Lesson: Microbodies

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Table of Contents

Chapter: Microbodies

- **Introduction**
    - **Peroxisomes**
        - **Structure**
        - **Functions**
            - **Fatty acid β-oxidation**
            - **Role of peroxisome in photorespiration**
            - **Conversion of fixed nitrogen to nitrogen-rich organic compounds**
            - **Other functions**
        - **Biogenesis**
        - **Peroxisome assembly**
- **Summary**
- **Exercise/ Practice**
- **Glossary**
- **References/ Bibliography/ Further Reading**

Introduction

Plant cells, protozoan cells, liver and kidney cells of vertebrates contain structurally simple and functionally diverse organelles called **Microbodies.** They were first reported at ultrastructural level in the proximal convoluted tubule of mouse kidney by Rhodin in 1954 and by Rouiller and Bernhard in 1956 in hepatic parenchymal cells. In plants these organelles were first reported by Porter and Caulfield in 1958. Christian Rene´ de Duve did pioneering work in the discovery and isolation of these subcellular organelles. De Duve separated these organelles on the basis of their sedimentation and density properties. The 1974 Nobel Prize for Physiology and Medicine was awarded to De Duve with Albert Claude and George Palade for this work.

The microbodies are composed of single membrane that surrounds the finely granular matrix. These organelles are home to diverse enzymatic reactions including several metabolic reactions that provide energy. They can be distinguished from other organelles by their richness in enzyme catalase. Their average diameter ranges from 0.1μm to 1.5μm.

Two types of microbodies have been distinguished :

- **Peroxisomes** –the peroxisomes are found in almost all eukaryotic cells and contain enzymes that oxidize molecules like fatty acids and amino acids. The byproduct of these oxidation reactions is hydrogen peroxide, which is converted to water and oxygen by an enzyme **catalase** present in the peroxisomes.
- **Glyoxysomes** – contain in addition to the enzymes found in peroxisomes the enzymes isocitrate lyase and/or malate synthetase the two enzymes of the glyoxylate cycle. In germinating seeds these organelles are involved in mobilization of fats.

Peroxisomes- Structure

Peroxisomes are ubiquitous single membrane (typical lipid bilayer) bound organelles that contain various metabolic enzymes including those involved in energy metabolism. Peroxisomes assume various forms. In rat liver these form large spheres of about 0.5 μm diameter with a paracrystalline core while in fibroblasts it consists of small 0.1-0.2 μm vesicles. Under some conditions in yeast cells and in liver cells these assume a tubular form, which is interconnected to the spherical elements. In 1965, Christian de Duve, while studying microbodies of rat liver, showed the presence of oxidases that transfer hydrogen

atom to molecular oxygen forming hydrogen peroxide. He coined the term peroxisomes, for the organelle because it produced and consumed hydrogen peroxide. It occurs in some animal cells and all photosynthetic cells of higher plants. In plants they perform wide range of functions like participation in lipid mobilization, metabolism of free oxygen radicals, synthesis of cholesterol and other lipids, catabolism of long chain fatty acids or conversion of fixed nitrogen into nitrogen-rich organic compounds and many others.

Peroxisomes appear circular in cross section, have a single membrane enclosing a granular matrix. These organelles do not have their own genome and all their proteins called **peroxins** are encoded by the nuclear genome. These are synthesized on free ribosomes in the cytosol and imported into the peroxisomes. Peroxisomes can replicate by division and can also be regenerated de novo even if entirely lost from the cell.

