. Of many techniques used to try to rupture spores with minimal organelle damage, passage through a syringe needle yielded the best results, with reproducible specific activities (Table 1). Most of the microbody and mitochondrial enzyme activities (ca 65% and 80%, respectively) were associated with

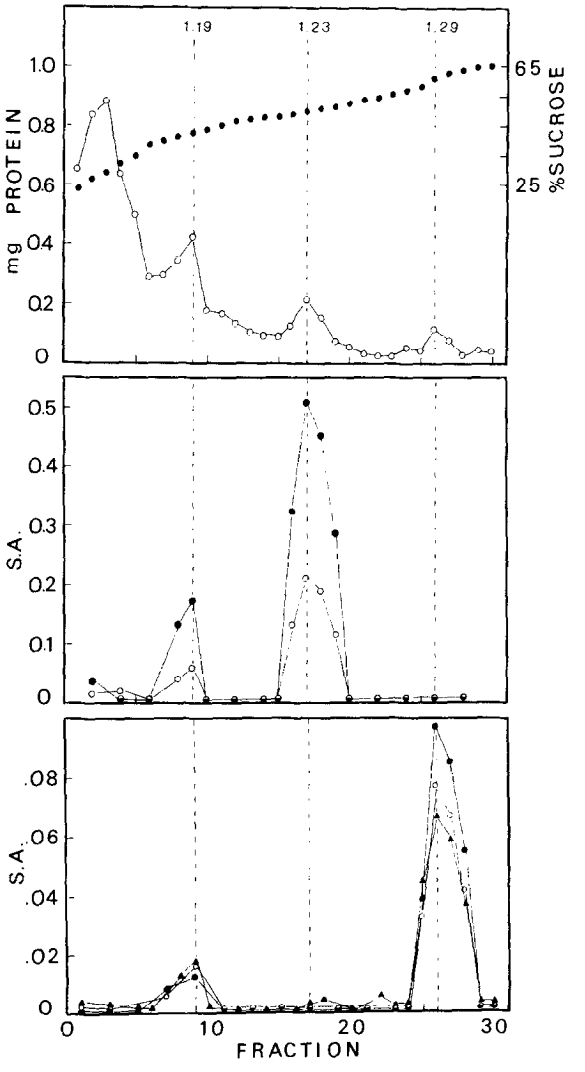

Fig. 1. Distribution of organelle protein (top: o——o), and activities of mitochondrial enzymes (middle: fumarase, ●——●; succinic dehydrogenase, o——o), and microbody enzymes (bottom: catalase, ●——●, x 200; isocitrate lyase, o——o; and malate synthase, ▲——▲) after sucrose density gradient separation of components of the 300 x g supernatant from zoospores. Dashed lines mark the peaks for mitochondrion-lipid-symphyomicrobody complexes (1.19 g/cm^3), mitochondria (1.23 g/cm^3) and the symphyomicrobodies (1.29 g/cm^3).

particulates. Homogenates freed of large debris (300 x g) and centrifuged through sucrose density gradients yielded four protein peaks (Fig. 1, top). Fractions 1-5 contained soluble protein. Fractions 7-10 contained mitochondria-lipid-symphyomicrobody complexes (see ref. 17 & 25 for structure) as determined both enzymatically and electron microscopically; they had a buoyant density of 1.19 g/cm^3. Fractions 16-19 (1.23 g/cm^3) were enriched for mitochondria (see ref. 25 for micrographs). The densest (1.29 g/cm^3) fractions, 25-28, contained symphyomicrobody material.

Isocitrate lyase, malate synthase, and catalase were associated with the symphyomicrobody fractions (Fig. 1, bottom), succinic dehydrogenase and fumarase with the mitochondrial fraction (Fig. 1, center). Table 2 shows mean buoyant densities of the symphyomicrobodies, microbodies, and mitochondria. Also listed (Table 1) are specific activities of enzyme markers associated with these isolated organelles, and the maximum and average purifications achieved for them.

The procedure used to isolate microbodies from sporangia yielded recoveries of ca. 60% for microbody enzymes in the 10,000 x g pellets, but only ca. 40% for mitochondrial enzymes. The profiles (Fig. 2) show that a shift in density (Table 2) occurred as the symphyomicrobody was fused out of smaller microbodies during zoosporogenesis. The profile (Fig. 2, top) for isocitrate lyase from sporangia collected after cleavage into spores but before spore release contrasts sharply with the profile (Fig. 2, bottom) for isocitrate lyase from sporangia harvested at the time of papilla formation, i.e., just before sporogenesis when

TABLE 2

The buoyant densities of isolated organelles

Symphyomicrobodies	Microbodies	Mitochondria
1.292 ± .0121 (*) (15)	1.222 ± .006 (5)	1.219 ± .010 (15)

(*) g/cm^3; mean ± S.D.; number of separate experiments, in brackets.

