Metabolic significance of microbodies in the yeasts Candida utilis and Hansenula polymorpha.
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All micro-organisms including yeasts require a carbon- and nitrogen source for the synthesis of cell material. For that purpose they may utilize a variety of compounds. Generally these compounds undergo several modifications in order for them to become incorporated into cell material. These modifications occur in the intermediary metabolism of the cell.

Certain carbon compounds are metabolized by yeasts in special compartments of the cell, namely peroxisomes. Apart from yeasts, these organelles have also been encountered in cells of animals and in plant tissue. They belong to a class of relatively simple subcellular organelles for which the general name microbody has been proposed. Peroxisomes are microbodies that are characterized by the presence of one or more hydrogen-peroxide producing oxidases, together with catalase which ensures degradation of hydrogen peroxide that may otherwise build up to toxic concentrations.

The introduction of this thesis (Chapter I) gives an overview of the properties of peroxisomes and other microbodies of cells of animals, plants and unicellular organisms. Their morphology and ultrastructure, their composition in relation to the function of these organelles and their biogenesis and turnover is discussed.

Chapter II describes the discovery of a novel peroxisomal enzyme, namely amine oxidase. The yeasts Candida utilis and Hansenula polymorpha have the capacity to utilize primary amines as a nitrogen source. These amines contain, apart from nitrogen, one or more carbon atoms and prior to the assimilation of the amine nitrogen into cell material, the nitrogen atom must be separated from the carbon moiety. In yeasts this reaction is catalyzed by the enzyme amine oxidase. By means of cytochemical staining techniques in conjunction with electron microscopy it was possible to localize the activity of this enzyme in peroxisomes. Peroxisomes of yeasts grown in the presence of methylamine as the sole nitrogen source had increased in size compared to cells grown in the presence of ammonium sulphate. In the latter cells no amine oxidase was detectable. During growth in the presence of primary amines as a nitrogen source, yeast peroxisomes therefore play an essential role in the nitrogen metabolism of the cell. All nitrogen must pass these organelles prior to further processing.

As indicated above in addition to nitrogen, amines also contain one or more carbon atoms. Surprisingly enough these yeasts are not able to utilize amines as the sole carbon source for growth. This is even more striking when for example the metabolism of ethylamine is compared with that of ethanol. Ethanol can be used as a carbon source and for that purpose is oxidized to acetaldehyde. From the level of acetaldehyde energy is generated and cell material is synthesized. Ethylamine is also oxidized to acetaldehyde and theoretically yeasts should be able to metabolize this acetaldehyde in a fashion similar to that produced from ethanol. Yet, growth on ethylamine is not
possible. The results discussed in chapter III demonstrate that growth on amines as a carbon source is prevented as a consequence of the regulation of the synthesis of amine oxidase in these yeasts. Ammonium ions act as a powerful repressor of the synthesis of this enzyme which results in an ultimate cessation of amine oxidation. Utilization of amines as a carbon source inevitably leads to excretion and accumulation of ammonia into the growth medium because the ratio of carbon (C) over nitrogen (N) is significantly lower in amines than in cell material (i.e. in ethylamine C:N = 2:1 versus cell material C:N = 7:1). As a consequence amine oxidase synthesis is blocked and sustained growth is impossible.

The yeast Hansenula polymorpha has the capacity to utilize methanol as the sole carbon source and it has been known for a number of years that the peroxisomal enzyme alcohol oxidase plays an essential role in the metabolism of this compound. Cells grown in media containing methanol as a carbon source and methylvamine as a nitrogen source synthesized two different hydrogen peroxide producing oxidases simultaneously, namely alcohol oxidase and amine oxidase. It was an interesting problem to investigate whether these two enzymes were located in different peroxisomes or in one organelle. The results presented in Chapter IV clearly demonstrate the occurrence of the latter situation. The peroxisomes formed in such cells are involved in carbon metabolism and nitrogen metabolism simultaneously. Cells grown on methanol and methylvamine contain large peroxisomes, in contrast to glucose/ammonium sulphate-grown cells, in which generally one small peroxisome can be observed. Chapter IV further describes that addition of glucose and ammonium sulphate to methanol/methylamine-grown H. polymorpha cells results in a rapid decrease in activity of alcohol oxidase and catalase. This is accompanied by a process of degradation of the large peroxisomes.