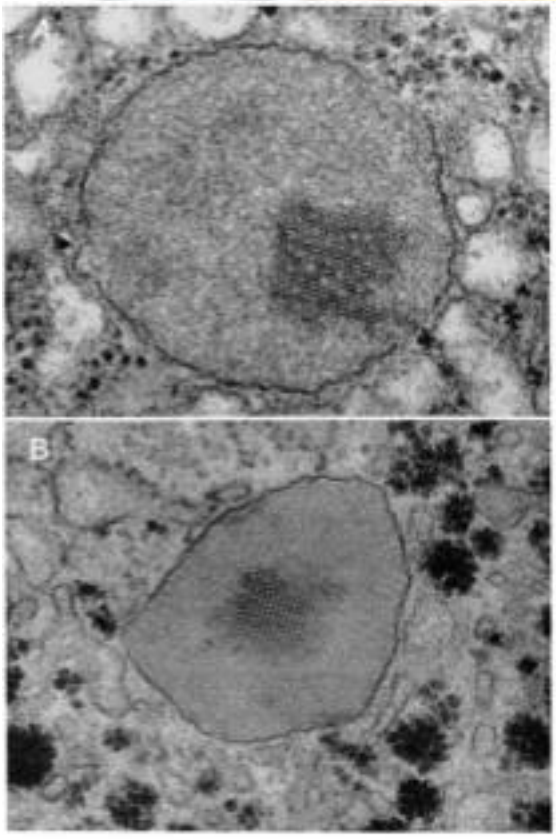


Figure: The core of the peroxisomes appears as crystalline, crystalloid or multilamelllated. Source: Duve, C.D. and Baudhun,P. 1966. Physiological reviews 46:303.

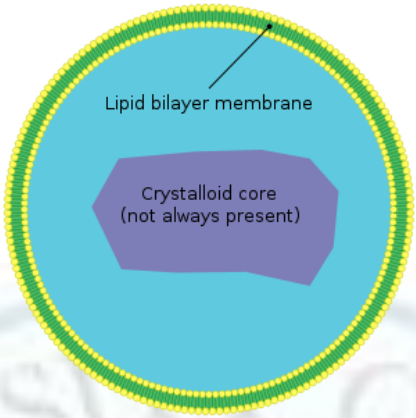


Figure: Structure of peroxisomes

Source:http://upload.wikimedia.org/wikipedia/commons/thumb/c/cb/Peroxisome.svg/300px-Peroxisome.svg.png

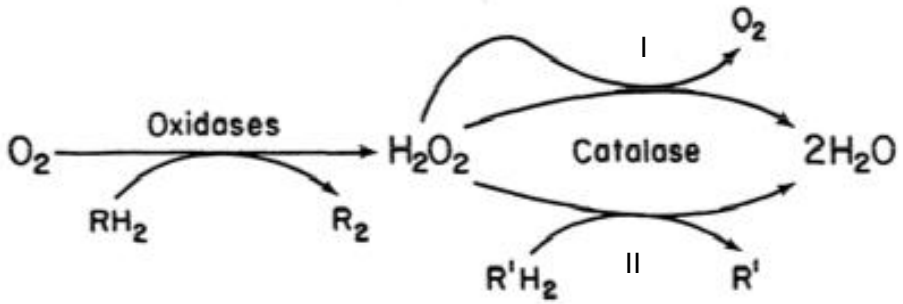
DID YOU KNOW?

- Transport of proteins into peroxisomes is facilitated by a specific sequence of three amino acids located at the carboxyl terminus of these proteins.
- Interestingly, if this sequence of amino acids is attached to a cytosolic protein, the protein gets transported into peroxisomes.
- If this sequence is lacking in a human being – a disease called Zellweger syndrome occurs which leads to severe protein deficiency in peroxisomes. Such patients suffer from severe abnormalities in brain, liver and kidney and die soon after birth.

Functions

Peroxisomes contain 50 different enzymes involved in a variety of biochemical pathways. Although initially identified in organelles that carried out oxidation reactions leading to the production and eventually destruction of hydrogen peroxide. Peroxisomes contain the enzymes – oxidases and catalases. The oxidases oxidizes the substrates (RH_2) and reduces oxygen to hydrogen peroxide (H_2O_2). The hydrogen peroxide is decomposed by catalase either by conversion to water (I) or by oxidation of another organic compound - $R'H_2$ (II).

Thus harmful H_2O_2 is broken down immediately within the organelle and never reaches the cytoplasm.



