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Review

Microbodies in fungi: a review

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SUMMARY

Microbodies are ubiquitous organelles in fungal cells, occurring in both vegetative hyphae and spores. They are bounded by a single membrane and may contain a crystalloid inclusion with subunits spaced at regular intervals. Typically, they contain catalase which reacts with the cytochemical stain 3,3′-diaminobenzidine to yield an electron-opaque product, urate oxidase, L-α-hydroxy acid oxidase and D-amino acid oxidase. Their fragility and the necessity to disrupt the tough fungal cell wall before isolating them make them difficult to isolate. Analysis of enzymes in purified or partially purified microbodies from fungi indicates that they participate in fatty acid degradation, the glyoxylate cycle, purine metabolism, methanol oxidation, assimilation of nitrogenous compounds, amine metabolism and oxalate synthesis. In organisms where microbodies are known to contain enzymes of the glyoxylate cycle, they are known as glyoxysomes; where they are known to contain peroxidatic activity, they are known as peroxisomes. In some cases microbodies contain enzymes for only a portion of a pathway or cycle. Thus, they must be involved in metabolic cooperation with other organelles, particularly mitochondria. The number, size and shape of microbodies in cells, their buoyant density and their enzyme contents may vary with the composition of the medium; their proliferation in cells is regulated by the growth environment. The isolation from the same organism of microbodies with different buoyant densities and different enzymes suggests strongly that more than one type of microbody can be formed by fungi.

MICROBODIES

In describing results of a study performed in the early 1950s, Rhodin used the term "microbody" for an organelle observed in electron micrographs of mouse kidney cells [45]. Microbodies were characterized by a single limiting membrane and a fine granular matrix. Subsequent studies of the ultrastructure of rat liver cells showed that some microbodies contain crystalloid inclusions.

As microbodies were isolated and characterized enzymatically for a number of eucaryotic organisms, including protozoa, algae, higher plants, animals, and fungi, it became apparent that the organelle contained hydrogen peroxide-generating oxidases and catalase [22]. On this basis, deDuve proposed the term 'peroxisome'. Beevers [4] found that microbodies in germinating castor bean seeds contained the fatty acid β-oxidation enzyme system and enzymes for the complete glyoxylate cycle. Therefore, he named the particles 'glyoxysomes'. A precise distinction between the two biochemical subclassifications of microbodies has not been made. However, where the function of microbodies is known, they are termed peroxisomes or glyoxysomes; where the function is not clear, they are termed microbodies.

All microbodies isolated to date are bounded by a single membrane (Fig. 1A,B) and typically contain catalase, urate oxidase, D-amino acid oxidase and L-α-hydroxy acid oxidase which serve as marker enzymes for the organelle. Some contain a crystalloid inclusion (Fig. 1B) which, in plant and animal cells, may represent a storage form of catalase [32,45]. Catalase shows a reaction product in electron micrographs when reacted with 3,3′-diaminobenzidine (DAB) (Fig. 1C) and thus is considered a cytochemical stain for microbodies. However, as noted below, marker enzymes may be absent in microbodies from some organisms and not all microbodies are DAB-positive.

Microbodies range in size from 0.1 to 1.7 μm in dia-

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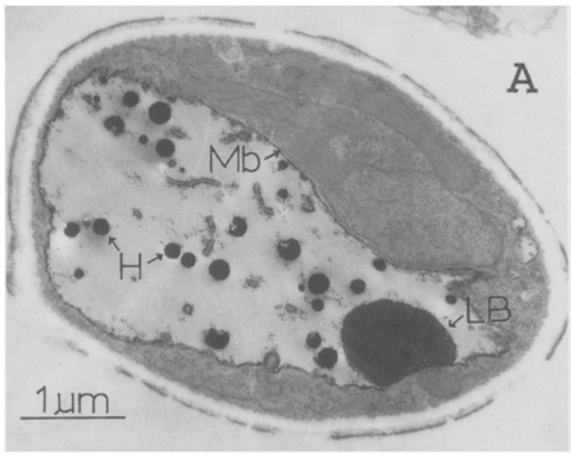

