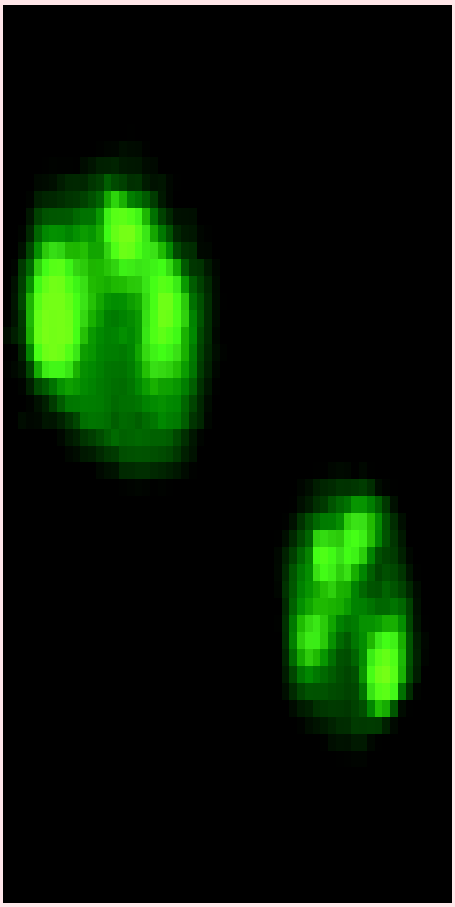
Contents

CHAPTER

9

Microbodies: Peroxisomes and Glyoxysomes



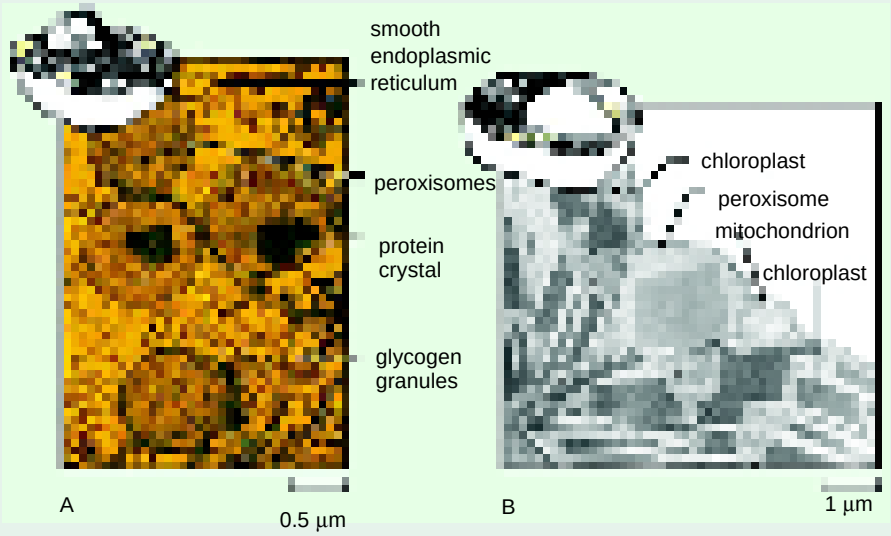
Peroxisomes illuminated by fluorscent protein.

The cells of Protozoa, fungi, plants, liver and kidney of vertebrates contain membrane-bound, spherical bodies of 0.2 to 1.5 μm diameter in close association of endoplasmic reticulum, and mitochondria or chloroplast, or both. These organelles have a central granular or crystalloid core containing some enzymes and are called **microbodies**. Microbodies use molecular oxygen like mitochondria, but instead of having cytochromes and capacity of ATP synthesis like them, they contain flavin-linked oxides and catalases for the hydrogen peroxide metabolism and also enzymes for fatty acid metabolism. Peroxisomes differ from mitochondria and chloroplasts in many ways. Most notably, these organelles are surrounded only by a single membrane, and they do not contain DNA (genome) or ribosomes. However, they resemble ER in being self-replicating membrane bound organelle.