These reactions can cause oxidative breakdown of various substrates including amino acids, alcohols, purines, uric acid and fatty acids.

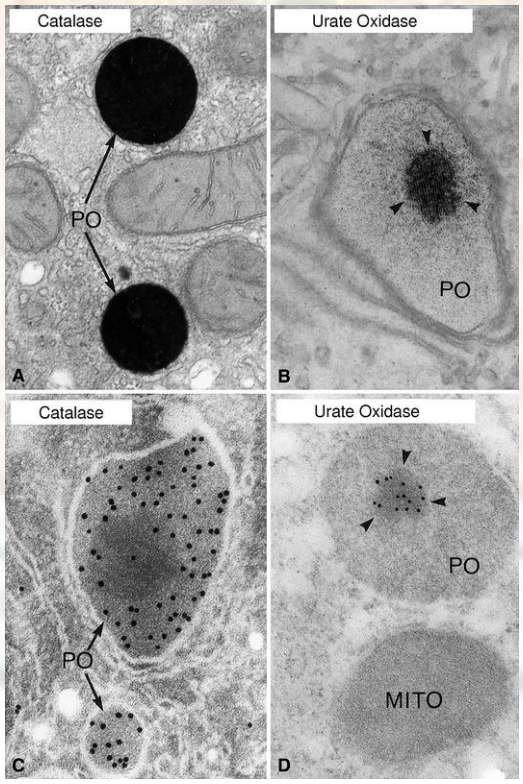


Figure: A.Cytochemical localization of catalase in rat hepatic peroxisomes stained with the alkaline diamino-benzidine technique. Note the uniform staining of the peroxisome matrix. Magnification, ×28,600.***B.***Cytochemical localization of urate oxidase in rat liver using the cerium method. Note the dark staining of the crystalline core (*arrowheads*). Magnification,