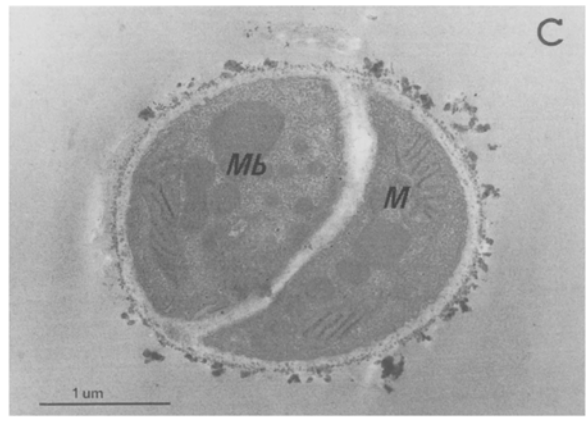

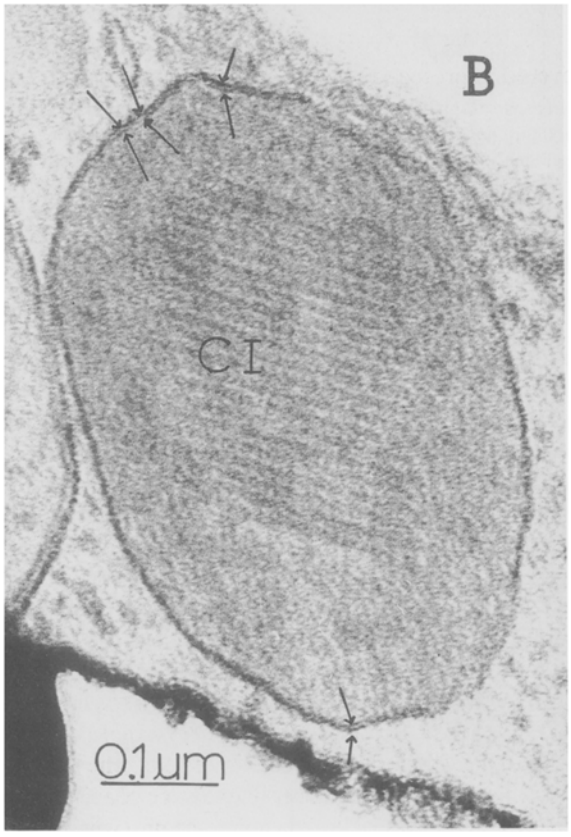

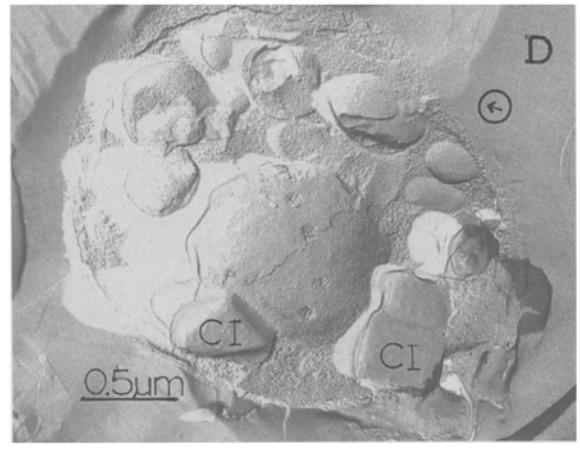

Fig. 1. Microbodies in cells of the fungus *Cladosporium resinae*. Cells were grown on kerosene. (A) Thin section through a hypha. Mb, microbody. (B) Thin section through a microbody. CI, crystalloid inclusion; arrows indicate microbody membrane. (C) Thin section through a hypha, stained with diaminobenzidine at pH 9.2. Sections were not post-stained, thus contrast is low. DAB reaction products are limited to the mitochondrial cristae and to microbodies. (D) Freeze-fracture replica of a spore. The arrow indicates the direction of shadowing. CI, structures believed to represent crystalloid inclusions. A, B, and D from ref. 100 and C from ref. 17, all by permission.

meter, and are limited by a single tripartite membrane, 6–8 nm in thickness (Fig. 1B), which is similar in phospholipid composition to that of the endoplasmic reticulum and which contains phosphatidylcholine, phosphatidylethanolamine, and phoshatidylinositol [25]. In contrast, integral proteins of peroxisomal membranes differ from those present in the endoplasmic reticulum [33,34]. Since microbodies do not contain DNA, synthesis of enzymes and organelle development is apparently controlled by the nucleus.

Over forty distinct enzymes have been localized within microbodies which are believed, therefore, to play a role in several metabolic pathways. There is an extensive literature on enzymes in microbodies of higher plants and animals. The objectives of the present review are to call the attention of microbiologists to microbodies and to summarize what is known of them in fungi. Because readers may have an interest in yeast or in filamentous fungi, we have introduced a somewhat artificial division by treating yeast and filamentous fungi separately. A brief description of microbodies from other organisms is presented and the reader is referred to reviews by de Duve [22], Huang et al. [46], Lazarow and Fujiki [55] and Tolbert and Essner [107] for a thorough coverage of microbodies in higher plants and animals.

Physiologic function

Enzymes of purine metabolism, the glyoxylate cycle, and the glycolate pathway are located within microbodies. Urate oxidase and allantoinase, enzymes involved in purine catabolism, have been found in peroxisomes isolated from rats, mice, birds, amphibians, protozoa, and plants. The ureides, allantoin and allantoic acid, are important nitrogen carriers in some plant species and may represent 10–99% of the total nitrogen present in xylem sap [105]. However, the complete purine degradative pathway has not been found in microbodies. Many of the enzymes required for the conversion of stored lipid to sugar in germinating plant seedlings are contained in microbodies. Glyoxysomes in tissues of oil-rich seeds, such as castor bean endosperm, house the enzymes for a complete glyoxylate cycle while the same organelle in the ciliate protozoan *Tetrahymena pyriformis* contains only the glyoxylate bypass enzymes, isocitrate lyase and malate synthase [67]. Microbodies also participate in photorespiration, a process which may greatly reduce the photosynthetic efficiency of C_3 plants. Leaf peroxisomes contain many of the enzymes for the glycolate pathway of photorespiration [108]. However, the pathway is incomplete, lacking several enzymes required for processing glycolate. Therefore, metabolic cooperation between microbodies and other organelles or the cytosol may be important in biochemical pathways housed partially within microbodies.

A complete β-oxidation enzyme system has been described in microbodies of castor bean, rat liver, and yeasts. The β-oxidation system in microbodies differs from that in mitochondria in that molecular oxygen serves directly for the reoxidation of $FADH_2$ with the concomitant production of hydrogen peroxide at the acyl CoA dehydrogenation step. In addition, the fatty acid β-oxidation system in hepatic peroxisomes oxidizes long chain fatty acyl CoAs, while the mitochondrial system oxidizes both short-chain and long-chain fatty acids [14]. In rat liver peroxisomes CoA is firmly bound to a matrix protein [120].

Two types of specialized microbodies have been discovered in protozoa. 'Hydrogenosomes' have been found in *Trichomonas foetus* which oxidize pyruvate to acetate and carbon dioxide coupled with the formation of ATP [68]. Hydrogenosomes have been reported in a number of trichomonads and rumen ciliates. All have a similar structure of microbodies [68]. Under anaerobic conditions protons serve as electron acceptors thereby generating molecular hydrogen. Also, the 'glycosome', a single-membrane-bound organelle which contains catalase and a portion of the glycolytic pathways, has been found in the protozoan *Trypanoplasma borelli* [74]. Such glycosomes contain nine enzymes involved in the conversion of glucose and glycerol to phosphoglyceric acid as well as enzymes involved in ether-lipid biosynthesis, purine salvage and CO_2 fixation [73]. Microbodies with similar functions have not been observed in fungi, and at least 16 enzymes reported in trypanosomal glycosomes [73] have not been found in fungal microbodies.

Biogenesis

Biogenesis of microbodies is currently a focus of intensive study, and this work has been reviewed [8,55,123]. Studies on biogenesis of microbodies and their components have been aided by the knowledge that formation of microbodies can be induced by certain growth conditions. These include induction in rat liver by feeding phthalates [3,39], in certain yeasts by growth on methanol and other substrates and in certain filamentous fungi by growth on hydrocarbons [100].

A given compound will not necessarily induce microbodies in all fungi. For example, microbodies and asso-

ciated enzymes of β-oxidation plus catalase and isocitrate lyase were induced in *Saccharomyces cerevisiae* by oleic acid, but not by uric acid, alkylated amines, amino acids, ethanol or acetate, which induce microbodies in other yeasts [128].

While early morphologic evidence suggested that microbodies are produced by budding from specialized regions of the endoplasmic reticulum, more recent investigations provide evidence that they arise from preexisting microbodies.

In support of this origin, all microbody enzymes investigated to date, including both membrane and matrix proteins, are synthesized mainly at their final sizes on free polysomes. The endoplasmic reticulum apparently does not participate in this process. After translation these enzymes are then transported through the cytosol and imported into preexisting microbodies. Information directing import and assembly of peroxisomal proteins presumable resides in their amino acid sequences since these proteins do not contain covalently linked carbohydrate or lipid. In vitro systems have been developed to show that microbody preparations from fungal cells and plant tissue take up precursor microbody proteins. Recently, it was reported that the import and assembly of alcohol oxidase into peroxisomes of the methylotrophic yeast *Candida boidinii* is inhibited by proton ionophores, indicating that energy is required for insertion and maturation of this microbody protein [6]. Moreover, purified peroxisomal membranes do not appear to have components in common with endoplasmic reticulum membranes [33].