HISTORICAL

Since the mid-1950s electron microscopists have observed small structures or bodies in cells that on morphological grounds have been aptly termed **microbodies**. **C.de Duve** and **P. Baudhuin** (1966) coined the term **peroxisome** for the microbodies of mammalian systems and studied their structure and function. **Leaf peroxisomes** were first isolated from spinach leaf homogenate (*i.e.*, from mesophyll cells) by **Tolbert's** group in Michigen in 1968. Glyoxylate cycle containing peroxisomes,

called **glyoxysomes**, were discovered in 1969 by **Beevers** in the endoplasm cells of germinating castor bean (*Ricinus*).



Peroxisome showing central crystalloid core in plant and animal cell.

MICROBODIES : STRUCTURE AND TYPES

Microbodies are spherical or oblate in form. They are bounded by a single membrane and have an interior or matrix which is amorphous or granular. Microbodies are most easily distinguished from other cell organelles by their content of **catalase** enzyme. Catalase can be visualized with the electron microscope when cells are treated with the stain **DAB** (*i.e.*, 3, 3′-diaminobenzidine). The product is electron opaque and appears as dark regions in the cell where catalase is present. By applying this technique microbodies have been observed by electron microscopy and subsequently isolated from various mammalian tissues such as liver, kidney, intestine and brain.

The technique of **isolation** of microbodies from animal and plant tissues includes the following steps : 1. Tissues are ground very carefully to save microbodies from disruption. 2. The homogenate is treated with differential centrifugation to obtain a fraction of the cell homogenate which is rich in microbodies. 3. The enriched fraction is subjected to isopycnic ultra-centrifugation on discontinuous or continuous sucrose density gradient.

Recent biochemical studies have distinguished two types of microbodies, namely **peroxisomes** and **glyoxysomes**. These two organelles differ both in their enzyme complement and in the type of tissue in which they are found. **Peroxisomes** are found in animal cells and in the leaves of higher plants. They contain catalases and oxidases (*e.g.*, D-amino oxidase and urate oxidase). In both they participate in the oxidation of substrates, producing hydrogen peroxide which is subsequently destroyed by catalase activity :

1. Reduced substrate → $FADH_2$ → H_2O_2
   Oxidized substrate ← FAD ← O_2
2. $H_2O_2 \rightarrow H_2O + O_2$

In plant cells, peroxisomes remain associated with ER, chloroplasts and mitochondria and are involved in photorespiration. **Glyoxysomes** occur only in plant cells and are particularly abundant in

germinating seeds which store fats as a reserve food material. They contain enzymes of glyoxylate cycle besides the catalases and oxidases. Glyoxysomes remain intimately associated with lipid bodies, the spherosomes and contain enzymes for fatty acid metabolism and gluconeogenesis (*i.e.*, formation of glucose from various non-carbohydrate precursors as succinate in this case). Apart from peroxisomes and glyoxysomes, a number of other terms have been used to describe microbodies, including **cytosomes**, **phragmosomes** and **crystal-containing bodies**. A detailed discussion of each type of microbody can be made as follows:

PEROXISOMES

Peroxisomes occur in many animal cells and in a wide range of plants. They are present in all photosynthetic cells of higher plants in etiolated leaf tissue, in coleoptiles and hypocotyls, in tobacco stem and callus, in ripening pear fruits and also in Euglenophyta, Protozoa, brown algae, fungi, liverworts, mosses and ferns.

Structure

Peroxisomes are variable in size and shape, but usually appear circular in cross section having diameter between 0.2 and 1.5μm (0.15 to 0.25 μm diameter in most mammalian tissues; 0.5 μm in rat liver cells). They have a single limiting unit membrane of lipid and protein molecules, which encloses their granular matrix. In some cases (*e.g.*, in the festuciod grasses) the matrix contains numerous threads or fibrils, while in others they are observed to contain either an amorphous nucleoid or a dense inner core which in many species shows a regular crystalloid structure (*e.g.*, tobacco leaf cell, **Newcomb** and **Frederick**, 1971). Little is known about the function of the core, except that it is the site of the enzyme urate oxidase in rat liver peroxisomes and much of the catalase in some plants (see **Hall** *et al.*, 1974).