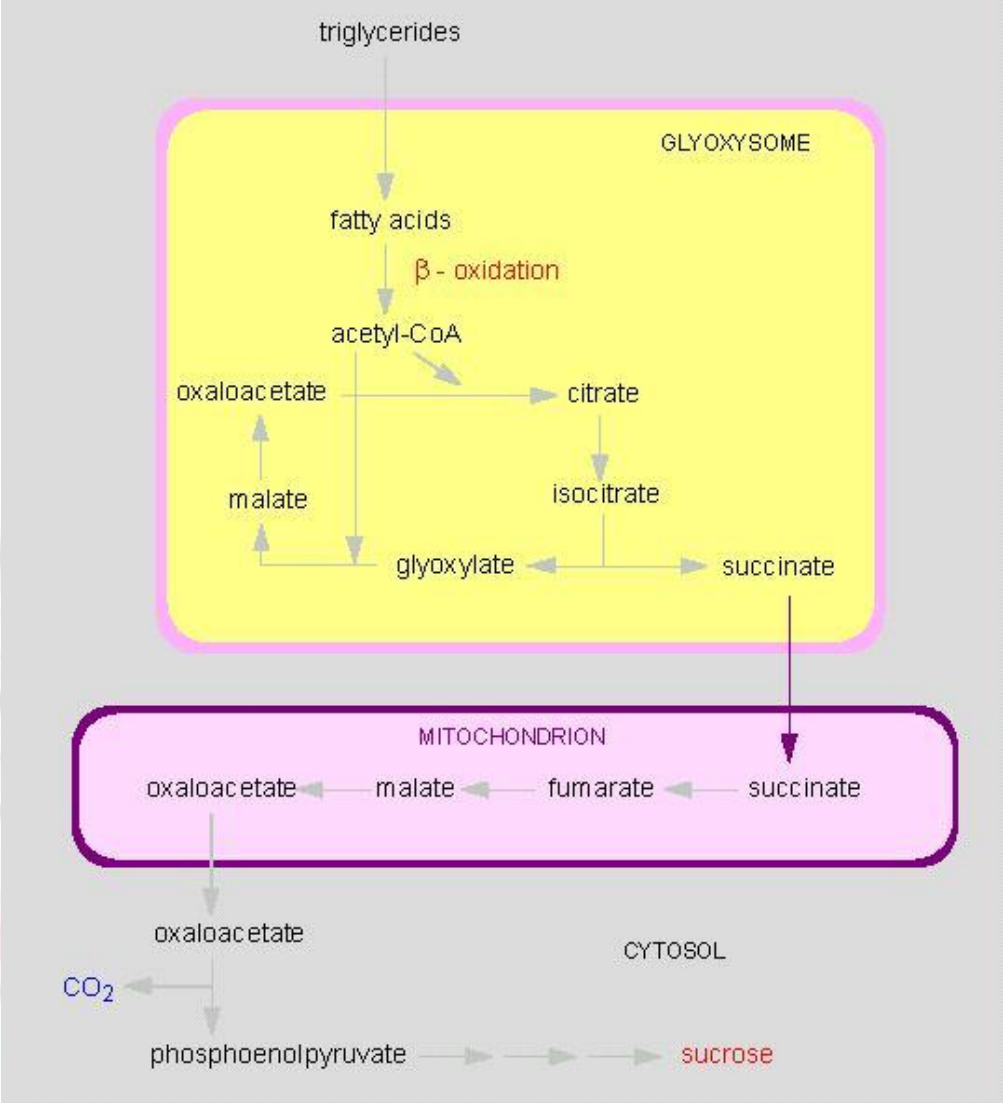
×50,400.**C.**Immunocytochemical localization of catalase in rat liver using the protein A-gold technique. Note the diffuse labelling of the matrix with gold particles and the sparing of the core region. Magnification, ×72,000.***D.*** Immunocytochemical localization of urate oxidase in rat liver using the protein A-gold technique. Note the exclusive labelling of the core with gold particles (*arrowheads*). Magnification, ×61,600.
Source:http://www.springerimages.com/img/Images/Springer/PUB=Springer-Verlag-Berlin-Heidelberg/JOU=00418/VOL=2008.129/ISU=4/ART=2008_396/MediaObjects/MEDIUM_418_2008_396_Fig2_HTML.jpg

In addition the peroxisomes play two important role in plants:

- Conversion of fatty acids to carbohydrates (β oxidation) providing energy and raw material for growth. In animals the process involves two organelles mitochondria and peroxisomes. In plants however oxidation of fatty acids occurs exclusively in peroxisomes.
- Photorespiration, which metabolizes phosphoglycolate a side product of photosynthesis.

Fatty acid β-oxidation

In plants peroxisomes are responsible for the breakdown of fatty acids to provide energy for growth in germinating seeds via series of reactions called as the glyoxylate cycle. These specialized peroxisomes are termed **glyoxysomes**. These contain in addition to the other enzymes the two enzymes of the glyoxylate cycle- isocitrate lyase and/or malate synthetase. In oil rich seeds like sunflower and castor, oil is converted to fatty acid and glycerol during germination. In glyoxysomes fatty acids are broken down by β oxidation and the intermediate products are further converted to sucrose.



Role of peroxisome in photorespiration

Photorespiration and photosynthesis are opposing processes that occur in plants. Both the processes are initiated in the chloroplasts but the in photorespiration leaf peroxisomes and mitochondria are also involved. Rubisco is a Calvin cycle enzyme which catalyses CO_2 fixation during photosynthesis. It is a unique enzyme which can function both as oxygenase and carboxylase. As a carboxylase it adds atmospheric CO_2 to Ribulose – 1,5- bisphosphate to form two molecules of 3-phosphoglycerate.As oxygenase, ribulose 1,5 bisphosphate is split into 1 molecule of 3-phosphoglycerate and 1 molecule of 2-phosphoglycolate. The phosphogycolate cannot be used in Calvin cycle and hence it is utilized in another pathway

devised by nature as photorespiration, which is driven by high oxygen and low carbon dioxide condition in the atmosphere.