Microbodies in disease

Microbodies are also involved in a lethal hereditary disease of humans. Zellweger's cerebrohepatorenal syndrome is characterized by liver and kidney tissue which is devoid of microbodies [97]. In addition, patients have low levels of several enzymes of lipid metabolism. This rare disorder is inherited as an autosomal recessive trait. Infants born with the disease usually die within six months. Symptoms include severe hypotonia, brain maturation defects, astrocytic accumulation of lipid, hepatomegaly with cirrhosis, and the presence of renal cysts [40]. A more thorough understanding of the function of microbodies in eucaryotic systems may eventually provide a means for treatment of metabolic disorders associated with microbody dysfunction.

FUNGAL MICROBODIES

Studies of ultrastructure on organisms representing virtually every group of fungi indicate that microbodies are ubiquitous in fungal cells and exhibit great diversity in morphology and function. Microbodies in fungal cells may vary in shape or number per cell as a function of the carbon or nitrogen source supplied in the growth medium as well as the developmental stage or growth phase of the organism. Morphological entities typical of microbodies have been demonstrated in freeze-fracture replica preparations (Fig. 1D).

Isolation of microbodies from fungi is difficult because of the tough fungal cell wall and the fragility of microbodies. Growth on certain substrates such as methanol or hydrocarbon appears to increase their fragility. In cell-free preparations, microbodies must be separated from mitochondria and the buoyant density of both organelles in preparations from fungi can be altered by the growth medium [12,48,54].

More than thirty enzymes have been reported in fungal microbodies (Table 1) which participate in several metabolic functions including fatty acid degradation, gluconeogenesis, purine metabolism, methanol oxidation, oxalate synthesis, and amine metabolism [46]. Although some of these enzymes have been partially characterized [143,147] most have simply been identified as present in microbodies. Although carnitine acetyl-transferase from *C. tropicalis* is not glycosylated [130], little is known about glycosylation of other peroxisomal enzymes in fungi. Undoubtedly, additional enzymes will be identified. These include citrate synthase [66] and the three enzymes which are 'auxiliary' to enzymes of β-oxidation and which function in degradation of polyunsaturated fatty acids: 2,4-dienoyl-CoA reductase, enoyl-CoA isomerase, and 3-hydroxy acyl-CoA epimerase which are localized in the matrix of glyoxysomes obtained from plant seedlings [5].

The distribution of enzymes among organelles may vary in fungi as it does in algae where *Mongeotia scalaris* contains thiolase and acyl-CoA-oxidizing enzymes in its peroxisomes, *Bumilleriopsis filiformis* contains them in its mitochondria, and *Ereosphaera vividis* has them in both organelles [140].

Yeast microbodies

Microbodies in yeasts were first isolated and characterized from strains of *Saccharomyces cerevisiae* [102].

TABLE 1

Enzymes in microbodies of fungi

Enzyme	EC number	Organism	Reference
Marker enzymes for microbodies			
Catalase	(EC 1.11.1.6)	Chytridiomycetes	
		Blastocladiella emersonii	[64]
		Botryodiplodia theobromae	[2]
		Entophlyctis sp.	[84]
		Entophlyctis variabilis	[85,87]
		Monoblepharella sp.	[23]
		Polyphagus euglenae	[88]
		Oomycetes	
		Phytophthora plamivora	[82,91]
		Ascomycetes	
		Aspergillus niger	[117]
		A. tamarii	[41]
		Candida albicans	[77]
		C. sp. N-16	[38]
		C. boidinii	[35,38]
		C. famata	[125]
		C. guillermondii	[77]
		C. intermedia	[77]
		C. lipolytica	[77]
		C. stellatoidea	[47]
		C. tropicalis pk 233	[51]
		C. utilis	[151]
		Cladosporium resinae	[17]
		Hansenula polymorpha	[38,130]
		Neurospora crassa	[53,104]
		Pichia trehalophila	[38]
		Saccharomyces cerevisiae	[81,102]
		Trichosporon cutaneum	[132]
		Basidiomycetes	
		Poria contigua	[9]
		Deuteromycetes	
		Sclerotium rolfsii	[1]
Urate oxidase	(EC 1.7.3.3)	*C. famata*	[125]
		C. tropicalis pk 233	[51]
		C. utilis	[151]
		H. polymorpha	[151]
		N. crassa	[104]
		S. cerevisiae	[81,102]
D-Amino acid oxidase	(EC 1.4.3.3)	*C. boidinii*	[35]
		C. tropicalis pk 233	[52]
		C. utilis	[150]
		H. polymorpha	[130]
		N. crassa	[104]
		S. cerevisiae	[81]
L-α-hydroxy acid oxidase	(EC 1.1.3.15)	*H. polymorpha*	[121]
		N. crassa	[81,102]
		S. cerevisiae	[30]

Table 1 (continued)

Enzyme	EC number	Organism	Reference
Nitrogen metabolism:			
Amine oxidase	(EC 1.4.3.4)	*A. niger*	[117]
		C. utilis	[150]
		H. polymorpha	[150]
		T. cutaneum	[132]
Glutamate-oxalacetate transaminase	(EC 2.6.1.1)	*C. tropicalis pk 233*	[36]
		T. cutaneum	[129]
Glyoxylate bypass:			
Isocitrate lyase	(EC 4.1.3.1)	*A. tamarii*	[41]
		B. emersonii	[64]
		B. theobromae	[2]
		C. lipolytica	[52]
		C. tropicalis pk 233	[52]
		C. utilis	[147]
		E. variabilis	[84]
		N. crassa	[72]
		S. cerevisiae	[128]
		S. rolfsii	[1]
		T. cutaneum	[129]
Malate synthase	(EC 4.1.3.2)	*B. emersonii*	[64]
		Coprinus lagopus	[75]
		Entophlyctis sp.	[84]
		H. polymorpha	[130]
		N. crassa	[53,105]
		S. rolfsii	[1]
		T. cutaneum	[129]
Citric acid cycle:			
Malate dehydrogenase	(EC 1.1.1.37)	*C. utilis*	[150]
		H. polymorpha	[130]
Oxidation of fatty acids:			
Acetyl CoA synthetase	(EC 6.2.1.1)	*C. tropicalis pk 233*	[141]
Long chain acyl CoA synthetase	(EC 6.2.1.3)	*C. lipolytica*	[141]
		C. tropicalis pk 233	[141]
Carnitine acetyltransferase	(EC 2.3.1.7)	*C. tropicalis pk 233*	[113]
		C. stellatoidea	[42]
Acyl CoA dehydrogenase	(EC 1.3.99.3)	*N. crassa*	[53]
Enoyl CoA hydratase	(EC 4.2.1.17)	*C. lipolytic*	[51]
		C. tropicalis pk 233	[51]
		N. crassa	[53]
		S. cerevisiae	[128]
Hydroxyacyl CoA dehydrogenase	(EC 1.1.35)	*C. lipolytica*	[51]
		C. tropicalis pk 233	[51]
		N. crassa	[53]
		S. cerevisiae	[128]
3-Oxoacyl CoA thiolase	(EC 2.3.1.16)	*C. lipolytica*	[51]
		C. tropicalis pk 233	[51]
		N. crassa	[53]
		S. cerevisiae	[128]
Acyl CoA oxidase	(EC 1.1.3.-)	*C. lipolytica*	[51]
		C. tropicalis pk 233	[51]
		S. cerevisiae	[128]