Functions of Peroxisomes

Peroxisomes are found to perform following two types of biochemical activities :

A. Hydrogen peroxide metabolism. Peroxisomes are so-called, because they usually contain one or more enzymes (*i.e.*, D-amino acid oxidase and urate oxidase) that use molecular oxygen to remove hydrogen atoms from specific organic substrates (R) in an oxidative reaction that produces hydrogen peroxide (H_2O_2) :

$$RH_2 + O_2 \rightarrow R + H_2O_2$$

Catalase (which forms 40 per cent of total peroxisome protein) utilizes the H_2O_2 generated by other enzymes in the organelle to oxidize a variety of other substances—including alcohols, phenols, formic acid and formaldehyde—by the "peroxidative" reaction:

$$H_2O_2 + R'H_2 \rightarrow R' + 2H_2O$$

This type of oxidative reaction is particularly important in liver and kidney cells, whose peroxisomes **detoxify** various toxic molecules that enter the blood stream. Almost half of alcohol one drinks is oxidized to acetaldehyde in this way. However, when excess H_2O_2 accumulates in the cell, catalase converts H_2O_2 to H_2O :

$$2H_2O_2 \rightarrow 2H_2O + O_2$$

H_2O_2 and aging. Most cytosolic H_2O_2 is produced by mitochondria and membranes of ER, although there are also H_2O_2-producing enzymes localized in the cytoplasmic matrix. Catalase acts as a "**safety valve**" for dealing with the large amounts of H_2O_2 generated by peroxisomes; however, other enzymes such as **glutathione peroxidase**; are capable of metabolizing organic hydroperoxides and also H_2O_2, in the cytosol (cytoplasmic matrix) and mitochondria. The production of superoxide anion (O_2^-) in mitochondria and cytosol (cytoplasmic matrix) is regulated mainly by the enzyme **superoxide dismutase**. All of these protective enzymes are present in high levels in aerobic tissues.

Recently, a possible relationship has been stressed between peroxides and free radicals (such as superoxide anion—O_2^-)with the process of aging. These radicals may act on DNA molecule to produce **mutations** altering the transcription into mRNA and the translation into proteins. In addition, free radicals and peroxides can affect the membranes by causing peroxidation of lipids and proteins. For these reasons reducing compounds such as vitamin E or enzymes such as superoxide dismutase could play a role in keeping the healthy state of a cell.

B. Glycolate cycle. Peroxisomes of plant leaves contain catalaze together with the enzymes of **glycolate pathway**, as glycolate oxidase, glutamate glyoxylate, serine-glyoxylate and asparate-α-ketoglutarate aminotransferases, hydroxy pyruvate reductase and malic dehydrogenase. They also contain FAD, NAD and NADP coenzymes. The glycolate cycle is thought to bring about the formation of the amino acids–glycine and serine–from the non-phosphorylated intermediates of photosynthetic carbon reduction cycle, *i.e.*, glycerate to serine, or glycolate to glycine and serine in a sequence of reactions which involve chloroplasts, peroxisomes, mitochondria and cytosol (**Tolbert**, 1971). The glycolate pathway also generates C_1 compounds and serves as the generator of precursors for nucleic acid biosynthesis.

Photorespiration. In green leaves, there are peroxisomes that carry out a process called **photorespiration** which is a light-stimulated production of CO_2 that is different from the generation of CO_2 by mitochondria in the dark. In photorespiration, **glycolic acid** (**glycolate**), a two-carbon product of photosynthesis is released from chloroplasts and oxidized into **glyoxylate** and **H2O2** by a peroxisomal enzyme called **glycolic acid oxidase**. Later on, glyoxylate is oxidized into CO_2 and **formate**:

$$CH_2OH.\ COOH + O_2 \longrightarrow CHO - COOH + H_2O_2$$
$$CHO — COOH + H_2O_2 \longrightarrow HCOOH + CO_2 + H_2O$$

Photorespiration is so-called because light induces the synthesis of glycolic acid in chloroplasts. The entire process involves intervention of two basic organelles : chloroplasts and peroxisomes.

Lastly, photorespiration is driven by atmospheric conditions in which the O_2 tension is high and the CO_2 tension low. Apparently O_2 competes with CO_2 for the enzyme **ribulose diphosphate carboxylase** which normally is the key enzyme in CO_2 fixation during photosynthesis. When O_2 is used by the enzyme, an unstable intermediate is formed which breaks down into **3-phosphoglycerate** and **phosphoglycolate**. The latter tends to increase the glycolate concentration by removal of its phosphate group and, therefore, more glycolate is available for additional oxidation and CO_2 release.