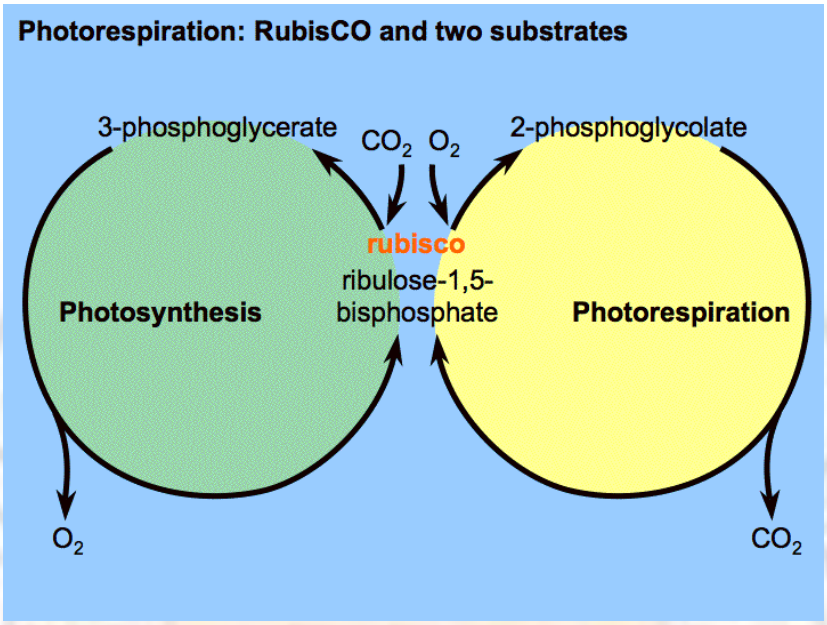


Figure: When the levels of CO_2 fall the Rubisco combines RUBP with O2 instead of CO2 producing one molecule of 3C PGA and one molecule of 2C Phosphoglycolate.

Source: http://plantphys.info/plant_physiology/images/photorespoverview.gif

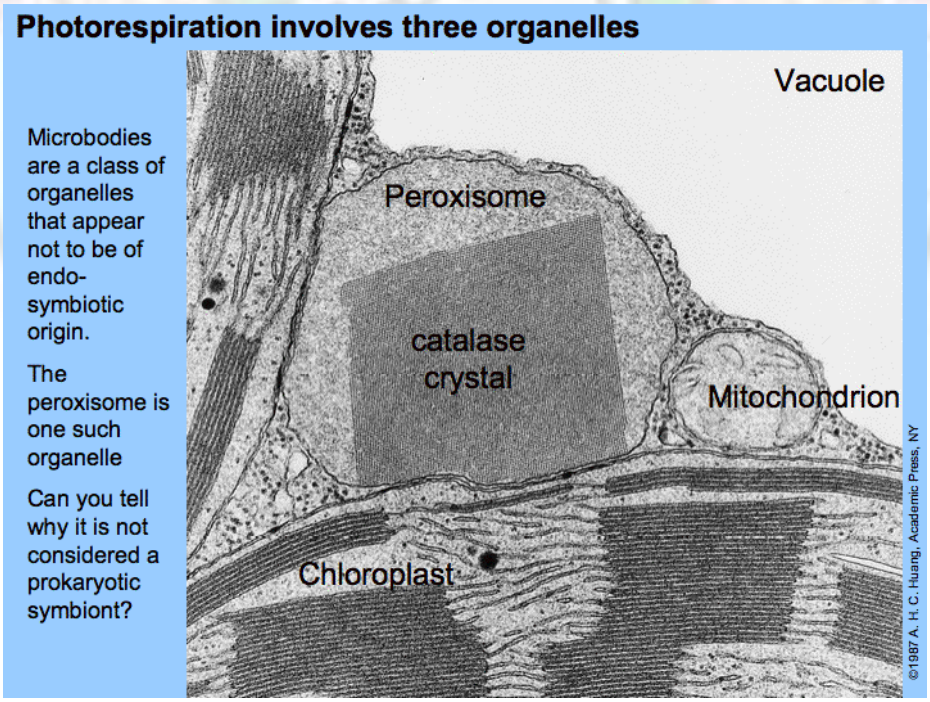


Figure: In plants electron micrographs reveal that three organelles chloroplast, leaf peroxisome and mitochondrion are closely associated demonstrating the relation between function and physical organization.