Table 1 (continued)

Enzyme	EC number	Organism	Reference
n-Alkane oxidation:			
Long chain alcohol dehydrogenase	(EC 1.1.1.1)	*C. maltosa*	[60]
		C. tropicalis pk 233	[140]
		C. stellatoidea	[42]
Long chain aldehyde dehydrogenase	(EC 1.2.1.3)	*C. maltosa*	[60]
		C. tropicalis pk 233	[140]
		C. stellatoidea	[42]
Other carbon compounds:			
Methanol oxidase	(EC 1.1.3.13)	*C. boidinii*	[35]
		H. polymorpha	[130]
		P. contigua	[9]
Dihydroxyacetone synthase (a special transketolase)	(EC 2.2.1.1)	*C. boidinii*	[48]
		H. polymorpha	[26]
Glucose oxidase	(EC 1.1.3.4)	*A. niger*	[117]
NAD: Glycerol-3-phosphate dehydrogenase	(EC 1.1.99.5)	*C. tropicalis pk 233*	[52]
Malate dehydroganse	(EC 1.1.1.37)	*C. tropicalis pk 233*	[44]
		T. cutaneum	[129]
Glyoxylate dehydrogenase	(EC 1.2.1.17)	*S. rolfsii*	[1]
NADP: Isocitrate dehydrogenase	(EC 1.1.1.42)	*C. tropicalis pk 233*	[52]
NAD: Glutamate dehydrogenase	(EC 1.4.1.3)	*T. cutaneum*	[129]
Other enzymes:			
Alkaline phosphatase	(EC 3.1.3.1)	*A. nidulans*	[30]
		N. crassa	[30]
In hydrogenosomes:			
Hydrogenase	(EC 1.18.3.1)	*N. patriciarum*	[143]
'Malic enzyme'	(EC 1.1.1.40)	*N. patriciarum*	[143]
Pyruvate: ferredoxin oxidoreductase	(EC 1.2.7.1)	*N. patriciarum*	[143]
Malate dehydrogenase (oxaloacetate reductase)	(EC 1.1.1.37)	*N. patriciarum*	[143]

They do not contain DNA [49]. Catalase, L-α-hydroxyacid oxidase, and malate synthase were detected in the isolated organelles (Table 1). Electron micrographs of serial sections of cells of *S. cerevisiae* grown on glucose contained one to four small microbodies. When cultures were shifted to a medium containing oleic acid, microbodies proliferated. As many as 14 microbodies were observed in a single cell, and they occupied 8–10% of the cytoplasmic volume [128]. Microbodies have now been isolated and characterized for a number of methanol- and hydrocarbon-utilizing yeasts. Catalase and flavin oxidases,such as urate oxidase, L-α-hydroxy acid oxidase, and D-amino acid oxidase are considered marker enzymes for microbodies in fungal cells [77,106].

Although crystalloids were present in peroxisomes in spheroplasts of *H. polymorpha* fixed with glutaraldehyde-osmium, but not in preparations fixed with $KMnO_4$ [132], crystalloid inclusions are considered a feature of yeast microbodies.

By comparing ^{31}P NMR spectra of microbody-rich cells of *C. utilis* and *H. polymorpha* with spectra from cells of the same organisms which contained few microbodies, the internal pH of microbodies was estimated to be 5.8–6.0 while the cytosol was at pH 7.1. Key enzymes in microbodies – alcohol oxidase, isocitrate lyase, malate synthase and catalase – were active at pH 6.0. It was suggested that a proton-translocating ATPase associated with the peroxisomal membrane may maintain the pH difference between cytosol and peroxisome matrix [70]. A membrane-associated ATPase was demonstrated in microbodies of *H. polymorpha*, *C. utilis* and *Trichosporon cutaneum*. The ATP-hydrolysis site was thought to be on the cytosol side of the membrane. In *H. polymorpha* the properties of the ATPase were similar to the properties of

the organism's mitochondrial ATPase associated with vacuoles or with plasma membranes in yeasts in regard to pH optimum and behavior towards inhibitors. The proton electrochemical gradient may play a role in transport of compounds across the microbody membrane [27]. These results are in contrast to findings for rat liver peroxisomes which appear to be permeable to small molecules, thus it is possible that ion gradients cannot be generated across their membranes [119].

Methanol-utilizing yeasts. Peroxisomes in yeasts play a key role in the metabolism of one-carbon compounds used as sole sources of carbon and energy and reviews of their roles are available [124,125]. Species of the yeast genera *Candida*, *Hansenula*, *Kloeckera*, *Pichia*, and *Torulopsis* grow on methanol [78,131]. Growth of yeasts on methanol is associated with increased synthesis of microbodies which contain alcohol oxidase, catalase, D-amino acid oxidase, dihydroxyacetone synthase and L-α-hydroxy acid oxidase [26,35,48,78,118,130]. Methanol is first oxidized to formaldehyde by a hydrogen peroxide-generating alcohol oxidase. In the cytosol, formate, followed by carbon dioxide, is produced through the action of the soluble proteins formaldehyde dehydrogenase and formate dehydrogenase, respectively. In *H. polymorpha* grown on methanol, H_2O_2 produced by methanol oxidase in microbodies is broken down by catalase in the microbodies. In a catalase-negative mutant H_2O_2 is broken down by cytochrome *c* peroxidase located in the intracristae space of mitochondria [134]. It is surprising that the highly toxic H_2O_2 can (presumably) diffuse out of the microbody, traverse some cytoplasmic material, and enter the mitochondrion without exerting its toxicity.

Cells of *H. polymorpha* grown on glucose contained one or a few small microbodies without crystalloid inclusions. After cells were transferred to methanol medium small microbodies divided to give additional microbodies which increased in size and developed crystalloid inclusions. These large microbodies gave rise to additional small microbodies [126].

Peroxisomes of methanol-utilizing yeasts contain prominent crystalloid inclusions and may occupy up to 80% of the total intracellular volume. Aging of cells is accompanied by an increase in the number and size of microbodies. In batch cultures the crystalloid inclusions increased as the culture aged, occupying nearly the entire microbody in stationary phase cells [132].