Photorespiration is a wasteful process for the plant cell, since, it significantly reduces the efficiency of the process of photosynthesis (*i.e.*, it returns a portion of fixed CO_2 to the atmosphere). It is a particular problem in C_3 plants that are more readily affected by low CO_2 tensions ; C_4 plants are much more efficient in this regard (see Chapter 11).

C. β-oxidation. Peroxisomes of rat liver cells contain enzymes of β-oxidation for the metabolism of fatty acids. They are capable of oxidizing palmitoyl-CoA (or fatty acyl-CoA) to acetyl-CoA, using molecular oxygen and NAD as electron acceptors (**Lazarow** and **de Duve**, 1976). The acetyl-CoA formed by this process is, eventually, transported to the mitochondria where it enters into the citric acid cycle. If, alternatively, acetyl-CoA remains in the cytosol, it is reconverted into fatty acids and ultimately to neutral fats. β-oxidation pathway of the peroxisomes is very similar to the one that occurs in mitochondria with one very important exception. In mitochondria, the flavin dehydrogenase donates its electrons to the respiratory chain. It does not react with molecular oxygen. In peroxisomes, the dehydrogenase reacts directly with O_2 and in so doing generates H_2O_2. Mitochondria contain no catalase and, therefore, cannot deal with the formation of toxic hydrogen peroxide. For peroxisomes this is not a problem.

D. Other functions. Mammalian cells do not contain D-amino acids, but the peroxisomes of mammalian liver and kidney contain **D-amino acid oxidase**. It is suggested that this enzyme is meant for D-amino acids that are found in the cell wall of the bacteria. Thus, the presumed role of this enzyme is to initiate the degradation of D-amino acid that may arise from breakdown and absorption of peptidoglycan material of intestinal bacteria.

Uric acid oxidase (uricase) is important in the catabolic pathway that degrades purines. Thus, peroxisomes are unusually diverse organelles and even in different cells of a single organism may contain very different sets of enzymes. They can also adapt remarkably to changing conditions. For example, yeast cells grown on sugar have tiny peroxisomes. But when some yeasts are grown on methanol, they develop large-sized peroxisomes that oxidize methanol; when grown on fatty acids, they develop large peroxisomes that break down fatty acids to acetyl-CoA (**Veenbuis**, *et al.*, 1983).

Biogenesis of Peroxisomes

At one time it was thought that the membrane 'shell' of the peroxisomes is formed by budding of the endoplasmic reticulum (ER), while the 'content' or matrix is imported from the cytosol (cytoplasmic matrix). However, there is now evidence suggesting that new peroxisomes always arise from pre-existing ones, being formed by growth and fission of old organelles similar to mitochondria and chloroplasts.

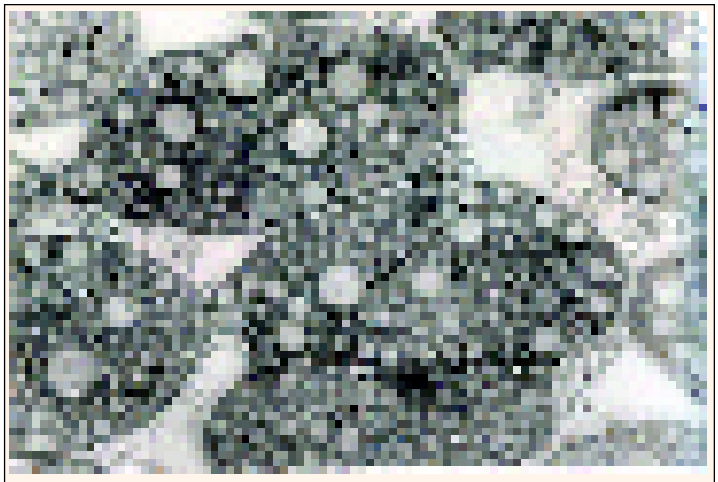
Thus, peroxisomes are a collection of organelles with a constant membrane and a variable enzymatic content. All of their proteins (both structural and enzymatic) are encoded by nuclear genes and are synthesized in the cytosol(cytoplasmic matrix) (*i.e.*, on the free ribosomes). The proteins present in either lumen or membrane of the peroxisome are taken up post-translationally from the cytosol (cytoplasmic matrix). For example, **catalase** enzyme is a tetrameric haeme-containing protein that is made in the cytosol (cytoplasmic matrix) as the haeme-free monomers ; the monomers are imported into the lumen of peroxisomes, where they assemble into tetramers in the presence of haeme. Catalase and many peroxisomal proteins are found to have a **signal sequence** (comprising of three amino acids) which is located near their carboxyl ends and directs them to peroxisome (**Gould**, **Keller** and **Subramani**, 1988). Peroxisomes contain **receptors** exposed on their cytosolic surface to recognize the signal on the imported proteins. All of the **membrane proteins** of the peroxisomes, including signal receptor proteins, are imported directly from the cytosol (cytoplasmic matrix). The lipids required to make new peroxisomal membrarne are also imported from the cytosol (cytoplasmic matrix), possibly being carried by **phospholipid transfer proteins** from sites of their synthesis in the ER membranes (**Yaffe** and **Kennedy**, 1983).