Source: http://plantphys.info/plant_physiology/images/photorespem.gif

Peroxisomes contain enzymes of glycolate pathway namely glutamate glyoxylate, serine glyoxylate, malic dehydrogenase and glycolate oxidase. Glycolate pathway returns around 75% of the reduced carbon in phosphoglycolate to the Calvin cycle by involving interaction between chloroplast, leaf peroxisome and mitochondrion. In photorespiration, a two-carbon product glycolate is released from chloroplast and oxidized to glyoxylate and H_2O_2 by a peroxisomal enzyme called glycolic acid oxidase.

The phoshoglycolate is converted to glycolate and transported to the nearby peroxisome. In the peroxisome the glycolate is oxidized to form glyoxylate and hydrogen peroxide. The hydrogen peroxide is converted to water and oxygen by the enzyme catalase present in the peroxisomes. The glyoxylate is converted to the amino acid glycine and transported to the mitochondria. Two molecules of glycine combine to form serine which is then transported to peroxisome. In the peroxisome the serine loses its amino group to α- ketoglutarate and is converted to hydroxypyruvate which is further reduced to glycerate by the enzyme hydroxypyruvate reductase. The glycerate is then transported to the chloroplast where it forms 3-phosphoglycerate. The phosphate molecule is derived from the ATP. Photorespiration thus instead of producing ATP utilizes the same and thus is considered to be energetically wasteful mechanism.