Ascospores of *H. polymorpha* typically contain 1–3 microbodies which do not appear to have a role in sporogenesis but can metabolize substrates present during germination [127]. Large crystalloid inclusions within microbodies of *Kloeckera* sp. are composed of alternating molecules of methanol oxidase and catalase [79]. In contrast, microbodies from *H. polymorpha* grown on methanol contain only alcohol oxidase as the structural element of peroxisomal crystalloids [124]. Catalase was removed from isolated peroxisomes by osmotic shock. The resultant peroxisomes retained their crystalloid structure and methanol oxidase activity. Microbodies in a methanol oxidase negative mutant of *H. polymorpha* did not contain crystalloid inclusions [126]. When preparations of peroxisomes from rat liver were reacted with antibody against urate oxidase followed by protein A-gold-silver, uricase was localized in crystalloid cores of peroxisomes. Similar preparations prepared using antibody against catalase showed it was located in the peroxisomal matrix [135]. This apparent contradiction regarding location of enzymes in microbodies remains to be clarified. The repeating unit of the crystalloid was suggested as containing four methanol oxidase molecules.

Similar immunocytochemical procedures have served to localize citrate synthase in the glyoxysomal membrane or peripheral matrix [96]. Monospecific antisera against the human liver peroxisomal enzymes catalase and three peroxisomal enzymes involved in β-oxidation of lipids were used to detect peroxisomes in tissues using light microscopy [59]. Such methods should be applied to fungal systems using electron microscopy to localize specific enzymes in microbodies as well as to address the question of whether there are different functional types of microbodies in fungal cells.

Nitrogen metabolism. Microbodies in yeasts exhibit great metabolic diversity; in addition to participating in methanol assimilation, microbodies function in assimilation of nitrogen. Microbodies in cells of *H. polymorpha* and *C. utilis* contained amine oxidase when grown on methanol with methylamine as sole nitrogen source [15]. The same organelle lacked amine oxidase when ammonium sulfate was supplied as the only nitrogen source.

Synthesis and degradation of amine oxidase and methanol oxidase can be regulated separately. *H. polymorpha* grown on methanol as carbon source together with methylamine as nitrogen source contained both methanol oxidase and amine oxidase and formed peroxisomes filled completely with crystalloid material. If cells were transferred to a glucose/ammonium sulfate medium, activity of both enzymes was lost and crystalloid peroxisomes were degraded. If methylamine was replaced by ammonium sulfate, amine oxidase synthesis was re-

pressed but neither extant enzyme nor peroxisomes were degraded. If methanol in the methanol/methylamine medium was replaced by glucose, the crystalloid peroxisomes were degraded and new peroxisomes were produced which contained amine oxidase [133]. Degradation of peroxisomes involves first formation of a number of electron-dense membranes around a single peroxisome (formation of an autophagosome). Contact between the autophagosome and a vacuole provides lytic enzymes from the vacuole for degradation of the peroxisome [121].

Amine oxidase is the first enzyme for nitrogen assimilation from methylamines in these organisms. When these yeasts were grown on medium containing ethanol as carbon source, microbodies housed the glyoxylate bypass enzymes isocitrate lyase and malate synthase [150]. Moreover, changing the sole nitrogen source altered the enzymatic content of microbodies. When D-alanine was supplied as a source of nitrogen, microbodies contained D-amino acid oxidase. However, when uric acid served as sole nitrogen source, uric acid oxidase was the only enzyme participating in nitrogen assimilation found in microbodies.

Transfer of methanol-limited cultures of *H. polymorpha* to a medium containing glucose resulted in degradation of methanol oxidase and catalase [10]. Thus, when enzymes in microbodies are not needed they can be degraded. When methanol-limited cells were exposed to an excess of methanol, a condition where continued activity of methanol oxidase can lead to accumulation of formaldehyde and formate which might lead to cell death, methanol oxidase and catalase were inactivated without being degraded. Evidence was presented which suggested that inactivation involved release of prosthetic groups from the enzymes: FAD from methanol oxidase and heme from catalase [10].

Candida utilis grew on D-alanine as sole carbon and nitrogen source and D-amino acid oxidase was localized in microbodies via direct cytochemistry [148]. In *C. utilis* the enzyme glutamate : oxaloacetate amino transferase (GOT) is exclusively in microbodies and cells do not form aspartase. During growth on aspartate as nitrogen source GOT is involved in aspartate catabolism, but when ammonium sulfate is the nitrogen source high GOT levels suggest a role in aspartate biosynthesis. Thus, it appears that microbodies can be involved in biosynthetic reactions as well as in catabolic reactions [149].

A number of yeast strains in the genera *Candida* and *Trichosporon* [63] can use primary amines as their sole source of carbon and nitrogen. Growth on a primary amine is accompanied by synthesis of microbodies which contain amine oxidase and catalase [151]. Transfer of cells of *T. cutaneum* from glucose to ethylamine was followed by synthesis of microbodies which arose by division of pre-existing microbodies. Metabolism of acetaldehyde produced from ethylamine requires glyoxylate cycle enzymes, and isocitrate lyase and malate synthase cosedimented with amine oxidase and catalase, as did malate dehydrogenase, glutamate : oxaloacetate amino transferase and NAD : glutamate dehydrogenase. Enzymes were localized in microbodies by cytochemical reactions and amine oxidase was localized there by immunocytochemistry involving immunogold labeling of the enzyme [129]. The action of amine oxidase on ethylamine in microbodies generates hydrogen peroxide which is degraded by catalase, ammonia which is used to generate aspartic acid via glutamate : oxaloacetate amino transferase, and acetaldehyde which is converted to acetyl CoA and metabolized via the glyoxylate cycle.

Transfer of *Candida famata* or *Trichosporon cutaneum* from a medium in which glucose or glycerol was the carbon source to one in which uric acid was the carbon and nitrogen source was followed by synthesis of microbodies which arose by growth and division of existing microbodies. The microbodies contained urate oxidase and catalase, but did not contain crystalline inclusions. When urate-grown cells were exposed to glucose, urate oxidase was inactivated but catalase was not and microbodies were not degraded [125].

