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Inducible Integral Membrane Proteins in Microbody-Membranes of *Hansenula Polymorpha*; β -Oxidation in Methylotrophic Yeasts

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specimen and the backscattered electron (BE) signal intensity is described by:

$$I(\text{be}) = I_0 \cdot q \cdot m \cdot G$$

with $I(\text{be})$ = BE signal intensity, I_0 = primary electron beam intensity, m = specimen mass thickness, G = factor depending on specimen composition, q = constant accounting for detecting system properties. The BE signal can be used to determine mass thickness for specimens in the range between 0.2 and 10 μm at a density of 1000 kg/m^3 . Below 0.2 μm the signal is too weak to be useful. In the same way the forward scattered electron (transmitted electron, TE) signal can be used to measure mass thicknesses in the lower range: 0.05 to 0.2 μm , since under certain conditions forward scattering of electrons by a thin specimen is at least 100 times more efficient than backward scattering.

The two methods for mass determination have been used in a transmission electron microscope EM400 equipped with a STEM attachment interfaced to an EDAX 9100/75 X-ray microanalytical system. Using quantitative X-ray microanalytical data the linearity of the relation between the elemental content and the measured droplet mass has been investigated for droplets containing known proportions of salts and sucrose, in a range of droplet volumes from 100 femtoliter to 1 nanoliter.

INDUCIBLE INTEGRAL MEMBRANE PROTEINS IN MICROBODY-MEMBRANES OF HANSENULA POLYMORPHA

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In yeasts the development and metabolic function of microbodies is largely dependent on growth conditions. In fact it is possible, simply by changing the composition of the growth medium, to induce microbody proliferation and to introduce new enzymes for new metabolic functions into the organelles. We have now studied microbody-membranes of H. polymorpha with respect to substructure and presence of unique, inducible peptides in relation to environmental conditions. Under all growth conditions used the organelles are surrounded by a single membrane of 8 nm which lacks ribosomes. Using standard freeze-etch procedures no large integral proteins (IMPs) were detected. However, when metabolically active cells were used

for spray-freezing, small particles could be detected on both fracture faces of the membrane. The presence of specific IMPs was confirmed biochemically. On PAA gels of isolated microbody-membranes different protein bands were observed in differently grown cells. Prominent bands were observed at the 30 kD, 33 kD, 40 kD and 47 kD positions. The IMP nature of some of these proteins was confirmed immunocytochemically.

β -OXIDATION IN METHYLOTROPHIC YEASTS

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Various yeasts can grow on oleic acid as the sole carbon source. Growth at the expense of this compound is associated with the synthesis of the complete set of β -oxidation enzymes, which are localized in peroxisomes. Also some methylotrophic yeasts (i.e. Candida boidinii) can utilize oleic acid for growth. Under these conditions only one type of peroxisome is present in the cells and the β -oxidation enzymes are in the same compartment as e.g. alcohol oxidase. Another methylotrophic yeast, Hansenula polymorpha, is unable to grow on oleic acid although the complete β -oxidation cycle is induced after a shift of glucose-grown cells into oleic-acid-containing media. In addition enhanced levels of catalase, isocitrate lyase, malate synthase and alcohol oxidase were found in these cultures. At the ultrastructural level such cells were characterized by the presence of many subcellular compartments of varied morphology. Some showed a crystalline substructure but most displayed an amorphous matrix and were surrounded by a number of membranous layers. Kinetic experiments revealed that these organelles originated from the small microbodies present in the glucose-grown inoculum cells. However, cytochemistry and immunocytochemistry revealed that the amorphous organelles could not be considered peroxisomes since all the proteins mentioned above were only present in the peroxisomes typified by the crystalline substructure. Presence of other compartments is explained by an excessive phospholipid synthesis induced by oleic acid. The possible role of peroxisomes in this process is not yet clear.