Symbiotic origin. One hypothesis (**de Duve**, 1969) holds that the peroxisomes is a vestige of an ancient organelle that carried out all of the oxygen metabolism in the primitive ancestors of eukaryotic cells. When the oxygen, produced by photosynthetic bacteria, first began to accumulate in the primitive atmosphere of earth, it would have been highly toxic to most types of cells. Peroxisomes may have served to lower the concentration of oxygen in such cells while also exploiting its chemical reactivity to carry out useful oxidative reactions. This hypothesis also holds that the later development of mitochondria rendered the peroxisome largely obsolete because many of the same reactions—which had formerly been carried out in peroxisomes without producing energy—were now coupled to ATP formation by means of oxidative phosphorylation.

GLYOXYSOMES

Glyoxysomes are found to occur in the cells of yeast, *Neurospora*, and oil rich seeds of many higher plants. They resemble with peroxisomes in morphological details, except that, their crystalloid core consists of dense rods of 6.0 μm diameter. They have enzymes for fatty acid metabolism and

gluconeogenesis, *i.e.*, conversion of stored lipid molecules of spherosomes of germinating seeds into the molecules of carbohydrates.



Glyoxysome localization within plant seedlings.

Functions

Glyoxysomes perform following biochemical activities of plants cells :

1. Fatty acid metabolism. During germination of oily seeds, the stored lipid molecules of spherosomes are hydrolysed by the enzyme lipase (glycerol ester hydrolase) to glycerol and fatty acids. The phospholipid molecules are hydrolysed by the enzyme phospholipase. The long chain fatty acids which are released by the hydrolysis are then broken down by the successive removal of two carbon or C_2 fragments in the process of **β-oxidation**.

β-Oxidation. During β-oxidation process, the **fatty acid** is first activated by enzyme fatty acid thiokinase to a **fatty acyl-CoA** which is oxidized by a FAD-linked enzyme fatty acyl-CoA dehydrogenase into **trans-2-enoyl-CoA**. Trans-2-enoyl-CoA is hydrated by an enzyme enoyl hydratase or crotonase to produce the **L-3- hydroxyacyl-CoA**, which is oxidized by a NAD linked L-3-hydroxyacyl-CoA dehydrogenase to produce **3-Keto acyl-CoA**. The 3-keto acyl-CoA looses a two carbon fragment under the action of the enzyme thiolase or β-keto thiolase to generate an **acetyl-CoA** and a new **fatty acyl-CoA** with two less carbon atoms than the original. This new fatty acyl-CoA is then recycled through the same series of reactions until the final two molecules of acetyl-CoA are produced. The complete β-oxidation chain can be represented as follows :

Fatty acid —(Activation: Fatty acid thiokinase+CoA)→ **Fatty acyl - CoA** —(Oxidation: Fatty acyl-CoA dehydrogenase + FAD)→ **trans-2-Enoyl-CoA**

trans-2-Enoyl-CoA —(Hydration (Enoyl-hydratase))↓ **L-3-hydroxyl-CoA**

L-3-hydroxyl-CoA —(Oxidation: L-3-hydroxy acyl-CoA dehydrogenase +NAD)← **3 keto acyl-CoA** —(Thiolytic cleavage: β-keto thiolase)← **Fatty acyl-CoA** + **Acetyl-CoA**

In plant seeds β-oxidation occurs in glyoxysomes (**Cooper** and **Beevers**, **1969**). But in other plant cells β-oxidation occurs in glyoxysomes and mitochondria. The glyoxysomal β-oxidation requires oxygen for oxidation of reduced flavorprotein produced as a result of the fatty-acyl-CoA dehydrogenase activity. In animal cells β-oxidation occurs in mitochondria.