Hydrocarbon-utilizing yeasts. The synthesis of microbodies is induced in cells of hydrocarbon-utilizing yeasts when they are grown on *n*-alkanes as sole carbon source [76] and the microbodies contain catalase [78] which is immunologically indistinguishable from cytoplasmic catalase [142]. The most common pathway for oxidation of *n*-alkanes is by oxidation at a terminal carbon to yield first a primary alcohol, then the corresponding aldehyde followed by a fatty acid which has the same number of carbons as the alkane. Thus, if growth on an *n*-alkane induces microbody formation, growth on the corresponding fatty acid might also induce microbodies. While this has not been tested, microbodies are induced in the non-hydrocarbon user *S. cerevisiae* by oleic acid [128]. Enzymes of the alkane hydroxylation system, cytochrome *P*-450 and NADPH : cytochrome *c* reductase, were not contained in microbodies from cells of *C. tropicalis* [52] or *C. maltosa* [60] grown on *n*-alkanes, but were located in microsomes. In *C. tropicalis*, catalase, D-amino acid oxidase, urate oxidase, NAD-linked glycerol-3-phos-

phate dehydrogenase, and a fatty acid β-oxidation system were localized in microbodies, while long-chain alcohol and aldehyde dehydrogenases were found in microsomal, mitochondrial, and peroxisomal fractions [116]. In *C. maltosa* the fatty alcohol oxidase and aldehyde dehydrogenase were localized in a fraction containing mitochondria and peroxisomes [7,60]. β-Oxidation occurs in microbodies [36]. Thus, initial oxidation of *n*-alkanes appears to occur in or on the endoplasmic reticulum. The fatty alcohol produced is oxidized further to a fatty acid, and then via β-oxidation in microbodies.

Purified catalase from *C. tropicalis* peroxisomes had a molecular mass of 210000 and was composed of four identical subunits of molecular mass 54000. Each subunit contained one molecule of heme [142]. Alkane-grown cells of *C. tropicalis* contained a mitochondrial and a peroxisomal carnitine acetyl transferase which were separated by DEAE Sephacel chromatography. The isozymes had similar kinetic properties and substrate specificities [113]. The inducible isocitrate lyase from peroxisomes of *C. tropicalis* was compared with its constitutive isozyme which is probably mitochondrial. Each enzyme contained two identical dimers of molecular mass 65000. The enzymes were indistinguishable immunochemically but had slight differences in amino acid composition [110].

Metabolic cooperation between mitochondria and microbodies of *C. tropicalis* is suggested since only isocitrate lyase and malate synthase of the glyoxylate cycle are contained in microbodies, whereas mitochondria house the other three glyoxylate cycle enzymes, aconitase, malate dehydrogenase and citrate synthase, which are common to the citric acid cycle and the glyoxylate cycle. Furthermore, peroxisomal and mitochondrial carnitine acetyltransferases were distinct in this organism [113]. This indicates that an acetyl-carnitine shuttle exists for the transport of acetyl units between the two organelles.

Growth on an alkane or on propionate was associated with appearance of microbodies which contained carnitine acetyltransferase in cells of *C. tropicalis*. The enzyme activity was 18–19 times higher in those cells than in cells grown on glucose and peroxisomal enzyme was immunologically distinct from mitochondrial enzyme [115].

Lastly, only 5% of the total catalase activity in cell extracts of *C. stellatoidea* prepared from *n*-hexadecane-grown cells could be sedimented, whereas 73% of this microbody marker in extracts from glucose-grown cells was particulate [47]. These results suggest that microbodies from hydrocarbon-grown cells were more fragile than those from glucose-grown cells. Microbodies were not separated from mitochondria by centrifugation through continuous gradients of metrizamide or sucrose.

In summary, yeast microbodies play a role in several physiologic processes including nitrogen assimilation, methanol and hydrocarbon utilization, fatty acid oxidation, and the glyoxylate cycle.

Microbodies in filamentous fungi

Microbodies are of physiologic importance in cells of filamentous fungi since they may participate in such fungal activities as plant and insect pathogenesis, zoospore motility, spore germination, hyphal growth, and hydrocarbon metabolism [61]. However, microbodies from filamentous fungi have rarely been isolated and fully characterized through combined biochemical and ultrastructural study. Isolation of microbodies from filamentous fungi is difficult since the organelle is fragile and it is enclosed within a tough cell wall. Moreover, isolation of microbodies using density gradient separation techniques has been hampered since the buoyant density of fungal microbodies is affected by growth conditions and the tonicity of the isolation medium [54,98].

A laboratory survey of 14 taxonomically diverse species of filamentous fungi showed that microbodies are ubiquitous and abundant. Synthesis of microbodies increased when fungi were shifted from a medium with glucose as the sole carbon source to one containing polypectate, carboxymethyl cellulose, or ethanol [62].

Microbodies also occur commonly in pathogenic fungi [61]. The plant pathogen *Sclerotium rolfsii* secretes oxalic acid which breaks down plant cell walls during infection. Two key enzymes for the production of oxalate, isocitrate lyase and glyoxylate dehydrogenase, are located in peroxisomes [1]. Microbodies are present in cells of the insect pathogen *Entomophaga aulicae* [69]. This organism grows in hyphal form on solid media or as a protoplast within the hemolymph of its host. Microbodies observed during the hyphal stage were ovoid and contained one or more crystalline inclusions whereas the same organelles in protoplasts lacked inclusions and were thin and elongate.

Microbodies are abundant in fungal spores. In zoospores of the aquatic chytridiomycete *Entophlyctis variabilis* microbodies stain with DAB [132] and are typically associated with lipid globules and other organelles [87]. This characteristic cluster of organelles composed of microbodies, a lipid globule, mitochondria, and endoplasmic reticulum was named the 'microbody-lipid globule complex' (MLC) [87]. The population of micro-

bodies and organelles associated with it was segregated among zoospores during their formation [84]. It has been found in other chytrids, including *Chytriomyces aureus* and *C. hyalinus* [24], *Synchytrium macrosporum* [65] and *Caulochytrium protostelioides* [89]. It has been suggested that the complexity of the MLC complex in chytridiomycetous fungal zoospores reflects phylogenetic relations in the group [23,86]. Microbodies isolated on discontinuous sucrose gradients contained catalase as well as the glyoxylate bypass enzymes, malate synthase and isocitrate lyase [84]. Analysis of serial thin sections of *E. variabilis* led to the conclusion that microbodies arose from either the endoplasmic reticulum or from other microbodies [87]. Another chytrid, *Polyphagus euglenae*, which parasitizes the alga *Euglena*, contains microbodies associated with lipid globules and mitochondria. Such structures were formerly regarded as chromatin extruded from the nucleus, but are now regarded as involved in lipid metabolism [88]. The microbodies contain catalase [90].

The formation of DAB reaction products is useful for visualizing catalase at the subcellular level. Microbodies in filamentous fungi may not stain with DAB [61], which may be due to use of phosphate buffer rather than cacodylate buffers [42]. Failure to yield reaction product may also occur if the reaction is run at pH 7.2 instead of 9.2 [91]. DAB reaction products may also be deposited due to the action of peroxidase or cytochrome *c* oxidase [83,85,94]. Thus, mitochondrial cristae and cleavage membranes contain DAB reaction product. The presence of reaction product at pH 9.2 but not at 7.2 suggests that the product is due to catalase. Powell [85] combined staining with DAB at pH 7.0 or 9.2 with the catalase inhibitor, aminothiazole, and the inhibitors of heme enzymes, sodium azide and sodium cyanide. The results supported the conclusion that DAB staining of microbodies is due to both catalase and peroxidase, mitochondrial staining is due to cytochrome *c*, and endoplasmic reticulum staining is due to peroxidase [85].