Chapter V discusses the role of yeast peroxisomes in the degradation of choline and ethanolamine. These important constituents of yeast phospholipid membranes can be utilized by these species as a sole nitrogen source. It is demonstrated that the enzyme responsible for the oxidation of primary amines, namely amine oxidase, also is involved in the metabolism of choline and ethanolamine.

In Chapter VI growth of the yeast C. utilis at the expense of the amino acid D-alanine is discussed. As in amines, D-alanine contains carbon atoms besides a nitrogen atom, but in contrast to amines D-alanine can be used by C. utilis as a carbon- and nitrogen source. It is demonstrated that the enzyme D-amino acid oxidase plays a key role in the metabolism of this compound and is located exclusively in peroxisomes. During growth on D-alanine these organelles are therefore involved both in the cell's carbon- and nitrogen metabolism. Such a phenomenon was also observed in H. polymorpha during growth in methanol/methylamine containing media. The major difference is, however, that in the case of D-alanine only one enzyme plays a role, whereas in the case of methanol/methylamine two different enzymes are involved. The carbon over nitrogen ratio in D-alanine is 3:1 so that growth on this substrate also results in the
excretion of ammonium ions. Free ammonium ions do not influence the synthesis of D-amino acid oxidase. It was demonstrated that the synthesis of this enzyme is regulated by induction.

Many micro-organisms, including yeasts, have the capacity to utilize C₂-compounds for growth. The basic elements for the synthesis of cell material are C₃-compounds, which are synthesized, at least partly, by means of a special metabolic pathway, the glyoxylate cycle. The glyoxylate cycle resembles in several aspects another important metabolic pathway in the cell, namely the TCA cycle, which plays an important role in the cell's energy supply. Three of the five glyoxylate cycle enzymes also play a role in the TCA cycle. The remaining two, isocitrate lyase and malate synthase, however, do not. The latter are regarded as key enzymes of the glyoxylate cycle. It is known that the TCA-cycle enzymes are located in mitochondria, organelles completely different from microbodies. For the localization of glyoxylate cycle enzymes in yeasts cytochemical staining techniques are not suited and for that reason another technique was applied in which protoplasts obtained from whole cells were homogenized gently and subsequently fractionated by means of differential and sucrose gradient centrifugation techniques (Chapter VII). The results showed that in yeasts grown on ethanol, the key enzymes of the glyoxylate cycle were localized in organelles which were identified as microbodies. Microbodies containing glyoxylate cycle enzymes are called glyoxysomes. Cells grown in media containing ethanol as a carbon source and D-amino acids or primary amines as a nitrogen source contained microbodies in which apart from glyoxysomal enzymes also peroxisomal enzymes were located. Under these growth conditions these organelles therefore were also simultaneously involved in the cell's carbon- and nitrogen metabolism. Moreover these results demonstrated that the differences between peroxisomes and glyoxysomes are not as strict as initially thought. The enzyme composition of these microbodies is completely determined by the growth conditions.

Finally, in Chapter VIII, a possible explanation is provided for the phenomenon that in yeasts microbodies always seem to be present. Not only under conditions where these organelles have an important function, but even under conditions where their metabolic function seems less obvious. The latter is for example the case during growth in media containing glucose as a carbon source and ammonium sulphate as a nitrogen source. It is demonstrated that microbodies of the yeasts C. utilis and H. polymorpha are the exclusive sites of the enzyme glutamate-oxaloacetate aminotransferase (GOT). In yeasts GOT has a unique function in the biosynthesis (and also in the degradation) of the amino acid L-aspartate. Since GOT is probably a constitutive enzyme, these organisms always contain microbodies.