In plant cells, the acetyl-CoA, the product of β-oxidation chain is not oxidized by Krebs cycle, because it remains spatially separated from the enzymes of Krebs cycle, instead of it, acetyl-CoA undergoes the **glyoxylate cycle** to be converted into succinate.

2. Glyoxylate cycle. The glyoxylate pathway occurs in glyoxysomes and it involves some of the reactions of the Krebs cycle in which citrate is formed from **oxaloacetate** and **acetyl-CoA** under the action of citrate synthetase enzyme. The citrate is subsequently converted into **isocitrate** by aconitase enzyme. The cycle then involves the enzymatic conversion of isocitrate to **glyoxylate** and **succinate** by isocitratase enzyme:

$$\text{Isocitrate} \xrightarrow{\text{Isocitratase}} \text{Glyoxylate} + \text{Succinate}$$

The glyoxylate and another mole of acetyl-CoA form a mole of **malate** by malate synthetse:

$$\text{Acetyl CoA} + \text{Glyoxylate} \xrightarrow{\text{Malate synthetase}} \text{Malate}$$

This malate is converted to oxaloacetate by malate dehydrogenase for the cycle to be completed. Thus, overall, the glyoxylate pathway invloves :

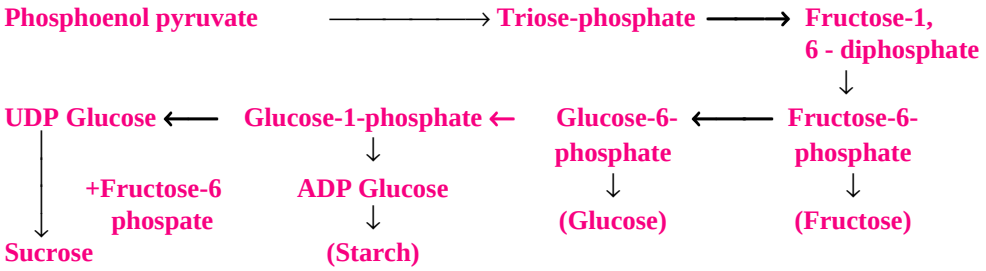
$$2\ \text{Acetyl-CoA} + NAD^+ \longrightarrow \text{Succintiate} + NADH + H+$$

Succinate is the end product of the glyoxysomal metabolism of fatty acid and is not further metabolized within this organelle.

The synthesis of hexose or gluconeogenesis involves the conversion of succinate to oxaloacetate, which presumably takes place in the mitochondria, since the glyoxysomes do not contain the enzymes fumarase and succinic dehydrogenase. Two molecules of **oxaloacetate** are formed from four molecules of acetyl-CoA without carbon loss. This oxaloacetate is converted to **phosphoenol pyruvate** in the phosphoenol pyruvate carboxykinase reaction with the loss of two molecules of CO_2:

$$2\ \text{Oxaloacetate} + 2ATP \rightleftharpoons 2\ \text{Phosphoenol pyruvate} + 2CO_2 + 2ADP$$

The phosphoenol pyruvate is converted into monosaccharides (*e.g.*, glucose, fructose), disaccharide (sucrose) and polysaccharide (starch) by following reation :



REVISION QUESTIONS

1. What are microbodies ? Describe the symbiotic origin theory of the peroxisomes.
2. Define the terms peroxisome and glyoxysome.
3. What would be the most likely role of peroxisome in cells lacking mitochondria ?
4. What is the function of the glyoxylate cycle ?
5. What is photorespiration and how do peroxisomes contribute ?
6. Write an account of structure, synthesis, function and histochemical localization of the catalase enzyme in eukaryotic cells.
7. Describe the mode of biogenesis and various functions of peroxisomes in animal cells.