Microbody-like structures called 'U-bodies' were isolated from zoospores of the Oomycete *Phytophthora palmivora* [82]. These bullet-shaped microbodies are lined on the inside by a marginal plate, possess rod-like elements within their matrix, and contain catalase. The presence of U-bodies in zoospores of this fungus was confirmed and ring-shaped and oblong microbodies were observed as well (Fig. 2). When sections were stained at pH 9.2, DAB reaction product filled the core of microbodies, but not the outer shell. U-bodies are considered a type of microbody [91].

Microbody-like organelles were described in dormant basidiospores of *Psilocybe cubensis* but cytochemical localization of catalase using DAB was unsuccessful [95]. Catalase and glyoxylate bypass enzymes were compartmentalized in the water mold *Blastocladiella emersonii* in a 'symphyomicrobody'; a larger organelle formed by the union of small microbodies during sporogenesis [64]. Electron microscopy showed that the symphyomicrobody was DAB-positive.

Direct cytochemistry applied to thin sections of cells of *Neurospora crassa* and *Aspergillus nidulans* showed deposits typical of alkaline phosphatase at microbody membranes [30]. Appropriate controls and an inhibitor were used, but it is not clear whether the enzyme activity was truly membrane-associated or derived from the cytoplasm.

Metabolism of ethanol and acetate. When filamentous fungi are grown on ethanol or acetate as sole carbon source, glyoxylate cycle enzymes are localized in microbodies. Microbodies isolated from *Aspergillus tamarii* grown on ethanol contained catalase, isocitrate lyase, and malate synthase [41]. Derepression of glyoxylate cycle enzyme activities was observed in *Neurospora crassa* after cells were transferred from sucrose medium to acetate medium [31].

Wanner and Theimer [104,139] found two types of microbodies in a plasmodioid wall-less slime mutant of *N. crassa* grown on acetate. Marker enzymes for microbodies were distributed between two particulate fractions which sedimented at different buoyant densities in sucrose density gradients. Catalase and urate oxidase sedimented at a density of 1.24 g/cm^3 whereas malate synthase and isocitrate lyase were found at 1.21 g/cm^3. Moreover, only the population of microbodies which contained catalase was DAB-positive. An inducible β-oxidation system was localized in glyoxysome-like microbodies in cells of *N. crassa* shifted from a sucrose to an acetate medium [53]. The results corroborate the work of Wanner and Theimer [139] in that enzymes of β-oxidation cosedimented with glyoxylate bypass enzymes on sucrose gradients, and were clearly resolved from catalase. The glyoxysome-like microbodies, which were deficient in catalase, also lacked acyl CoA-oxidase, a characteristic enzyme of β-oxidation systems in microbodies. Rather, acyl CoA-dehydrogenase, indicative of mitochondrial β-oxidation, was detected. Thus, more than one type of microbody may be present in cells of filamentous fungi. This aspect should be explored. Moreover, catalase is not always a marker enzyme. The usefulness of catalase as a

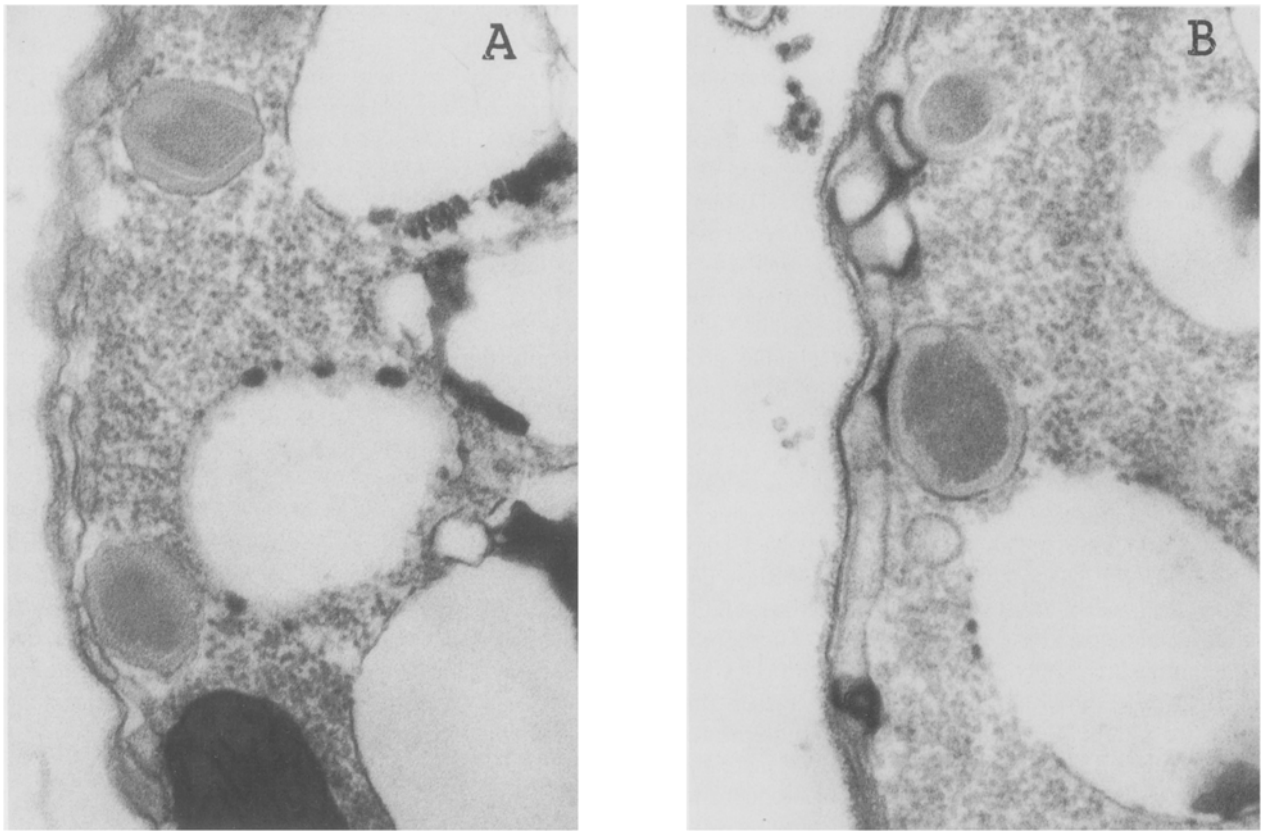

Fig. 2. U-bodies in zoospores of the plant pathogen *Phytophthora palmivora*. (A) Diaminobenzidine (DAB) reaction at pH 7.2. Reaction product is absent in U-bodies, but the U-shaped band and crystalline substructure are visible. (B) DAB reaction at pH 9.2. Reaction product is in the core but not the outer rim of U-body. Courtesy of M.J. Powell, Botany Dept., Miami Univ., Oxford, OH 45056.

marker enzyme is further decreased by reports that in animal cells other organelles contain the enzyme [93,134] and that in *S. cerevisiae* catalase is present in vacuoles and in the cytosol [101].