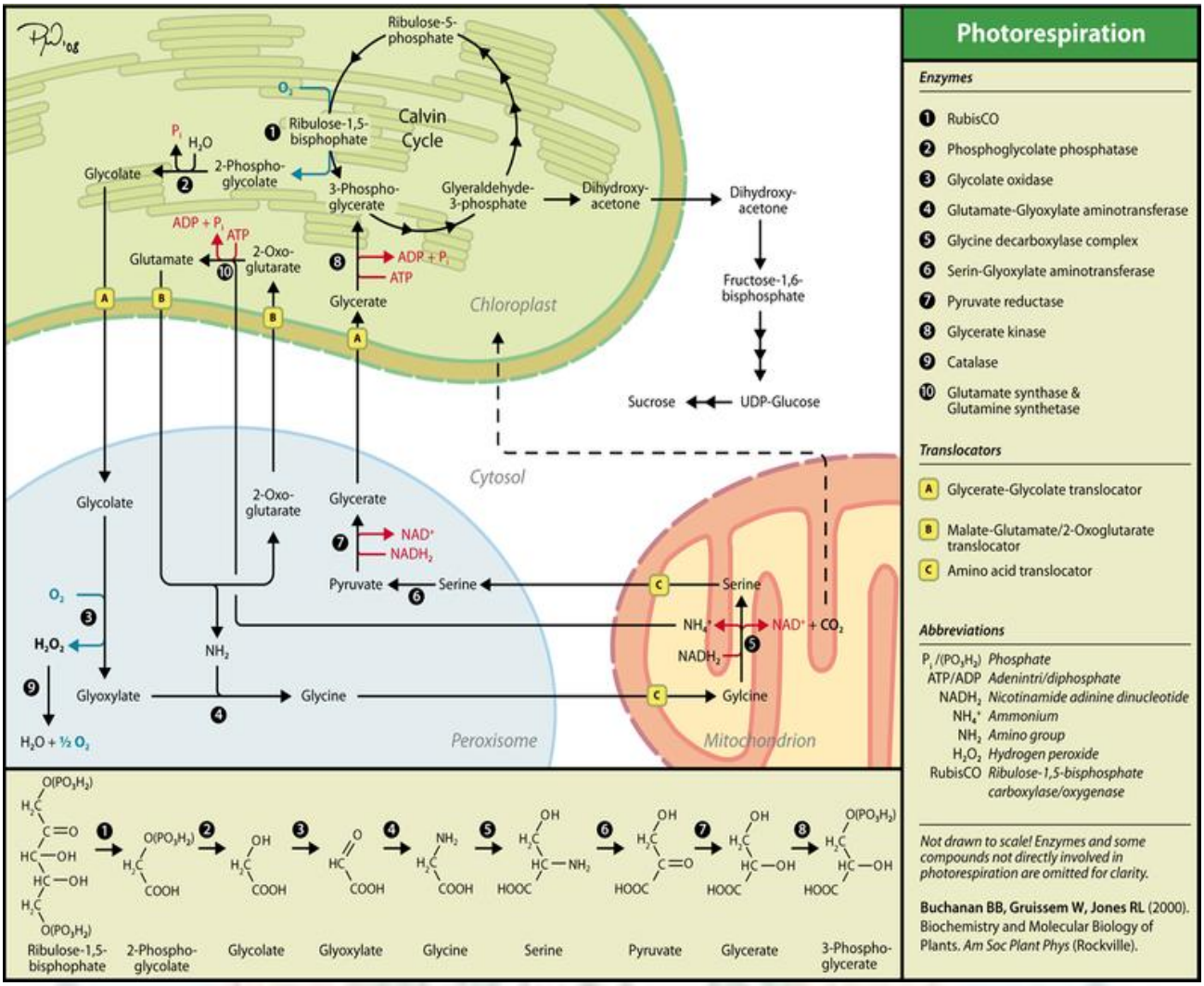


Figure: The photorespiration pathway

Source:http://upload.wikimedia.org/wikipedia/commons/thumb/2/2c/Photorespiration_eng.png/795px-Photorespiration_eng.png

This process reduces efficiency of photosynthesis, potentially reducing photosynthetic output by 25% in C3 plants. Photorespiration involves a complex network of enzyme reactions that exchange metabolites between chloroplasts, leaf peroxisomes and mitochondria.

The oxygenation reaction of RuBisCO is a wasteful process because 3-Phosphoglycerate is created at a reduced rate and higher metabolic cost compared with RuBP carboxylase activity. While photorespiratory carbon cycling results in the formation of G3P eventually, there is still a net loss of carbon (around 25% of carbon fixed by photosynthesis is re-

released as CO2) and nitrogen, as ammonia. The ammonia must be detoxified at a substantial cost to the cell. Photorespiration also incurs a direct cost of 2ATP and one NAD(P)H. While it is common to refer to the entire process as photorespiration, technically the term refers only to the metabolic network which acts to rescue the products of the oxygenation reaction (phosphoglycolate)

Conversion of fixed nitrogen to nitrogen-rich organic compounds

Several legumes fix nitrogen in organic intermediates that become ureides, nitrogen rich compounds useful in translocating nitrogen to the growing and storage organs of the plants. One of the last steps in ureide production, i.e. conversion of uric acid to allantoin is catalyzed by urate oxidase, a hydrogen peroxide generating enzyme packed with catalase in peroxisomes of interstitial cells.

Other functions

- Biosynthesis of lipids and amino acid lysine
- Synthesis of cholesterol and Dolichol in animals
- In liver cells the synthesis of bile acids
- Synthesis of plasmalogens (an important phospholipid that is a membrane component of heart and brain tissues.

GLYOXYSOMES AND LEAF PEROXISOMES CAN BE INTERCONVERTED
Microbodies were first isolated from plants in 1967 by Breidenbach and Beevers and were called as Glyoxysomes because they contained the enzymes for Glyoxylate cycle (for eg. Isocitrate lyase and malate synthetase). The same population of micro bodies can change its enzyme constitution depending on the developmental stage and activity that has to be performed. It is seen that cotyledons of oil seeds, after greening undergo senescence and at this stage leaf peroxisomal activity disappears and glyoxysomal activity reappears. Such a conversion is also seen in oil storing cotyledon seeds of cotton and legumes etc. following exposure to illumination. The functions of the microbodies are changed from lipid metabolism to photorespiratory metabolism.