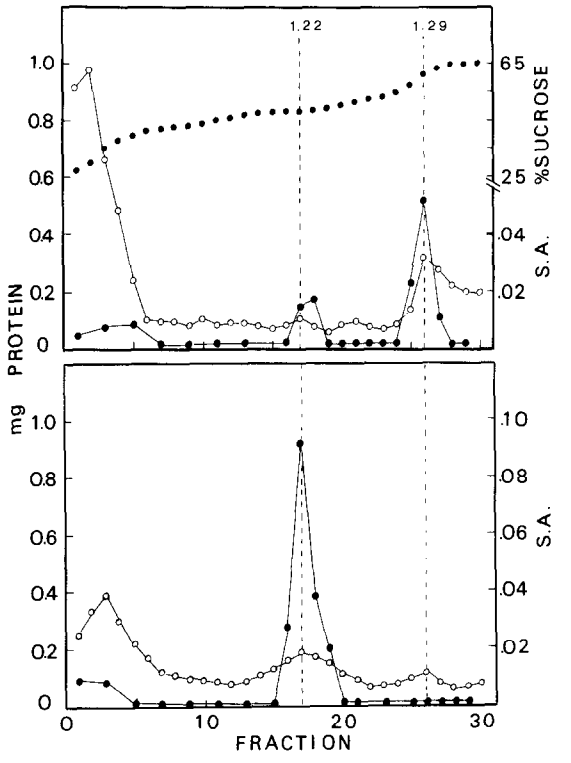

Fig. 2. Distribution of protein (o——o) and isocitrate lyase activity (● ——●) after sucrose density gradient separation of components of the 10,000 x g pellet from zoosporangia. The dashed lines show the shift in density of the peak activities for symphyomicrobodies (top) and individual microbodies (bottom).

symphyomicrobodies had not yet formed. The distribution of catalase and malate synthase followed that of isocitrate lyase; the shift in density measured with these three markers was also demonstrable in discontinuous sucrose gradients (not shown). The mitochondrial enzyme markers derived from sporangia undergoing papilla formation were located at a buoyant density of ca. 1.20 g/cm^3.

DISCUSSION. The zoospores of B. emersonii contain a single large symphyomicrobody (17,25), so called because it is formed by union of small microbodies (formerly "sb granules"; 26) during sporogenesis (17,25). The small microbodies isolated from zoosporangia just before sporogenesis have a mean buoyant density of 1.222 g/cm^3, are 0.2 - 0.3 μ in diam, and carry catalase, isocitrate lyase, and malate synthase activities. However, if microbodies are isolated from

zoosporangia 2 h later, at the time of sporogenesis but before spore release, two changes associated with symphyogenesis occur: a large increase in microbody size, and an increase in buoyant density to 1.292 g/cm^3.

This increase in density may result from elevated sucrose uptake due to the larger size of the new organelle, of from addition of proteins at the time of symphyogenesis. Changes in buoyant density have been reported for microbodies in other organisms. The density of developing wheat leaf peroxisomes increases from 1.18 g/cm^3 to 1.25 g/cm^3 (27), the explanation offered being acquisition of new protein. Shifts in microbody density in Arum (28) and developing soybean cultures (29) have also been described. Changes in microbody size have also been detected, e.g., in Phaseolus (30). However, in none of these or other instances (7,9) has a fusion of microbodies been involved.

The B. emersonii symphyomicrobody has been shown ultracytochemically (17) and biochemically (this report) to contain catalase, hence it might be classified as a peroxisome. But since it also carries malate synthase and isocitrate lyase, it might be functioning as a glyoxysome. This idea is supported by indirect evidence, such as the close physical relationship (25) among lipid globules, mitochondrion and symphyomicrobody. Furthermore, 64% of the triglycerides in zoospores are used up after 5 h of swimming (31); they are thought to be the major component of the lipid globules, which also decrease (32). When we analyzed homogenates of spores that had been swimming 5 h, the two glyoxylate cycle enzyme activities had increased three-fold whereas catalase had not changed.

If the B. emersonii symphyomicrobody is a glyoxysome, how does it compare with microbodies isolated from other fungi? There is a paucity of information about isolated fungal microbodies. What little there is indicates that at least some glyoxylate cycle enzymes are associated with particulates. The latter vary considerably, however, in buoyant density and enzyme content. In yeast (13) and Coprinus (15), particulates containing glyoxylate cycle enzymes had lower buoyant densities than mitochondrial fractions. Isocitrate lyase from yeast was bimodally distributed between mitochondrial and microbody fractions but malate

synthase was almost entirely associated with the microbody fraction (13). In Neurospora (14) both malate synthase and isocitrate lyase were associated with particulates of greater buoyant density than that carrying mitochondrial enzymes. The particulates from Coprinus and Neurospora were classified as glyoxysomes; the yeast organelle was called a peroxisome since catalase and glycolate oxidase were associated with it. We think the symphyomicrobody of B. emersonii zoospores may be functioning as a glyoxysome.

Although plant microbodies seem to develop from endoplasmic reticulum (7,9), it is also a fact (33; Cantino, unpublished observations) that at one stage in the life cycle of B. emersonii, after a zoospore encysts, microbody-like structures are generated by fission of the symphyomicrobody. Further studies of the formation and decay of this novel organelle should contribute new insights on microbody ontogeny. Work is in progress to further characterize the symphyomicrobody chemically, enzymatically and physiologically.

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