Oxidases in microbodies. There are two reports of oxidases in microbodies of filamentous fungi. Glucose oxidase, an enzyme important in industry, was detected in microbodies of *Aspergillus niger* [117]. When methylamine was supplied as sole source of nitrogen, peroxisomes of this fungus contained amine oxidase. Methanol oxidase, as well as catalase, is located in microbodies of the brown rot fungus *Poria contigua* [9].

Microbodies and hydrocarbon metabolism. Cladosporium resinae is a major contaminant of light hydrocarbon fuels such as kerosene, jet fuels and diesel fuels [18,28,57,103] where it can cause operational problems in the fuel systems of jet aircraft [80] and ocean-going vessels [71]. *C. resinae* uses branched and non-branched alkanes, alkenes, cycloalkanes and aromatic hydrocarbons as sole carbon sources, but it grows best on *n*-alkanes with chain lengths of 10 to 18 carbons [15,56,103]. Enzymes for oxidation of *n*-alkanes are constitutive and they can act on short, intermediate and long chain substrates [137,138].

Cells of *C. resinae* grown on glucose contained few microbodies while in thin sections of cells grown on *n*-alkanes microbodies made up to 3 to 4% of the cytoplasmic area. Microbodies in sections of whole cells were DAB-positive and ranged in diameter from 250 to 650 nm. They were limited by a tri-laminar membrane which ranged in thickness from 6.2 to 9.7 nm. Some microbodies contained crystalloid inclusions, which had a regular lattice structure with a periodic spacing of 13.0 nm (Fig. 1B). Transfer of cells from medium containing glucose as carbon source to one containing

n-alkanes as carbon source led to synthesis of microbodies and a doubling of the specific activity of catalase [16,100]. Thus, microbodies appear to be involved in growth on *n*-alkanes, but they are probably not involved in the initial oxidation of alkanes since the ability to oxidize *n*-alkanes to the corresponding fatty acid is present in cells grown on glucose, which contain few microbodies [137,138]. Catalase has been localized within microbodies using DAB [17]. In a different strain of *C. resinae* microbodies were formed by cells grown on glucose or on *n*-alkanes [109]. This is consistent with the view that microbodies are present in most fungal cells and proliferate under certain environmental conditions.

Microbodies were isolated from spheroplasts of hydrocarbon-grown *C. resinae* and partially purified by differential and density gradient centrifugation [11]. A fraction enriched in the microbody marker enzyme catalase contained intact microbodies which stained with DAB. Similar fractions from cells grown on glucose did not contain microbodies or catalase. Urate oxidase, a second marker for microbodies, was not detected in cell extracts [12] demonstrating that it is not always a marker enzyme for microbodies. These preparations should prove useful in determining the relationship between microbodies and the metabolism of hydrocarbons by *C. resinae*.

n-Alkanes are metabolized to fatty acids, which are presumably metabolized via β-oxidation yielding acetate. Derepression of glyoxylate cycle enzymes is observed in many filamentous fungi grown on substrates which are presumably metabolized via the glyoxylate cycle [30,61]. It seems important to examine such organisms to determine if synthesis of microbodies accompanies derepression of glyoxylate cycle enzymes. In addition, a thorough search for microbodies should be made among fungi which grow on hydrocarbons, as suggested by the fact that microbodies were not observed in thin sections of a *Penicillium* sp. grown on *n*-hexadecane [19].

Hydrogenosomes. Hydrogenosomes of protozoa contain pyruvate : ferredoxin oxidoreductase and hydrogen [13,58,99,144]. Hydrogenosomes isolated from microbody-like organelles in the anaerobic rumen fungus *Neocallimastix patriciarum* contained hydrogenase, 'malic enzyme', pyruvate : ferredoxin oxidoreductase and NADPH : ferredoxin oxidoreductase (Table 1). They did not contain D-amino acid oxidase or NADH oxidase and thus were not peroxisomes, nor were they glycosomes because they did not contain hexokinase or 3-phosphoglycerate kinase [145], and catalase was not detected [89]. It is clear that the term microbody includes a group of morphologically similar organelles which may have diverse functions.

DISCUSSION AND AREAS FOR FURTHER WORK

Compared to knowledge of microbodies in animal cells and cells of higher plants, relatively little is known about fungal microbodies. Presumably, microbodies in fungi arise from existing microbodies as they do in higher organisms, but this must be established. The observations that a single fungus can yield populations of microbodies with different densities, each of which has its own reaction to DAB, as well as its own array of marker enzyme and other enzymes [53,104,139] suggests strongly that what are called microbodies may represent a group of functionally distinct fungal organelles.

The need to disrupt or remove the tough fungal wall and the fragility of microbodies has hindered progress. Moreover, few studies have combined cytological and biochemical aspects with cell fractionation.

The location of catalase in other organelles [93,142] and the possibility of obtaining false positive and false negative reactions with DAB have decreased the usefulness of catalase as a marker enzyme.

The continued application of immunocytochemical techniques should allow enzyme localization without cell disruption and fractionation. Likewise, use of serial thin sections through microbodies coupled with application of DAB staining [87] and immunocytochemical stains for individual enzymes may help to resolve questions of whether there are discrete functional groups of microbodies. Similarly, 'shift-up' experiments may be useful, wherein cells grown on a substrate such as glucose are shifted to a substrate such as acetate or ethylamine which would presumably elicit the formation of a new or additional population of microbodies. Likewise, application of inhibitors of specific enzymes, such as aminothiazole which inhibits catalase [43,90], may help to elucidate the functions of specific enzymes in microbodies.

There is very little information on genetic or physiologic regulation of enzymes in microbodies. There are well-developed genetic systems for several fungi, notably *Neurospora* and *Saccharomyces*, so that the powerful tools of genetics can be directed to these questions. A clone carrying partial cDNA for peroxisomal catalase has been prepared from *Candida tropicalis* grown on *n*-alkanes [112]. cDNA libraries have been made for cells in which peroxisomes were induced: alkane-grown *C. tropicalis* [92] and methanol-grown *Pichia pastoris* [29]. A genomic

library is available for *C. tropicalis* grown on oleate [50].

The nature and function of crystalloids in fungal microbodies should be addressed as should properties and functions of the limiting membrane and the non-crystalloid 'matrix' material inside the membrane. Such basic aspects as the nature of and requirements for interactions with other organelles should be examined.

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