Biogenesis

It is seen that new peroxisomes arise from pre-existing ones and grow by importing lipids, membrane proteins and matrix proteins from cytosol. Peroxisomes contain receptors on their cytosolic surface to recognize the signal on the imported proteins.

Another hypothesis, proposed by Christian de Duve states that peroxisomes represent an ancient class of respiratory particles which has been rendered obsolete as its function was taken over by mitochondria which was more efficient. They are retained just to perform above mentioned functions.

Interesting Facts

- Human congenital diseases are associated with the absence of peroxisomes and/or with the dysfunction of their enzymes
- Many chemicals (drugs,industrial pollutants) induce a marked proliferation of peroxisomes
- Prolonged treatment with most proliferators induce malignant hepatic tumors
- Estradiol has depressive effect on peroxisomes in fish hepatocytes.

DID YOU KNOW

All peroxisomal proteins are synthesized from nuclear genome and are called peroxins (example: Pex1,Pex2 etc.)

- Approximately,85 genes in human genome are known to code for peroximal proteins, most of which are metabolic enzyme.
- These proteins resemble the eukaryotic proteins unlike the endosymbiont derived organelles where the proteins resemble the prokaryotic proteins.

Peroxisome assembly

- Peroxisome assembly begins on rough endoplasmic reticulum, where two peroxins, namely Pex3 and Pex19 are localized.
- Interaction between these two proteins causes Pex3/ Pex19- containing vesicles to bud off from ER.
- These vesicles fuse with pre- existing peroxisomes or with one another to form new peroxisomes.
- Pex3,Pex19 and other peroxisomal proteins then act as receptors for import of other proteins, which are translated on free cytosolic ribosomes and then transported into peroxisomes as completed and folded polypeptides.
- They are targeted to peroxisomes by either of the two pathways which are conserved from yeasts to humans. Peroxisome targeting signal 1(PTS-1)-→ simple amino acid sequence ser-lys-leu at their carboxy terminus. Peroxisome targeting signal-2(PTS-2)→ a sequence of nine amino acids at their amino terminus. Both PTS-1 and PTS-2 are recognized by distinct cytosolic receptors and are passed through a channel in peroxisomal membrane into the matrix.

For details visit: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3082194/

Summary

Peroxisomes are small organelles, bound by single membrane that contain enzymes involved in a variety of metabolic reactions including fatty acid oxidation, the glyoxylate cycle and photorespiration. They are found in both animal and plant tissues and contain a number of flavin oxidases that produce hydrogen peroxide. The potentially harmful peroxides are degraded by peroxisomal catalase. Glyoxysomes are found in germinating seeds and contain enzymes of the glyoxylate cycle on addition to other peroxisomal enzymes. Peroxisomal proteins are synthesized in the cytosol and are imported into the peroxisomes.

Exercise

1. What are microbodies ?
2. Distinguish between peroxisomes and glyoxysomes.
3. What is photorespiration and what is the role of peroxisomes in it?

4. In what ways are peroxisomes similar to mitochondria and in what ways they are unique?

5. Discuss the role of catalase in peroxisomal activities.

Glossary

Catalase: An enzyme responsible for hydrogen peroxide metabolism

Glyoxylate cycle: A metabolic pathway which replenishes two carbon metabolites; it is associated with glyoxysomes.

Peroxisome: Single membrane bound intracellular organelle that contains a granular matrix and enzyme involved in hydrogen peroxide metabolism.

Peroxins: Peroxisomal proteins synthesized from nuclear genome.

Photorespiration: Uptake of oxygen and release of carbon di oxide by photosynthetic cells or whole plant in light.

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Links

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